Loloatins A−D, Cyclic Decapeptide Antibiotics Produced in Culture by a Tropical Marine Bacterium

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Loloatins A (**1**) to D (**4**), a family of new cyclic decapeptide antibiotics, have been isolated from laboratory cultures of a tropical marine bacterium recovered from the Great Barrier Reef in Papua New Guinea. The structures of loloatins A−D were elucidated via a combination of spectroscopic analyses and chemical degradation. Loloatins A−D exhibit in vitro antimicrobial activity against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and drug-resistant *Streptococcus pneumoniae*.

Cultured marine microorganisms represent a promising new source of bioactive metabolites with potential for development into drugs for treating human disease.[1] One area where new drugs are desperately needed is in the treatment of antibiotic-resistant strains of human bacterial pathogens.[2] As part of an ongoing program designed to discover new antimicrobial metabolites produced by microorganisms obtained from marine habitats,[3] it was found that laboratory cultures of the isolate MK-PNG-276A recovered from reefs off Loloata Island, Papua New Guinea, gave crude extracts with potent activity against several Gram-positive human pathogens. Bioassay-guided fractionation of MK-PNG-276A culture extracts led to the isolation of the four new cyclic decapeptide antibiotics, loloatins A (**1**) to D (**4**), whose structures are reported below.

GC analysis of cellular fatty acids indicated that the marine bacterial isolate MK-PNG-276A was an unidentified species, possibly in the genus *Bacillus*. To generate sufficient quantities of the MK-PNG-276A secondary metabolites for chemical studies, the bacterium was grown in moderate scale culture as confluent lawns on trays of solid agar containing marine salts and nutrients. The solid agar cultures were harvested by gently scraping the cells from the surface of the agar. Freshly harvested cells were lyophylized and then extracted with MeOH to yield a crude intracellular extract that exhibited Gram-positive antimicrobial activity. Fractionation of the EtOAc-soluble portion of the cell extract by sequential application of Sephadex LH20 chromatography and reversed-phase HPLC yielded pure samples of loloatins A−D (**1**−**4**).

The structure of loloatin B (**2**), the most abundant of the four peptides, was elucidated by detailed analysis of HRMS and NMR data obtained for the natural product **2** and its *N*-acetyl methyl ester derivative. Absolute configurations of the component amino acids in loloatin B (**2**) were determined by acid hydrolysis and chiral GC analysis of the derivatized amino acids. A complete description of the structure elucidation of **2** is published elsewhere.[4] The NMR assignments (Tables 1 and 2) and MS fragmentation patterns (Table 3) for **2** are included here for comparison purposes.

Loloatin A (**1**) was obtained as an optically active ($[\alpha]_D$ −88°) white solid that gave a $[M + H]^+$ peak in the HRFABMS at *m*/*z* 1273.6631 appropriate for a molecular formula of $C_{65}H_{84}N_{12}O_{15}$. The ^1H and ^{13}C NMR data obtained for loloatin A (**1**) were very similar to those of loloatin B (**2**) (Tables 1 and 2) suggesting that the two molecules were closely related. Differences in the ^1H NMR spectrum of loloatin A (**1**) compared to that of loloatin B (**2**) included the absence of an NH resonance in the region of δ 10.81 ppm and a series of aromatic resonances in the region δ 7.0−7.5 that were all assigned to a tryptophan residue in **2**, and the appearance of two new doublets at δ 6.61 and 6.90 ppm, each integrating for two protons. One of the doublets was chemical shift degenerate with *meta* proton resonance of the Tyr residue (δ 6.61) previously identified in **2.** The aromatic region of the ^{13}C NMR spectrum of loloatin A (**1**) contained only 16 resonances instead of the 20 observed in **2**. Taken together, the NMR and MS evidence suggested that the tryptophan residue

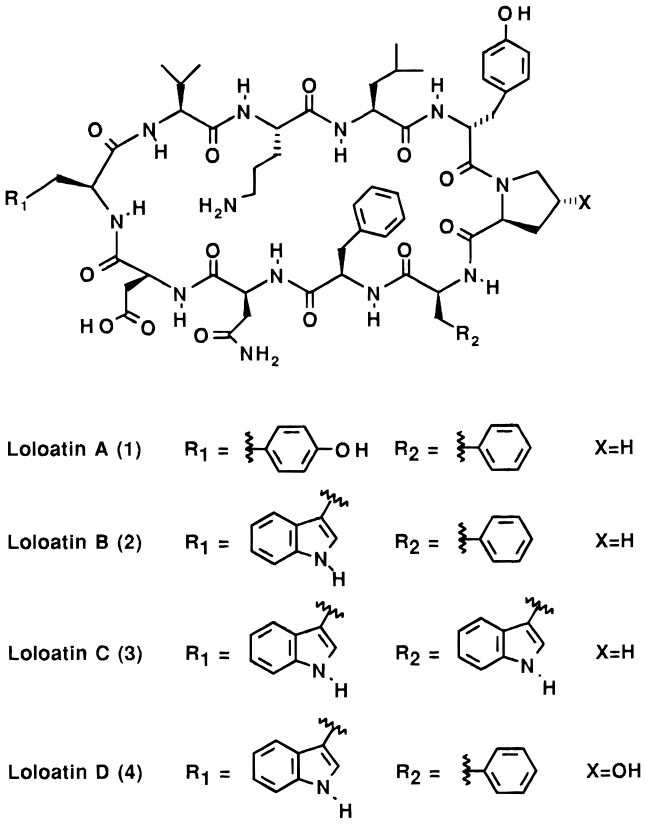

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Table 1. ^{1}H NMR Data (500 MHz) for Loloatins A (**1**) to D (**4**) recorded in DMSO-d_6

	loloatin A (**1**) Pro5 Phe6 Tyr10	loloatin B (**2**) Pro5 Phe6 Trp10	loloatin C (**3**) Pro5 Trp6 Trp10	loloatin D (**4**) 4-OH-Pro5 Phe6 Trp10
Val 1				
NH	7.50 br d (8)	7.52 d (8)	7.58 br d (8)	7.52 d (7.6)
αCH	4.58 m	4.56 m	4.60 m	4.58 m
βCH	1.99 m	2.01 sept (7.0)	2.03 m	2.01 sept (7)
γCH_3	0.92 d (7)	0.93 d (7)	0.99 d (7)	0.93 d (7)
γCH_3	0.91 d (7)	0.93 d (7)	0.99 d (7)	0.93 d (7)
Orn2				
NH	8.89 br d (7)	8.88 d (8.9)	8.90 br s	8.89 d (9.5)
αCH	5.29 m	5.27 m	5.32 m	5.27 m
βCH_2	1.75 m	1.8 m	1.8 m	1.8 m
γCH_2	1.69 m	1.7 m	1.7 m	1.7 m
δCH_2	2.80 m	2.8 m, 2.9 m	2.85 m	2.8 m, 2.9 m
δNH_3	7.45 br s	7.45 br s	7.48 br s	7.45 br s
Leu3				
NH	8.00 br d	7.92 br s	8.04 br s	7.89 d (7.3)
αCH	4.58 m	4.55 m	4.55 m	4.54 m
βCH_2	1.37 m, 1.23 m	1.35 m, 1.25 m	1.45 m, 1.27 m	1.35 m, 1.25 m
γCH	1.48 m	1.5 m	1.5 m	1.5 m
δCH_3	0.94 d (7)	0.93 d (7)	0.96 d (7)	0.93 d (7)
δCH_3	0.92 d (7)	0.93 d (7)	0.95 d (7)	0.93 d (7)
Tyr4				
NH	9.22 s	9.21 s	9.20 s	9.17 s
αCH	4.22 m	4.22 m	4.20 m	4.21 m
βCH_2	2.75 m	2.70 m, 2.81 m	2.70 m, 2.87 m	2.70 m, 2.81 m
*o*CH	6.98 d (8.2)	6.98 d (8.6)	6.98 d (7.8)	6.98 d (8.6)
*m*CH	6.61 d (8.2)	6.61 d (8.6)	6.62 d (7.8)	6.61 d (8.6)
*p*COH	7.45 br s	7.45 br s	7.48 br s	7.45 br s
Pro5/HOPro				
αCH	4.08 d (7.9)	4.07 d (7.6)	4.03 d (7.6)	4.11 d (8.2)
βCH_2	1.48 m, 1.22 m	1.43 m, 1.25 m	1.35 m, 1.18 m	1.50 m, 1.25 m
$\gamma CH_2/\gamma$CHOH	1.07 m, 0.41 m	1.07 m, 0.41 m	1.0 m, 0.30 m	3.10 m
δCH_2	2.21 m, 3.30 m	2.20 m, 3.30 m	2.14 m, 3.27 m	2.20 m, 3.43 m
Phe6/Trp6				
NH	7.23 d (9.5)	7.23 d (9.5)	7.21 br d	7.23 d (9.5)
αCH	4.50 m	4.5 m	4.48 m	4.55 m
βCH_2	2.23 m	2.25 m	2.45 m, 2.30 m	2.25 m
*o*CH/C	7.18	7.18		7.18
*m*CH/CH	7.18	7.18	7.78 d (7.6)	7.18
*p*CH/CH	7.10	7.10	6.94	7.10
/CH			7.07	
/CH			7.32 d (7.3)	
/NH			10.64 br s	
/CH			7.03	
Phe7				
NH	9.06 d (8.8)	9.05 d (9)	9.18 d	9.06 d (10.3)
αCH	5.57 m	5.57 m	5.60 m	5.55 m
βCH_2	2.73 m, 2.99 m	2.75 m, 3.02 m	2.78 m, 3.01 m	2.75 m, 3.02 m
*o*CH	7.18	7.18	7.24	7.18
*m*CH	7.07	7.1	7.08	7.1
*p*CH	7.10	7.1	7.22	7.1
Asn8				
NH	9.01 d (7)	9.03 d (6)	9.06 d (3)	9.03 d (6.9)
αCH	4.46 m	4.46 m	4.48 m	4.47 m
βCH_2	3.40 m, 3.01 m	3.37 m, 3.0 m	3.39 m, 3.0 m	3.37 m, 3.0 m
NH_2	8.05 s, 7.45 br s	8.04 br s, 7.45	8.08 s, 7.48 br s	8.04 br s, 7.45
Asp9				
NH	8.35 d (3)	8.32 d (4.2)	8.35 d (3)	8.33 d (4.2)
αCH	4.24 m	4.28 m	4.25 m	4.26 m
βCH_2	2.35 m	2.35 m, 2.2 m	2.77 m, 2.44 m	2.35 m, 2.2 m
Tyr10/Trp10				
NH	8.57 d (8.5)	8.63 d (9.8)	8.66 d (9.2)	8.63 d (9.5)
αCH	4.32 m	4.5 m	4.52 m	4.49 m
βCH_2	2.92 m	3.15 m	3.18 m	3.15 m
*o*CH/C	6.90 d (7.6)			
*m*CH/CH	6.61 d (7.6)	7.5	7.46	7.5
*p*COH/CH	7.56 br s	7.0	7.02	7.0
/CH		7.03	7.01	7.03
/CH		7.32 d (8)	7.26 d (8)	7.32 d (8)
/NH		10.81 br s	10.83 br s	10.81 br s
/CH		7.02	7.03	7.02

Table 2. ^{13}C NMR Data (100 MHz) for Loloatins A (**1**) to D (**4**) recorded in DMSO-d_6

	A (**1**) Pro5 Phe6 Tyr10	B (**2**) Pro5 Phe6 Trp10	C (**3**) Pro5 Trp6 Trp10	D (**4**) HO-Pro5 Phe6 Trp10
Val 1				
αCH	56.9	57.0	57.0	56.9
βCH	31.4	31.4	31.6	31.4
γCH_3	18.6	18.7	18.9	18.6
γCH_3	18.0	18.0	18.1	18.0
CO	169.9	169.9	170.0	170.0
Orn2				
αCH	50.5	50.5	50.4	50.3
βCH_2	30.9	30.9	31.0	30.8
γCH_2	22.7	22.6	22.8	22.7
δCH_2	38.6	38.5	38.6	38.5
CO	170.2	170.3	na[a]	170.2
Leu3				
αCH	50.1	50.2	50.2	50.1
βCH_2	41.4	41.5	41.5	41.3
γCH	24.5	24.5	24.7	24.4
δCH_3	22.1	22.2	23.0	22.8
δCH_3	22.9	23.0	22.0	22.1
CO	171.7	171.8	na	171.8
Tyr4				
αCH	54.4	54.3	54.6	54.3
βCH_2	34.8	34.7	34.9	34.8
*i*C	125.9	126.0	126.0	126.0
*o*CH	130.2	130.2	130.0.3	129.9
*m*CH	114.9	114.9	114.9	114.9
*p*COH	156.3	156.2	156.3	156.2
CO	na	171.5	na	171.4
Pro5/4−HOPro5				
αCH	59.6	59.5	59.7	58.2
βCH_2	28.4	28.3	28.5	36.7
$\gamma CH_2/\gamma$CHOH	21.9	21.9	22.0	66.4
δCH_2	45.7	45.8	45.9	52.7
CO	169.2	169.2	169.2	169.2
Phe6/Trp6				
αCH	53.4	53.4	52.7	53.4
βCH_2	37.0	37.5	34.9	36.9
*i*C/C	138.4	138.5	110.8	138.3
*o*CH/C	128.8	128.8	127.2	129.1
*m*CH/CH	127.6	127.4	119.1	127.8
*p*CH/CH	126.1	126.1	118.4	126.1
/CH			120.9	
/CH			111.4	
/C			136.0	
/CH			123.7	
CO	172.2	172.1	171.6	172.1
Phe7				
αCH	52.7	52.7	53.4	52.7
βCH_2	40.5	39.5	39.5	39.5
*i*C	137.3	137.4	137.5	137.4
*o*CH	129.1	129.2	129.3	129.2
*m*CH	127.8	127.6	127.8	127.6
*p*CH	126.0	126.0	126.2	126.0
CO	170.5	170.5	170.5	170.5
Asn8				
αCH	49.0	49.0	49.1	49.0
βCH_2	35.1	35.2	36.0	35.2
γCO	173.1	173.0	173.2	173.0
CO	na[a]	170.2	na[a]	170.2
Asp9				
αCH	52.1	52.0	52.1	52.0
βCH_2	34.9	34.7	34.9	34.5
γCO	171.4	na[a]	na[a]	na[a]
CO	170.3	170.1	170.2	170.1
Tyr10/Trp10				
αCH	56.2	55.5	55.6	55.4
βCH_2	37.1	28.4	28.3	28.2
*i*C/C	127.8	110.5	110.5	110.5
*o*CH/C	129.7	127.0	127.2	127.1
*m*CH/CH	114.8	118.0	118.1	118.0
*p*COH/CH	155.7	118.3	117.9	118.2
/CH		120.7	120.8	120.7
/CH		111.2	111.0	111.2
/C		136.0	136.1	136.0
/CH		123.0	123.1	122.8
CO	171.0	171.0	171.0	170.9

[a] na = not assigned: The carbonyl regions of the ^{13}C NMR spectra of **1** to **4** were poorly dispersed making it impossible to accurately count the total number of resonances or determine all of their chemical shifts. In addition, an absence of observed HMBC correlations meant that it was not possible to unambiguously assign resonances to the carbonyl carbons listed as na.

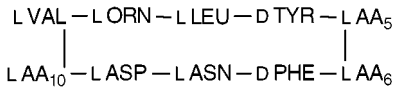
in loloatin B (**2**) was replaced by a second tyrosine residue in loloatin A (**1**).

Detailed analysis of the COSY, HOHAHA, HMQC, and HMBC data confirmed that loloatin A (**1**) contained two phenylalanine residues, two tyrosine residues, and one residue each of valine, leucine, proline, ornithine, asparagine, and aspartate (Tables 1 and 2). Hydrolysis of **1** with 6N HCl containing thioglycolic acid and examination of the PFPA−IPA ester derivatives of the liberated amino acids via chiral GC analysis established the presence of L-phenylalanine, D-phenylalanine, D-tyrosine, L-tyrosine, L-valine, L-leucine, L-proline, L-ornithine, and L-aspartic acid (from Asp and Asn). The similarity in the NMR data for peptides **1** and **2** indicated that the amino acid sequence in loloatin A (**1**) was identical to that in loloatin B (**2**), except for the substitution of Tyr in **1** for Trp in **2**. This was confirmed by mass spectrometric analysis (Table 3). The MS−MS data for **1** were consistent with initial cleavage of the ring at the Tyr1−CO/Pro-N bond to give a linear decapeptide that sequentially loses Leu−Tyr (*m*/*z* 998), Orn−Leu−Tyr (*m*/*z* 883), and Tyr−Val−Orn−Leu−Tyr (*m*/*z* 621). FABMS peaks at *m*/*z* 245 and 377 could be assigned to the protonated fragments Pro−Phe and Phe−Asn−Asp, respectively.

Loloatin C (**3**) was obtained as an optically active ($[\alpha]_D$ −75.5°) white solid that gave a $[M + H]^+$ peak in the HRFABMS at *m*/*z* 1335.6527 appropriate for a molecular formula of $C_{69}H_{86}N_{14}O_{14}$. Once again, the ^1H and ^{13}C NMR data obtained for loloatin C (**3**) were very similar to those of loloatin B (**2**) suggesting closely related peptides. The ^1H NMR spectrum of loloatin C (**3**) contained two indole NH resonances (Trp6, δ 10.64; Trp10, δ 10.83 ppm) instead of just one, and added complexity was apparent in the aromatic region, suggesting the presence of two tryptophan residues. A pair of two-proton doublets at δ 6.62 and 6.98 ppm in the ^1H NMR spectrum of **3** confirmed that a tyrosine residue was still present. The ^{13}C NMR spectrum of loloatin C (**3**) contained 24 aromatic resonances, nine of which were assigned by an APT experiment to quaternary carbons. Subtraction of the eight aromatic quaternary and 12 aromatic methine carbons accounted for by one tyrosine and two tryptophan residues left one quaternary and three methine aromatic resonances, suggesting a phenylalanine residue. Detailed analysis of the COSY, HOHAHA, HMQC, and HMBC data confirmed that loloatin C (**3**) contained two tryptophan residues and one residue each of valine, leucine, proline, ornithine, phenylalanine, tyrosine, asparagine, and aspartate (Tables 1 and 2). Hydrolysis of **3** with 6N HCl containing thioglycolic acid and analysis of the PFPA−IPA ester derivatives of the liberated amino acids via chiral GC analysis identified L-tryptophan, D-tyrosine, D-phenylalanine, L-valine, L-leucine, L-proline, L-ornithine, and L-aspartic acid (from Asp and Asn).

The similarities in the ^1H NMR chemical shifts for the α-methine and NH protons in the spectra of **2** and **3** suggested that the amino acid sequence in **3** was identical to the sequence of peptide **2**, except for replacement of one of the Phe residues with Trp. MS−MS analysis confirmed the assigned sequence shown in **3**. Thus, the fragmentation pattern was consistent with initial cleavage of the ring at the Tyr−CO/Pro-N bond to give a linear decapeptide that sequentially loses Leu−Tyr (*m*/*z* 1060) and Trp−Val−Orn−Leu−Tyr (*m*/*z* 660). FABMS peaks at *m*/*z* 284 and 377 could be assigned to the protonated fragments Pro−Trp and Phe−Asn−Asp, respectively (Table 3). The observation of Pro−Trp and Phe−Asn−Asp fragments demonstrated that the Phe6 residue in loloatin B (**2**) was replaced

Table 3. FABMS Parent and Fragment Ions for Loloatins A−D (**1**−**4**)



fragment	loloatin A (**1**) AA_5 = Pro AA_6 = Phe AA_{10} = Tyr	loloatin B (**2**) AA_5 = Pro AA_6 = Phe AA_{10} = Trp	loloatin C (**3**) AA_5 = Pro AA_6 = Trp AA_{10} = Trp	loloatin D (**4**) AA_5 = HO−Pro AA_6 = Phe AA_{10} = Trp
$[AA_5-AA_6$-F-N-D$-AA_{10}$-V-O-L−Y$]^+$	1274	1297	1336	1313
$[AA_5-AA_6$-F-N-D$-AA_{10}$-V−O$]^+$	998	1019	1060	
$[AA_5-AA_6$-F-N-D$-AA_{10}$−V$]^+$	883	905		
$[AA_5-AA_6$-F-N-D$-AA_{10}]^+$	784	807		
$[AA_5-AA_6$-F-N−D$]^+$	621	621	660	637
$[AA_5-AA_6$-F−N$]^+$	506	506		522
$[AA_5-AA_6$−F$]^+$		392	431	408
$[AA_5-AA_6]^+$	245	245	284	261
[F−N-D]+	377	377	377	377

Table 4. Minimum Inhibitory Concentrations (μg/mL) of Loloatins A (**1**) to D (**4**)

test organism	loloatin A (**1**)	loloatin B (**2**)	loloatin C (**3**)	loloatin D (**4**)
Staphylococcus aureus	4	2	0.5	8
methicillin-resistant *S. aureus*	4	2	0.5	8
Enterococcus faecalis	4	2	1	8
vancomycin-resistant *E. faecalis*	4	2	1	8
Enterococcus faecium	4	2	2	8
vancomycin-resistant *E. faecium*	4	2	2	8
Streptococcus pneumoniae	1	1	<0.25	4
penicillin-resistant *S. pneumoniae*	2	1	<0.25	4
Escherichia coli	>32	>32	1	>32
Pseudomonas aeruginosa	>32	>32	>32	>32
Pseudomonas cepacia	>32	>32	>32	>32
Xanthomonas matophilia	>32	>32	>32	>32
Candida albicans	8	8	8	16

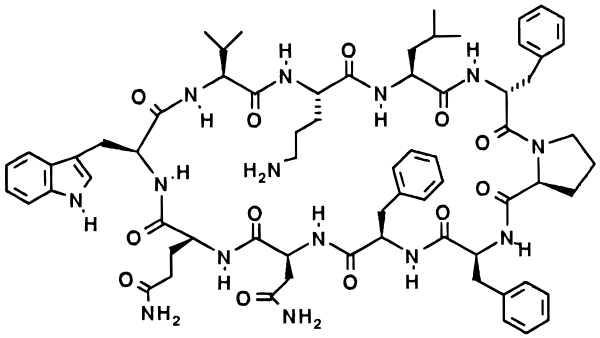
by a Trp residue in loloatin C (**3**). Because the only Phe residue in loloatin C (**3**) had the D configuration, we have assumed that the corresponding Phe7 residue in loloatin B (**2**) also was D and, therefore, the Phe6 residue in **2** was L.

Loloatin D (**4**) was obtained as a white solid that gave a $[M + H]^+$ peak in the HRFABMS at *m/z* 1312.6368 appropriate for a molecular formula of $C_{67}H_{85}N_{13}O_{15}$, differing from that of loloatin B (**2**) simply by the addition of one oxygen atom. The ^{1}H and ^{13}C NMR data obtained for loloatin D (**4**) (Tables 1 and 2) were mostly very similar to those of loloatin B (**2**), supporting the assumption that the molecules were closely related. The major difference in the NMR data for the two compounds was the presence of a CH resonance at δ 66.4 in the ^{13}C spectrum of loloatin D (**4**), which was assigned to a carbinol methine. COSY and HOHAHA data indicated that the carbinol methine was at the C-4 position of a proline residue, suggesting that loloatin D (**4**) simply contained a 4-hydroxyproline residue in place of the proline residue in loloatin B (**2**). Hydrolysis of loloatin D with 6N HCl containing thioglycolic acid and examination of the PFPA-IPA ester derivatives of the liberated amino acids via chiral GC analysis confirmed the presence of L-phenylalanine, D-phenylalanine, D-tyrosine, L-tryptophan, L-valine, L-leucine, L-ornithine, L-aspartic acid (from Asp and Asn), and *trans*-4-hydroxy-L-proline, supporting the proposed structure **4**. This structure was confirmed by mass spectrometric analysis (Table 3). The MS−MS data for loloatin D (**4**) was consistent with initial cleavage of the cyclic peptide at the Tyr−CO/hydroxyPro-N bond to give a linear decapeptide that sequentially loses Trp−Val−Orn−Leu−Tyr (*m/z* 637) and Asp−Trp−Val−Orn−Leu−Tyr (*m/z* 522). FABMS peaks at *m/z* 261, 377, and 408 could be assigned to the protonated fragments 4-hydroxyPro−Phe, Phe−Asn−Asp, and 4-hydroxyPro−Phe−Phe, respectively.

Loloatins A−C (**1**−**3**) showed potent antibiotic activity against methicillin-resistant strains of *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* sp. (VRE), and penicillin-resistant *Streptococcus pneumoniae* (Table 4). Interestingly, only loloatin C (**3**) showed antibacterial activity against the Gram-negative bacterium *Escherichia coli*, and loloatin D (**4**) was four-fold less active than loloatins A−C against Gram-positive bacteria. These latter results demonstrate that rather subtle changes in the cyclic decapeptide structure can have a significant impact the antimicrobial activity.

The loloatins share structural features with the tyrocidines [i.e., tyrocidine A (**5**)] isolated from a *Bacillus brevis* species.[5] The loloatins and tyrocidines together compose a family of antibiotic cyclic decapeptides containing four aromatic amino acid residues, two of which have the D configuration. However, the loloatins, unlike the tyrocidines, have zwitterionic character due to the presence of both ornithine and aspartic acid residues. The conformation of tyrocidine A (**5**) has been studied in solution and in the solid state, and it has been shown to possess an antiparallel β-sheet conformation with a type II′ β-turn at D-Phe−Pro and a type I β-turn at Gln−Tyr.[6] Comparison of the ^{1}H chemical shifts of the amide protons of tyrocidine A and loloatin B reveals that, regardless of the side chain, there is little variation in the actual chemical shifts of the amide protons between corresponding amino acids in each sequence. This evidence, along with the trans-annular ROESY correlations observed between ornithine and D-phenylalanine residues in loloatin B (**2**), suggests that the loloatins possess the same solution conformation as the tyrocidines.

There have been extensive investigations into the role of the tyrocidines in the life cycle of *B. brevis*, and evidence to date suggests that the tyrocidines play a regulatory role during sporulation.[7] Tyrocidine A (**5**) also appears to relax superhelical chromosomal DNA, reducing torsional tension, thereby inducing "packaging" of the DNA when the bacteria enter the sporulation phase.[8] The tyrocidines have also been shown to interact with phospholipid membranes, creating an ion channel through the membrane.[9] This interruption of membrane function may explain the antimicrobial action of the tyrocidines against other species of bacteria. Examination of the MICs of loloatins A−C (**1**−**3**) shows they are at least as potent against Gram-positive bacteria as tyrocidine C, the most potent antibiotic in the tyrocidine family.[10] There are no reports of tyrocidines showing activity against strains of Gram-negative bacteria. Therefore, loloatin C (**3**) appears to be the first member of this cyclic decapeptide antibiotic family to possess Gram-negative activity.



Tyrocidine A (5)

Experimental Section

General Experimental Procedures. NMR data were collected on Bruker AM400 and AMX500 spectrometers. All spectra were recorded in DMSO-d_6. Proton spectra were referenced to internal residual DMSO-d_5 (δ 2.49), and carbon spectra were referenced to the DSMO methyl carbon resonance (δ 39.5). FABMS data were collected on a Kratos Concept IIHQ hybrid mass spectrometer with cesium ion secondary ionization and a magnetic sector mass analyzer. Samples were dissolved in a MeOH−thioglycerol matrix, and spectra were obtained using a source voltage of 8 kV and a cesium ion gun voltage of 12 kV. Fragment ion peaks were confirmed via secondary MS−MS using a quadrapole mass analyzer. IR spectra were measured on a Galaxy Series 3000 FT-IR spectrophotometer using poly(tetrafluoroethylene) (PTFE) film plates. Optical rotations were measured on a JASCO J-710 spectropolarimeter (1-cm quartz cell).

Isolation of Loloatins. The marine bacterium MK-PNG-276A was isolated during a collecting expedition off of Loloata Island, Papua New Guinea. GC analysis of cellular fatty acids indicated that MK-PNG-276A was an unknown species possibly within the genus *Bacillus*. MK-PNG-276A has been cryopreserved and deposited in the marine microbial culture collections at SeaTek and UBC. Moderate scale cultures of MK-PNG-276A were grown as confluent lawns for 5 days at 16 °C on trays of solid trypticase soy agar supplemented with NaCl to a final concentration of 1%. The cultures were harvested by gently scraping the cells from the agar surface. Lyophilized cells (61.5 g dry wt) were exhaustively extracted with three 600-mL portions of MeOH that were combined, filtered, and reduced in vacuo to give a brown/gray tar. This tar was dissolved in 750 mL of MeOH−H_2O (1:4) and sequentially extracted with hexanes (3 × 250 mL) and EtOAc (3 × 250 mL). The combined EtOAc extracts were reduced in vacuo to give a taupe/brown crystalline solid (5.5 g). Fractionation of the taupe/brown solid via Sephadex LH-20 chromatography (MeOH) gave an early-eluting fraction that showed antibiotic activity against MRSA and *Enterococcus sp.* Further purification of this fraction by preparative reversed-phase column chromatography and reversed-phase HPLC chromatography (9:1 MeOH−H_2O containing 0.1% TFA) gave pure loloatin A (**1**) (281 mg), loloatin B (**2**) (1.87 g, 3% dry wt of cells), loloatin C (**3**) (39 mg), and loloatin D (**4**) (8 mg) as amorphous solids.

Loloatin A (1), cyclic (L-asparaginyl−L-aspartyl−L-tyrosyl−L-valyl−L-ornithyl−L-leucyl−D-tyrosyl−L-prolyl−L-phenylalanyl−D-phenylalanyl): isolated as a white solid (281 mg); mp 229−232 °C; IR (thin film on PTFE membrane) ν_{max} cm^{-1}, 3275 (br m), 3032 (w), 3070 (w), 2958 (w), 1637 (br s), 1537 (br m), 1454 (br w), 1251 (br m); $[\alpha]_D$ −88° (EtOH);UV (EtOH) λ_{max} (ϵ) 224 (21 000), 278 (3400); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 1273.63082 (calcd for M + H/$C_{65}H_{85}N_{12}O_{15}$, 1273.62604), 621.26866 (calcd for $C_{31}H_{37}N_6O_8$, 621.26741), 506.24050 (calcd for $C_{27}H_{32}N_5O_5$, 506.24046), 392.19821 (calcd for $C_{23}H_{26}N_3O_3$, 392.19752), 377.14716 (calcd for $C_{17}H_{21}N_4O_6$, 377.14617), 245.12933 (calcd for $C_{14}H_{17}N_2O_2$, 245.12907).

Loloatin B (2), cyclic (L-asparaginyl−L-aspartyl−L-tryptophanyl−L-valyl−L-ornithyl−L-leucyl−D-tyrosyl−L-prolyl−L-phenylalanyl−D-phenylalanyl): isolated as a white solid (1.87 g); mp 229−233 °C; IR (thin film on PTFE membrane) ν_{max} cm^{-1}, 3275 (br,m), 3070 (m), 3032 (w), 1637 (br s), 1537 (br m), 1454 (w); $[\alpha]_D$ −80° (EtOH); UV (EtOH) λ_{max} (ϵ) 220 (43 000), 280 (5900); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 1296.64232 (calcd for M + H/$C_{67}H_{86}N_{13}O_{14}$, 1296.64203), 621.26944 (calcd for $C_{31}H_{37}N_6O_8$, 621.26741), 506.24105 (calcd for $C_{27}H_{32}N_5O_5$, 506.24046), 392.19848 (calcd for $C_{23}H_{26}N_3O_3$, 392.19752), 377.14717 (calcd for $C_{17}H_{21}N_4O_6$, 377.14617), 245.12922 (calcd for $C_{14}H_{17}N_2O_2$, 245.12907).

Loloatin C (3), cyclic (L-asparaginyl−L-aspartyl−L-tryptophanyl−L-valyl−L-ornithyl−L-leucyl−D-tyrosyl−L-prolyl−L-tryptophanyl−D-phenylalanyl): isolated as a tan/white solid (39 mg); mp 239−243 °C; IR (thin film on PTFE membrane) ν_{max} cm^{-1}, 3273 (br m), 3080 (w), 2960 (w), 1633 (br s), 1537 (br m), 1516 (br m); $[\alpha]_D$ −76° (EtOH); UV (EtOH) λ_{max} (ϵ) 223 (66 000), 280 (10 000); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 1335.65267 (calcd for M + H/$C_{69}H_{87}N_{14}O_{14}$, 1335.65293), 545.25262 (calcd for $C_{29}H_{33}N_6O_5$, 545.25136), 431.20811 (calcd for $C_{25}H_{27}N_4O_3$, 431.20842), 377.14580 (calcd for $C_{17}H_{21}N_4O_6$, 377.14617), 284.14057 (calcd for $C_{16}H_{18}N_3O_2$, 284.13997).

Loloatin D (4), cyclic (L-asparaginyl−L-aspartyl−L-tryptophanyl−L-valyl−L-ornithyl−L-leucyl−D-tyrosyl−L-*trans*-4-hydroxyprolyl−L-phenylalanyl−D-phenylalanyl): isolated as a white solid (8 mg); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 1312.63677 (calcd for M + H/$C_{67}H_{86}N_{13}O_{15}$, 1312.63694), 637.26276 (calcd for $C_{31}H_{37}N_6O_9$, 637.26232), 522.23714 (calcd for $C_{27}H_{32}N_5O_6$, 522.23537), 408.19091 (calcd for $C_{23}H_{26}N_3O_4$, 408.19243), 377.14612 (calcd for $C_{17}H_{21}N_4O_6$, 377.14617), 261.12402 (calcd for $C_{14}H_{17}N_2O_3$, 261.12398).

Total Acid Hydrolysis and GC Analysis. Loloatins A (**1**), B (**2**), C (**3**), and D (**4**) (1.0 mg each) were hydrolyzed individually with 3 mL of 6N HCL containing 1% thioglycollic acid (to reduce oxidation of tryptophan and tyrosine residues) for 8 h at 105 °C under N_2. The hydrolysates were reduced to dryness using a stream of dry N_2, and the reaction mixtures were then esterified with HCl−*i*-PrOH at 100 °C for 45 min and reduced to dryness. The esterified mixtures were reacted with 50 mL of pentafluoropropionic anhydride in 250 mL of CH_2Cl_2 at 100 °C for 15 min in a sealed vial, the sample was evaporated under N_2 and then redissolved in 500 mL CH_2Cl_2. Racemic mixtures as well as optically pure L-amino acid standards were derivatized in a similar fashion. The amino acid standards and the hydrolysate were analyzed on a 25-m chiralsil-Val Heliflex column with FID detection using the following conditions: He carrier, detector temperature 275 °C, injector temperature 250 °C, injector split ratio 25:1, initial oven temperature 90 °C, initial time 5 min, program rate 4 °C/min, final oven temperature 200 °C, final time 27.5 min.

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