Pergamon

Tetrahedron Letters 40 (1999) 4687–4690

TETRAHEDRON LETTERS

Koshikamide A_1, a New Cytotoxic Linear Peptide Isolated from a Marine Sponge, *Theonella* sp.[1]

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Received 5 March 1999; revised 16 April 1999; accepted 23 April 1999

Abstract: Koshikamide A_1, a new cytotoxic linear peptide, was isolated from a marine sponge, *Theonella* sp. Its structure was elucidated by spectroscopic and chemical methods.

Keywords: sponge; cytotoxicity; peptide

Lithistid sponges are a rich source of bioactive peptides, often containing unusual amino acids [1]. Sponges of the genus *Theonella* are particularly interesting from the viewpoints of unusual structural features as well as biological activities. In the course of our search for bioactive metabolites from Japanese marine invertebrates, we encountered a marine sponge of the genus *Theonella*[2] collected from southwestern Japan that showed cytotoxicity against P388 murine leukemia cells. Bioassay-guided isolation furnished a cytotoxic compound named koshikamide A_1 which was a linear decapeptide. Here we describe the isolation and structure elucidation of the new peptide.

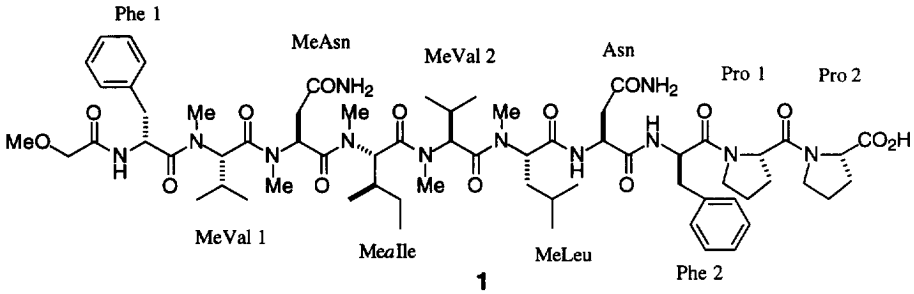
The $CHCl_3$-soluble portion of the EtOH extract of the frozen sponge (280g wet weight) was partitioned between *n*-hexane and MeOH/H_2O (9:1). The aqueous MeOH phase was successively fractionated by ODS

[1] Bioactive Marine Metabolites Series 93. Part 92, Tsukamoto S, Yamashita T, Matsunaga S, Fusetani N., *J. Org. Chem.*, in press.

[2] The sponge was collected at a depth of 10 m off Shimo-Koshiki-jima Island, (129°244'N, 31°244'E). Although closely related to *Theonella swinhoei*, it was clearly different from *T. swinhoei*; it had longer strongyles, coarser ectosomal phyllotriaenes, and two sizes of acanthose rhabds. The desmas were notzygosed but reached the surface. The sponge was deposited at the Zoologial Museum of the University of Amsterdam (ZMA POR. 13011).

0040-4039/99/$ - see front matter
PII: S0040-4039(99)00844-8

flash chromatography [aq. MeOH (stepwise gradient)], gel-filtration [Sephadex LH-20; CH_2Cl_2-MeOH (1:1)], and reversed-phase HPLC [CAPCELL PAK C_{18} UG120; liner gradient H_2O-EtOH-TFA from (50 : 50 : 0.05) to (30:70:0.05)] to afford 35.1 mg of koshikamide A_1 as a colorless solid (0.012% yield based on wet weight).



1

Koshikamide A_1 (**1**)[3] had a molecular formula of $C_{66}H_{100}N_{12}O_{15}$ as determined by HR-FABMS [$(M+Na)^+$, *m/z* 1323.7334 (Δ +0.5 mmu)] and NMR data (Table 1). Interpretation of the COSY, HOHAHA, HMQC, and HMBC spectra in DMSO-d_6, together with amino acid analysis revealed that **1** contained one residue each of Asn, MeAsn, MeIle, and MeLeu, and two residues each of Pro, Phe, and MeVal, in addition to a methoxyacetyl group. Interestingly, Asn, MeLeu, two Pro, one of two Phe, and MeVal residues exhibited doubled NMR signals in a ratio of 1:1.[4] Each set of signals gave rise to the same sequence of MeVal/MeLeu/Asn/Phe/Pro1/Pro2 on the basis of HMBC and ROESY analyses.[5] Detailed NMR analysis proved that the two conformers were due to the *cis/trans* isomerism of a prolyl amide bond between Phe2 and Pro1, which was evident from chemical shifts of β- and γ-carbons of Pro1: *cis* [29.8 (C_β) and 21.9 (C_γ) ppm] and *trans* [27.7 (C_β) and 24.0 (C_γ) ppm] [2], and from ROESY cross peaks between Phe2 α and Pro1 δ, δ 4.26/3.41 [*cis*-isomer] and δ 4.75/3.16 and 3.49 [*trans*-isomer]. The amide bond between Pro1 and Pro2 exclusively adopts *trans*-geometry. The remaining portion of the molecule was determined by an HMBC experiment (Table 1), leading to the gross structure of methoxyacetyl/Phe1/Asn/MeLeu/MeVal/MeIle/MeAsn/MeVal/Phe2/Pro1/Pro2. This sequence was also supported by FAB-MS/MS analysis (Fig. 1).

Marfey analysis [3] of the acid hydrolysate disclosed that the absolute configuration of Phe was D, while that of the remaining amino acids was L. The presence of *Mea*Ile in **1** was confirmed by comparison of the ^1H NMR spectrum of the amino acid isolated from the acid hydrolysate with those of authentic MeIle and *Mea*Ile.[6]

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[3] $[\alpha]_D^{25}$ -156 ° (*c* 0.19, MeOH) ; IR (film) 3410, 3339, 2962, 2930, 2874, 1635, 1521, 1456, 1404, 1269, 1201, 1122, 1006, 700 cm^{-1} ; UV (MeOH) 212 nm (ε 2.9 x 10^4).

[4] NMR data of the *cis* isomer for residues 6-10 are as follows (Chemical shifts of residues 1-5 were identical with those of the trans-isomers.): ^1H NMR (DMSO-d_6) MeLeu δ 5.11 (α), 1.44 (β), 1.60 (β), 1.16 (γ), 0.78 (δ), 0.84 (δ'), 2.77 (*N*-Me); Asn δ 4.40 (α), 2.16 (β), 2.23 (β), 6.89 and 7.15 ($CONH_2$), 7.54 (NH); Phe2 δ 4.26 (α), 2.73 (β), 2.77 (β), 7.03 (C2, C6), 7.20 (C3, C5), 7.15 (C4), 8.40 (NH); Pro1 δ 4.91 (α), 1.96 (β), 2.29 (β), 1.79 (γ), 3.41 (δ); Pro2 δ 4.30 (α), 1.90 (β), 2.11 (β), 1.90 (γ), 3.48 (δ), 3.69 (δ). ^{13}C NMR (DMSO-d_6) MeLeu δ 169.9 (CO), 53.5 (α), 36.2 (β), 24.3 (γ), 20.9 (δ), 23.3 (δ'), 30.1 (*N*-Me); Asn δ 170.8 (CO), 49.6 (α), 37.7 (β), 171.3 ($CONH_2$); Phe2 δ 169.3 (CO), 51.6 (α), 36.6 (β), 138.1 (C1), 128.8 (C2, C6), 128.0 (C3, C5), 126.2 (C4); Pro1 δ 57.6 (α), 29.8 (β), 21.9 (γ), 46.6 (δ); Pro2 δ 172.9 (CO), 58.7 (α), 28.4 (β), 24.5 (γ), 46.0 (δ). NMR data for the *trans* isomer, see Table 1.

[5] The carbonyl carbon of Pro1 did not give any cross peaks in the HMBC experiments. However, the linkage of the three residues from the C-terminus was established on the basis of the ROESY data, which disclosed not only the sequence but also the conformaitonal isomerism.

[6] ^1H NMR (CD_3OD) L-*N*-MeIle δ 3.54 (d, 1.5), 2.70 (s), 1.94 (brd.), 1.65 (sep., 6.2), 1.36 (sep., 6.9), 1.02 (d, 6.9), 0.98 (t, 7.3); L-*allo*-*N*-MeIle 3.66 (d, 3.1), 2.71 (s), 2.03 (brd.), 1.56 (sep., 6.9), 1.28 (m), 1.06 (d, 6.9), 0.98 (t, 7.3).

Table 1. ^{1}H and ^{13}C NMR Data for Koshikamide A_1 (1) in DMSO-d_6

		δH	δC	HMBC			δH	δC	HMBC
methoxyAc	OMe	3.20	58.4	70.9	MeLeu*	CO		170.0	
	CH_2	3.74	70.9	58.4, 168.9		α	5.10	53.4	30.3, 36.2, 170.0
	CO		168.9			β	1.46	36.2	21.1, 24.4
Phe 1	CO		171.2				1.65		
	α	4.93	50.2	36.6, 137.2, 168.9, 171.2		γ	1.16	24.4	
	β	2.86	36.6	50.2, 129.3, 137.2, 171.2		δ	0.78	21.1	23.2, 24.4
		3.00		50.2, 129.3, 137.2, 171.2		δ'	0.84	23.2	21.1, 24.4
	C1		137.2			*N*-Me	2.81	30.3	53.4, 170.1
	C2, 6	7.28	129.3	36.6, 126.5, 129.3	Asn*	CO		169.8	
	C3, 5	7.25	129.3	126.5, 129.3, 137.2		α	4.53	49.5	37.4, 169.8, 171.4
	C4	7.18	126.5	129.3		β	2.24	37.4	49.5, 169.8, 171.4
	NH	8.01		36.6, 50.2, 168.9			2.32		49.5, 169.8, 171.4
MeVal 1	CO		168.7			$CONH_2$	6.91	171.4	37.4
	α	4.89	58.2	17.8, 19.6, 26.5, 29.8, 168.7, 171.2			7.25		
	β	2.15	26.5	17.8, 19.6, 58.2		NH	7.71		170.0
	γ	0.57	17.8	19.6, 26.5, 58.2	Phe 2*	CO		168.2	
	γ'	0.79	19.6	17.8, 26.5, 58.2		α	4.75	51.6	38.1, 136.8, 168.2, 169.8
	N-Me	2.88	29.8	58.2, 171.2		β	2.76	38.1	51.6, 129.3, 136.8
MeAsn	CO		169.3				2.88		51.6, 129.3, 136.8
	α	5.66	50.0	30.4, 169.3, 171.0		C1		136.8	
	β	1.99	34.4	50.0, 169.3, 171.0		C2, C6	7.14	129.3	38.1, 126.5, 129.3
	β'	2.82		50.0, 169.3, 171.0		C3, C5	7.24	128.1	128.1, 136.8
	$CONH_2$	6.82	171.0	34.4		C4	7.19	126.5	129.3
		7.30				NH	7.84		169.8
	N-Me	2.75	30.4	50.0, 168.7	Pro 1*	CO		-	-
MeaIle	CO		169.0			α	4.48	57.4	24.0
	α	5.04	56.2	13.4, 25.3, 29.3, 32.3, 169.0		β	1.74	27.7	24.0, 46.7
	β	2.09	32.3				1.98		
	β'	0.64	13.4	25.3, 32.3, 56.2		γ	1.65	24.0	57.4
	γ	0.97	25.3	10.5, 13.4, 32.3			1.85		
		1.23				δ	3.16	46.7	
	δ	0.81	10.5	25.3, 32.3			3.49		
	N-Me	2.79	29.3	56.2, 169.3	Pro 2*	CO		173.2	
MeVal 2	CO		170.1			α	4.22	58.3	24.5, 28.4, 173.2
	α	5.05	57.6	17.7, 19.4, 26.7, 29.4, 169.0, 170.1		β	1.84	28.4	24.5, 46.2, 173.2
	β	2.15	26.7	17.7, 19.4, 57.6			2.11		24.5, 173.2
	γ	0.72	17.7	19.4, 26.7, 57.6		γ	1.92	24.5	28.4, 46.2
	γ'	0.83	19.4	17.7, 26.7, 57.7		δ	3.52	46.2	28.4
	N-Me	2.79	29.4	57.6, 169.0			3.68		24.5, 28.4

*The data for the *trans* isomer.

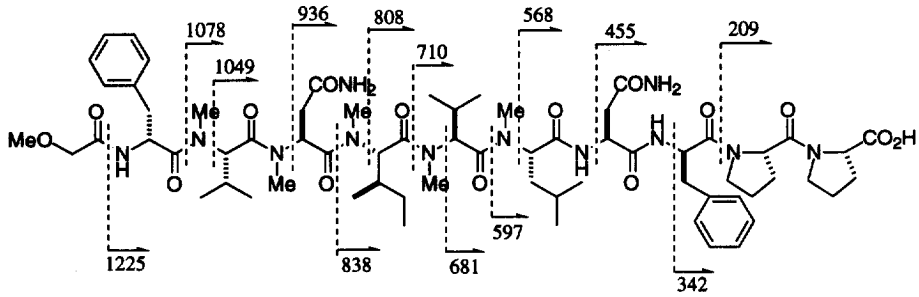

Fig. 1. FAB-MS/MS fragmentation (negative mode) of koshikamide A_1 (1).

Koshikamide A_1 showed moderate cytotoxicity against P388 leukemia cells with an IC_{50} value of 2.2 μg/mL and has the methoxyacetylated *N*-terminus, which is reminiscent of theonellapeptlides, tridecapeptide lactones, from other *Theonella* sponges [4]. Component amino acids of theonellapeptolides are characterized by the presence of β-alanine residues, which are not present in **1**. Although linear peptides with *N*-methyl amino acids have been isolated from the mollusks [5] and blue green alga [6], the presence of five contiguous *N*-methyl amino acid residues is, to the best of our knowledge, unprecedented [7].

Acknowledgment

We are indebted to Professor Paul J. Scheuer, University of Hawaii for reading the manuscript. Thanks are also due to the crew of R/V Toyoshiomaru for assistance in collecting the sponge and to T. Araki for cytotoxicity test. This work was partly supported by a grant-in-aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

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