Communications to the Editor

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STRUCTURE OF THEONELLAPEPTOLIDE ID, A NEW BIOACTIVE PEPTOLIDE FROM AN OKINAWAN MARINE SPONGE, THEONELLA SP. (THEONELLIAE)

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A new peptolide named theonellapeptolide Id (1) has been isolated from an Okinawan marine sponge of Theonella sp. (Theonelliae) together with some minor peptolides (theonellapeptolides Ia, Ib, Ic, and Ie). These peptolides inhibit development of the fertilized eggs of the sea urchin Hemicentrotus pulcherrimus. The structure of theonellapeptolide Id (1) has been determined on the basis of chemical and physicochemical evidence. Theonellapeptolide Id (1) is rare example of a peptolide characteristically comprising N-methyl and D amino acids in high ratio.

KEYWORDS —— Theonella sp.; marine sponge; Theonelliae; theonellapeptolide Id; peptolide; D amino acid; N-methyl amino acid; sea urchin fertilized egg development inhibition

In search of new bioactive substances from marine organisms,[1] we have investigated the chemical constituents of a marine sponge of Theonella sp. (Theonelliae) which was collected in July in the coral reef of Zamami-jima, Okinawa Prefecture. We have so far isolated a macrolide[2] and five new peptolides named theonellapeptolides Ia, Ib, Ic, Id, and Ie which inhibit development of the fertilized eggs of the sea urchin Hemicentrotus pulcherrimus[3] at 2, 2, 2, 50, and 10 μg/ml concentrations, respectively. This paper deals with the structure of the major peptolide theonellapeptolide Id (1).[4]

The acetone extract of the fresh marine sponge was partitioned into an AcOEt-H_2O mixture and the AcOEt soluble portion was first subjected to silica gel column chromatography ($CHCl_3$-MeOH) to separate the macrolide fraction and the two peptolide fractions. The more polar peptolide fraction was further purified by HPLC (Cosmosil $5C_{18}$, $CHCl_3$-CH_3CN-H_2O) to afford theonellapeptolides Ia, Ib, Ic, Id (1), and Ie (0.04, 0.08, 0.05, 2.2, and 0.29% respectively) from the AcOEt soluble portion.

Theonellapeptolide Id (1), colorless crystals, mp 168-169°C (CH_3OH-H_2O), $C_{70}H_{125}O_{16}N_{13}$, $[\alpha]_D^{20}$ -68° (MeOH), FAB-MS m/z 1404 $(M+H)^+$, was shown by its IR spectrum to have amide groups (3330, 3030, 1680, 1540 cm^{-1}) and a lactone group (1740

cm^{-1}). Complete acidic hydrolysis (6N HCl, 110°C, 24 h) of theonellapeptolide Id (1) provided eight amino acids [Thr (1), βAla (3), Val (1), Leu (2), aIle (1)] (determined by amino acid analysis) and five N-methyl amino acids [Me-Ala (1), Me-Val (1), Me-Leu (1), Me-Ile (1), Me-aIle (1)] [determined by detailed analysis of the various partial hydrolysates of 1 (vide infra)]. The analysis also revealed that the N-terminal Val of 1 was protected by a methoxyacetyl moiety.

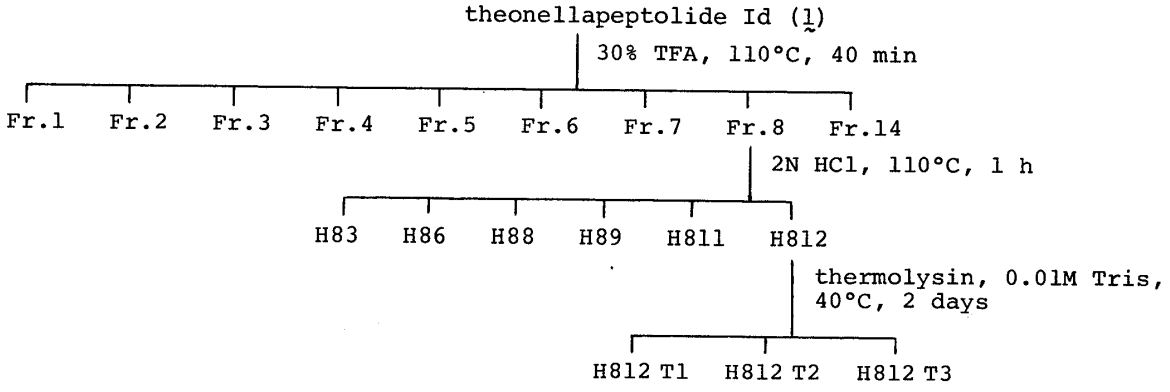
The ^1H NMR spectrum ($CDCl_3$-CD_3OD)[5] of theonellapeptolide Id (1) showed signals assignable to eight amide protons (δ 8.70, 1H d, J=9.5 Hz; δ 8.51, 1H d, J=9.5 Hz; δ 8.43, 1H d, J=9.2 Hz; δ 8.35, 1H d, J=8.5 Hz; δ 7.42, 1H d, J=8.9 Hz; δ 7.38, 1H br d,[6] J=ca. 5.2 Hz; δ 7.22, 1H br d,[6] J=7.6 Hz; δ 7.18, 1H t-like), five N-methyl groups (δ 3.30, 3.29, 3.24, 3.20, 2.75, all 3H s), and one methoxy group (δ 3.43, 3H s). The ^{13}C NMR spectrum ($CDCl_3$-CD_3OD) of 1 showed signals due to thirteen amide carbons and one lactone carbon (δc 169.5-175.4, all s) and one methoxyacetyl group (δc 71.9, t, $CH_3O\underline{C}H_2CO$-). Since theonellapeptolide Id (1) was negative in the Ninhydrin test and was unaffected by diazomethane treatment and its partial hydrolysis yielded methoxyacetyl-Val (11) (vide infra), 1 has been identified as a peptolide in which the C-terminal amino acid participates in the lactone ring and the N-terminal amino acid (Val) has a methoxyacetyl group attached to it.

In order to determine the amino acid sequence, theonellapeptolide Id (1) was hydrolyzed with 30% aq. trifluoroacetic acid (TFA) (110°C, 40 min) to afford various fragments (Fr.1 - Fr.8 and Fr.14), which were separated by HPLC [Zorbax ODS Anal 0.25 m x 9.4 φ; eluent: solvent A= isoPrOH-CH_3CN (7:3)-0.1% TFA, solvent B= H_2O-0.1% TFA; gradient: A (0→100%) and B (100→0%) in 30 min]. The structures of Fr.1 - Fr.5 have been elucidated as 2 - 6 by FAB-MS (JMS-HX 100, positive) and ^1H NMR (D_2O) analyses and by chemical analysis of their dansyl (DNS) derivatives. Thus, the presence of a Leu→Me-Ile→βAla→aIle→Me-Val sequence in 1 has been determined. Comparison of FAB-MS data has shown that Fr.6 (7) is a des-methoxyacetyl derivative of Fr. 8 (9), while Fr.7 (8) is a C-terminal (Leu) less derivative of Fr.8 (9). The DNS derivation method has revealed that the N-terminal of Fr.8 (9) is Me-Ala.

Acidic hydrolysis of Fr.8 (9) (2N HCl, 110°C, 1 h) yielded fragments H83, H86, H88, H89, H811, and H812. The structures of H83 (11), H86 (12), H88 (13), H89 (14), and H811 (15) were determined by FAB-MS and ^1H NMR analyses and by the DNS derivation method. Fragment H812 was further subjected to enzymatic hydrolysis [0.01M aq. tris(hydroxymethyl)aminomethane hydrochloride (pH 7.75), thermolysin (excess, ca. 1/100 weight of H812, 40°C, 2 days] to furnish fragments H812T1, H812T2, and H812T3. The structures of these fragments (17, 18, 19) were determined by examination of their physicochemical data and by the DNS method. Thus, the structure of H812 (16) has been elucidated.

The ester linkages branching at Thr in H86 (12), H88 (13), H811 (15), and H812 (16) were further confirmed by the ^1H NMR signals due to the β-H of Thr residues in these fragments. They were observed at δ 5.58 (dd, J=2.5, 6.5 Hz) in H86 (12), δ 5.50 (m) in H88 (13), δ 5.64 (m) in H811 (15), and δ 5.49 (m) in H812 (16), whereas the signals due to the β-H of Thr's in H812T1 (17) and H812T2 (18) were observed at δ 4.11 (m) and δ 4.31 (m).

Based on the elucidated amino acid sequences of H83 (11), H86 (12), H88 (13),



Fr.1 (2): βAla → aIle

Fr.2 (3): Me-Ala → βAla → Leu

Fr.3 (4): βAla → aIle → Me-Val

Fr.4 (5): Me-Ile → βAla → aIle

Fr.5 (6): Leu → Me-Ile → βAla → aIle

Fr.6 (7): Me-Leu ← Val
↓
Thr → βAla → Leu
|
O – Me-aIle ← Leu ← βAla ← Me-Ala

Fr.7 (8): Me-Leu ← Val – $COCH_2OCH_3$
↓
Thr → β-Ala
|
O – Me-aIle ← Leu ← βAla ← Me-Ala

Fr.8 (9): Me-Leu ← Val – $COCH_2OCH_3$
↓
Thr → βAla → Leu
|
O – Me-aIle ← Leu ← βAla ← Me-Ala

Fr.14 (10): Me-Leu ← Val
↓
Thr → βAla → Leu → Me-Ile → βAla → aIle
|
O – Me-aIle ← Leu ← βAla ← Me-Ala ← Me-Val

H83 (11): CH_3OCH_2CO-Val

H86 (12): Me-Leu
↓
Thr → βAla
|
O – Me-aIle

H88 (13): Thr → βAla
|
O – Me-aIle ← Leu

H89 (14): Me-Leu ← Val

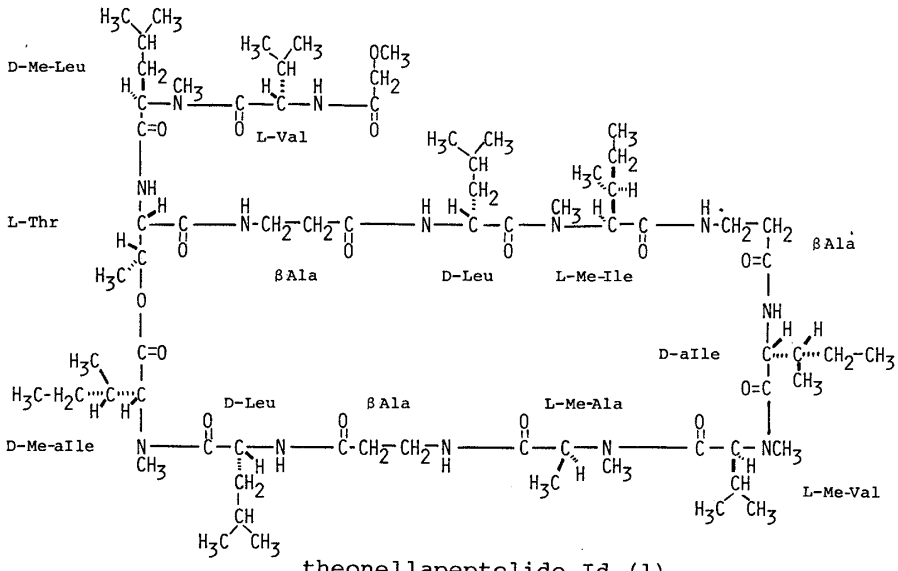
H811 (15): Thr → βAla
|
O – Me-aIle ← Leu ← βAla

H812 (16): Me-Leu ← Val
↓
Thr → βAla → Leu
|
O – Me-aIle ← Leu

H812 T1 (17): Thr → βAla → Leu

H812 T2 (18):
Val → Me-Leu → Thr → βAla → Leu

H812 T3 (19): Me-aIle ← Leu



theonellapeptolide Id (1)

H89 (14), H811 (15), H812 (16), and Fr.2 (3), the structure of Fr.8 (9) has been determined. Consequently, the structures of Fr.6 (7) and Fr.7 (8) are substantiated and the amino acid sequence of theonellapeptolide Id (1) has been determined as shown and Fr.14 (10) is shown to be a des-methoxyacetyl derivative of 1.

Finally, the absolute configurations of the constituent amino acids were determined by CD analysis of the amino acids liberated by acidic hydrolysis (6N HCl, 110°C, 24 h) of Fr.2 (3), Fr.3 (4), Fr.4 (5), H89 (14), H812T1 (17) and H812T3 (19).

Theonellapeptolide Id (1) is a rare example of a peptolide characteristically comprising N-methyl amino acids and D amino acids in high ratio. We are currently studying the structure of other theonellapeptolides: Ia, Ib [m/z 1391 $(M+H)^+$], Ic [m/z 1404 $(M+H)^+$], and Ie [m/z 1418 $(M+H)^+$].

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REFERENCES AND NOTES

1) I. Kitagawa, M. Kobayashi, T. Inamoto, M. Fuchida, and Y. Kyogoku, Chem. Pharm. Bull., 33, 5214 (1985), and the preceding papers from our laboratory.
2) The physicochemical properties of the macrolide are quite similar to those of swinholide A, a macrolide polyketide isolated from the marine sponge Theonella swinhoei by S. Carmely and Y. Kashman, Tetrahedron Lett., 26, 511 (1985).
3) N. Fusetani, Y. Kato, K. Hashimoto, T. Komori, Y. Itakura, and T. Kawasaki, J. Nat. Prod., 47, 997 (1984).
4) A peptolide named theonellamine B was isolated from an Okinawan marine sponge, Theonella sp., and the structure has been proposed by H. Nakamura, J. Kobayashi, Y. Nakamura, Y. Ohizumi, and Y. Hirata, at the Annual Meeting of the Pharmaceutical Society of Japan held at Chiba, Apr. 4, 1986. A direct comparison of the sponge specimen and the peptolide has not yet been carried out. However, the structure proposed at the Meeting is not identical with our present proposal for theonellapeptolide Id (1).
5) The ^1H NMR spectra were measured at 500 MHz and the ^{13}C NMR spectra at 125 MHz.
6) The fact that two of three βAla amide protons were observed as br d may suggest the cyclic nature of theonellapeptolide Id (1).
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