Septocylindrins A and B: Peptaibols Produced by the Terrestrial Fungus *Septocylindrium* sp. LL-Z1518[⊥]

Mia Y. Summers,* Fangming Kong, Xidong Feng, Marshall M. Siegel, Jeffrey E. Janso, Edmund I. Graziani, and Guy T. Carter

Department of Chemical and Screening Sciences, Wyeth Research, 401 N. Middletown Road, Pearl River, New York 10965

Received November 14, 2006

Two new peptaibols, septocylindrin A (**1**) and septocylindrin B (**2**), related to the well-studied membrane-channel-forming peptaibol alamethicin, were obtained from a terrestrial isolate of the fungus *Septocylindrium* sp. Both **1** and **2** are linear 19-amino acid peptides with a modified phenylalanine C-terminus. Analysis of the HRMS data indicated that they differ only in the 18th residue, where **1** contains Glu and **2** contains Gln. The structures of these two peptaibols were determined by extensive NMR and HRMS analysis. The absolute configurations of amino acids present in **1** were determined using Marfey's methodology. Both compounds were isolated through bioassay-guided fractionation and exhibited significant antibacterial and antifungal activity.

Peptaibols comprise a large class of peptides characterized by a high number of α-aminoisobutyric acid (Aib) residues, an acetylated N-terminus, and a 2-amino alcohol C-terminus.[1] They include such compounds as alamethicins, zervamicins, antiamoebins, and chrysospermins.[1,2] Peptaibols are commonly isolated from fungi and exhibit antibacterial and antifungal activity.[2] These antibiotics are also known for their membrane-modifying and pore-forming abilities. They are generally known to promote voltage-dependent ion channel formation in lipid bilayer membranes. Alamethicin is one of the best studied models for peptaibol membrane channel activity.[3,4]

Alamethicin has been shown to be a mixture of chromatographically separable components.[5] The structures of alamethicins I and II were revised in 1977 using ^{13}C NMR spectroscopy and high-resolution field desorption mass spectrometry and were shown to contain a phenylalaninol C-terminus.[6] Structural studies of alamethicins have shown that Aib residues aid in forming the helical structures of peptaibols.[7] Aggregation due to the amphipathic nature of alamethicins causes formation of helical bundles with hydrophobic exteriors that contact the lipid membranes. The hydrophilic interior of the channels contain Gln-7, Glu/Gln-18, and Gln-19 residues, which appear to be important for conductance.[7] Alamethicins are some of the most potent antibiotics in this class against Gram-positive bacteria and exhibit cytotoxic activity against wall-less eubacteria and diverse mammalian cell lines.[8] The mechanism of these activities is considered to be predominantly through its pore-forming ability.[8,9] Other biological activities of peptaibols include hemolysis, uncoupling of oxidative phorsphorylation in mitochondria, inhibition of amoeba cell growth, and antimycoplasmic activities.[10]

Results and Discussion

Two new peptaibols, septocylindrin A (**1**) and septocylindrin B (**2**), were isolated from *Septocylindrium* sp. LL-Z1518, a terrestrial fungus from the Wyeth culture collection. The compounds were purified using bioassay-guided fractionation, through initial elution from HP-20 resin followed by HPLC purification. Their structures were determined using 1D and 2D NMR and HRESI-FTICR-MS (high-resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry) techniques. Septocylindrin A (**1**) and septocylindrin B (**2**) are structurally similar to the well-studied membrane-channel-forming peptaibol alamethicin, with 19 amino acids and an extended C-terminus. More specifically, they are comparable to alamethicins F30 and F50, which differ only in the 18th residue, where F30 has Glu and F50 has Gln.[5] Analysis of HRMS data indicated that **1** and **2** have the molecular formulas $C_{94}H_{155}N_{23}O_{25}$ [$(M + H)^+$: 2007.16649, calcd 2007.16372, Δ = 2.77 mmu] and $C_{94}H_{156}N_{24}O_{24}$ [$(M + H)^+$: 2006.18009, calcd 2006.17971, Δ = 0.38 mmu], respectively. Owing to signal overlap in the NMR spectra, sequential MS/MS fragmentation information was instrumental in elucidating the sequences of these peptaibols. The difference of 0.98640 amu between the two compounds was attributed to a difference in the peptide sequences, where the 18th residue in **1** is Glu and in **2** is Gln [calcd (Glu − Gln): 129.04259 − 128.05858 = 0.98401 amu, Δ = 2.39 mmu]. This difference was confirmed by HRMS analysis of the MS/MS fragments.

Initial analysis of the ^1H and ^{13}C NMR spectra of **1** indicated a peptide structure (Table 1). In addition, a multiplicity-edited ^{15}N HSQC experiment showed 18 NH protons and 3 NH_2 units. The high number of putative amino acid residues was confirmed by analysis of the carbonyl region of the ^{13}C NMR spectrum, which showed 22 carbons with chemical shifts between δ 169 and 177. Eight quaternary carbons between δ 55.6 and 56.2 suggested the presence of eight Aib units. Two Ala units were identified through HMBC correlations from the α protons at δ 3.93 and 3.96 to the methyl carbons at δ 16.2 and 16.1, respectively.

The multiplicity-edited HSQC spectrum showed significant overlap of signals in the geminal dimethyl region (^{13}C δ 22−26 and ^1H δ 1.39−1.52), which were attributed to the Aib residues. Analysis of HMBC, multiplicity-edited HSQC, COSY, and TOCSY experiments allowed unambiguous assignment of the Pro-2, Gln-7, Val-9, Gly-11, Leu-12, Pro-14, Val-15, and Gln-19 residues.

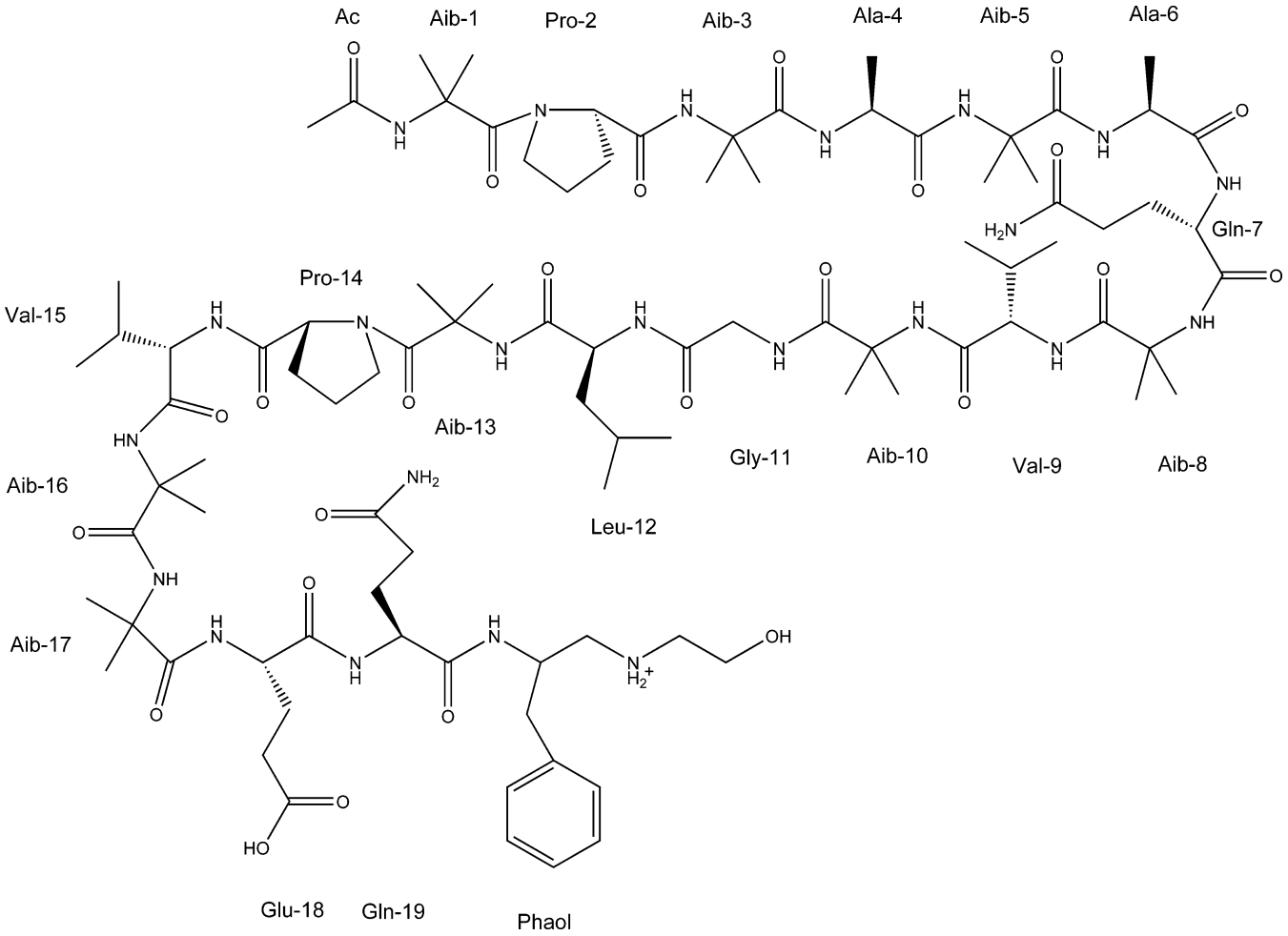
Assignment of the Glu-18 residue was more difficult due to severe overlap of signals for Gln-7 and Gln-19 in the NOESY spectrum. However, analysis of MS fragmentation data and careful examination of the NOESY spectrum indicated the presence of signals from a third amino acid overlapped in this region that could be attributed to the Glu-18 residue. It was known that two Gln units were present from the δ 6.63, 7.04 (Gln-19) and δ 6.74, 7.17 (Gln-7) signals in the ^{15}N NMR spectrum. The NOESY spectrum showed correlations from the δ 7.83 (Gln-19) and 7.85 (Gln-7) NH protons to the δ 3.85 and 3.79 α protons, respectively. These residues showed NOESY correlations from each NH proton to two neighboring sets of methylene protons in the δ 2 region. A third NH proton at δ 7.79 (Glu-18) with the corresponding α proton at δ 3.81 was also found overlapping the first two sets of methylene

[⊥] Dedicated to the late Dr. Kenneth L. Rinehart of the University of Illinois at Urbana−Champaign for his pioneering work on bioactive natural products.

* Author to whom correspondence should be addressed. Tel: 845-602-5493. Fax: 845-602-5687. E-mail: summerm@wyeth.com.

10.1021/np060571q CCC: $37.00
Published on Web 02/09/2007

Chart 1



1

1 Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phaol$^+$

2 Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Phaol$^+$

protons in the δ 2 region. There was no correlation seen from any of these three NH protons to NH_2 protons of Gln or the OH proton of Glu; therefore it could not be distinguished which of the three amino acids was Glu. However, the presence of Glu was confirmed by the observation of only three NH_2 units in the ^{15}N HSQC experiments, which were accounted for in Gln-7, Gln-19, and the C-terminus and also confirmed by MS fragmentation patterns. In Figure 1a, Glu was confirmed at position 18 in **1** by the difference between the fragment at *m*/*z* 496.27626 and 367.23409. The accurate mass difference of 129.04217 is consistent with a Glu residue (calcd 129.04259, $\Delta = -0.42$ mmu).

Assignments for the C-terminus of **1** as shown in Figure 2 were made as follows. ^1H, ^{13}C, HSQC, and HMBC data clearly showed the presence of a phenyl ring. Analysis of the HMBC spectrum showed correlations from the methylene protons at δ 2.87 and 2.58 (H_2-5) to the adjoining nonprotonated aromatic carbon at δ 137.9 (C-6, Figure 2). These methylene protons were also coupled in the TOCSY spectrum to the α proton at δ 4.42 (H-4). This α proton showed an HMBC correlation to another methylene carbon at δ 50.1 (C-3) with attached protons at δ 3.26 and 3.17. TOCSY correlations from the NH proton at δ 7.12 to both H_2-3 and H_2-5 completed the C-3 to C-9 portion of the spin system as shown in Figure 2. A key NOESY correlation from the δ 7.12 NH proton to the Gln-19 α proton at δ 3.85 linked the end unit with the rest of the peptide.

The OH proton signal at δ 5.22 was coupled in the TOCSY spectrum to two NH protons at δ 7.87 and 8.19 (NH_2^+). This OH proton also had TOCSY correlations to two methylene protons at δ 3.08 (H_2-2). The C-2 methylene protons correlated in the TOCSY spectrum to methylene protons at δ 3.70 (H_2-1) and also showed HMBC correlations to methylene carbons at δ 56.6 (C-1) and δ 50.1 (C-3), confirming the connection between the reduced phenylalanine unit to the amino alcohol. TOCSY correlations from the H-2, H-3, and H-5 methylenes as well as the H-4 α proton at δ 4.42 to the ammonium NH signals at δ 7.87 and 8.19 further confirmed the location of the amino salt and secured the structure of the phenylalanine-amino alcohol portion of the molecule. The downfield shifts of the NH_2 proton signals of this amino salt in the ^{15}N HSQC spectrum and the broad peak shapes of the δ 7.87 and 8.19 signals in the ^1H NMR spectrum were consistent with the presence of this positively charged end unit. This indicated that the isolated septocylindrium A (**1**) was a cation.

Despite extensive signal overlap, the peptide backbone structure was determined using multiplicity-edited HSQC and HMBC NMR experiments. To further confirm the linear sequence of **1**, mass spectrometric fragmentation studies were performed. The combination of NMR and high-resolution mass spectrometric analysis has proven to be an effective method for characterizing the sequence of peptaibols, as demonstrated in structure revision of alamethicins I and II.[6]

Table 1. ^{1}H and ^{13}C NMR Data for **1** in DMSO-d_6

residue		^{13}C	^{1}H (mult., *J* Hz)[a]	residue		^{13}C	^{1}H (mult., *J* Hz)[a]
Ac	C═O	170.3		Leu-12	C═O	173.9	
	CH_3	22.4	2.00		1	51.5	4.31
Aib-1[b]	C═O	173.5			2	39.8	1.52, 1.81
	1	55.6			3	24.0	1.79
	2	23.4	1.44		4	22.8	0.88
	3	23.4	1.39		5	20.9	0.84 (d, 6.3)
	NH		8.81 (s)		NH		7.75
Pro-2	C═O	173.6		Aib-13[b]	C═O	172.9	
	1	62.8	4.12 (t, 8.3)		1	56.2	
	2	28.0	2.26, 1.70		2	22.4	1.52
	3	25.9	1.98, 1.81		3	25.9	1.43
	4	48.3	3.42, 3.82		NH		8.24 (s)
Aib-3[b]	C═O	176.2		Pro-14	C═O	174.1	
	1	55.7			1	62.7	4.29
	2	22.4	1.45		2	28.0	1.70, 2.22
	3	25.6	1.45		3	25.6	1.93
	NH		7.58		4	48.5	3.65, 3.73
Ala-4	C═O	175.5		Val-15	C═O	173.4	
	1	51.5	3.93		1	62.3	3.61
	2	16.2	1.42		2	28.5	2.25
	NH		7.77		3	18.8	0.90
Aib-5[b]	C═O	173.5			4	19.5	0.99 (d, 6.3)
	1	55.7			NH		7.47
	2	22.5	1.45				
	3	25.8	1.45	Aib-16[b]	C═O	174.1	
	NH		7.77		1	55.8	
Ala-6	C═O	175.0			2	22.3	1.41
	1	51.7	3.96		3	25.4	1.48
	2	16.1	1.39		NH		7.83
	NH		7.58	Aib-17[b]	C═O	175.6	
Gln-7[c]	C═O	175.5			1	55.8	
	1	56.0	3.79		2	22.5	1.40
	2	25.6	1.96, 2.03		3	25.6	1.47
	3	31.0	2.15, 2.29		NH		7.73
	C═O (4)	173.1		Glu-18[c]	C═O	174.0	
	NH_2		6.74 (s), 7.17		1	55.7	3.81
	NH		7.85		2	31.2	1.97, 2.08
					3	29.9	2.43, 2.48
Aib-8[b]	C═O	175.5			C═O (4)	173.6	
	1	55.8			OH		n.d.
	2	22.3	1.47		NH		7.79
	3	25.7	1.40	Gln-19[c]	C═O	171.7	
	NH		7.76		1	55.0	3.85
Val-9	C═O	173.1			2	25.6	1.87, 1.95
	1	62.3	3.63		3	30.1	2.42, 2.49
	2	28.7	2.18		C═O (4)	173.0	
	3	19.6	1.01 (d, 6.3)		NH_2		6.63 (s), 7.04 (s)
	4	19.1	0.89		NH		7.83
	NH		7.28				
Aib-10[b]	C═O	175.5		Phaol[d]	NH		7.12
	1	55.7			1	56.6	3.70
	2	22.3	1.44		2	49.0	3.08
	3	25.6	1.44		3	50.1	3.17, 3.26
	NH		8.00 (s)		4	46.2	4.42
Gly-11	C═O	169.7			5	38.0	2.58, 2.87
	1	43.6	3.52, 3.78		6	137.9	
	NH		8.13		7	129.2	7.28
					8	128.0	7.23 (t, 7.5)
					9	126.3	7.17
					OH		5.22
					NH_2^+		7.87, 8.19

[a] Signals were obscured in the ^{1}H NMR spectrum and were assigned using 2D NMR experiments. [b] Due to signal overlap, the Aib signals are interchangeable. [c] Due to signal overlap, the peptide bond N−H and C═O, C-1, C-2, and C-3 signals of these units are interchangeable. [d] Phaol = 2-(2-amino-3-phenylpropylamino)ethanol.

The initial MS fragmentation of **1** (*m*/*z* 2007.16649) occurred between Aib-13 and Pro-14 to form daughter ions at *m*/*z* 1189.69349 and 818.47693, as shown in Figure 3. Both **1** and **2** gave identical *m*/*z* 1189 fragments, within experimental error, leading to the elemental composition of $C_{55}H_{93}N_{14}O_{15}$ [calcd 1189.69447, **1**: 1189.69349, $\Delta = -0.98$ mmu; **2**: 1189.69281, $\Delta = -1.66$ mmu].

FTMS/MS analysis of the intact *m*/*z* 1189.69540 ion of **1** [calcd 1189.69447, $\Delta = 0.93$ mmu] resulted in sequential fragmentation of Aib-13 through Pro-2, as shown in Figure 4. Accurate mass analysis of each fragment and differences in mass between fragments confirmed the sequence of this portion of the peptide. A similar sequential fragmentation pattern was also observed from Aib-8 to Pro-2. The accurate mass difference between the *m*/*z* 310.17592 fragment of Ac to Aib-3 and its inner Pro-2/Aib-3 fragment at *m*/*z* 183.11275 confirmed the Ac−Aib end unit as the N-terminus.

The second set of related daughter ions of **1** and **2** were isolated at *m*/*z* 818.47735 and 817.49319, respectively (Figure 1). Again,

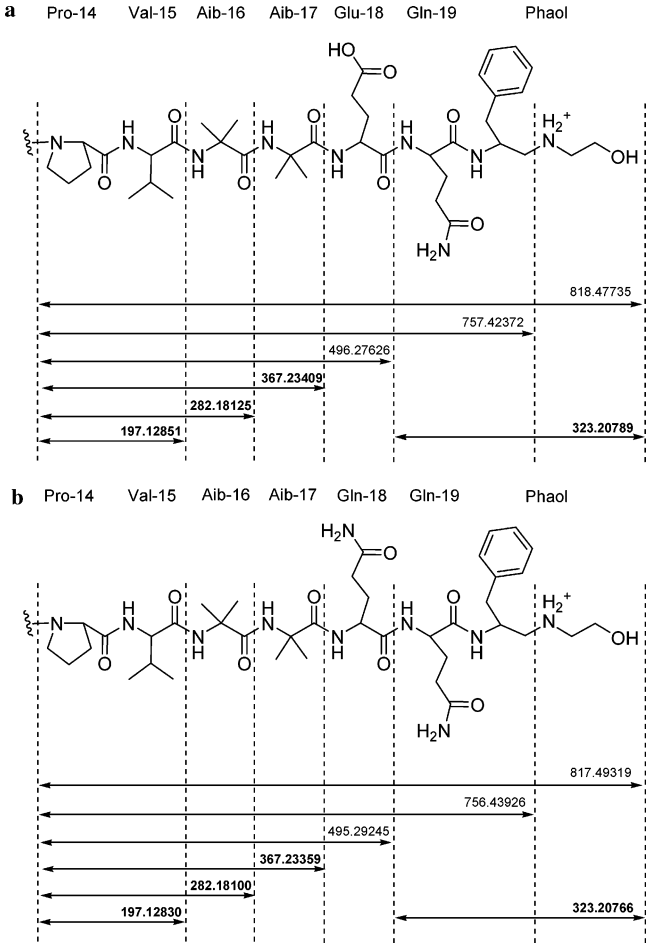


Figure 1. (a) FTMS/MS fragmentation of the *m*/*z* 818 daughter ion of **1**. (b) FTMS/MS fragmentation of the *m*/*z* 817 daughter ion of **2**.

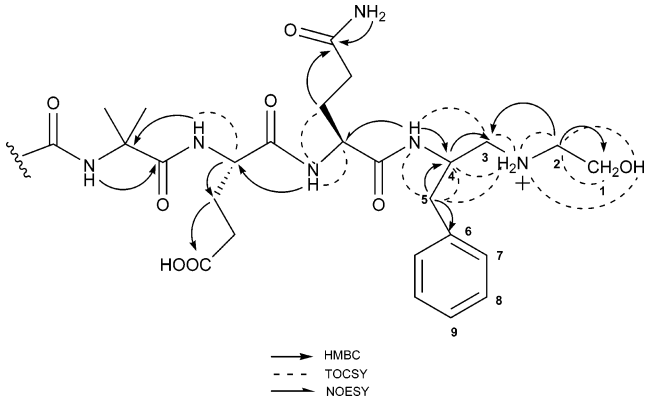


Figure 2. Selected HMBC, TOCSY, and NOESY NMR correlations for the C-terminus backbone of **1**.

through precise FTMS/MS measurement of adjacent amino acids and their fragments, accurate masses from *m*/*z* 197 to 367 showed identical amino acid composition for these two peptaibols. The remaining three MS/MS fragments at *m*/*z* 496−495, 757−756, and 818−817 show mass differences between **1** and **2**. The accurate mass values for fragments at *m*/*z* 496.27626 in **1** and *m*/*z* 495.29245 in **2** were used to attributed the difference to the 18th residue, which is a Glu residue in **1** and a Gln residue in **2**. This mass difference between the two peptaibols was carried through to the next fragment at *m*/*z* 757.42372 in **1** and *m*/*z* 756.43926 in **2**, and finally to the major fragment of the parent compound at *m*/*z* 818.47735 in **1** [$C_{39}H_{64}N_9O_{10}$, calcd 818.47706, $\Delta = 0.29$ mmu] and *m*/*z* 817.49319 in **2** [$C_{39}H_{65}N_{10}O_9$, calcd 817.49305, $\Delta = 0.14$ mmu]. These data confirm the results of the NMR analysis for **1**, where the position-18 amino acid assignment is a Glu residue. All other residues assigned by HRMS were also consistent with the NMR results.

The absolute configurations of the commonly available amino acids present in **1** were determined using Marfey's methodology. The acid hydrolyzate of **1** was treated with Marfey's reagent, 5-fluoro-2,4-dinitrophenyl-L-alanine amide (FDAA), and the resulting FDAA derivatives were analyzed by reversed-phase HPLC. Peaks in the chromatogram were identified by comparing the retention times with those of the FDAA derivatives of the authentic amino acids. The Marfey's reagent derivative of the amino acids liberated from **1** showed peaks matching L-Gln, L-Glu, L-Val, L-Pro, L-Ala, and L-Ile. The absolute configuration of the C-terminus Phaol unit of **1** is likely to be the same *S* configuration as that of aibellin, another peptaibol reportedly containing a Phaol end unit, as the ^1H and ^{13}C chemical shifts assigned for C-4 in both compounds are very similar.[11]

MIC values were measured for septocylindrins A (**1**) and B (**2**), and the results are shown in Table 2. Both **1** and **2** showed significant antibacterial and antifungal activity. The MIC values are in the same range as those of alamethicins and similar peptaibols, zervamicins.[4,8,12]

In summary, septocylindrins A (**1**) and B (**2**) are linear 19-amino acid peptides with a Phaol [Phaol = 2-(2-amino-3-phenylpropylamino)ethanol] C-terminus. The commonly available amino acids in **1** all possess the L-configuration. Compound **1** has the same sequence as alamethicin I, except for the Phaol C-terminus. Although uncommon in peptaibols, this Phaol C-terminus has been previously reported for aibellin, a 20-residue peptaibol isolated from the fungus *Verticimonosporium ellipticum*, which has efficiency-enhancing activity on rumen fermentation.[11] The Phaol end unit has also been reported in compounds WSS1060 and WSS1061 isolated from *Diplospora longispora*. WSS1060 and WSS1061 are reported to be chitin synthetase inhibitors as well as antifungal agents.[13] Septocylindrins A (**1**) and B (**2**) have significant antibacterial and antifungal activity, which is consistent with known peptaibols. With the similarity of these peptaibols to alamethicin, it is presumed that the mechanism of antibacterial and antifungal action might also be through a helical structure and pore-forming ability.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco P-1020 polarimeter. IR spectra were recorded on a Nicolet Nexus 470 instrument in attenuated total reflectance (ATR) mode. ^1H, ^{13}C, and TOCSY experiments were performed on a Bruker Avance 400 MHz spectrometer, while HMBC, NOESY, and multiplicity-edited HSQC experiments were acquired on a Bruker Avance 500 MHz spectrometer equipped with a cryoprobe. NMR spectra were recorded in DMSO-d_6 solution, and the chemical shifts were referenced relative to the corresponding solvent signals ($\delta_H = 2.50$ for ^1H NMR, $\delta_C = 39.5$ for ^{13}C NMR). The delay for the long-range C−H coupling for the HMBC experiment was 63 ms. A mixing time of 500 ms was used in the phase-sensitive NOESY experiment.

Mass spectrometric data were acquired on a Bruker-Daltonics Apex II FTICR mass spectrometer equipped with a Bruker-Magnex 9.4 T superconducting magnet and an Analytica ESI source. The ESI source was operated in the nanoelectrospray mode. Typically, 5 μL of sample was loaded into the nanoelectrospray tip (New Objective, Woburn, MA) and a high voltage (about −700 V) was applied between the tip and the glass capillary. FTMS/MS experiments were performed using SORI-CID (sustained off-resonance irradiation with collision-induced dissociation). The protonated precursor ion was isolated using a correlated sweep and then was selectively activated to collide with Ar gas molecules pulsed into the ultrahigh-vacuum region containing the analyzer cell.

HPLC was performed using a Varian Prostar preparative HPLC system equipped with a PDA (photodiode array) detector.

Fermentation, Extraction, and Isolation. The producing organism, *Septocylindrium* sp. LL-Z1518, is a terrestrial fungus that was found

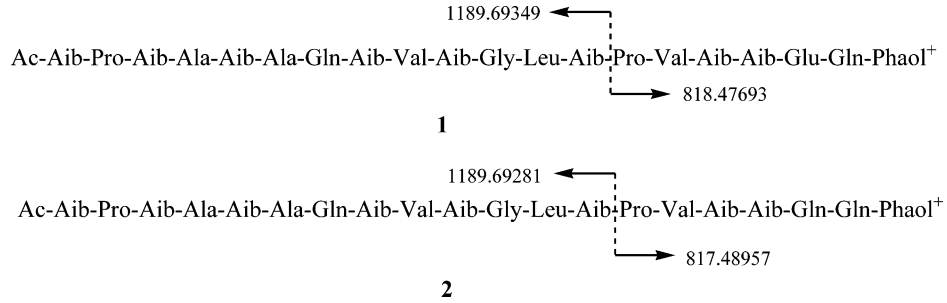


Figure 3. FTMS/MS fragmentation of parent ions of **1** (*m*/*z* 2007.16649) and **2** (*m*/*z* 2006.18009).

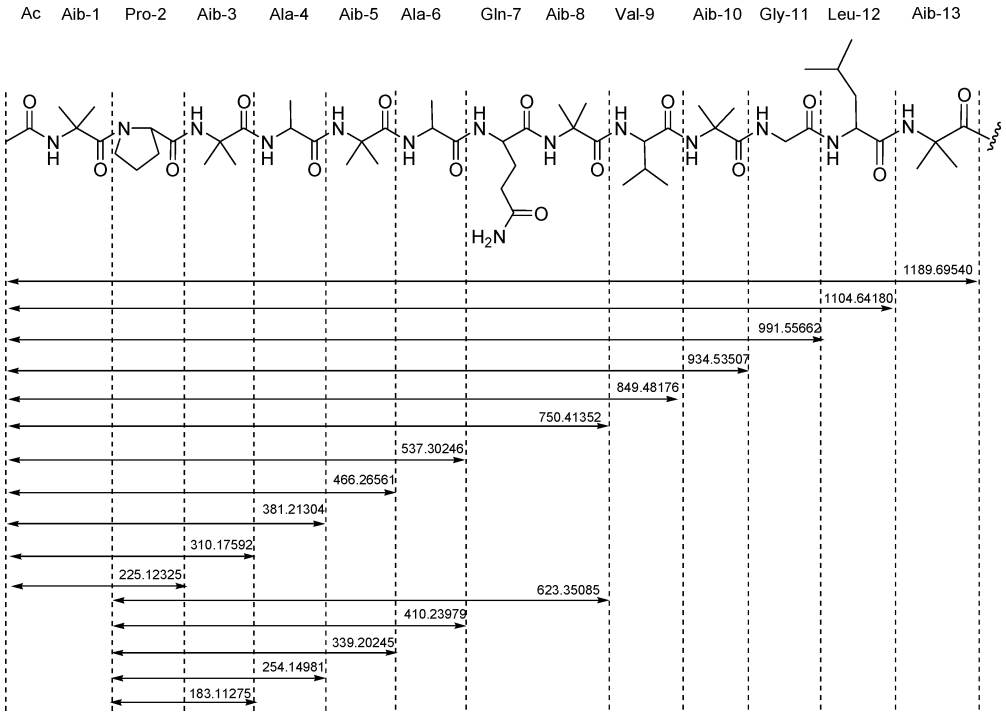


Figure 4. FTMS/MS fragmentation of the *m*/*z* 1189 daughter ion of **1**.

Table 2. Biological Activity Data for Septocylindrin A (**1**) and Septocylindrin B (**2**)

organism	MIC (μg/mL) septocylindrin A	septocylindrin B
Staphylococcus aureus, wild type	32	8
Staphylococcus aureus, methicillin-resistant	128	16
Enterococcus faecium, vancomycin-resistant	16	8
Escherichia coli imp, membrane permeability mutant	32	8
Escherichia coli, wild type	>128	>128
Candida albicans	32	16

growing on *Peziza* sp., isolated in Delaware County, PA, in 1950. A viable culture of this microorganism is maintained in the Wyeth culture collection. *Septocylindrium* sp. LL-Z1518 was grown on an agar plate and transferred to 25 × 150 mm tubes containing two glass beads and 11 mL of Difco potato dextrose broth. The first stage was grown at 22 °C with aeration. After 4 days incubation, the first stage was inoculated into 1 L of potato dextrose broth in a 2.8 L Fernbach flask. The production fermentation was incubated under the same conditions as the seed and harvested after 8 days.

The culture was centrifuged to separate the broth from the cells. The antibiotics were recovered from the clarified broth by adsorption onto 5% Diaion HP-20 resin (Mitsubishi) followed by desorption from the resin using a stepwise MeOH−water gradient (25% to 100% MeOH in 25% increments). The 75% and 100% MeOH fractions were combined on the basis of bioactivity results, dried under reduced pressure, and further purified by reversed-phase HPLC (C_{18}; 20 × 250 mm; 10 μm) using a gradient of 25% to 95% over 30 min and then held at 95% acetonitrile in water, to yield approximately 5 mg each of **1** and **2** (t_R = 63 and 65 min, respectively).

Septocylindrin A (1): white, amorphous powder; $[\alpha]^{25}_D$ −16 (*c* 0.3, MeOH); UV (MeOH) end absorption; IR (attenuated total reflectance) ν_{max} 3320, 2924, 2859, 1734, 1661, 1539, 1455, 1373, 1056, 1033, 1013, 909, 732 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) data, see Table 1; HRESIFTMS *m*/*z* 2007.16649 (calcd for [M + H] $^+$, $C_{94}H_{156}N_{23}O_{25}$, 2007.16372, Δ = 2.77 mmu).

Septocylindrin B (2): white, amorphous powder; $[\alpha]^{25}_D$ −23 (*c* 0.4, MeOH); UV (MeOH) end absorption; IR (attenuated total reflectance) ν_{max} 3320, 2923, 2861, 1713, 1659, 1540, 1455, 1371, 1055, 1033, 1013, 909, 732 cm^{-1}; HRESIFTMS *m*/*z* 2006.18009 (calcd for [M + H]$^+$, $C_{94}H_{157}N_{24}O_{24}$, 2006.17971, Δ = 0.38 mmu).

Determination of Absolute Configuration. Standards. A standard amino acid (0.2 mg, Sigma) was dissolved in 30 μL of water and then treated with 1% 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA) in acetone (60 μL) and 6% triethylamine in 30 μL of water at 40 °C for 1 h. After cooling to room temperature, the derivative was analyzed by reversed-phase HPLC detected by UV absorption at 340 nm. The column [Luna C_{18}(2), 5 μm, 4.6 × 150 mm] was eluted with a linear gradient of (A) CH_3CN and (B) 0.05% aqueous TFA from 10% to 40% (A) over 30 min followed by isocratic elution with 40% (A) at a flow rate of 1 mL/min. The standards gave the following retention times in min: 18.7 for FDAA; 19.3 for L-Gln, 20.0 for D; 22.5 for L-Glu, 24.1 for D; 35.4 for L-Leu, 41.1 for D; 30.6 for L-Val, 35.2 for D; 25.4 for L-Pro, 27.3 for D; 24.4 for L-Ala, 27.8 for D; 34.5 for L-Ile, 40.0 for D.

Peptides. Septocylindrin A (**1**, 1 mg) was dissolved in 6 N HCl (0.5 mL) and heated at 108 °C in a sealed vial overnight to yield the corresponding amino acids. The cooled reaction mixture was evaporated

to dryness under reduced pressure, and HCl was removed from the residual acid hydrolyzate by repeated evaporation from frozen water (1 mL). The amino acid mixture was then treated in the same manner as the standards above (1% FDAA and 6% Et_3N). The mixture of FDAA derivatives was filtered, and the filtrate was diluted with water and analyzed by HPLC. The FDAA derivatives of the amino acids liberated from **1** showed peaks at 18.7, 19.3, 22.4, 24.5, 25.6, 30.9, and 34.7 min, matching the retention times of FDAA, L-Gln, L-Glu, L-Ala, L-Pro, L-Val, and L-Ile, respectively.

Antimicrobial Assay System. Antimicrobial activity was measured by a broth dilution method as previously described.[14]

Acknowledgment. The authors thank R. T. Williamson for acquiring the NMR data and D. Arias for bioassay support.

Supporting Information Available: ^1H, ^{13}C, TOCSY, HMBC, NOESY, and multiplicity-edited HSQC NMR experiments for septocylindrin A (**1**), along with IR and mass spectra for septocylindrin A (**1**) and septocylindrin B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Degenkolb, T.; Berg, A.; Gams, W.; Schlegel, B.; Grafe, U. *J. Pept. Sci.* **2003**, *9*, 666−678.
(2) Chugh, J. K.; Wallace, B. A. *Biochem. Soc. Trans.* **2001**, *29*, 565−570.
(3) Bechinger, B. *J. Membr. Biol.* **1997**, *156*, 197−211.
(4) Duclohier, H.; Wroblewski, H. *J. Membr. Biol.* **2001**, *184*, 1−12.
(5) Martin, D. R.; Williams, R. J. P. *Biochem. J.* **1976**, *153*, 181−190.
(6) Pandey, R. C.; Cook, J. C., Jr.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 8469−8483.
(7) Marsh, D. *Biochem. J.* **1996**, *315*, 345−361.
(8) Beven, L.; Helluin, O.; Molle, G.; Duclohier, H.; Wroblewski, H. *Biochim. Biophys. Acta* **1999**, *1421*, 53−63.
(9) Duval, D.; Cosette, P.; Rebuffat, S.; Duclohier, H.; Bodo, B.; Molle, G. *Biochim. Biophys. Acta* **1998**, *1369*, 309−319.
(10) Oh, S.-U.; Yun, B.-S.; Lee, S.-J.; Kim, J.-H.; Yoo, I. D. *J. Antibiot.* **2002**, *55*, 557−564.
(11) Kumazawa, S.; Kanda, M.; Aoyama, H.; Utagawa, M.; Kondo, J.; Sakamoto, S.; Ohtani, H.; Mikawa, T.; Chiga, I.; Hayase, T. *J. Antibiot.* **1994**, *47*, 1136−1144.
(12) Argoudelis, A. D.; Dietz, A.; Johnson, L. E. *J. Antibiot.* **1974**, *27*, 321−327.
(13) Nosawa, O.; Kawauchi, H.; Sugawara, T.; Chen, C.-H.; Chu, I.-W. JP11140096, 1999.
(14) Singh, M. P.; Kong, F. M.; Janso, J. E.; Arias, D. A.; Suarez, P. A.; Bernan, V. S.; Petersen, P. J.; Weiss, W. J.; Carter, G.; Greenstein, M. *J. Antibiot.* **2003**, *56*, 1033−1044.

NP060571Q