Sporovexins A−C and a New Preussomerin Analog: Antibacterial and Antifungal Metabolites from the Coprophilous Fungus *Sporormiella vexans*

Ashish G. Soman,[†] James B. Gloer,*[,†] Brenda Koster,[‡] and David Malloch[‡]

Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, and Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S1A1

Received December 9, 1998

Sporovexins A−C (**1**−**3**) and 3′-*O*-desmethyl-1-epipreussomerin C (**4**) have been isolated from liquid cultures of the coprophilous fungus *Sporormiella vexans* (JS 306). The structures of these new metabolites were elucidated on the basis of MS and NMR analysis. Compounds **1** and **4** show antifungal activity against competitor fungi, as well as antibacterial effects.

Interspecies antagonism within fungal communities has been frequently observed and reported.[1−3] Such interactions may play an important role in patterns of colonization and successional changes within fungal ecosystems. Our studies of antagonistic coprophilous (dung-colonizing) fungi have led to the discovery of a variety of new antifungal agents.[3−7] During our continuing studies in this area, liquid cultures of *Sporormiella vexans* (Auerswald) Ahmed and Cain (JS 306) (Sporormiaceae) were examined chemically. These efforts resulted in the isolation of three new *p*-hydroxybenzoic acid derivatives (sporovexins A−C; **1**−**3**) and a new preussomerin analogue, 3′-*O*-desmethyl-1-epipreussomerin C (**4**). Details of this work are presented here.

Silica gel chromatography of the EtOAc extract from *S. vexans* liquid cultures, followed by semipreparative reversed-phase HPLC, afforded compound **1** as the major antifungal component. A molecular ion at *m*/*z* 238 was observed in the EI mass spectrum of **1**, and the negative-ion HRESI mass spectrum contained an ion at *m*/*z* 259.0581 [$C_{12}H_{12}O_5$-Na $(M + Na - 2H)^-$; Δ 0.1 mmu]. These data suggested a molecular formula of $C_{12}H_{14}O_5$. Although the ^{13}C NMR spectrum revealed only 10 resonances, two sets of signals (δ 115.0 and 132.5) were ultimately assigned to degenerate carbons on the basis of symmetry, accounting for all 12 carbons. ^1H NMR and DEPT results provided evidence for all but two (exchangeable) protons. The ^1H and ^{13}C NMR data for **1** (Table 1) indicated the presence of a monooxygenated, para-disubstituted benzene ring and a 4-oxy-2-methylbutanoic acid unit.

Results of HMBC experiments verified the identities of these structural units. In addition, an HMBC correlation observed between the oxygenated methylene protons (H_2-8) and the oxygenated aromatic carbon C-4 allowed connection of the 4-oxy-2-methylbutanoic acid unit to the para-disubstituted benzene ring via an ether linkage. The remaining CHO_2 unit, the second exchangeable hydrogen, and the final degree of unsaturation must be accounted for by a second COOH group. Based on these data, the structure of sporovexin A was assigned as **1**. The absolute stereochemistry at C-10 was not determined. Relatively few naturally occurring O-substituted *p*-hydroxybenzoic acid derivatives have been reported in the literature. The closest known analogue to **1**, 4-prenyloxybenzoic acid, has been reported from both plant and fungal sources.[8]

Analysis of ^1H, ^{13}C, and DEPT NMR spectra of sporovexin B (**2**) suggested the molecular formula $C_{12}H_{14}O_6$, differing from that of compound **1** by an additional oxygen atom. This formula was confirmed by HREIMS. The EI mass spectrum contained a fragment ion corresponding to loss of water not observed as a major fragment in the spectrum of **1**. In addition, the non-oxygenated methylene signal observed in the ^{13}C NMR spectrum of **1** (δ 33.5) was replaced by an oxygenated methine carbon resonance in the spectrum of **2** (δ 72.3). Correspondingly, the ^1H NMR spectrum differed from that of compound **1** by the appearance of a new oxygenated methine multiplet (δ 4.11) in place of the CH_2 signals at δ 2.16 and 1.90 observed in the spectrum of **1**. These observations, together with the expected changes in coupling patterns and NMR data otherwise closely paralleling those for **1**, led to the assignment of structure **2** for sporovexin B. The stereochemistry at C-9 and C-10 was not determined.

The EI mass spectrum of the third related metabolite, sporovexin C (**3**), contained a molecular ion at *m*/*z* 309, and negative-ion HRESI mass spectral analysis led to assignment of the molecular formula $C_{15}H_{19}NO_6$. DEPT data indirectly indicated the presence of three exchangeable protons. The ^1H NMR spectrum revealed the same two isolated spin-systems found in compound **1**, and HMBC correlations of the H_2-8 protons with the oxygenated aromatic carbon again confirmed an ether linkage of the 4-oxy-2-methylbutanoic acid unit to the aromatic ring. The ^1H NMR spectrum also contained signals corresponding to a third spin-system consisting of two mutually coupled methylene triplets at δ 2.60 and 3.60. The chemical shifts and HMBC correlations of these new methylene units (Table 1) were suggestive of an N-acylated β-alanine subunit. The presence of the β-alanine group was further supported by intense EIMS fragment ions at *m*/*z* 88 and $[M - 88]^+$. This subunit accounted for the remaining atoms and unsaturations required by the molecular formula. Correlation of the signal for the aromatic ring protons H-2/H-6 to the carbonyl that must acylate the β-alanine unit (C-7) led to assignment of the structure as shown in **3**.

Analysis of compound **4** by HRFABMS and ^{13}C NMR suggested the molecular formula $C_{20}H_{14}O_8$, indicating the presence of 14 unsaturations. The ^1H NMR spectrum contained resonances indicating the presence of 1,2,3-trisubstituted and 1,2,3,4-tetrasubstituted aromatic rings, and an α-hydroxy epoxide. Comparison of the data with those in our spectral library suggested a close resemblance to the preussomerin class of compounds.[6,9,10] In fact, the

* To whom correspondence should be addressed. Tel.: (319) 335-1361. Fax: (319) 335-1270. E-mail: james-gloer@uiowa.edu.
[†] University of Iowa.
[‡] University of Toronto.

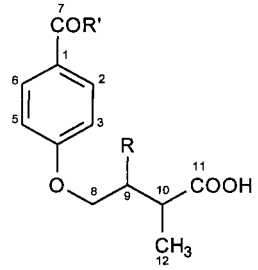
10.1021/np980563c CCC: $18.00
Published on Web 03/26/1999

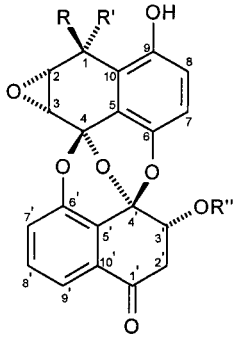
Table 1. NMR Data for Sporovexins A (**1**), B (**2**), and C (**3**)[a]

	1[b]			**2**[c]		**3**[d]		
C#	δ_H (mult., *J* in Hz)	δ_C	HMBC correlations (H → C#)	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C	HMBC correlations (H → C#)
1		123.6			123.2 [f]		127.4	
2/6	7.97 (d, 8.7)[e]	132.5	4, 6/2, 7	7.86 (d, 8.7)[e]	131.3	7.75 (d, 8.7)[e]	129.8	4, 6/2, 7
3/5	7.02 (d, 8.7)[e]	115.0	1, 4, 5/3	6.99 (d, 8.7)[e]	114.3	6.95 (d, 8.7)[e]	114.9	1, 4, 5/3
4		163.7			162.2		162.8	
7		167.3			167.1 [f]		169.6	
8a	4.16 (ddd, 13, 9.7, 6.4)	66.8	4, 9, 10	4.04 (dd, 10, 3.9)	70.4	4.08 (br t, 6.5)	66.8	4, 9, 10
8b	4.15 (ddd, 13, 9.7, 6.5)		4, 9, 10	3.98 (dd, 10, 6.2)				
9	2.16 (m) 1.90 (m)	33.5	8, 10, 11, 12	3.93 (m)	70.4	2.15 (m) 1.87 (m)	33.8	8, 10, 11, 12
10	2.71 (ddq, 7.2, 7.0, 6.8)	36.5	8, 9, 11, 12	2.60 (m)	43.0	2.67 (m)	37.4	8, 9, 11, 12
11		177.2			175.6[f]		180.0 [g]	
12	1.23 (d, 7.1)	17.5	9, 10, 11	1.06 (d, 7.2)	13.1	1.22 (d, 7.1)	17.4	9, 10, 11
13						3.60 (br t, 7)	36.8	7, 14, 15
14						2.60 (br t, 7)	34.7	13, 15
15							175.5	

[a] All NMR spectra were recorded at 600 MHz (^{1}H) and 75 MHz (^{13}C). [b] Acetone-d_6 solutions. [c] DMSO-d_6 solutions. [d] Methanol-d_4 solutions. [e] These multiplets appeared as distorted doublets as a result of magnetic inequivalence. [f] These chemical shift values were obtained from selective INEPT experiments. [g] Obtained from HMBC data.

Chart 1





1 R = H; R' = OH

2 R = R' = OH

3 R = H; R' = $NH\overset{13}{C}H_2\overset{14}{C}H_2\overset{15}{C}OOH$

4 R = OH; R' = R'' = H

5 R = H; R' = OH; R'' = CH_3

^{13}C NMR spectrum of **4** is almost identical to that of preussomerin C (**5**), except for the absence of the methoxy signal, a corresponding upfield shift in the C-3′ signal, and significant differences in the shift values for C-1 and C-10. Analysis of HMBC data, chemical shifts, and ^{1}H NMR *J* values verified the structure of **4** as a 3′-*O*-desmethyl analogue of preussomerin C. The only other difference between **4** and **5** appears to be a stereochemical change in the epoxy alcohol moiety at positions 1−3. The absolute stereochemistry of the preussomerins was originally assigned on the basis of X-ray crystallography and chemical degradation experiments conducted on preussomerin A.[9] Preussomerins A−D all possess this same epoxy alcohol moiety, and all were originally assigned the same stereochemistry at positions 1−3 on the basis of nearly identical NMR data (e.g., δ values for C-1 all within 0.5 ppm; δ values for C-10 all within 0.7 ppm).[6] However, the chemical shift of C-1 in **4** is 7.2 ppm upfield from its position in compound **5** in the same solvent, while the shift of C-10 is 4.4 ppm downfield from its position in **5**. Some differences in relevant ^{1}H δ-values were also noted. Moreover, the *J* value between H-1 and H-2 was measured as 2.9 Hz in **4**, but only 0.5 Hz in **5**. Finally, a noticeable four-bond ^{1}H−^{1}H coupling between H-1 and H-3 (0.7 Hz) was observed in the spectrum of **4**, but not in the spectrum of **5**, which would be consistent with a difference in relative orientation of H-1 and H-3. Unfortunately, NOESY data have not been useful in establishing the stereochemistry of this class of compounds.[6] On the basis of all of these data, together with the fact that the data otherwise match those of **5** very closely, the structure of **4** was assigned as 3′-*O*-desmethyl-1-epipreussomerin C. It is conceivable that the relative configurations at C-1, 2, and 3 are all inverted relative to those shown in **4** (i.e., that it is the epoxide stereochemistry that is actually inverted relative to the corresponding positions in **5**), but it was not possible to determine this with the available data. Based on the specific spectral differences, together with the fact that some known preussomerins contain keto groups at C-1, it was felt that stereostructure **4** is more likely.

Preussomerins have been reported to show activity as antifungal agents[6,9] and as *Ras* farnesyl-protein transferase inhibitors.[10] It is intriguing to note that both prior reports of this unusual class of compounds have also arisen through studies of other coprophilous fungal isolates from the family Sporormiaceae.

Sporovexin A (**1**) was active in a standard disk assay[11] against *Bacillus subtilis* (ATCC 6051), affording a zone of inhibition of 9 mm at 100 μg/disk. It also showed activity in a centerpoint inoculation disk assay[12] against the coprophilous fungus *Ascobolus furfuraceus* (NRRL 6460), causing 90% reduction in radial growth rate at 200 μg/disk. At the same level, 3′-*O*-desmethyl-1-epipreussomerin C (**4**) caused a 40% reduction in radial growth rate of *A.*

furfuraceus. Compounds **1**−**4** were found to be inactive in a standard disk assay versus *Candida albicans* (ATCC 10453) at 200 μg/disk. Members of the genus *Sporormiella* have afforded a number of interesting bioactive metabolites in recent years.[13−17] However, to our knowledge, there have been no prior reports of metabolites from *S. vexans*.

Experimental Section

General Experimental Procedures. HPLC employed a 5-μm Beckman Ultrasphere C_{18} column (1.0 × 25 cm): flow rate, 2.0 mL/min; solvent system, 10−50% MeCN in 0.1% HCOOH/H_2O for 40 min; monitored by UV absorption at 215 nm. NMR spectra were recorded using CD_3COCD_3, DMSO-d_6, or CD_3OD solutions, and were referenced to the corresponding solvent signals. Selective INEPT experiments were optimized for ${}^nJ_{CH}$ = 7 Hz. HMQC and HMBC experiments were optimized for ${}^1J_{CH}$ = 150 Hz and ${}^nJ_{CH}$ = 8 Hz, respectively. MS data were recorded on a VG ZAB-HF or a Fisons Autospec mass spectrometer. Descriptions of other NMR parameters, as well as NMR, UV, IR, and other analytical instrumentation, have been provided elsewhere.[18]

Fungal Culture. The culture of *S. vexans* employed in this work was a subculture of an isolate originally obtained from a sample of porcupine dung collected by D.M. near St. Andrews Marine Research Station in New Brunswick, Canada, in August, 1995. The culture was assigned the accession number JS 306 in the D. Malloch culture collection at the University of Toronto. The dung sample was incubated in a laboratory moist chamber, and ascomata of *S. vexans* were observed approximately 2 weeks later. Five 2-L Erlenmeyer flasks, each containing 400 mL of potato dextrose broth (Difco) were inoculated with several 1-cm^2 agar plugs taken from stock cultures maintained on potato dextrose agar. Flask cultures were incubated at 25−28 °C and aerated by agitation on an orbital shaker at 150 rpm for a period of 26 days.

Extraction and Isolation. The culture filtrate was extracted with EtOAc (4 × 1 L), and the organic phase was dried ($MgSO_4$) and evaporated to give 220 mg of crude extract. The EtOAc extract was chromatographed on a silica gel column (2 × 45 cm) with a stepwise gradient from 0 to 40% (v/v) MeOH in CH_2Cl_2. Two antifungal fractions eluting with 10% and 20% MeOH in CH_2Cl_2 (51 and 60 mg, respectively) were further purified by semipreparative reversed-phase HPLC. The first fraction afforded sporovexin A (**1**; 16.6 mg, t_R 37 min) and 3′-*O*-desmethyl-1-epipreussomerin C (**4**; 2.9 mg, t_R 29.5 min), and the second fraction yielded sporovexin B (**2**; 1.7 mg, t_R 25.3 min) and sporovexin C (**3**; 2.4 mg, t_R 29.8 min).

Sporovexin A (1): pale yellow solid; mp 177−179 °C; $[\alpha]_D$ −22° (*c* 0.4 g/dL, MeOH, 25 °C); UV λ_{max} (MeOH) 251 nm (ε 3400); IR ν_{max} (CaF_2) 3352, 1708, 1690, 1607 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 238 (M^+, rel int. 5), 221 (2), 165 (3), 138 (100), 121 (77); negative-ion HRESI obsd [M + Na − 2H]$^-$ at *m*/*z* 259.0581, calcd for $C_{12}H_{12}O_5Na$, 259.0582.

Sporovexin B (2): pale yellow oil; $[\alpha]_D$ −12° (*c* 0.1 g/dL, MeOH, 25 °C); UV λ_{max} (MeOH) 252 nm (ε 3800); IR ν_{max} (CaF_2) 3400, 1708, 1685, 1609 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 254 (M^+, rel int. 3), 236 (2), 151 (13), 138 (100), 121 (61); HREIMS obsd at *m*/*z* 254.0777, calcd for $C_{12}H_{14}O_6$, 254.0786.

Sporovexin C (3): colorless oil; $[\alpha]_D$ -11° (*c* 0.15 g/dL, MeOH, 26 °C); UV λ_{max} (MeOH) 250 nm (ε 4900); IR ν_{max} (CaF_2) 3375, 1718, 1702, 1684, 1636, 1559, 1459 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 309 (M^+, rel int. 2), 221 (2), 209 (12), 191 (8), 121 (100), 88 (23); negative-ion HRESI obsd [M − H]$^-$ at *m*/*z* 308.1150, calcd for $C_{15}H_{18}O_6N$, 308.1129.

3′-*O*-Desmethyl-1-epipreussomerin C (4): colorless oil; $[\alpha]_D$ −123° (*c* 0.25 g/dL, MeOH, 28 °C); UV λ_{max} (MeOH) 251 (ε 5400), 307 (4200) nm; IR ν_{max} (CH_2Cl_2) 3630, 3055, 2855, 1698, 1597, 1460 cm^{-1}; ^1H NMR (600 MHz, CD_3COCD_3) δ (mult, *J* in Hz, H#) 8.51 (br s, 9-OH), 7.54 (dd, 7.7, 1.1, H-9′), 7.44 (br t, 8, H-8′), 7.17 (dd, 8.3, 1.1, H-7′), 6.89 (d, 8.8, H-8), 6.73 (d, 8.8, H-7), 5.53 (m, H-1), 5.02 (br s, 3′-OH), 4.69 (br dt, 5.4, 2.9, H-3′), 4.22 (br d, 6.8, 1-OH), 3.86 (dd, 4.0, 0.7, H-3), 3.76 (dd, 4.0, 2.9, H-2), 3.36 (dd, 18, 3.1, H-2′a), 2.85 (dd, 18, 2.7, H-2′b); ^{13}C NMR (75 MHz, CD_3COCD_3) δ194.5 (C-1′), 152.0 (C-6′), 151.1 (C-9), 143.7 (C-6), 131.9 (C-10′), 131.4 (C-8′), 122.2 (C-7′), 121.7 (C-10), 121.6 (C-5′), 120.0 (C-9′), 119.3 (C-8), 117.6 (C-7), 116.2 (C-5), 96.3 (C-4), 94.6 (C-4′), 70.9 (C-3′), 62.1 (C-1), 52.5 (C-3), 51.8 (C-2), 43.2 (C-2′); EIMS *m*/*z* 382 (M^+, rel int. 12), 366 (21), 348 (47), 319 (28), 191 (6), 176 (12), 91 (14), 83 (17), 69 (21), 44 (100); HRFABMS (thioglycerol) obsd *m*/*z* 383.0762 (M + H), calcd for $C_{20}H_{15}O_8$, 383.0767.

Acknowledgment. We thank the National Institutes of Health (R01 AI 27436) for financial support.

References and Notes

(1) Wicklow, D. T. In *The Fungal Community: Its Organization and Role in the Ecosystem*; Wicklow, D. T., Carroll, G. C., Eds.; Marcel Dekker: New York, 1981; Vol. 2, Chapter 19, pp 351−375.
(2) Widden, P. In *The Mycota IV*; Wicklow, D. T.; Söderström, B., Eds.; Springer-Verlag: Heidelberg, 1997; Chapter 9, pp 135−147.
(3) Gloer, J. B. In *The Mycota IV*; Wicklow, D. T.; Söderström, B., Eds.; Springer-Verlag: Heidelberg, 1997; Chapter 16, pp 249−268.
(4) Weber, H. A.; Baenziger, N. C.; Gloer, J. B. *J. Org. Chem.* **1988**, *53*, 4567−4569.
(5) Weber, H. A.; Gloer, J. B. *J. Nat. Prod.* **1988**, *51*, 879−883.
(6) Weber, H. A.; Gloer, J. B. *J. Org. Chem.* **1991**, *56*, 4355−4360.
(7) Wang, Y.; Gloer, J. B.; Scott, J. A.; Malloch, D. *J. Nat. Prod.* **1993**, *56*, 341−344.
(8) Nozawa, K.; Takada, M.; Udagawa, S.-I.; Nakajima, S.; Kawai, K.-I. *Phytochemistry* **1989**, *28*, 655−656.
(9) Weber, H. A.; Baenziger, N. C.; Gloer, J. B. *J. Am. Chem. Soc.* **1990**, *112*, 6718−6719.
(10) Singh, S. B.; Zink, D. L.; Liesch, J. M.; Ball, R. G.; Goetz, M. A.; Bolessa, E. A.; Giacobbe, R. A.; Silverman, K. C.; Bills, G. F.; Pelaez, F.; Cascales, C.; Gibbs, J. B.; Lingham, R. B. *J. Org. Chem.* **1994**, *59*, 6296−6302.
(11) Johnson, L. F.; Curl, E. A.; Bond, J. H.; Fribourg, H. A. *Methods for Studying Soil−Microflora−Plant Disease Relationships*; Burgess Publishing: Minneapolis, 1960; p 71.
(12) Gloer, J. B.; Truckenbrod, S. M. *Appl. Environ. Microbiol.* **1988**, *54*, 861−864.
(13) Weber, H. A.; Swenson, D. C.; Gloer, J. B.; Malloch, D. *Tetrahedron Lett.* **1992**, *33*, 1157−1160.
(14) Bills, G. F.; Pelaez, F.; Polishook, J. D.; Diez-Matas, M. T.; Harris, G. H.; Clapp, W. H.; Dufresne, C.; Byrne, K. M.; Nallin-Omstead, M.; Jenkins, R. G.; Mojena, M.; Huang, L.; Bergstrom, J. D. *Mycol. Res.* **1994**, *98*, 733−739.
(15) Hensens, O. D.; Helms, G. L.; Turner-Jones, E. T.; Harris, G. H. *J. Org. Chem.* **1995**, *60*, 1772−1776.
(16) Wang, Y.; Gloer, J. B.; Scott, J. A.; Malloch, D. *J. Nat. Prod.* **1995**, *58*, 93−99.
(17) Harris, G. H.; Dufresne, C.; Joshua, H.; Koch, L. A.; Zink, D. L.; Salmon, P. M.; Goklen, K. E. Kurtz, M. M.; Rew, D. J.; Bergstrom, J. D.; Wilson, K. E. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2403−2408.
(18) Alfatafta, A. A.; Gloer, J. B.; Scott, J. A.; Malloch, D. *J. Nat. Prod.* **1994**, *57*, 1696−1702.

NP980563C