Marine Natural Products. XXXIII.[1] Theonellapeptolide IId, a New Tridecapeptide Lactone from the Okinawan Marine Sponge *Theonella swinhoei*

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Following the characterization of theonellapeptolides Ia—Ie (2—6), another new tridecapeptide lactone named theonellapeptolide IId (1) was isolated from the Okinawan marine sponge *Theonella swinhoei*. The structure of 1 has been determined on the bases of chemical and physicochemical examinations which included an HPLC–CD combined analysis of the amino acid composition. Theonellapeptolide IId (1) prevented fertilization of the sea urchin *Hemicentrotus pulcherrimus* at the concentration of 25 μg/ml or greater but did not affect early embryonic development of fertilized eggs up to the gastrula stage.

Keywords *Theonella swinhoei*; marine sponge; theonellapeptolide; peptide lactone; ionophoretic activity; fertilization prevention

As a part of our continuing studies in search of new biologically active substances from marine organisms,[1,2] we have been investigating the chemical constituents of the Okinawan marine sponge *Theonella swinhoei*. This sponge contains a wide variety of constituents. We have so far isolated five bioactive tridecapeptide lactones named theonellapeptolides Ia—Ie,[3-6] four potent cytotoxic dimeric macrolides named swinholides A, B, and C, and isoswinholide A (swinholides in Fig. 1),[7-10] two new 3-keto-4-methylene steroids named theonellasterone and conicasterone (sterones in Fig. 1), and a Diels–Alder type dimeric steroid named bistheonellasterone[11] and elucidated their chemical structures.

These constituents were separated from the ethyl acetate-soluble portion (AcOEt extract) of the sponge and detected as prominent spots on the thin-layer chromatogram (TLC), as depicted in Fig. 1. Theonellapeptolides Ia (**2**), Ib (**3**), Ic (**4**), Id (**5**), and Ie (**6**) moved with the same *Rf* value on TLC under the described conditions. The same TLC showed the presence of another peptide mixture (detected by the Dragendorff reagent) designated theonellapeptolide II, which moved with a higher *Rf* value on TLC than that of the theonellapeptolide I mixture. The theonellapeptolide II mixture comprised several related compounds and was further separated by high-performance liquid chromatography (HPLC) to provide the major constituent, named theonellapeptolide IId (**1**). Here we present a full account of the structure elucidation of theonellapeptolide IId (**1**).[12]

The fast atom bombardment mass spectrum (FAB-MS) of theonellapeptolide IId (**1**) showed a quasi-molecular ion $(M+H)^+$ peak at m/z 1390, and the infrared (IR) spectrum suggested the presence of amide groups (3320, 1620, 1500 cm^{-1}) and a lactone carbonyl moiety (1750 cm^{-1}) in **1**. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed signals assignable to four *N*-methyl groups [δ 3.11, 3.14, 3.15, 3.17 (each 3H, s)] and one methoxyacetyl group [δ 3.43 (3H, s), 3.96 (2H, s)]. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1**, which closely resembled that of theonellapeptolide Id (**5**), showed signals ascribable to thirteen amide carbons, a lactone carbonyl, a β-carbon in a threonine moiety (δ_C 72.1), and a methoxyacetyl group [δ_C 60.4 (q), 73.1 (t)].

Theonellapeptolide IId (**1**) was not detected by the ninhydrin test and was unaffected by diazomethane treatment, as was theonellapeptolide Id (**5**). Thus, it has been presumed that **1** is a tridecapeptide lactone in which the N-terminal is blocked with a methoxyacetyl group and the C-terminal is connected through a lactone linkage to the β-hydroxyl group of the threonine moiety (Thr) [δ 5.23 (1H, m, due to the β-proton of Thr)], as is seen in the structure of theonellapeptolide Id (**5**).

In order to elucidate the amino acid composition, theonellapeptolide IId (**1**) was first subjected to complete hydrolysis with 6 N aqueous hydrochloric acid by heating at 110 °C for 24 h in a sealed tube. The hydrolysate was then analyzed by HPLC as described in our previous papers.[4,5] The absolute configurations of the component amino acids thus obtained were determined by measuring the circular dichroism (CD) spectrum (taken in 0.5 N aqueous hydrochloric acid) of each amino acid[4,5] which was eluted from the HPLC column. The amino acid composition of **1** has been determined as comprising

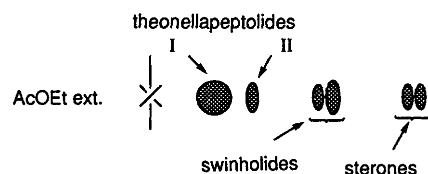

Fig. 1. The TLC Pattern of the AcOEt-Soluble Constituents

Plate: Silica gel $60F_{254}$. Solvent: $CHCl_3$–MeOH (8 : 1). Detection: 1% $Ce(SO_4)_2$–10% H_2SO_4.

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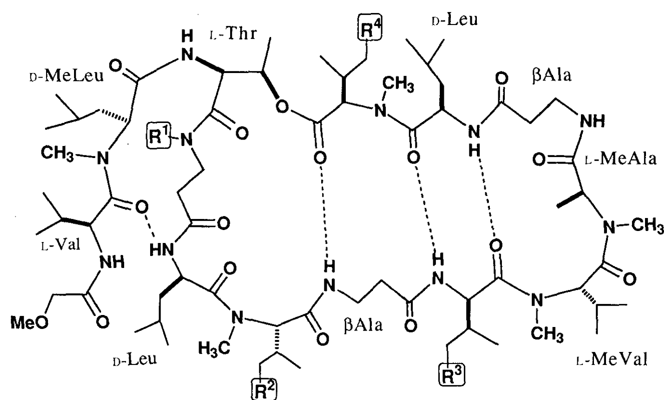
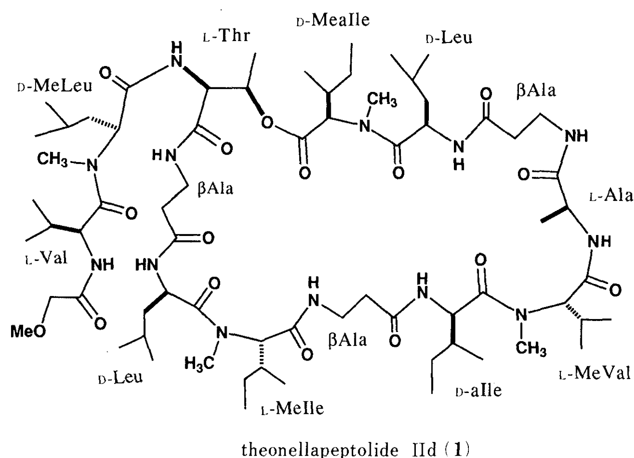


theonellapeptolide	R^1	R^2	R^3	R^4
Ia(2)	H βAla	CH_3 L-MeIle	H D-Val	CH_3 D-MeaIle
Ib(3)	H βAla	H L-MeVal	CH_3 D-aIle	CH_3 D-MeaIle
Ic(4)	H βAla	CH_3 L-MeIle	CH_3 D-aIle	H D-MeVal
Id(5)	H βAla	CH_3 L-MeIle	CH_3 D-aIle	CH_3 D-MeaIle
Ie(6)	CH_3 MeβAla	CH_3 L-MeIle	CH_3 D-aIle	CH_3 D-MeaIle



theonellapeptolide IId (1)

Fig. 2. Structures of Theonellapeptolides

β-alanine (βAla) (3 mol), L-alanine (L-Ala) (1), L-Thr (1), L-valine (L-Val) (1), *N*-methyl-L-valine (L-MeVal) (1), D-alloisoleucine (D-aIle) (1), D-leucine (D-Leu) (2), *N*-methyl-D-alloisoleucine (D-MeaIle) (1), *N*-methyl-L-isoleucine (L-MeIle) (1), *N*-methyl-D-leucine (D-MeLeu) (1).

Next, theonellapeptolide IId (**1**) was treated with sodium methoxide in methanol to furnish the acyclic methyl ester **7**, which was generated by methanolysis of the lactone ring of **1**. The FAB-MS of **7** provided, in addition to the quasi-molecular ion $(M+H)^+$ peak at *m/z* 1422, several fragment ion peaks which were consistent with the amino acid sequence of **1** as shown in Fig. 3. The findings described above have led us to presume that theonellapeptolide IId (**1**) possesses a structure in which the *N*-methyl-L-alanine (L-MeAla) moiety in theonellapeptolide Id (**5**) is replaced with L-Ala.

In order to verify this presumption, theonellapeptolide IId (**1**) was subjected to alkaline hydrolysis and subsequent partial acidic hydrolysis. Thus, **1** was treated with a 3:2 mixture of 2 N aqueous potassium hydroxide and dioxane to furnish the acyclic tridecapeptide acid **8**, which showed a shifted one-proton signal at δ 4.30 (m) assignable to the β-proton of the threonine moiety. The acyclic acid (**8**) was further treated with 30% aqueous trifluoroacetic acid (TFA) at 110 °C for 40 min in a sealed tube, and the resulting mixture of partial hydrolysates was separated by HPLC (Cosmosil $5C_{18}$-AR) to furnish twenty partial hydrolysates: *i.e.* IId-OH-H1—IId-OH-H20. On the other hand, treatment of **8** with 30% aqueous TFA–dioxane (1:1) at 110 °C for 40 min in a sealed tube furnished thirteen partial hydrolysates: *i.e.* IId-OH-D1—IId-OH-D13. Among these partial hydrolysates, IId-OH-H3, IId-OH-H5, IId-OH-H6, IId-OH-H18, IId-OH-D5, IId-OH-D6, IId-OH-D7, and IId-OH-D8 were useful for construction of the structure of theonellapeptolide IId (**1**), as shown in Chart 1. The amino acid sequence of each hydrolysate was elucidated as described in our previous papers.[4,5)]

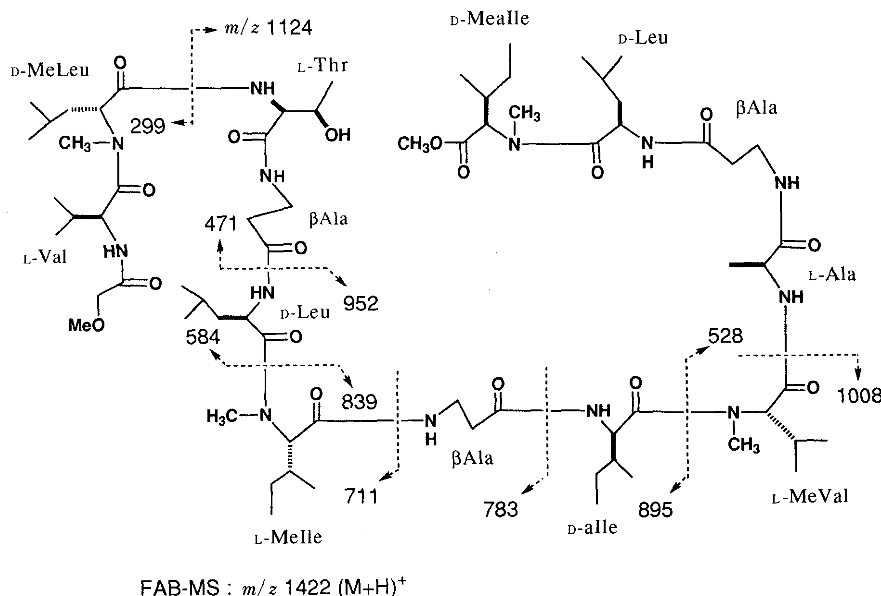


Fig. 3. Mass Spectral Fragmentation Pattern of the Acyclic Methyl Ester 7

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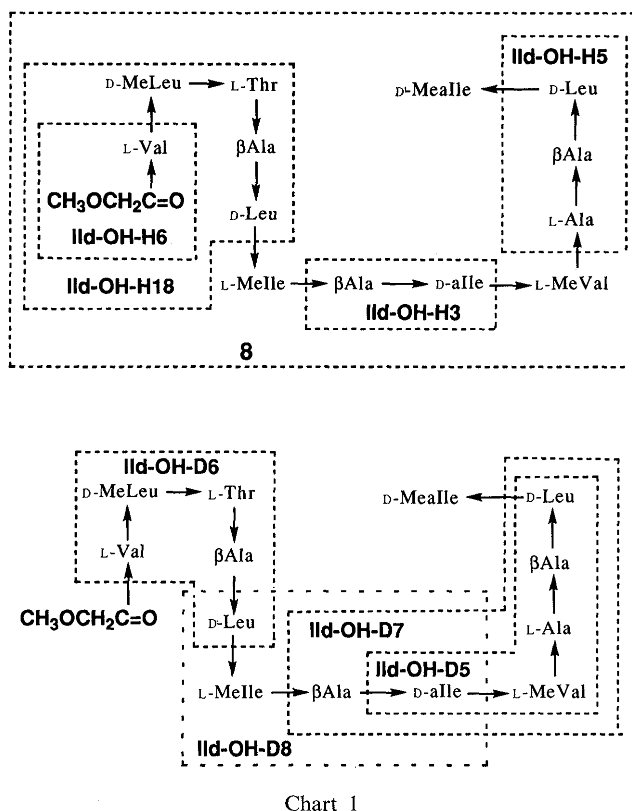

Chart 1

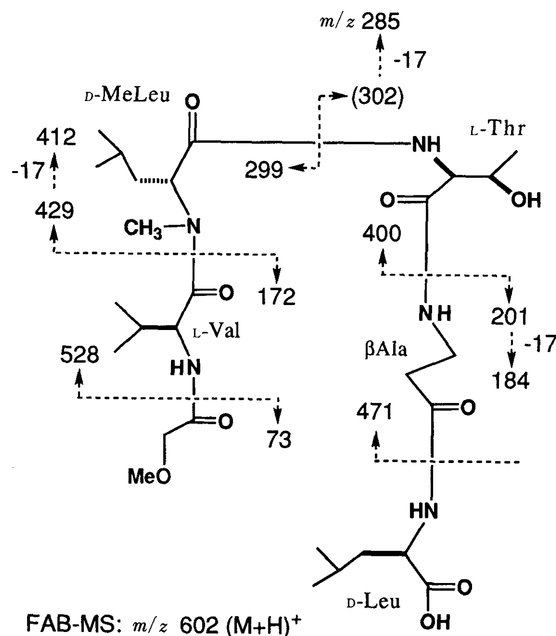

Fig. 4. MIKES Fragmentation Pattern of IId-OH-H18

The partial hydrolysates were subjected to FAB-MS and ^{1}H-NMR analyses and then to HPLC–CD amino acid analysis.[4,5] For example, the N-terminal amino acid of IId-OH-H5, which contains 1 mol each of βAla, L-Ala, and D-Leu (Chart 1), was elucidated by examination of the solvent-induced shift[13] in the ^{1}H-NMR spectra of IId-OH-H5. The α-proton and α-methyl proton signals of the Ala moiety in IId-OH-H5 were observed at higher field ($\Delta\delta$ 0.59 and 0.26, respectively) in the spectrum taken in NaOD–D_2O (pH 14) than in the spectrum taken in D_2O. In addition to this finding, analysis of the fragmentation pattern of FAB-MS led to the conclusion that the amino acid sequence of IId-OH-H5 was D-Leu←βAla←L-Ala. The order of the Thr and βAla moieties in IId-OH-H18 was elucidated from the mass analyzed ion kinetic spectrum (MIKES) of a quasi-molecular ion observed at m/z 602 $(M+H)^+$, as shown in Fig. 4. Thus, fragment ion peaks, derivable from the fission of the peptide linkage between the Thr and βAla moieties, were observed at m/z 400, 201, and 184.

From the accumulated evidence mentioned above, the structure of theonellapeptolide IId has been determined as **1**, an L-Ala analogue of theonellapeptolide Id (**5**), which possesses L-MeAla as the tenth amino acid from the N-terminal, L-Val.

As mentioned above, theonellapeptolide IId (**1**) moves faster than theonellapeptolide Id (**5**) on ordinary silica gel TLC (Fig. 1), although the structures of the two peptolides differ only in the one amino acid constituent. From the behavior of these tridecapeptide lactones on TLC, it has been presumed that the exchange of L-MeAla in Id (**5**) to L-Ala as in IId (**1**) may lead to a considerable difference in the three-dimensional conformations of **1** and **5**. We found several years ago that theonellapeptolide Id (**5**) exhibited ion-transport activities for Na^+, K^+, and Ca^{2+} ions.[14,15]

In order to get a better understanding of the relationship between these peptolide ring conformations and ionophoretic activities, we next examined the ionophoretic activity of theonellapeptolide IId (**1**) in comparison with that of theonellapeptolide Id (**5**). As shown in Figs. 5 and 6, the examinations were carried out 1) by using a liquid membrane-type apparatus (W-08)[16] which was constructed by us for measurement of ion-transport and ion-binding activities and 2) by employing a human erythrocyte membrane method[15] for measurement of ion-permeability. It was confirmed with the apparatus W-08 that **5** showed Ca^{2+} ion-binding activity and Ca^{2+} ion-transporting activity across human erythrocyte membrane. However, surprisingly, **1** was found not to exhibit ion-transport activity but to show very weak ion-binding activity with the apparatus W-08. Recently, Jefford and his group reported an X-ray crystallographic analysis of theonellapeptolide Id (**5**)[17] (Fig. 7). Compound **5** was characterized by a *cis* peptide linkage between the L-MeAla and the L-MeVal moieties, which were respectively the ninth and tenth amino acid constituents from the N-terminal L-Val. On the bases of these findings and although the three-dimensional conformation of **1** is not yet known, it seems likely that lack of the *N*-methyl residue in the tenth amino acid (L-MeAla in **5**) may result in significant change of the peptide-lactone conformation.

We have also examined the allomone activity of theonellapeptolide IId (**1**). It was found that **1** prevented fertilization of the sea urchin *Hemicentrotus pulcherrimus* at the concentration of 25 μg/ml or more, while it did not affect early embryonic development of fertilized eggs up to the gastrula stage. During these experiments, it was also shown that sperm-egg fusion was not affected by treatment with **1**, while formation of the fertilization envelope in the

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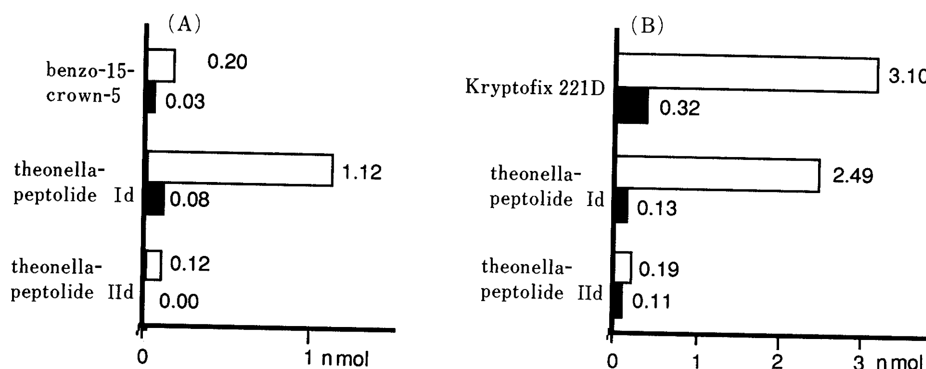

Fig. 5. Na^{+} (A) and Ca^{++} (B) Ion-Transport (■) and Ion-Binding (□) Activities of Theonellapeptolides Id (**5**) and IId (**1**), in Comparison with Those of Benzo-15-crown-5 and Kryptofix 221D, by Using the W-08 Apparatus (Liquid-Membrane Type)

Initial concentration of sample: 0.01 M in decanol, after 10 h.

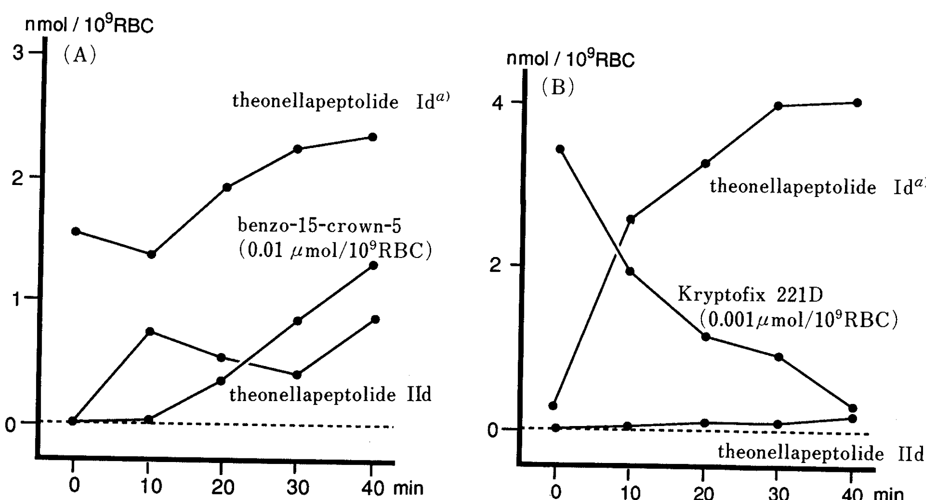

Fig. 6. Na^{+} (A) and Ca^{++} (B) Ion-Transport Activities of Theonellapeptolides Id (**5**) and IId (**1**), in Comparison with Those of Benzo-15-crown-5 and Kryptofix 221D, by the Human Erythrocyte Membrane Method

Initial concentration of sample: 0.005 μmol/10^{9} red blood cells (RBC). *a*) Hemolysis observed.

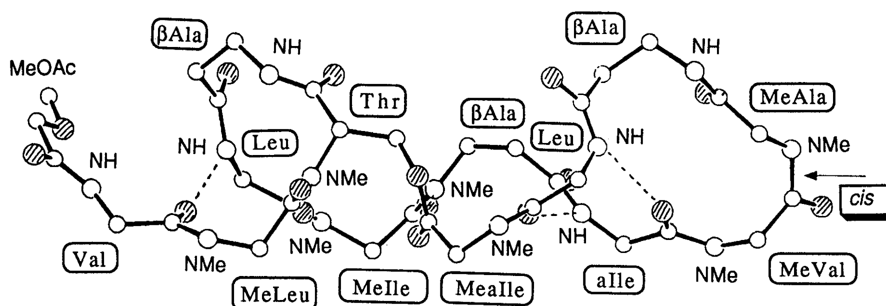

Fig. 7. Backbone Conformation of Theonellapeptolide Id (**5**) as Elucidated by X-Ray Analysis[17]

fertilized egg was prevented. Furthermore, we found that **1** prevented the motility of sperm within 1 min at the concentration of 25 μg/ml. These remarkable effects on echinoderm gametes were specific to **1**, and theonellapeptolide Id (**5**) affected neither fertilization of sea urchin gametes nor early embryonic development of fertilized eggs up to the gastrula stage even at the concentration of 100 μg/ml. Consequently, theonellapeptolide IId (**1**) may be a rare example of a tool for analyzing gamete-specific cellular functions which are normally not present in somatic cells. The details will be reported elsewhere.

Experimental

Instruments for obtaining physical data and experimental conditions for chromatography were the same as described in the previous paper.[5] The FAB-MS were obtained with a JEOL JMS SX-102 mass spectrometer and a JMA DA-6000 data system. The primary FAB gas was Xe gas. The sample was dissolved in glycerol–thioglycerol as a matrix, and injected. Helium gas was used as the secondary collision gas to obtain

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MIKES.

Isolation of Theonellapeptolide IId (1) Fresh whole animal of the marine sponge *Theonella swinhoei* (2 kg, collected in July in Zamami-jima, Okinawa Prefecture) was cut up and extracted with acetone at room temperature. Removal of the solvent under reduced pressure from the combined extracts provided the acetone extract, which was partitioned into an ethyl acetate–water mixture. The ethyl acetate phase was separated and evaporated under reduced pressure to furnish the ethyl acetate-soluble portion (20 g). The ethyl acetate-soluble portion was subjected repeatedly to column chromatography (Kieselgel 60, 1-hexane : AcOEt = 1 : 2 and $CHCl_3$: MeOH = 10 : 1) to furnish two peptide fractions; the less polar one was named theonellapeptolide II (460 mg) and more polar one, theonellapeptolide I (1.25 g). The less polar peptide fraction (460 mg) was further subjected to HPLC (Cosmosil $5C_{18}$ 10 mm × 25 cm, $CHCl_3$: CH_3CN : H_2O = 1 : 20 : 6) to furnish theonellapeptolide IId (**1**) (40 mg) (0.20% from the ethyl acetate-soluble portion).

Theonellapeptolide IId (**1**), an amorphous solid; $[\alpha]_D$ −27° (MeOH, c = 1.0, 25 °C). FAB-MS m/z: 1390 $(M+H)^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3320, 3040, 1750, 1620, 1500. ^1H-NMR (500 MHz, CD_3OD) δ : 3.11, 3.14, 3.15, 3.17 (each 3H, s) (four N-methyl protons), 3.43 (3H, s), 3.96 (2H, s) (a methoxyacetyl), 5.23 (1H, m). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 171.0, 171.3, 171.6, 172.5 (2C), 172.6, 173.5, 173.7, 173.8, 175.1, 175.2, 175.4, 175.6, 175.9 (each s), 73.1 (t), 60.4 (q), 72.1 (d).

Complete Acidic Hydrolysis of Theonellapeptolide IId (1) Followed by HPLC–CD Analysis Theonellapeptolide IId (**1**) (5 mg) was treated with 6 N aqueous HCl (0.5 ml) and, after degassing, the whole mixture was heated in a sealed tube at 110 °C for 24 h. After cooling, the reaction mixture was evaporated *in vacuo* to give an amino acid mixture. A small portion of the amino acid mixture was subjected to HPLC analysis of amino acids as described in our previous papers.[4,5] The absolute configurations of the constituent amino acids were determined by measuring the CD spectrum of each amino acid (in 0.5 N aqueous HCl) collected by repeated HPLC separation.

Sodium Methoxide Treatment of Theonellapeptolide IId (1) A solution of **1** (20 mg) in MeOH (2 ml) was treated with 28% NaOMe in MeOH (0.2 ml) at room temperature for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with ethyl acetate. The ethyl acetate solution was taken, washed with brine, and evaporated under reduced pressure to furnish a product (20 mg). The product was purified by silica gel column chromatography ($CHCl_3$: MeOH : H_2O = 30 : 3 : 1, lower phase) to give the methyl ester **7** (8 mg). **7**, $[\alpha]_D$ −22° (MeOH, c = 0.7, 21 °C). FAB-MS: as given in Fig. 3. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3320, 1710, 1620, 1510. ^1H-NMR (500 MHz, CD_3OD) δ : 4.30 (1H, m, Thr β-H).

Alkaline Hydrolysis of Theonellapeptolide IId (1) A solution of **1** (400 mg) in dioxane (6 ml) was treated with 2 N aqueous KOH (4 ml) and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was poured into ice-water and adjusted to pH 3, then the whole was extracted with ethyl acetate. The ethyl acetate solution was taken, washed with brine, and evaporated under reduced pressure to furnish a product (390 mg). The product was purified by silica gel column chromatography ($CHCl_3$: MeOH : H_2O = 30 : 3 : 1, lower phase) to give the acyclic acid **8** (320 mg). **8**, $[\alpha]_D$ −21° (MeOH, c = 1.0, 21 °C). FAB-MS m/z: 1409 $(M+H)^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3310, 3020, 1660, 1510. ^1H-NMR (500 MHz, CD_3OD) δ : 4.30 (1H, m, Thr β-H).

Partial Acidic Hydrolysis of 8 Giving IId-OH-H1—H18 A solution of **8** (100 mg) in 30% aqueous TFA (4 ml) was degassed and heated in a sealed tube at 110 °C for 40 min. The reaction mixture was evaporated under reduced pressure to give a mixture of partial hydrolysates. The partial hydrolysates were separated by HPLC [Cosmosil $5C_{18}$ 10 mm × 25 cm, eluted with 2-propanol (PrOH)–CH_3CN–H_2O (7 : 3 : 20) containing 0.1% TFA] to furnish eighteen fractions: IId-OH-H1—IId-OH-H18. IId-OH-H3, FAB-MS m/z: 203 $(M+H)^+$; amino acid composition: βAla (1), aIle (1); ^1H-NMR (270 MHz, D_2O) δ : βAla [3.14 (2H, t, J = 6.7 Hz, α-H), 2.65 (2H, t, J = 6.7 Hz, β-H)], aIle [4.30 (1H, d, J = 4.7 Hz, α-H), 1.89 (1H, m, β-H), 1.65 (2H, m, γ-H), 0.80 (6H, m, β,γ-CH_3)]. IId-OH-H5, FAB-MS m/z: 274 $(M+H)^+$, 203, 131 $(Leu)^+$, 143 $(M-Leu)^+$; amino acid composition: βAla (1), Ala (1), Leu (1); ^1H-NMR (270 MHz, D_2O (pH 7)) δ : βAla [3.23 (2H, t, J = 6.7 Hz, α-H), 2.44 (2H, t, J = 6.7 Hz, β-H)], Ala [3.89 (1H, m, α-H), 1.32 (3H, d, J = 7.0 Hz, α-CH_3)], Leu [4.23 (1H, m, α-H), 1.54 (3H, m, β,γ-H), 0.79 (6H, m, γ-CH_3 × 2)]; (D_2O (pH 14)) δ : Ala [3.30 (1H, m, α-H), 1.06 (3H, d, J = 7.0 Hz, α-CH_3)]. IId-OH-H6, FAB-MS m/z: 190 $(M+H)^+$; amino acid composition: Val; ^1H-NMR (270 MHz, D_2O) δ : Val [4.12 (1H, d, J = 5.9 Hz, α-H), 2.11 (1H, m, β-H), 0.81, 0.84 (both 3H, d, J = 6.9 Hz, β-CH_3)], methoxyacetyl [3.96 (2H, s), 3.32 (2H, s)]. IId-OH-H18, FAB-MS m/z: 602 $(M+H)^+$, 299, 172; amino acid composition: βAla (1), Thr (1), Val (1), Leu (1), MeLeu (1); ^1H-NMR (270 MHz, D_2O) δ : βAla [3.39 (2H, t, J = 6.7 Hz, α-H), 2.41 (2H, t, J = 6.7 Hz, β-H)], Thr [4.18 (2H, m, α,β-H), 1.27 (3H, d, J = 6.7 Hz, β-CH_3)], Val [4.02 (1H, d, J = 4.3 Hz, α-H), 2.01 (1H, m, β-H)], MeLeu and Leu [1.53 (6H, m, β,γ-H), 3.04 (3H, s, N-CH_3)], methoxyacetyl [3.96 (2H, s), 3.33 (3H, s)].

Partial Acidic Hydrolysis of 8 Giving IId-OH-D1—D8 A solution of **8** (250 mg) in 30% aqueous TFA–dioxane (1 : 1) (4 ml) was degassed and heated in a sealed tube at 110 °C for 40 min. The reaction mixture was evaporated under reduced pressure to give a mixture of partial hydrolysates. The partial hydrolysates were separated by HPLC [Cosmosil $5C_{18}$-AR 10 mm × 25 cm, eluted with PrOH–CH_3CN–H_2O (7 : 3 : 20) containing 0.1% TFA] to furnish eight fractions: IId-OH-D1—IId-OH-D8. IId-OH-D5, FAB-MS m/z: 500 $(M+H)^+$; amino acid composition: βAla (1), Ala (1), aIle (1), Leu (1), MeVal (1); ^1H-NMR (270 MHz, D_2O) δ : βAla [3.36 (2H, t, J = 6.7 Hz, α-H), 2.50 (2H, t, J = 6.7 Hz, β-H)], Ala [4.18 (1H, q, J = 7.0 Hz, α-H), 1.32 (3H, d, J = 7.0 Hz, α-CH_3)], aIle [4.55 (1H, d, J = 11.2 Hz, α-H), 2.25 (1H, m, β-H), 1.46 (2H, m, γ-H)], Leu [4.35 (1H, t, J = 6.8 Hz, α-H), 1.64 (3H, m, β,γ-H)], MeVal [4.47 (1H, m, α-H), 3.09 (3H, s, N-CH_3), 2.07 (1H, m, β-H)]. IId-OH-D6, FAB-MS m/z: 530 $(M+H)^+$, 227; amino acid composition: βAla (1), Thr (1), Val (1), Leu (1), MeLeu (1); ^1H-NMR (270 MHz, D_2O) δ : βAla [3.40 (2H, t, J = 6.7 Hz, α-H), 2.43 (2H, t, J = 6.7 Hz, β-H)], Thr [4.24 (2H, m, α,β-H), 1.01 (3H, d, J = 7.0 Hz, β-CH_3)], Val [2.22 (1H, m, β-H)], Leu and MeLeu [4.35 (2H, m, α-H), 3.01 (3H, s, N-CH_3), 1.54 (6H, m, β,γ-H)]. IId-OH-D7, FAB-MS m/z: 571 $(M+H)^+$; amino acid composition: βAla (2), Ala (l), aIle (1), Leu (1), MeVal (1); ^1H-NMR (270 MHz, D_2O) δ : βAla [3.45, 3.24 (both 2H, t, J = 6.7 Hz, α-H), 2.73, 2.51 (both 2H, t, J = 6.7 Hz, β-H)], Ala [4.21 (1H, q, J = 7.0 Hz, α-H), 1.31 (3H, d, J = 7.0 Hz, α-CH_3)], aIle [4.54 (1H, d, J = 10.8 Hz, α-H), 2.25 (1H, m, β-H), 1.35 (2H, m, γ-H)], Leu [4.37 (1H, m, α-H), 1.66 (3H, m, β,γ-H)], MeVal [4.37 (1H, m, α-H), 3.16 (3H, s, N-CH_3), 1.90 (1H, m, β-H)]. IId-OH-D8, FAB-MS m/z: 443 $(M+H)^+$; amino acid composition: βAla (1), aIle (1), Leu (1), MeIle (1); ^1H-NMR (270 MHz, D_2O) δ : βAla [3.46 (2H, t, J = 6.7 Hz, α-H), 2.54 (2H, t, J = 6.7 Hz, β-H)], aIle [4.58 (1H, d, J = 11.3 Hz, α-H), 2.09 (1H, m, β-H), 1.24 (2H, m, γ-H)], Leu [4.47 (1H, dd, J = 10.2, 3.3 Hz, α-H), 1.73 (3H, m, β,γ-H), 0.99, 0.97 (both 3H, d, J = 6.6 Hz, γ-CH_3)], MeIle [4.29 (1H, d, J = 4.4 Hz, α-H), 1.93 (1H, m, β-H), 1.73, 1.24 (both 1H, m, γ-H)].

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