Konbamide, a Novel Peptide with Calmodulin Antagonistic Activity from the Okinawan Marine Sponge *Theonella* sp.

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A novel peptide, konbamide **1**, with calmodulin antagonistic activity has been isolated from the Okinawan marine sponge *Theonella* sp. and the structure elucidated to be a unique hexapeptide with an ureido bond on the basis of spectroscopic data, including two-dimensional NMR techniques and FAB MS–MS analysis (FAB = fast atom bombardment).

Recently several peptides with unique chemical structures and interesting biological activities have been isolated from marine sponges of the genus *Theonella*.[1] During our studies on bioactive substances from Okinawan marine organisms,[2] we have now isolated a novel calmodulin antagonist, konbamide **1**, from the methanol extract of the sponge *Theonella* sp. The structure of konbamide **1** has proved to be a unique hexapeptide containing the previously unknown amino acid, 2-bromo-5-hydroxytryptophan, and possessing an unusual ureido linkage. This communication deals with the isolation and structural elucidation of **1**.

The sponge, collected off Konbu, Okinawa, was extracted

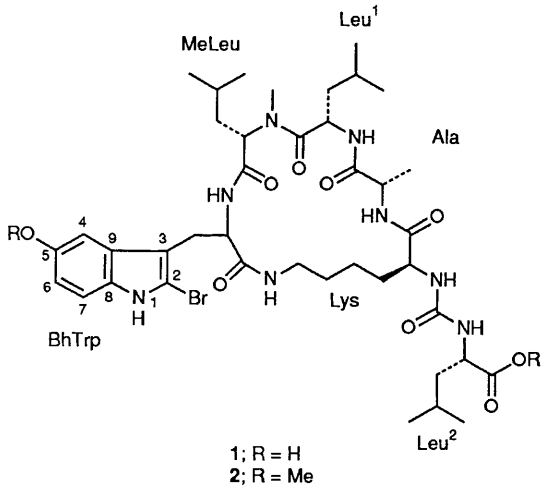
Table 1 ^1H and ^{13}C NMR spectral data of konbamide **1** in [$^2H_6$]Me_2SO

Position		^1H		J/Hz	^{13}C	
BhTrp	CO				170.7	s
	NH	8.29	d	9.0		
	α	4.61	ddd	11.3, 9.0, 3.5	53.7	d
	β	2.77	dd	14.5, 11.3	28.0	t
		3.22	dd	14.5, 3.5		
	1-NH	11.15	s			
	2				109.3	s
	3				109.4	s
	4	6.80	d	2.1	102.3	d
	5				150.6	s
	6	6.57	dd	8.6, 2.1	111.7	d
	7	7.01	d	8.6	111.1	d
	8				130.6	s
	9				127.6	s
MeLeu	CO				169.3	s
	NMe	2.07 (3H)	s		27.7	q
	α	4.72	m		57.9	d
	β	1.25	m		36.5	t
		1.65	m			
	γ	1.32	m		23.8	d
	Me	0.85[a] (3H)	d	6.4	21.1[a]	q
	Me′	0.86[a] (3H)	d	5.7	11.5	q
Leu[1]	CO				172.4	s
	NH	8.74	d	6.2		
	α	4.72	m		47.2	d
	β	1.16	m		39.2	t
		1.71	m			
	γ	1.86	m		24.1	d
	Me	0.92 (3H)	d	6.5	23.3	q
	Me′	0.86[a] (3H)	d	5.7	22.8[a]	q
Ala	CO				173.4	s
	NH	6.99	d	5.7		
	α	4.18	m		48.1	d
	Me	1.31 (3H)	d	7.0	17.1	q
Lys	CO				172.0	s
	α-NH	6.42	d	7.4		
	α	3.93	m		54.1	d
	β	1.60 (2H)	m		31.6	t
	γ	1.24 (2H)	m		28.9	t
	δ	1.40 (2H)	m		28.2	t
	ε	2.77	m		38.3	t
		3.55	m			
	ε-NH	7.44	dd	7.8, 3.3		
Leu[2]	CO_2H				174.0	s
	NH	6.26	d	9.0		
	α	4.03	dd	9.0, 5.0	57.0	d
	β	1.12	m		24.8	t
		1.36	m			
	γ	1.73	m		36.8	d
	Me	0.88 (3H)	d	6.5	15.8	q
	Me′	0.84[a] (3H)	d	7.0	23.1[a]	q
	CO (ureido)				157.5	s

[a] Signals may be interchanged.

with methanol and the methanol extract was partitioned between ethyl acetate and water. The ethyl acetate-soluble fraction was subjected to silica gel flash column chromatography with 5–50% methanol in chloroform followed by reversed-phase HPLC on ODS (methanol : water : trifluoroacetic acid, 75 : 25 : 0.1) to give konbamide {**1**; 0.0012% yield based on wet sponge; $[\alpha]_D^{21}$ −43° (*c* 0.042, MeOH); IR (KBr) ν_{max}/cm^{-1} 3400, 1640, 1520 and 1200; UV (MeOH) λ_{max}/nm 222 (ε 18000), 278 (5800), 298 (4200) and 310 (2900)}.

The positive ion FABMS of **1** gave quasi-molecular ions at *m/z* 877 and 879 $(M + H)^+$ with an intensity ratio of *ca.* 1 : 1 and a characteristic ion at *m/z* 799 due to dehalogenation,[3] indicative of the presence of one bromine atom. The molecular formula was established as $C_{40}H_{61}N_8O_9Br$ by HRFABMS (HR = high resolution) [*m/z* 877.3801 $(M + H)^+$



for $C_{40}H_{62}N_8O_9Br$, calc. 877.3824]. From the ^1H NMR spectrum konbamide **1** was inferred to be a peptide and suggested either to be cyclic or to be without an *N*-terminus by the fact that **1** was negative to the ninhydrin test. The standard amino acid analysis of the hydrolysate of **1** (6 mol dm^{-3} HCl, 110°C, 20 h) revealed the presence of 2 mol of leucine and 1 mol each of alanine and lysine. Compound **1** showed strong-red colouration with Fast Red B salt,[4] indicating the presence of a tryptophan derivative. The ^1H and ^{13}C NMR signals of **1** were assigned on the basis of extensive application of two dimensional NMR techniques [^1H–^1H COSY, NOESY, HMQC (heteronuclear multiple quantum coherence),[5] HMBC (heteronuclear multiple bond correlation),[6] and HOHAHA (homonuclear Hartmann–Hahn)[7]] and presented in Table 1. The HOHAHA spectrum in particular was quite efficient for interpreting the intra-residue proton connectivities of each amino acid. From these NMR data the presence of *N*-methylleucine (MeLeu) and 2-bromo-5-hydroxytryptophan (BhTrp) was clearly revealed. For MeLeu the HMBC correlation peaks were observed from *N*-methyl protons to α-carbon and from α-proton to *N*-methyl carbon, while for BhTrp the ^1H and ^{13}C chemical shifts corresponded well to those of 2-bromo[8] or 5-hydroxyindole derivatives.[9] The HMBC and NOESY (nuclear Overhauser effect spectroscopy) spectra afforded information relevant to the amino acid sequence to establish the connectivity of the cyclic pentapetide moiety [cyclo-(BhTrp-MeLeu-Leu[1]-Ala-Lys)].† The α-CH and α-NH of Lys showed the HMBC correlations with the sp^2 carbon resonating at δ 157.5, to which the HMBC cross-peak was also observed from α-CH of Leu[2]. The NOESY spectrum showed a cross-peak between α-NH(Lys) and NH(Leu[2]). These observations suggested that Leu[2] is attached to α-NH of Lys through an ureido bond and a carboxy group is present at the side-chain terminal. Treatment of **1** with diazomethane yielded a methyl ester **2**, in which the hydroxy group in BhTrp was also methylated [$\delta_H(CDCl_3)$ 3.88 and 3.66, MeO × 2]. The FABMS of **2** showed intense peaks at *m/z* 873 and 875 (1 : 1) due to a stable hydantoin ion generated by loss of methanol between the carboxy group of Leu[2] and α-NH of Lys, which corroborated the presence of the ureido bond.[10] Furthermore, the FAB MS–MS technique was utilized to confirm the structure. The collisionally activated dissociation[11] spectrum of the molecular ion of **1** (*m/z* 877) provided evidence for the amino acid sequence[12] as well as the existence

† The HMBC spectrum showed the sequential cross peaks of NH(BhTrp)/CO(MeLeu), NMe(MeLeu)/CO(Leu[1]), NH(Leu[1])/CO(Ala), NH(Ala)/CO(Lys) and ε-NH(Lys)/CO(BhTrp), whereas in the NOESY spectrum the sequential correlations were observed for NH(BhTrp)/α-H(MeLeu), NMe(MeLeu)/α-H(Leu[1]), NH(Leu[1])/α-H(Ala), NH(Ala)/α-H(Lys) and ε-NH(Lys)/α-H(BhTrp).

of the ureido bond.‡ The chiral GC–MS analysis (Chirasil-Val, Alltech; 25 m × 0.32 mm) of the *N*-trifluoroacetyl–methyl ester derivatives of the peptide hydrolysate showed that Ala, Lys, and two Leu residues of **1** bore the L configurations. The MeLeu residue was revealed to be L by a chiral HPLC study (Chiralpak WH, Daicel Chemical Industries, Ltd; 4.6 × 250 mm; eluent: 1.0 mmol dm^{-3} $CuSO_4$; 50 °C).§ From the results described above the structure of konbamide was concluded to be **1**.

It is well known that calmodulin,[13] a Ca^{2+}-binding protein, regulates many cellular functions as a key mediator of signal transduction in mammalian cells. Konbamide **1** exhibited calmodulin antagonistic activity with the value of the 50% inhibitory concentration of calmodulin-activated brain phosphodiesterase of 10^{-4} mol dm^{-3}. This unique peptide might be produced by symbiotic microorganisms such as microalgae, bacteria, or fungi, since peptides with unusual amino acids and/or peptide linkages have often been isolated from terrestrial microorganisms.

We thank Mr Z. Nagahama for his help in collecting the sponge, Dr H. Hirota, The University of Tokyo, for NMR measurements and helpful discussions, GC–MS and NMR laboratory, Faculty of Agriculture, Hokkaido University, for GC–MS determination, and Professor T. Shioiri, Nagoya City University, for the generous gift of *N*-methylleucine derivatives.

Received, 16th April 1991; Com. 1/01777B

‡ The following substantial daughter ions were observed: *m/z* 748 [cyclo-(BhTrp-MeLeu-Leu[1]-Ala-Lys)-αNH-CO(ureido)]$^+$+2H, 720 [cyclo(BhTrp-MeLeu-Leu[1]-Ala-Lys)-αNH]$^+$+2H, 408 (BhTrp-Lys)$^+$, 380 [(BhTrp-Lys) − CO]$^+$, 312 (Ala-Leu[1]-MeLeu)$^+$ + H and 241 (Leu[1]-MeLeu)$^+$ + H.

§ The absolute configuration of BhTrp residue remains to be defined.

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