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Croissamide, a proline-rich cyclic peptide with an *N*-prenylated tryptophan from a marine cyanobacterium *Symploca* sp.

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ABSTRACT

Croissamide, a proline-rich cyclic peptide that contains an *N*-prenylated tryptophan, was isolated from a marine cyanobacterium *Symploca* sp. Its gross structure was determined by spectroscopic analyses, and the absolute configuration was established based on chiral HPLC analyses of acid hydrolysates.

To date, many proline-rich cyclic peptides have been discovered from various marine organisms such as sponges, ascidians and so on. These compounds have attracted increasing interest because of their biological activities [1], including cytotoxicity [2], antitubercular activity [3], anti-HIV activity [4], repellent (antifouling) activity [5] and inhibitory activity toward NO production [6]. Among marine creatures, cyanobacteria are known to be prolific producers of peptidic natural products, and several proline-rich cyclic peptides, including wewakapeptin A [7], wewakazole B [8], pahayokolide A [9] and trichormamide A [10], have been isolated from marine cyanobacteria. Against this background, we investigated the secondary metabolites of a marine cyanobacterium *Symploca* sp. and isolated croissamide (**1**), a cyclic peptide containing 11 α-amino acids, including five prolines and one *N*-prenylated tryptophan. Here we report the isolation and structure determination of croissamide (**1**).

Marine cyanobacterial samples (1600 g, wet weight) were collected at Minna Island (called "croissant island" due to its crescent shape), Okinawa. Based on morphological observations, the cyanobacterium was identified as *Symploca* sp. (see Supplementary Data for details). This sample was extracted with methanol, and 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 by reversed-phase column chromatography (ODS silica gel, MeOH–H_2O) and repeated reversed-phase HPLC to give croissamide (**1**, 10.4 mg) [11,12].

The molecular formula of **1** was found to be $C_{67}H_{92}N_{12}O_{11}$ by HRESIMS (*m*/*z* 1241.7104, calcd for $C_{67}H_{93}N_{12}O_{11}$ $[M+H]^+$ 1241.7087). The NMR data for **1** are summarized in Table 1. The ^{1}H NMR spectrum revealed the presence of a double doublet signal (δ 6.15, *J* = 17.7, 10.6 Hz) and two doublet signals (δ 5.14, *J* = 17.7 Hz, δ 5.15, *J* = 10.6 Hz) corresponding to a mono-substituted alkene. In the ^{13}C NMR spectrum, 11 carbonyl signals (δ 172.2, 172.1, 171.6, 170.9, 170.4, 170.1, 169.5, 169.1, 169.0, 168.8 and 166.6) were observed. Based on further analyses of the ^{1}H NMR, ^{13}C NMR, COSY, TOCSY, HMQC, HMBC and NOESY spectra, the presence of 11 α-amino acids: glycine (Gly), alanine (Ala), two leucines (Leu), phenylalanine (Phe), five prolines (Pro) and *N*-prenylated-tryptophan (*N*-Pre-Trp) was confirmed. The location of a prenyl group was determined as shown in Fig. 1 based on two NOESY correlations: H4 of *N*-Pre-Trp/H15 of *N*-Pre-Trp and H4 of *N*-Pre-Trp/H16 of *N*-Pre-Trp. Although significant overlap of the methylene signals derived from the five proline residues was observed on the ^{1}H NMR spectrum, we were able to distinguish among them based on analyses of the TOCSY spectrum.

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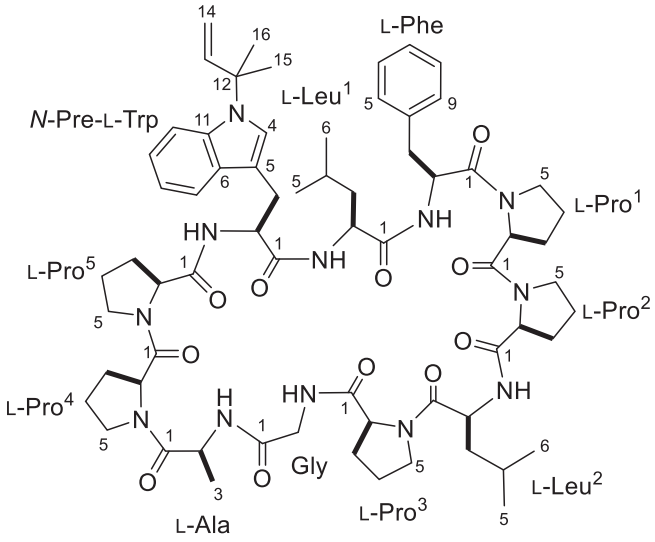

croissamide (1)

The sequences of these partial structures were determined based on HMBC and NOESY data (Table 1 and Fig. 1). Five HMBC correlations, NH of Leu^2/C-1 of Pro^2, NH of Gly/C-1 of Pro^3, NH of Ala/C-1 of Gly, NH of Leu^1/C-1 of *N*-Pre-Trp, and NH of Phe/C-1 of Leu^1, were observed. Moreover, 11 NOESY correlations, H2 of Pro^1/H5a of Pro^2, H2 of Pro^1/H5b of Pro^2, H2 of Leu^2/H5a of Pro^3, H2 of Leu^2/H5b of Pro^3, H2 of Pro^3/NH of Gly, H2 of Pro^4/H5a of Pro^5, H2 of Pro^4/H5b of Pro^5, H2 of Pro^5/NH of *N*-Pre-Trp, H3a of Pro^5/NH of *N*-Pre-Trp, H3b of Pro^5/NH of *N*-Pre-Trp and NH of Leu^1/H2 of *N*-Pre-Trp, were observed. Based on these observations, the presence of two partial sequences, Pro^1 – Pro^2 – Leu^2 – Pro^3 – Gly – Ala and Pro^4 – Pro^5 – *N*-Pre-Trp – Leu^1 – Phe, was clarified. In addition, based on the molecular formula and the degree of unsaturation, **1** was considered to be a cyclic peptide: Pro^1 and Pro^4 must be connected to Phe and Ala, respectively. Thus, the gross structure of **1** was determined as shown in Fig. 1.

The absolute configuration of **1** was determined as follows. The stereochemistry of all the α-amino acid residues was assigned to be L-form based on chiral HPLC analyses of the hydrolysate of **1**. With regard to *N*-Pre-Trp, Trp was obtained due to elimination of the prenyl group during acid hydrolysis, which was used to determine the stereochemistry.

In several solvents such as $CDCl_3$ and CD_3OD, croissamide (**1**) existed as a complex mixture of several conformers, probably due to restricted rotation of the amide bonds in the five proline residues. However, in DMSO-d_6, a single conformer of **1** was observed. Thus, we examined the conformation of each amide bond in the five proline residues in DMSO-d_6. According to previous reports, it is possible to determine the geometries of amide

Table 1
NMR data for croissamide (**1**) in DMSO-d_6.[a]

Unit	Position	δ_C[b]	δ_H[c] (*J* in Hz)	COSY	Selected HMBC (H → C)	Selected NOESY
Leu^1	1	170.9				
	2	52.2	4.16, m	3a, 3b, NH	1	
	3a	41.0	1.11, m	2, 3b, 4		
	3b		1.24, m	2, 3a, 4		
	4	24.5	1.27, m	3a, 3b, 5, 6		
	5	21.5	0.71, d (6.3)	4		
	6	22.3	0.80, d (6.3)	4		
	NH		8.23, d (10.1)	2	1 (*N*-Pre-Trp)	2 (*N*-Pre-Trp)
Phe	1	169.1[d]				
	2	50.6	4.70, m	3a, 3b, NH	1	
	3a	38.9	2.76, dd (13.1, 7.1)	2, 3b	4, 5, 9	
	3b		2.85, dd (13.1, 7.1)	2, 3a	4, 5, 9	
	4	137.2				
	5/9	129.4	7.16, m	6, 8		
	6/8	128.0	7.23, m	5, 9, 7		
	7	126.2	7.17, m	6, 8		
	NH		7.32, m	2	1 (Leu^1)	
Pro^1	1	169.5				
	2	58.3	4.61, brd (8.4)	3a, 3b	1	5a (Pro^2), 5b (Pro^2)
	3a	30.1	1.76, m	2, 3b, 4		
	3b		2.16, m	2, 3a, 4		
	4	21.5	1.78–1.84, m	3a, 3b, 5a, 5b		
	5a	46.3	3.29, m	4, 5b		
	5b		3.69, m	4, 5a		
Pro^2	1	171.6				
	2	59.1	4.40, m	3a, 3b	1	
	3a	27.6	1.21, m	2, 3b, 4a, 4b		
	3b		1.87, m	2, 3a, 4a, 4b		
	4a	25.4	1.94, m	3a, 3b, 4b, 5a, 5b		
	4b		2.10, m	3a, 3b, 4a, 5a, 5b		
	5a	47.5	3.42, m	4a, 4b, 5b		2 (Pro^1)
	5b		3.53, m	4a, 4b, 5a		2 (Pro^1)
Leu^2	1	170.1				
	2	48.0	4.68, m	3a, 3b, NH	1	5a (Pro^3), 5b(Pro^3)
	3a	43.1	1.20, m	2, 3b, 4		
	3b		1.27, m	2, 3a, 4		
	4	23.5	1.50, m	3a, 3b, 5, 6		
	5	23.3	0.56, d (6.2)	4		
	6	21.8	0.65, d (6.2)	4		
	NH		7.81, d (9.1)	2	1 (Pro^2)	

(*continued on next page*)