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THEONELLAMINE B, A NOVEL PEPTIDAL Na,K-ATPASE INHIBITOR, FROM AN OKINAWAN MARINE SPONGE OF THE GENUS THEONELLA.[1,2]

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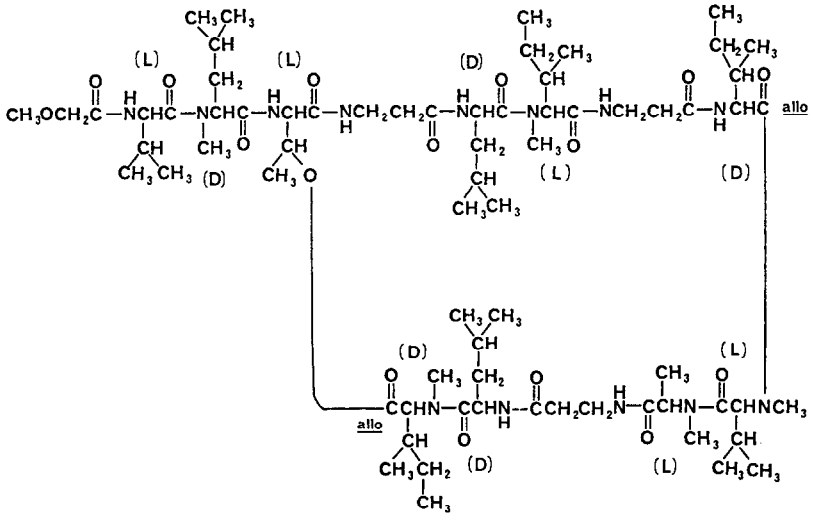
Abstract: A novel tridecadepsipeptide, theonellamine B, has been isolated from an Okinawan marine sponge of the genus Theonella as a specific inhibitor against Na,K-ATPase. Its structure has been determined to be a lactone of methoxyacetyl-L-Val-D-MeLeu-L-Thr-β-Ala-D-Leu-L-MeIle-β-Ala-D-allo-Ile-L-MeVal-L-MeAla-β-Ala-D-Leu-L-allo-MeIle on the basis of chemical reaction and spectral data including two dimensional homo- and heteronuclear shift correlation experiments.

Though large numbers of novel natural products have been isolated from marine sponges,[3] only four bioactive peptides have been reported.[4] In our continuing search for physiologically active substances from marine sources, we recently found several enzyme modulators from Okinawan sponges.[5] This report deals with the isolation and structure elucidation of theonellamine B, an unusual tridecadepsipeptide with a 37-membered ring. This is the first peptide that inhibits Na,K-ATPase (50% inhibitory concentration in pig brain Na,K-ATPase, 7×10^{-6} M).

The 1-butanol soluble portion of 70% ethanolic extracts of a sponge of the genus Theonella collectd in Okinawa Island was subjected to column chromatographies on



silica gel and alumina ($CHCl_3$-MeOH). Further purification was carried out by means of HPLC on an ODS column (0.2M NaCl in 9 : 1 MeOH-H_2O) to afford pure theonellamine B as colorless powder (0.16% from wet sponge, mp 149-151 °C); $[\alpha]_D^{25}$ -57.2 ° (c 1.0, MeOH). Upon hydrolysis (6 N HCl, 110 °C, 40 h) it liberated 10 amino acids; β-alanine (3 residues, β-Ala), N-methyl-L-alanine (1, MeAla), L-valine (1, Val), N-methyl-L-valine (1, MeVal), L-threonine (1, Thr), D-leucine (2, Leu), N-methyl-D-leucine (1, MeLeu), D-allo-isoleucine (1, a-Ile), N-methyl-L-isoleucine (1, MeIle) and N-methyl-D-allo-isoleucine (1, Me-a-Ile).[6] Since this peptide was negative to ninhydrin reagent and showed an IR band (KBr) at 1740 cm^{-1}, it is speculated that the N-terminus be acylated and the hydroxyl group of Thr be esterified, the later of which was supported by a low field signal at δ 5.01 in ^1H NMR. Fast atom bombardment mass spectrum (FABMS) of **1** [m/z 1405.6, $(M+H)^+$] revealed the presence of an additional unit having a molecular weight of 73 dalton, which was evidenced by ^1H and ^{13}C NMR signals at δ_H 3.22 (3H, s) and 3.74 (2H, AB center, J=15Hz) and δ_C 58.77 (q), 71.52 (t) and 168.7 (s). These data allowed us to assign the unknown portion to methoxyacetyl (MA) group. Treatment of **1** with DBU in toluene (100 °C, 2 h) yielded an unsaturated linear peptide, whose FABMS (m/z 1426.4, M^++Na) and amino acid analyses (loss of Thr) gave further evidence that the hydroxyl group of Thr residue and an N-terminus were acylated by C-terminus carbonyl and MA groups, respectively.

Hydrolysis of **1** in 50% formic acid (100 °C, 5 h) afforded three peptide fragments, I [mp 142-148 °C, FABMS m/z 1310.1, $(M+H)^+$], II [mp 119-124 °C, FABMS m/z 998.5, $(M+H)^+$], and β-alanyl-allo-isoleucine. Amino acid analyses of I (loss of MeVal) and II (loss of MeVal, a-Ile, β-Ala and MeIle) suggested the presence of a sequence composed of MeIle, β-Ala-a-Ile and MeVal in **1**. Two dimensional ^1H-^1H and ^1H-^{13}C shift correlation experiments of **1** in $CDCl_3$ made it possible to assign both proton signals and protonated carbon signals to each amino acid residues except signals for Me-a-Ile.[8] The detailed negative NOE experiments allowed to distinguish MeIle from Me-a-Ile, and allowed us to construct partial structures A-G as shown in Figure 1.

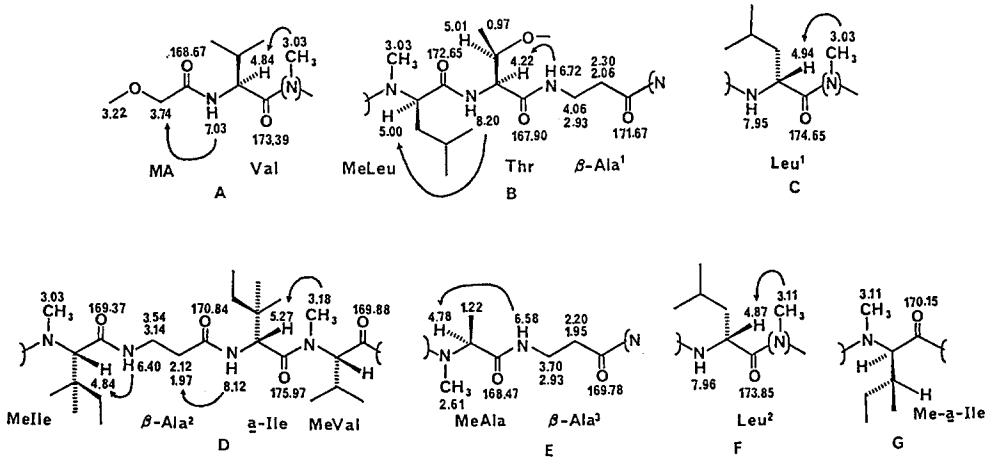


Fig.1 Partial structures A, B, C, D, E, F, and G, a part of ^1H and ^{13}C NMR data for **1** in $CDCl_3$ and observed NOE at -20 °C (→).

Recently, peptide sequencing by ^{1}H-^{13}C shift correlation via long range couplings has been shown to be effective,[9] especially in the case of unusual peptides containing N-methyl amino acids.[10] In the ^{1}H-^{13}C shift correlation spectrum of **1** measured in $CDCl_3$, all methyl proton signals could be assigned by four sets of long range couplings between α-protons of N-methylated amino acid residues and the corresponding N-methyl carbons. Similarly 14 carbonyl carbon signals were assigned by long range couplings to α-, NH or N-methyl protons (Figure 2). Five carbony carbon signals were coupled to both N-methyl proton signals and α-proton signals of their adjacent amino acid residues, indicating the presence of sequences of Val-MeLeu, a-Ile-MeVal-MeAla, Leu-MeIle, and Leu-Me-a-Ile, thus sequences of A-B, C-D-E and F-G were established. Furthermore, correlation observed between a carbony carbon signal of β-Ala[3] and an NH proton signal of Leu residue evidenced that the partial structure E was attached to F. From these data, the peptide sequence, A-B-C-D-E-F-G was obtained for theonellamine B (**1**).

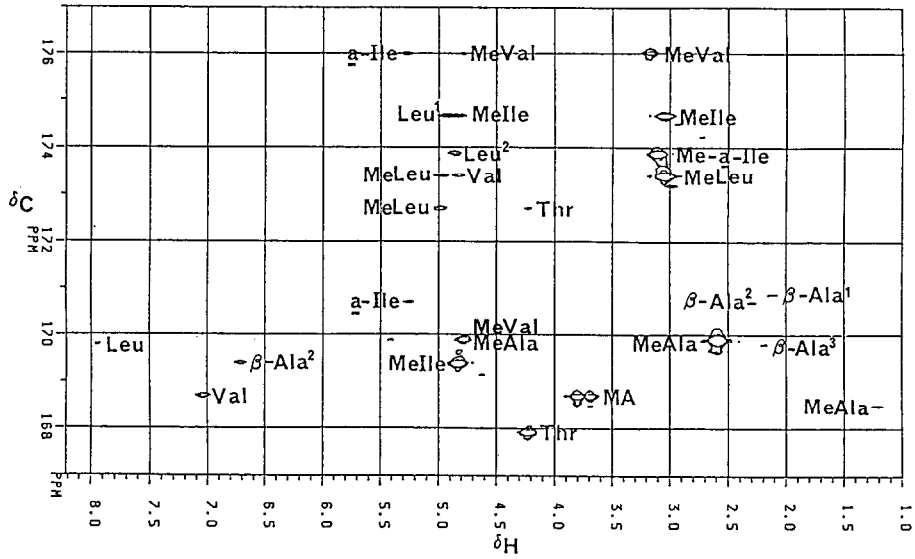


Figure 2. A part of 400 MHz ^{1}H-^{13}C shift correlation spectrum of **1** optimized for 6 Hz proton coupling constants of the carbonyl carbon signals in $CDCl_3$ (100 mg/0.6 ml).[11] The shift correlation peaks from NH proton signals (δ 6.5 -8.0), α-proton signals (δ 3.5-5.5 and 2.2-2.5) and N-methyl proton signals (δ 2.5-3.5) are indicated by abbreviation of their residues.

Unusual depsipeptides with N-methyl amino acid residues are frequently encountered in peptide antibiotics[12] as well as marine peptides.[4,13] It is the question whether theonellamine B is synthesized by the sponge or by some symbiotic or associated microorganisms. The detailed chemical and pharmacolgical studies on theonellamine B and its related compounds are in progress.

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References and Notes

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