Isolation and Structure Elucidation of Perthamide B, a Novel Peptide from the Sponge *Theonella* sp.

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Abstract: A sponge of the genus *Theonella* collected off Perth, Australia, contained a cyclic octapeptide, perthamide B, which weakly inhibited the binding of [^{125}I]IL-1β to intact EL4.6.1 cells with an IC_{50} of 27.6 μM. The inhibition of binding could however, not be separated from the cytotoxic effects of perthamide B. The structure was elucidated by spectroscopic methods.

Over the past two decades marine sponges have been revealed to be an excellent source of bioactive peptides.[1] Examples include the potent cytotoxic peptides jaspamide[2] from *Jaspis* sp.; polydiscamide A[3] from *Discodermia polydiscus*; the geodiamolides C-F[4] from *Pseudaxinyssa sp.*; the discokiolides[5] from *Discodermia kiiensis*; hymenastin 1[6] from a *Hymeniacidon* sp.; theonellamide F,[7] and orbiculamide[8] from *Theonella* spp. A number of enzyme inhibitors have been discovered including the phospholipase A_2 inhibiting discodermins[9] from *Discodermia kiiensis*; a potent protein phosphatase inhibitor, motopurin[10] from *Theonella swinhoei;* a Ca^{+2}ATPase inhibitor, keramamide A,[11] from *Theonella sp.*, and the cyclotheonamides[12] from *Theonella sp.* which are potent inhibitors of thrombin, trypsin and plasmin. These compounds are especially interesting due to the presence of novel amino acids in the majority of these compounds as well as their potent bioactivities.

As part of our continuing research to define novel natural products with therapeutic potential which act via inhibition of binding of ligand to its receptor, a number of extracts of marine organisms were screened for their ability to inhibit binding of [^{125}I]IL-1β to intact EL4.6.1 cells. An extract of the sponge *Theonella sp.*[13] (Demospongiae, Lithistida, Discodermiidae, *Theonella* sp.) collected at a depth of 15 m by SCUBA near Perth, off Cape Vlamingh in Western Australia, was found to inhibit binding in this assay. Bioassay guided purification of the active components was undertaken and the compound which we call perthamide B was isolated. This paper will describe the isolation and structure elucidation of this metabolite.

The frozen sponge was extracted exhaustively with MeOH and the extract was concentrated to dryness. The extract was partitioned between *n*-butanol and water. The active organic partition was further chromatographed on an LH-20 stationary phase eluted with methanol. The active fractions from this separation were combined and chromatographed by HPLC using a Vydac Protein and Peptide C-18 column eluted with 35% phosphate buffer (10 mM, pH 6.5) in MeOH to obtain pure perthamide B[14] (0.03% of wet weight).

Both the ^{1}H and ^{13}C NMR spectra (Table 1) suggested that perthamide B was a peptide. Additionally the DEPT experiment indicated the presence of 15 CH, 8 CH_2,7 CH_3 and 14 quaternary carbons. HRFABMS suggested a formula of $C_{44}H_{65}N_{10}O_{14}{}^{81}Br$ ($M+H^+$, obsvd. 1039.3946, calc. 1039.3924) which requires 17 sites of unsaturation. The NMR spectra suggested the presence of a 1,3,4 trisubstituted aromatic ring, of which the ^{1}H NMR signals appeared at δ 6.81 (d J = 8.1 Hz), 7.25 (br d, J = 8.1 Hz) and 7.33, a trisubstituted olefin and ten amide carbonyls. This accounts for fifteen of the sites of unsaturation and suggested that perthamide B had two additional rings. The individual amino acids were assigned based upon comparison to literature chemical shifts and interpretation of the 2-D COSY, TOCSY (Fig. 1), HMQC and HMBC experiments as described below. Full NMR data will be reported elsewhere.

The presence of an Asn unit was suggested by the COSY, TOCSY and HMBC data. Most important were the TOCSY correlations observed between the NH and both the α-H and β-CH_2 protons; and the HMBC correlations observed between the $CONH_2$ and the β-CH_2. A 3-amino-2-hydroxy-6 methyloctanoic acid moiety (Hamo) was assigned based upon interpretation of the ^{1}H and ^{13}C chemical shift data and the 2-D COSY, TOCSY and HMBC data in which correlations between the α-H through the η-H were observed (selected TOCSY correlations include: NH to the α-H, β-H, γ-H and ζ-CH_3; ζ-CH_3 to γ-H, NH and ζ-CH_2; ζ-CH_2 to η-CH_3). Related α-hydroxy-β-amino acids with modified side chains have been reported from the blue green alga *Calothrix fusca*[15] and the fungus *Streptomyces viridochromogenes.*[16] The 2,3-dehydro-2-aminobutyric acid (Dhb) was assigned based upon the TOCSY correlations observed between the NH and γ-CH_3 and between the γ-CH_3 and the β-H. Also important were the HMBC correlations observed between the γ-CH_3 and both the α and β carbons. An enhancement observed between the γ-CH_3 and the NH in a 1D difference nOe experiment suggested the E configuration of the double bond. The *N*-Me Gly was proposed based on the ^{1}H, ^{13}C and the HMBC data.

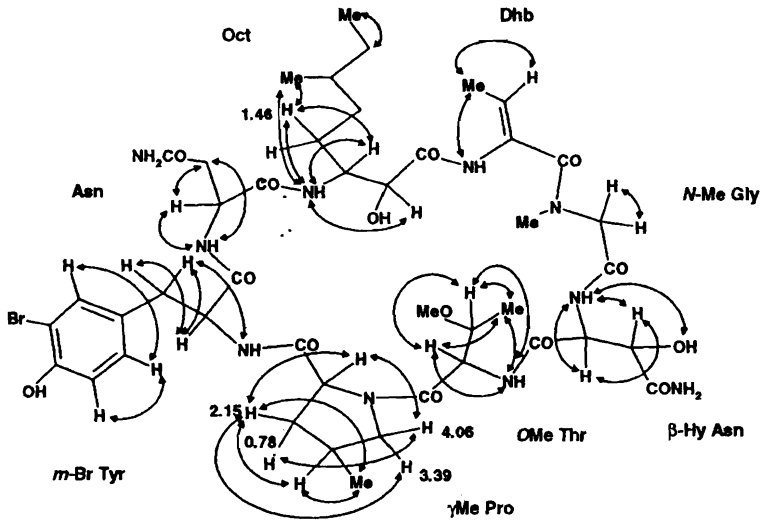

Fig.1 Selected TOCSY correlations of perthamide B

Table 1. ^{1}H and ^{13}C NMR Data[a] of Perthamide B in DMSO-d_6

Amino Acid / C#	^{1}H	^{13}C	Amino Acid / C#	^{1}H	^{13}C
Asn			β OH	5.64 (br, s)	--
α	4.66 (1H, m)	48.1	NH	7.25	--
β	3.08 (1H, dd, 4.7, 16.3) 2.37 (1H, br d, 16.3)	37.0	$CONH_2$	7.56 (br s), 7.33	173.8
NH	7.14 (1H, d, 10.2)	--	CO	--	168.5
$CONH_2$	7.64 (1H, br, s) 7.33 (1H)	172.6	***O*Me Thr**		
CO	--	170.7	α	4.84 (1H, br s)	55.6
Octanoic moiety			β	4.13 (1H, m)	72.6
α	4.09 (1H, d, 1.6)	72.7	γ	1.18 (3H., d, 6.0)	14.5
β	4.0 (1H, m)	52.0	NH	8.66 (1H, d, 10)	--
γ	1.46 (1H, m), 1.08 (1H, m)	23.7	OMe	3.23 (3H, s)	54.9
δ	1.09 (2H, m)	32.0	CO	--	[b]
ε	1.17 (1H, m)	33.5	**γMe Pro**		
ζ	1.02 (1H, m), 1.22 (1H, m) 0.75 (3H, d, 6.5)	28.9 15.9	α	3.91 (1H, dd, 6.6, 11.2)	63.5
η	0.76 (3H, t, 7.4)	11.1	β	2.15 (1H, m), 0.78 (1H, m)	36.4
NH	6.32 (1H, d, 9.3)	--	γ	2.24 (1H, m)	33.5
OH	4.85 (1H, br s)	--	δ	3.39 (1H, m), 4.06 (1H, m)	53.2
CO	--	169.5	Me	1.05 (3H, d, 6.3)	18.6
Dhb			CO	--	171.0
α	--	132.2	***m*-Br Tyr**		
β	5.80 (1H, q, 6.9)	126.4	α	4.18 (1H, dd, 12.0, 4.7)	55.6
γ	1.58 (3H, d, 6.9)	12.2	β	2.93 (1H, br d, 13.0), 2.87 (1H, d, 13.0)	30.5
NH	8.7 (1H, s)	--	1'	--	127.1
CO	--	167.3	2'	7.33 (1H, br s)	132.7
***N*-Me Gly**			3'	--	110.7
α	3.42 (1H, d, 17.5) 4.26 (1H, d, 17.5)	51.7	4'	--	154.1
N-Me	2.82 (3H, s)	35.1	5'	6.81 (1H, d, 8.1)	116.8
CO	--	[b]	6'	7.25 (1H, br d, 8.1)	130.5
β Hy Asn			CO	--	[b]
α	5.01 (1H, m)	54.5	NH	7.06 (br s)	
β	4.22 (1H,d, 3.7)	71.3			

[a]Recorded on a Bruker AMX-500. [b]Carbonyls at δ 171.2, 170.4, 167.5 are unassigned.

The β-hydroxyAsn unit was assigned based upon comparison of the ^{1}H and ^{13}C NMR data with that reported by Fusetani et. al.[7] An *O*-methyl-threonine was assigned based upon the TOCSY and HMBC data. Especially important were the HMBC correlations observed between the OCH_3 protons and the β-C as well as between the α-H and the β-CH_3 group. The presence of a γ-methyl proline unit was proposed based upon the COSY, TOCSY and HMBC data. The most useful HMBC correlations were those observed between: the CO and both the α and β protons; the β-C and the δ-H; and finally the γ-CH_3 protons and the β-C, γ-C and δ-C. The final amino acid, *m*-bromotyrosine unit was assigned based upon

comparison of the ^{13}C chemical shift data to that of known compounds and interpretation of the 2-D COSY and HMBC data. An HMBC correlation observed between H-2' of the benzene ring and the β-C of the side chain confirmed the assignment of the bromine position.

The amino acids were connected based upon HMBC correlations and nOe data. The major interresidue HMBC correlations observed were: Asn CO/Octanoic NH; Octanoic CO: Dhb NH; Dhb CO:N-Me of NMeGly. The other connectivities were made based on the 1D nOe difference experiments: (**irradiated proton**: enhanced proton) **βHydroxy Asn NH**: NMe Gly α-H; ***O*-Methyl Threonine NH**: βHydroxy Asn α-H; **OMe of *O*-Methyl Threonine**: γ-methyl proline δ-H. The 2D ROESY experiment showed correlations between the H-5' of the *m*-bromotyrosine and the Me of the γ-methyl proline indicating that γ-methyl proline and *m*-bromotyrosine are connected. Additional nOe results of (**irradiated proton**: enhanced proton) **Asn NH**: Octanoic NH; **Dhb NH**: *N*-MeGly α-H further confirmed the proposed structure. The final connectivity between Asn and the *m*-bromotyrosine was made based upon the molecular formula which indicated the presence of one additional ring. The relative and absolute stereochemistry of perthamide B are currently under investigation. Perthamide B showed a moderate inhibition of binding of [^{125}I]IL-1β to intact EL4.6.1 cells with an IC_{50} of 27.6 μM. However the binding could not be differentiated from the toxicity of the compound.

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