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Barangamide A, a new cyclic peptide from the Indonesian sponge *Theonella swinhoei*

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Abstract

Barangamide A has been isolated from the sponge *Theonella swinhoei*, collected at Baranglompo Island, Indonesia, and the structure elucidated by interpretation of spectral data and application of Marfey's method. Barangamide A is a cyclic undecapeptide possessing three *N*-methylated amino acids, three β-alanine and the same amino acid sequence with the ring portion of the known theonellapeptolide IId isolated also in the present work. The theonellapeptolides have been reported to have moderate cytotoxicity, whereas barangamide A was inactive at the same concentration.

Keywords: cyclic peptide, sponge, cytotoxicity, Marfey's method

A number of small peptides have been described from marine sponges. They have attracted considerable attention because of their unique structures, rich physiological properties and thus of potential as important drugs [1]. Among them, theonellapeptolide Id (**1**), a tridecapeptide lactone isolated from an Okinawan *Theonella* sponge, is a rare example having a high proportion of *N*-methyl amino acids and of D isomers [2]. It inhibits the development of fertilized sea urchin eggs and exhibits moderate cytotoxicity, ion-transport activity for Na^+ and K^+ ions [3], and Na^+,K^+-ATPase inhibitory activity [4]. Seven theonellapeptolides have so far been reported [2,3,5,6].

In our continuing search for bioactive compounds from marine sources, we have recently investigated the chemical constituents of the Indonesian sponge *Theonella swinhoei* and isolated three peptides, one new cyclic peptide named barangamide A (**3**), and two known depsipeptides, theonellapeptolides Id (**1**) and IId (**2**). In this report we describe the isolation and structure elucidation of **3**.

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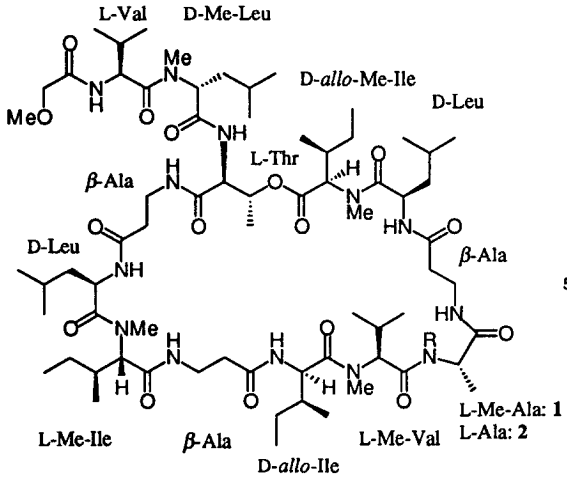
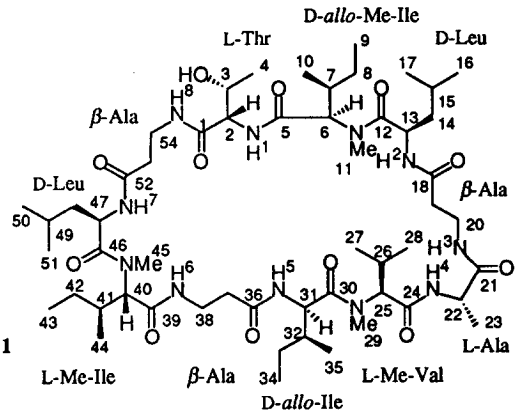
The sponge (600 g dry weight) was collected at Baranglompo Island, Indonesia by SCUBA and dried in the sun prior to transporting to Okinawa. It was extracted successively with heptane, ethyl acetate, acetone, and methanol. The residue (14.6 g) of the acetone extract, which showed cytotoxicity at 10 μg/mL, was separated by column chromatography on silica gel (CH_2Cl_2-MeOH) followed by ODS (MeOH-H_2O). The fractions eluted with MeOH were combined (3.22 g) and further separated by HPLC on ODS using MeCN-MeOH-H_2O (16:3:1) then MeOH-H_2O (88:12) as eluents to afford 11 mg of barangamide A (**3**)[1] as a glassy solid, along with theonellapeptolides Id (**1**) and IId (**2**) as major components.



1: Theonellapeptolide Id (R = Me)
2: Theonellapeptolide IId (R = H)



3: Barangamide A

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[1] Barangamide A (**3**): $[\alpha]_D^{25}$ -38.4° (*c* 0.3, MeOH); IR (film) ν_{max} 3270, 2917, 1660, 1633 cm^{-1}; ^{13}C NMR (125 MHz, $CDCl_3$) **L-Thr:** δ 174.5 (s), 52.7 (d), 64.6 (d), 21.9 (q), **D-*allo*-Me-Ile:** 169.3 (s), 64.7 (d), 32.4 (d), 26.4 (t), 11.4 (q), 15.3 (q), 29.0 (q), **D-Leu:** 174.3 (s), 47.2 (d), 40.8 (t), 24.5 (d), 23.3 (q), 21.2 (q), **β-Ala:** 172.7 (s), 34.7 (t), 32.8 (t), **L-Ala:** 172.4 (s), 47.6 (d), 15.8 (q), **L-Me-Val:** 172.0 (s), 62.2 (d), 27.7 (d), 19.8 (q), 19.1 (q), 31.3 (q), **D-*allo*-Ile:** 173.6 (s), 53.3 (d), 37.6 (d), 25.6 (t), 11.1 (q), 14.5 (q), **β-Ala:** 170.4 (s), 34.3 (t), 36.5 (t), **L-Me-Ile:** 169.2 (s), 60.6 (d), 31.9 (d), 25.1 (t), 9.7 (q), 16.8 (q), 31.7 (q), **D-Leu:** 175.7 (s), 49.1 (d), 39.5 (t), 24.3 (d), 23.4 (q), 21.8 (q), **β-Ala:** 171.8 (s), 34.0 (t), 39.2 (t); ^1H NMR (500 MHz, $CDCl_3$) **L-Thr:** δ 4.83 (m; H-2), 4.05 (m; H-3), 1.31 (d, 6; H_3-4), 8.58 (d, 9.5; NH-1), 5.92 (d, 6.5; OH), **D-*allo*-Me-Ile:** 4.91 (d, 10.5; H-6), 2.27 (m; H-7), 1.51 (dq, 15, 7.5; H-8), 1.14 (m; H-8'), 0.99 (t, 7.5; H_3-9), 0.87 (d, 6.5; H_3-10), 2.65 (s; H_3-11), **D-Leu:** 4.81 (m; H-13), 1.99 (m; H-14), 1.29 (m; H-14'), 1.80 (m; H-15), 0.89 (d, 7; H_3-16), 0.89 (d, 7; H_3-17), 9.09 (d, 6.5; NH-2), **β-Ala:** 2.59 (dd, 15, 4; H-19), 1.83 (m; H-19'), 3.88 (m; H-20), 3.13 (brt, 13; H-20'), 7.77 (d, 10; NH-3),, **L-Ala:** 4.19 (dq, 9, 6.5; H-22), 0.74 (d, 6.5; H_3-23), 8.61 (d, 9; NH-4), **L-Me-Val:** 4.84 (d, 10.5; H-25), 2.16 (m; H-26), 0.86 (d, 6.5; H_3-27), 0.96 (d, 6; H_3-28), 3.30 (s; H_3-29), **D-*allo*-Ile:** 4.90 (t, 10; H-31), 1.94 (m; H-32), 1.37 (m; H-33), 1.06 (m; H-33'), 0.88 (t, 7; H_3-34), 0.91 (d, 6.5; H_3-35), 8.07 (d, 10; NH-5), **β-Ala:** 2.35 (m; H_2-37), 3.74 (m; H-38), 3.44 (m; H-38'), 6.36 (brd, 7.5; NH-6), **L-Me-Ile:** 4.97 (d, 10; H-40), 2.20 (m; H-41), 1.37 (m; H-42), 1.06 (m; H-42'), 0.85 (t, 7.5; H_3-43), 1.11 (d, 6; H_3-44), 2.98 (s; H_3-45), **D-Leu:** 4.44 (dt, 10, 5; H-47), 1.84 (m; H-48), 1.45 (ddd, 13, 8.5, 5; H-48'), 1.91 (m; H-49), 1.03 (d, 6.5; H_3-50), 0.99 (d, 6.5; H_3-51), 8.88 (d, 5; NH-7), **β-Ala:** 2.31 (m; H-53), 1.96 (m; H-53'), 3.51 (m; H-54), 3.27 (m; H-54'), 9.14 (d, 6; NH-8).

The molecular formula of barangamide A (**3**) was determined as $C_{54}H_{97}N_{11}O_{12}$ (12 degrees of unsaturation) by high-resolution FABMS (MH^+ *m/z* 1092.7374 Δ -2.2 mmu). The peptidic nature of **3** was indicated by both ^1H (eight NH resonances at δ 6.2-9.2, three NMe resonances at δ 2.62, 2.98, 3.30) and ^{13}C NMR (eleven C=O resonances at δ 169-176). Since **3** has 11 amide carbonyl carbons and no other double bonds, it should have one ring as supported also by a negative result with ninhydrin. The eleven amino acid residues as revealed by NMR data were β-Ala (x3), Ala, Ile, Me-Ile (x2), Leu (x2), Thr, and Me-Val. Ile and one of Me-Ile were in an *allo*-form as shown by comparison of the ^1H NMR of the acid hydrolysate (6N HCl, 110 °C, 24 h) of **3** with those of authentic samples. The amino acid sequence was determined by detailed analysis of the HMBC and NOE correlations to complete the planar structure for **3** (Figure 1). Application of the Marfey's method [7] enabled us to determine the chirality of the residues as L-Ala, D-*allo*-Ile, L-Me-Ile, D-*allo*-Me-Ile, D-Leu (x2), L-Thr, and L-Me-Val.[2)]

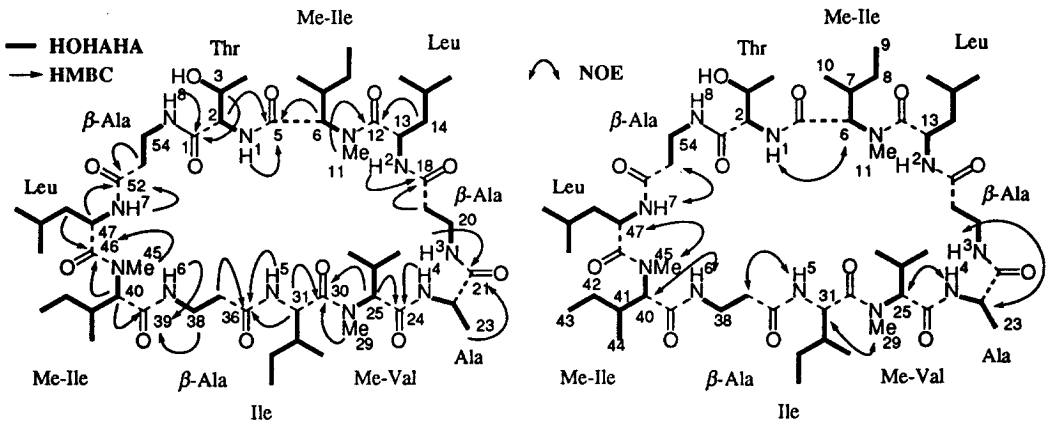

Figure 1. Partial structures and connectivity for barangamide A (**3**).

The positions of the two isomeric Me-Ile units (C5-11, C39-45) were assigned as follows. Authentic samples of isoleucine and *allo*-isoleucine showed small vicinal coupling constants (4 Hz) for H-2 and H-3, indicating no rotation barrier around C2-C3 axis. On the contrary, **3** showed large values for the corresponding coupling constants ($J_{H6,H7}$=10.5 Hz and $J_{H40,H41}$=10 Hz) in the Me-Ile units, suggesting restricted rotation and *anti* relationship of these protons (Figure 2). This contention was reinforced by no NOEs between these protons (H-6 and H-7; H-40 and H-41). In ROESY and NOEDS experiments, the NMe (C-11) showed significant NOE with H_3-10 and no NOE with H_2-8 and H_3-9, suggesting that this unit (C5-11) is the *allo*-Me-Ile (**a** in Figure 2). Similar NOE relationship was observed for the *allo*-Ile unit (**b**). The remaining C39-45 unit was assigned as Me-Ile by NOE between H_3-45 and H_2-42 and by no

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[2)] Authentic samples of the *N*-methyl amino acids were prepared by the method described in reference 8.

NOE between H_3-45 and H_3-44 (**c**) as shown in Figure 2. We reached the same conclusion with **1** when it was examined by NMR in the same manner. Thus the structure of barangamide A (**3**) is determined as shown.

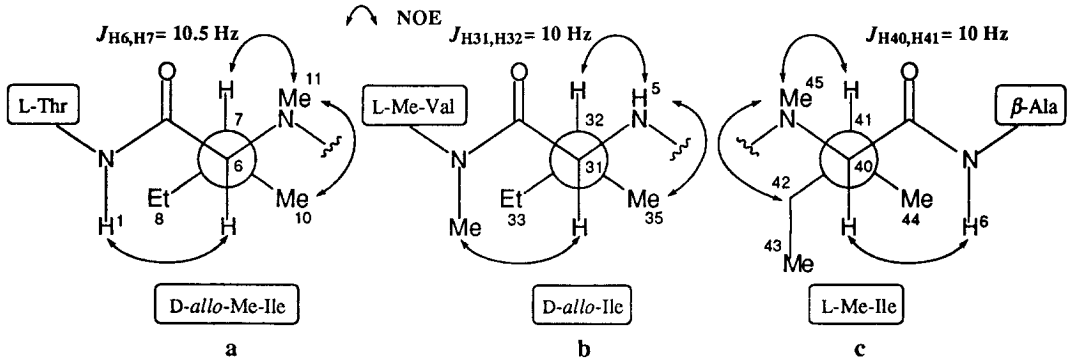

Figure 2. NOE correlations and assignment of three isoleucine units in **3**.

The amino acid sequence of barangamide A (**3**) is identical with the cyclic portion of theonellapeptolide IId (**2**). The difference between **2** and **3** is arisen by the difference in the cyclization modes with the threonine residue. In **2**, as the hydroxyl group is involved in the formation of a lactonic linkage, the amino group is used to extend the side chain. It is interesting to note that the difference between **2** and **3** appears to significantly affect their biological activities. In a preliminary assay no cytotoxicity has been observed with **3**, while theonellapeptolides Ia-Ie have been reported to be cytotoxic against L1210 (IC_{50} 1.3-2.4 μg/mL) [3]. Further chemical and biological studies are under way.

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