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Pupukeamide, a Linear Tetrapeptide from a Cephalaspidean Mollusk *Philinopsis speciosa*

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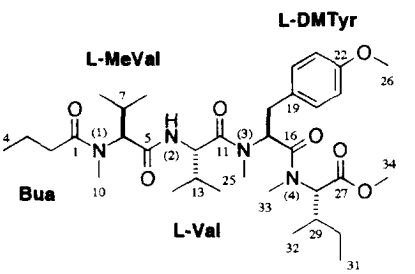
Abstract: A linear peptide pupukeamide (**1**) was isolated from a cephalaspidean mollusk *Philinopsis speciosa*. Its structure was elucidated by spectroscopic and chemical methods.

Introduction

A carnivorous cephalaspidean mollusk *Philinopsis speciosa* is is known to feed on other mollusks.[1] We have previously isolated a cyclic depsipeptide kulolide[2] and its congeners[3] from this animal. Further investigation of this animal yielded a linear depsipeptide, pupukeamide (**1**),[4] which in contrast to the cyclic kulolides, did not show cytotoxicity against P388 cells. However, its characteristic features, *N*-methylamino acids, an *N*,*O*-dimethyl Tyr unit, and an *N*-terminal fatty acid, are reminiscent of majusculamides A and B previously isolated from the blue-green alga *Lyngbya majuscula*.[5] This fact reveals unexpected biogenetic relationships and prompted us to initiate feeding experiments.[1] Isolation and structure elucidation of pupukeamide (**1**) is the subject of this paper.



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Results and Discussion

Philinopsis speciosa (300 animals; 9.0 kg, wet weight) collected on mid-summer nights in 1994 at Shark's Cove, Pupukea, O'ahu was extracted with EtOH, then $CHCl_3$/MeOH (1:1). The organic layer of the combined extracts was separated by solvent partition, ODS flash chromatography, gel-filtration, and repetitive ODS HPLC which yielded pupukeamide (**1**; 12.5 mg; 1.4 x 10^{-4} yield based on wet weight).[6]

Table. 1. NMR Data for Pupukeamide (1) in CD_3CN.

	#	^{13}C	^1H	HMBC
Bua	1	175.2		
	2	36.1	2.30 m	C: 1, 3, 4
	3	19.2	1.58 hex 7.4	C: 1, 2, 4
	4	14.2	0.91 t 7.4	C: 2, 3
MeVal	5	170.6		
	6	63.0	4.50 d 11.2	C: 1, 5, 7, 8, 9, 10
	7	26.5	2.17 m	C: 6
	8	20.1	0.85 d 6.4	C: 6
	9	18.8	0.78 d 6.6	C: 6
	10	31.1	2.87 s	C: 1, 6
Val	11	172.4		
	12	54.3	4.61 dd 6.5, 9.1	C: 5, 11, 13, 14, 15
	13	31.9	1.88 octet 6.5	C: 12, 14, 15
	14	20.0	0.81 d 6.5	C: 12, 13, 15
	15	17.4	0.72 d 6.5	C: 12, 13, 14
	NH		6.56 d 9.1	C: 5
DMTyr	16	171.8		
	17	54.9	5.65 dd 7.7, 7.5	C: 11, 16, 18, 19
	18a	34.8	3.06 dd 14.0, 7.5	C: 16, 19, 20, 24
	18b		2.80 dd 14.0, 7.7	
	19	130.2		
	20	131.2	7.09 d 8.8	C: 18, 22, 24
	21	114.6	6.79 d 8.8	C: 19, 22, 23
	22	159.4		
	23	114.6	6.79 d 8.8	C: 19, 21, 22
	24	131.2	7.09 d 8.8	C: 18, 20, 22
	25	31.4	3.01 s	C: 11, 17
	26	55.8	3.73 s	C: 22
DMIle	27	171.9		
	28	61.7	4.69 d 10.8	C: 16, 27, 29, 30, 32, 33
	29	33.8	1.94 m	
	30a	25.4	1.21 ddq 3.3, 13.4, 7.2	
	30b		0.90 m	C: 32
	31	10.7	0.78 t 7.2	C: 29, 30
	32	16.1	0.88 d 6.6	C: 29, 30
	33	32.1	2.82 s	C: 16, 28
	34	52.3	3.60 s	C: 27

Positive ion HR-FABMS analysis of pupukeamide (matrix: glycerol) revealed a molecular formula of $C_{34}H_{56}N_4O_7$ [$(M+H)^+$ *m/z* 633.4183 (Δ -4.5 mmu)]. The ^1H NMR spectrum of **1** showed three *N*-methyls and two methoxy groups but only one NH proton. This NH proton was assigned to Val on the basis of 2D NMR spectra including COSY, HMQC[7] and HMBC.[8] Spin systems similar to Val, Ile, and Tyr were also

found, but these residues lacked NH protons. They had *N*-methyl groups instead. Further analysis of 2D NMR data allowed us to assign the remaining two *O*-methyls to Ile and Tyr units. Therefore, **1** embraces *N*-methylvaline (MeVal), *N*,*O*-dimethyltyrosine (DMTyr), and *N*,*O*-dimethylisoleucine (DMIle) residues.

The last residue of this peptide was a fatty acid with terminal methyl group. In the COSY spectra, correlation could be seen from this methyl (δ 0.91 t 7.4; H4) to methylene protons at 1.58 ppm (H3) which also showed a correlation to methylene protons at 2.30 ppm (H2). The chemical shifts of these protons (H2) and carbon attached to this proton (36.1 ppm; C2), together with HMBC correlations from H2 and H3 to carbonyl carbon at 175.2 ppm, were indicative of 1-butyrate (Bua) at the *N*-terminus. These five residues when joined linearly by five peptide bonds equal the experimental mass weight of 632 da.

Sequencing was straightforward. Unambiguous HMBC correlations between residues could be seen between H6 and H10/C1, H12 and NH2/C5, H17 and H25/C11, H28 and H33/C16 to complete the sequence of Bua-MeVal-Val-DMTyr-DMIle.

The absolute stereochemistry of all residues was determined by Marfey's method[9,10] and proved to be L.

Pupukeamide (**1**) is a linear tetrapeptide isolated from a cephalaspidean mollusk *Philinopsis speciosa*. The structure of **1** containing *N*-methyl amino acids, an *N*,*O*-dimethyl Tyr, and an *N*-terminal fatty acid is reminiscent of majusclamides A and B from *Lyngbya majuscula*.[5] We frequently encountered *Stylocheilus longicaudus* during many years of collecting *P. speciosa*.[2] We recently confirmed that *P. speciosa* will indeed feed on this sea hare.[1] Together with our earlier observation[12] that *S. longicaudus* feeds on the bluegreen alga *Lyngbya*, we have been able to establish ecological links by tracing the chemistry through three trophic levels.

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References and Notes

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6. **Pupukeamide (1):** colorless amorphous solid; $[\alpha]_D^{20}$ -89° (*c* 1.0, MeOH); IR ($CHCl_3$) 1720, 1660, 1630 cm^{-1}; UV (MeOH) 261.5 nm (ε 1100); HR-FABMS $C_{34}H_{56}N_4O_7$ *m/z* 633.4183 (Δ -4.5 mmu); ^1H and ^{13}C NMR (CD_3CN) see Table. 1.
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