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Preliminary Structure Determination of the theonellapeptolide Ie from the marine sponge *Theonella swinhoei* Using NMR Methods

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Abstract : A known theonellapeptolide **Ie**, previously reported in other research group, was isolated from the methanolic extract of the Philippine sponge *Theonella swinhoei*. The planar structure of this compound was determined on the basis of NMR methods including HMBC and selective HMBC experiments. This is fast and efficient for the dereplication of natural products compared with the MS studies of fragments obtained from complete and partial hydrolysis. The sequence of thirteen amino acids including six N-methyl amino acids in the compound was clearly determined from correlations of extensive HMBC experiments.
Key words : theonellapeptolide, selective HMBC, *Theonella swinhoei*, small peptide

INTRODUCTION

A number of small peptides have been reported from marine sponges. They are so structurally unique and biologically active that they have been paid considerable attention because of potential as important drugs.[1] Among them, a series of theonellapeptolide, isolated from the marine sponge *Theonella swinhoei*, is a rare example with a high proportion of N-methyl amino acids. It exhibited moderate cytotoxicity, ion-transport activity for Na^+ and K^+ ion[2] and Na^+, K^+ - ATPase inhibitory activity.[3] More than ten theonellapeptolides have so far been reported. [2, 4-8]

In our search for bioactive compounds from marine organisms, we have recently investigated the chemical constituents of a Philippine sponge *Theonella swinhoei* and isolat-

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ed two known macrolides, named swinholide **A** and swinholide **A** carboxylic acid, and two known depsipeptides, theonellapeptolides **Id**, **Ie**.

In most cases, the structure of theonellapeptolides was established by MS studies on the fragments obtained from complete and partial hydrolysis. However, for the dereplication of natural peptides isolated from organisms, a fast and efficient way to determine the sequence of amino acids is needed. Extensive analyses of NMR data in an intact molecule are available to establish of the amino acids sequence of small peptides. Though a peptide with fairly congested carbonyl carbons makes the NMR analyses difficult, the selective HMBC experiment solves the problem.[9-10]

We here report our preliminary determination of structure of the theonellapeptolide **Ie** through a combination of NMR spectra.

EXPERIMENTAL

NMR experiments

The ^{1}H and 2D NMR spectra were recorded on a Bruker Biospin AVANCE 800 spectrometer installed at Korea Basic science Institute (KBSI), in Ochang Korea, while ^{13}C NMR spectrum was recorded on a Jeol JNM-ECP 500 at WonKwang University, in Iksan. All the spectra were measured in acetonitrile-d_3 at 24°C and referenced to residual solvent signals at 1.94ppm for ^{1}H and at 118.7ppm for ^{13}C in the nitrile group. The assignment of the protons and carbons in the compound was carried out on the basis of COSY, TOCSY, HSQC, HMBC, and selective HMBC spectra.

Long-range ^{1}H-^{13}C correlation experiments were used with the heteronuclear multiple bond coherence (HMBC) sequence with a delay of *62.5ms*. A selective HMBC experiment was done with selective low powered Q5 carbon pulse of 500μsec unambiguously to assign fairly congested carbonyl carbon resonances in the ^{13}C spectrum (128 × 1024).

Extraction and isolation

The sponge (500g dry weight) collected at Liloan Island, Philippine by SCUBA was dried in the shadow and transported to our laboratory under vacuum package. This specimen

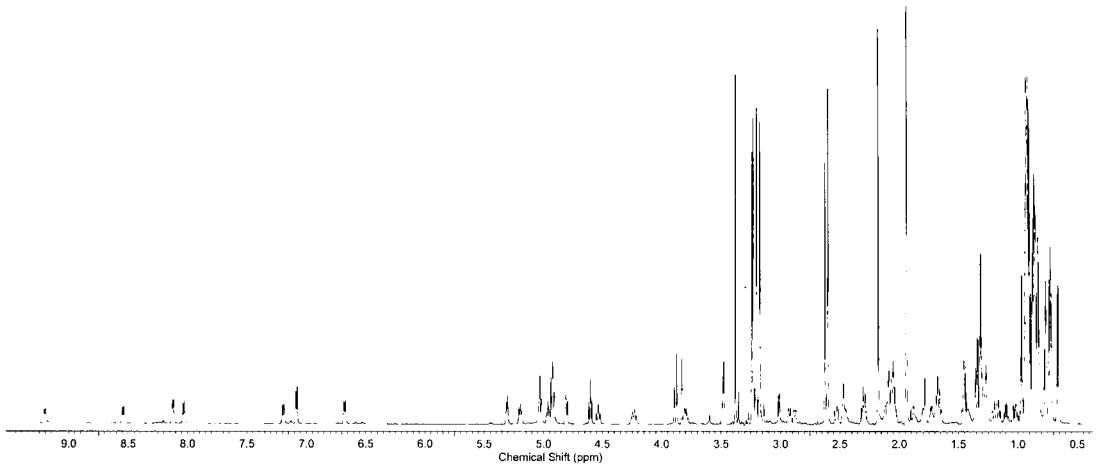


Fig.1 ^{1}H spectrum for compound **1** obtained in acetonitrile-d_3 at 800MHz.

was extracted with MeOH solvent (2L) twice for 4 days. The methanolic extract (20g) was partitioned between CH_2Cl_2 and H_2O and the organic fraction successively re-partitioned into 15% aqueous MeOH and hexane. The methanol soluble material was subjected on reverse-phased silica flash chromatography and eluted with the solvent of decreasing polarity (H_2O : MeOH = 50 : 50 → 10 : 90, 100% MeOH, 100% acetone) to afford seven fractions. A fraction of 90% aq. MeOH was separated on HPLC using a mixture solvent of 95% MeOH and 5% H_2O to give macrolide compounds, swinholide **A** and swinholide **A** carboxylic acid. Two theonellapeptolides were isolated from a fraction of 100% MeOH on reverse phased HPLC (YMC ODS-A 250 × 10 mm column, Waters RI detector) using 100% MeOH solvent at a retention time of approximately 45 min. Purification of theonellapeptolide **Ie** was carried out a further HPLC with 92% aq. MeOH as eluant solvent.

RESULT AND DISCUSSION

Compound **1** (theonellapeptolide **Ie**), colorless crystal and *m/z* 1419 $[M + H]^+$ in the LRFABMS, exhibited absorption bands from the amide groups (3318, 1626 and 1542 cm^{-1}) and lactone group (1733 cm^{-1}) in the IR spectrum. The ^{1}H NMR spectrum of **1** measured in acetonitrile-d_3 showed signals consisting of seven doublet protons in the downfield range,

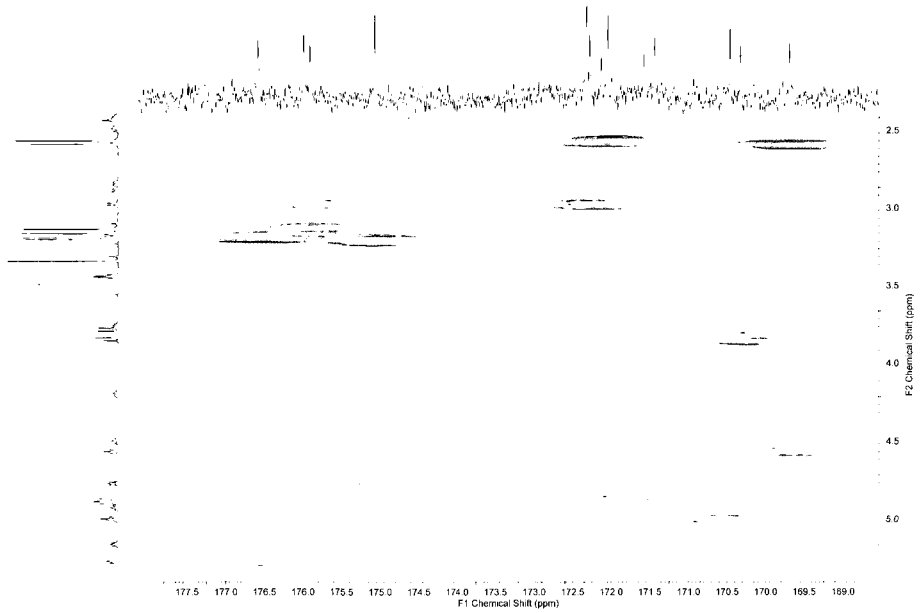
Table 1. NMR Spectral data of **1** (theonellapeptolide Ie) in CH_3CN.

		Theonellapeptolide Ie	
		$\delta^{13}C$, multi	$\delta^{1}H$ (multi, Hz)
MeOAc (1)	OMe	60.0, q	3.38 (br s)
	CH_2	72.8, t	3.82 (AB, 14.9) / 3.88 (AB, 14.9)
	CO	170.32, s	
Val (2)	NH		7.09 (d, 9.0)
	α-CH	55.2, d	4.81 (dd, 9.0, 6.7)
	β-CH	32.2, d	2.04 (m)
	CH_3	18.3, q	0.87 (d, 7.0)
	CH_3	12.7, q	0.92 (d)
	CO	175.05, s	
N-Me-Leu (3)	NCH_3	32.5, q	3.24 (s)
	α-CH	56.3, d	5.03 (dd, 12.5, 3.1)
	β-CH_2	38.6, t	1.20 (ddd, 12.5, 12.5, 3.1) / 2.05 (m)
	γ-CH	26.2, d	1.42 (m)
	CH_3	24.2, q	0.90 (d)
	CH_3	21.6, q	0.73 (d, 6.7)
	CO	172.27, s	
Thr (4)	NH		9.21 (d, 9.8)
	α-CH	53.8, d	4.60 (dd, 9.8, 9.8)
	β-CH	71.0, d	5.20 (m)
	CH_3	19.0, q	0.97 (d, 6.3)
	CO	169.69, s	
N-Me-β-Ala (5)	NCH_3	35.2, q	2.63 (s)
	α-CH_2	45.7, t	2.62 (d, 12.5) / 4.54 (dd, 12.5, 12.5)
	β-CH_2	35.1, t	2.06 (m) / 2.30 (dd, 12.5, 12.5)
	CO	172.12, s	
Leu[1] (6)	NH		8.04 (d, 8.6)
	α-CH	49.0, d	4.96 (m)
	β-CH_2	41.2, t	1.16 (ddd, 10.5, 10.5, 3.1) / 1.66 (m)
	γ-CH	25.9, d	1.79 (m)
	CH_3	24.0, q	0.85 (d, 7.1)
	CH_3	21.5, q	0.88 (d, 6.7)
	CO	175.98, s	
N-Me-Ile[1] (7)	NCH_3	31.8, q	3.18 (s)
	α-CH	61.0, d	4.94 (d, 11.4)
	β-CH	33.1, d	2.07 (m)
	γ-CH_2	25.8, t	1.03 (m) / 1.29 (m)
	γ-CH_3	20.1, q	0.91 (d)
	δ-CH_3	10.0, q	0.77 (t, 7.4)
	CO	171.57, s	

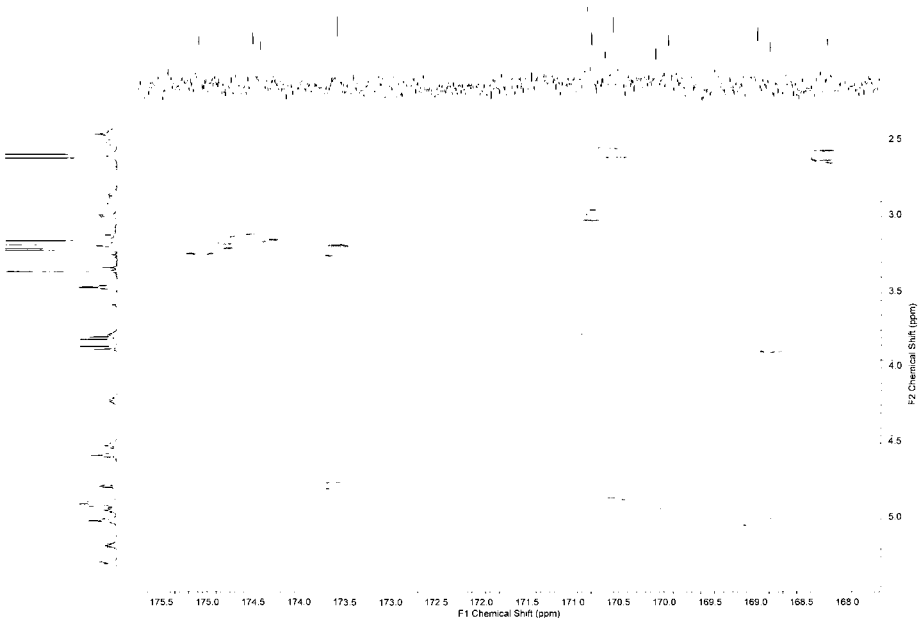
Table 1. (*continued*)

		Theonellapeptolide Ie	
		δ^{*} ^{13}C, multi	δ^{*} ^{1}H (multi, Hz)
β-Ala[1] (8)	NH		7.20 (d, 9.0)
	α-CH_2	35.6, t	2.88 (dd, 11.7, 9.0) / 4.23 (dd, 11.7, 11.7)
	β-CH_2	35.5, t	2.11 (m) / 2.52 (dd, 11.7, 11.1)
	CO	171.43, s	
Ile (9)	NH		8.55 (d, 9.4)
	α-CH	53.6, d	5.31 (dd, 9.4, 2.7)
	β-CH	38.6, d	1.73 (dd, 2.7, 7.0)
	γ-CH_2	28.0, t	1.10 (q, 7.0) / 1.35 (q, 7.0)
	γ-CH_3	14.8, q	0.67 (d, 7.0)
	δ-CH_3	12.8, q	0.92 (dd, 7.0, 7.0)
	CO	176.57, s	
N-Me-Val (10)	NCH_3	32.0, q	3.23 (s)
	α-CH	58.5, d	4.92 (d, 11.0)
	β-CH	29.5, d	2.30 (m)
	CH_3	20.0, q	0.84 (d, 6.7)
	CH_3	20.6, q	0.86 (d, 6.3)
	CO	172.03, s	
N-Me-Ala (11)	NCH_3	29.95, q	2.60 (s)
	α-CH	57.7, d	5.04 (q, 7.0)
	β-CH_3	15.6, q	1.32 (d, 7.0)
	CO	170.45, s	
β-Ala[2] (12)	NH		6.68 (d, 7.8)
	α-CH_2	37.4, t	2.90 (dd, 14.1, 2.7) / 3.80 (m)
	β-CH_2	39.5, t	2.09 (2H, m)
	CO	172.31, s	
Leu[2] (13)	NH		8.13 (d, 9.0)
	α-CH	49.2, d	4.93 (m)
	β-CH_2	40.7, t	1.28 (m) / 1.68 (m)
	γ-CH	25.7, d	1.69 (m)
	CH_3	21.2, q	0.92 (d)
	CH_3	16.4, q	0.875 (d, 7.2)
	CO	175.89, s	
N-Me-Ile[2] (14)	NCH_3	40.3, q	3.20 (s)
	α-CH	70.5, d	3.01 (d, 9.8)
	β-CH	35.7, d	2.46 (m)
	γ-CH_2	29.94, t	0.91 (m) / 1.88 (d, 7.1)
	γ-CH_3	15.1, q	0.74 (d, 7.0)
	δ-CH_3	24.5, q	0.93 (d, 7.1)
	CO	172.27, s	

* Referenced to residual solvent CH_3CN $\delta_H = 1.94$, $\delta_{CN} = 118.7$



(a)



(b)

Fig. 2. HMBC spectra of compound **1** in the F1-dimension having the amide carbonyl carbons (a) conventional HMBC spectrum, (b) selective HMBC spectrum.

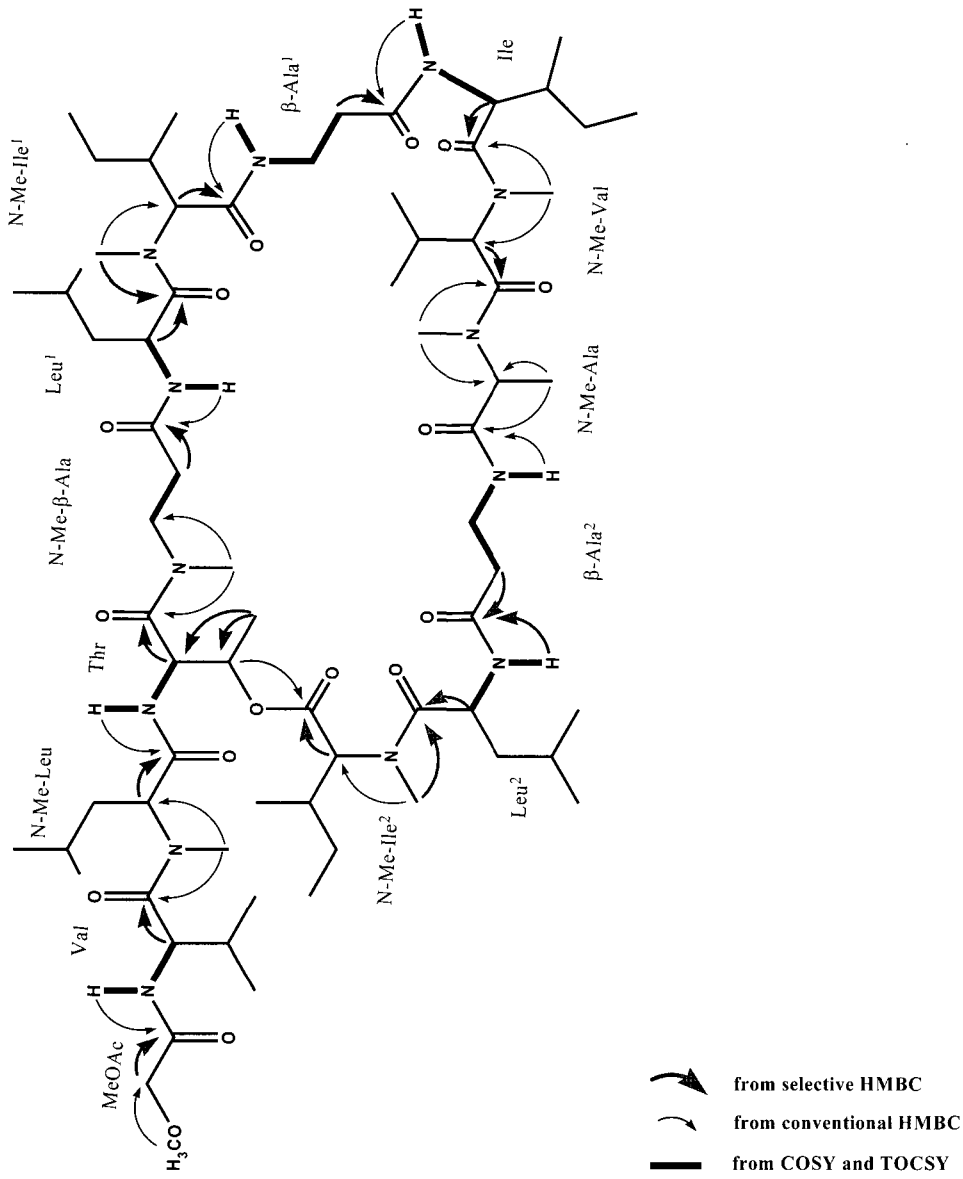


Fig. 3. The planar structure of **1** determined from HMBC, COSY, and TOCSY correlations.

overlapped doublet / triplet protons between 3.7 and 5.4 ppm, and severe crowded methyl peaks. A further observation of ^{1}H NMR spectrum also revealed the presence of six N-methyl groups and a methoxy group. (Fig. 1) These findings reflected a pattern that is typical for peptides. The ^{13}C NMR spectrum showed signals attributable to thirteen amide carbons and one lactone carbon. Thus, compound **1** indicated a tridecapeptide containing six N-methyl amino acids.

Careful analyses of 1D and 2D NMR data including COSY, TOCSY, HSQC, and HMBC revealed thirteen amino acid residues along with methoxyacetyl moiety ; Val, Thr, Ile, Leu (× 2), β-Ala (× 2), N-Me-Ile (× 2), N-Me-Ala, N-Me-Val, N-Me-Leu, and N-Me-β-Ala and led to assignments of all the protons and carbons as listed in the Table 1. In the HMBC spectrum, the singlet methoxy proton at δ 3.38 showed the correlation to the methylene carbon at δ 72.8, and the methylene protons further correlated to the amide carbon at δ 170.32 which also afforded a long-range coupling to the amide proton of Valine (Val). This indicated the attachment of a methoxyacetyl group with the N-terminal amino acid of **1**.

Next the amino acid sequence of **1** was established by the detailed analysis of the conventional HMBC and selective HMBC data. As shown in Fig. 2(a), the conventional HMBC correlations were obscured fairly congested carbonyl carbon resonances in the F1-dimension. On the other hand, the selective HMBC experiment which focused on a narrow band of interest in the F1-dimension presented much better resolved correlations because of higher digital resolution. With the help of the unambiguous HMBC correlations, together with COSY, and TOCSY cross peaks, the planar structure of **1** could be completed as shown in Fig. 3.

Unfortunately, compound **1** was found to be theonellapeptolide **Ie** derived from an Okinawan marine sponge of *Theonella* sp.[11]

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