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Urumamide, a novel chymotrypsin inhibitor with a β-amino acid from a marine cyanobacterium *Okeania* sp.

Yuki Kanamori, Arihiro Iwasaki, Shinpei Sumimoto, Kiyotake Suenaga *

Department of Chemistry, Keio University, 3-14-1, Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan

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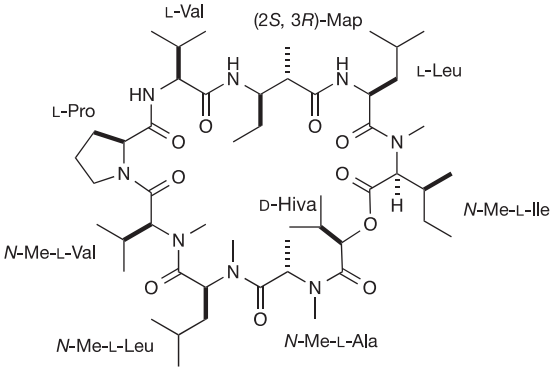
ABSTRACT

Urumamide, a novel cyclic depsipeptide that contains a β-amino acid, was isolated from a marine cyanobacterium *Okeania* sp. Its gross structure was determined by spectroscopic analyses, and the absolute configuration was established based on Marfey's analyses and chiral HPLC analyses of hydrolysis products. Biologically, urumamide inhibited the growth of human cancer cells. In addition, urumamide inhibited chymotrypsin.

Marine cyanobacteria produce many interesting peptides that are biosynthesized by nonribosomal peptide synthetases (NRPS).[1] These peptides are noteworthy not only because of their remarkable structures but also because of their biological activities, such as their ability to inhibit the growth of human cancer cells and/or serine proteases. In our continuing search for new compounds from marine cyanobacteria, we have isolated related peptides, including bisebromoamide,[2] kurahyne,[3] kurahamide,[4] and maedamide.[5]

Here, we report the discovery of urumamide (**1**), a cyclic depsipeptide composed of seven α-amino acids, one α-hydroxy acid, and one β-amino acid, 2-methyl-3-aminopentanoic acid (Map), from a marine cyanobacterium *Okeania* sp. In addition, **1** inhibited the growth of human cancer cells and inhibited chymotrypsin, but not elastase or trypsin. To the best of our knowledge, this is the first report to show that a Map-containing peptide exhibits chymotrypsin–inhibitory activity. Several related peptides containing a Map moiety, such as lyngbyastatin 1,[6] majusculamide C,[7] and guineamide A,[8] have been discovered in marine cyanobacteria. In addition, some types of cyclic depsipeptides have recently been isolated from cyanobacteria, including medusamide A,[9] companeramide A,[10] and precarriebowmide.[11]

The marine cyanobacterial samples (800 g, wet weight) were collected at Ikei Island, Okinawa, and extracted with methanol. The extract was filtered, concentrated, and partitioned between EtOAc and H_2O. The EtOAc–soluble material was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to fractionation with reversed-phase column chromatography (ODS silica gel, MeOH–H_2O) and repeated reversed-phase HPLC to give urumamide (**1**) (11.8 mg) as a colorless oil.



urumamide (**1**)

The molecular formula of **1** was found to be $C_{51}H_{90}N_8O_{10}$ by HRESIMS (*m*/*z* 975.6838, calcd for $C_{51}H_{91}N_8O_{10}$ $[M+H]^+$ 975.6858). The NMR data for **1** are summarized in Table 1. The ^1H NMR spectrum revealed the presence of four singlets corresponding to *N*-methyl amide substituents (δ 3.12, 3.11, 3.01,

* Corresponding author.

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Table 1
NMR data for urumamide (**1**) in CD_3OD[a]

Unit	Position	δ_C[b]	δ_H[c] (*J* in Hz)	COSY	HMBC (H→C)	Selected NOESY
Val	1	174.3				
	2	62.9	3.86, dd (4.8, 8.1)	3, NH	1, 3, 5	
	3	30.6	2.02, m	2, 4, 5	1, 2, 4, 5	
	4	20.9	1.16, d (6.5)	3	2, 3, 5	
	5	20.0	1.08, m	3	2, 3, 4	
	NH		8.36, d (4.8)	2	2, 3, 1 (Pro)	2 (Pro)
Map	1	176.0				
	2	45.7	2.46,m	3, 6	1, 3, 4, 6	
	3	55.9	3.77, m	2, 4a, 4b, NH	1, 2	
	4a	21.5	0.93, m	3		
	4b		1.61, m	3, 5	3, 5	
	5	11.9	0.85, t (7.2)	4b	3, 4	
	6	13.5	1.09, m	2	1, 2, 3	
	NH		8.62, d (9.7)	3	3, 1 (Val)	
Leu	1	176.8				
	2	51.5	4.79, m	3a, 3b, NH		
	3a	39.7	1.38, m	2, 3b, 4	4	
	3b		1.98, m	2, 3a	2, 4, 6	
	4	26.5	1.87, m	3a, 5, 6		
	5	24.9	0.90, m	4	3, 4, 6	
	6	20.7	0.98, m	4	3, 4, 5	
	NH		7.94, d (8.1)	2	2, 3, 1 (Map)	
N-Me-Ile	1	173.3				
	2	61.9	5.39, d (6.3)	3	1, 3, 4, 6, *N*-Me, 1 (Leu)	
	3	36.8	2.24, m	2, 4a, 4b, 6	4, 5	
	4a	28.8	1.39, m	3, 4b, 5	2, 3, 5, 6	
	4b		1.57, m	3, 4a, 5	2, 3, 5, 6	
	5	12.4	0.95, m	4a, 4b	3, 4	
	6	16.9	1.09, m	3	2, 3, 4	
	N-Me	32.9	3.12, s		2, 1 (Leu)	2 (Leu)
Hiva	1	171.2				
	2	76.4	5.29, d (9.0)	3	3, 5, 1 (*N*-Me-Ile)	
	3	31.8	2.24, m	2, 4, 5	1, 2, 4	
	4	18.0	0.99, m	3	2, 3, 5	
	5	19.2	1.11, m	3	2, 3, 4	
N-Me-Ala	1	172.5				
	2	51.9	5.55, m	3	1, 3, *N*-Me, 1 (Hiva)	
	3	14.7	1.23, d (6.7)	2	1, 2	
	N-Me	30.7	3.01, s		2, 1 (Hiva)	
N-Me-Leu	1	173.0				
	2	53.9	5.57, m	3a, 3b	1, 3, 4, *N*-Me	*N*-Me (*N*-Me-Val)
	3a	38.0	1.33, m	2, 3b, 4	4, 5	
	3b		1.75, m	2, 3a	2, 4, 5	
	4	26.6	1.50, m	3a,5, 6	3	
	5	20.9	0.99, m	4	3, 4, 6	
	6	24.3	1.06, d (6.7)	4	3, 4, 5	
	N-Me	33.3	2.69, s		2. 1 (*N*-Me-Ala)	
N-Me-Val	1	172.4				
	2	60.1	5.19, d (11.2)	3	1, 3, 4, 5, *N*-Me	2 (Pro)
	3	28.8	2.24, m	2, 4, 5	2	
	4	19.5	0.91, m	3	2, 3, 5	
	5	18.7	0.98, m	3	2, 3, 4	
	N-Me	31.2	3.11, s		2, 1 (*N*-Me-Leu)	2 (*N*-Me-Leu)
Pro	1	174.0				
	2	60.9	4.93, m	3	3, 4, 5	NH (Val), 2 (*N*-Me-Val)
	3	32.7	2.09, m	2, 4a, 4b	4, 5	
	4a	23.2	1.90, m	3, 4b	3	
	4b		2.09, m	3, 4a	3, 5	
	5a	48.0	3.47, m	4a, 4b, 5b	3, 4	
	5b		3.51, m	4a, 4b, 5a		

[a] ^1H–^{13}C connectivities were determined by the HMQC method.
[b] Measured at 100 MHz.
[c] Measured at 400 MHz.

2.69). In addition, three broad signals (δ 8.62, 8.36, 7.94) of amide protons were observed. In the ^{13}C NMR spectrum, nine carbonyl signals (δ 176.8, 176.0, 174.3, 174.0, 173.3, 173.0, 172.5, 172.4, 171.2) were observed. Based on further analyses of the ^1H NMR, ^{13}C NMR, COSY, HMQC, HMBC, DEPT 135, and DEPT 90 spectra, urumamide was confirmed to contain seven α-amino acids: valine (Val), leucine (Leu), *N*-Me-isoleucine (*N*-Me-Ile), *N*-Me-alanine (*N*-Me-Ala), *N*-Me-leucine (*N*-Me-Leu), *N*-Me-valine (*N*-Me-Val), and proline (Pro). In addition, the presence of two unusual residues, one α-hydroxy acid and one β-amino acid, was also clarified as

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