Pergamon

Tetrahedron Letters, Vol. 35, No. 5, pp. 719-720, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $6.00+0.00

0040-4039(93)E0286-S

Polytheonamides, Unprecedented Highly Cytotoxic Polypeptides, from the Marine Sponge *Theonella swinhoei* 1. Isolation and Component Amino Acids[1]

Toshiyuki Hamada, Takeo Sugawara, Shigeki Matsunaga, and Nobuhiro Fusetani*
Laboratory of Marine Biochemistry
Faculty of Agriculture, The University of Tokyo
Bunkyo-ku, Tokyo 113, Japan

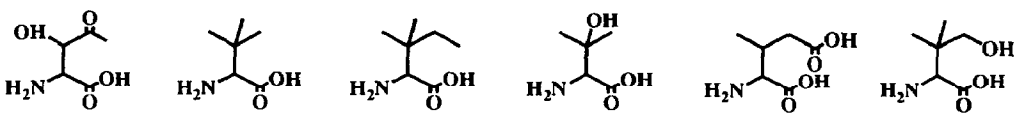
Abstract: Highly cytotoxic polypeptides, polytheonamides A-C, have been isolated from the marine sponge *Theonella swinhoei*. Interpretation of the 2D NMR data of the acid hydrolyzate of polytheonamide B as well as amino acid analysis led to identification of Ala, Asp, Thr, *a*Thr, Ser, Glu, Val, Gly, Ile, *t*-Leu, β-methylGlu, β-methylIle, β-hydroxyVal, and β-hydroxyAsp. 2D NMR data of polytheonamide B suggested the presence of γ-hydroxy-*t*-Leu, which was a new amino acid.

Sponges of the order Lithistida are a rich source of diverse bioactive peptides containing unusual amino acid residues.[2] Most remarkable is *Theonella swinhoei* collected off Hachijo-jima Island, from which we have isolated cyclic peptides cyclotheonamides,[3] orbiculamides;[4] a linear peptide nazumanide A;[5] and the non-peptides onnamides.[6] During our cytotoxicity-directed fractionation of the lipophilic extract of *T. swinhoei*, we found a new class of linear polypeptides named polytheonamides A, B, and C. We report the isolation and amino acid composition of polytheonamides in this paper. The structures of polytheonamides will be reported in an accompanying paper.

The Et_2O soluble portion of the EtOH extract of *T. swinhoei* was subjected to solvent partitioning between 90% MeOH and *n*-hexane to remove non-polar oil; the aqueous MeOH phase was fractionated by ODS flash chromatography. The cytotoxic fraction eluted with CH_2Cl_2/MeOH (1:1) was further purified on Sephadex LH-20, followed by ODS-HPLC to yield polytheonamides A, B, and C (3.8, 5.2, and 1.0 x 10^{-4} % of wet weight, respectively).

Polytheonamides were negative to ninhydrin reagent. However, upon spraying with ninhydrin after *in situ* hydrolysis on TLC plates and heating with H_2SO_4 they gave an orange spot, suggesting a cyclic or *N*-terminal-blocked peptide, which was also confirmed by NMR data. The ^1H NMR spectrum of polytheonamide B in DMSO-d_6 exhibited many amide and α-methine signals as well as huge aliphatic methyl signals; the ^{13}C NMR spectrum was also reminiscent of a peptide composed of many aliphatic amino acids.

Amino acid analysis of the total acid hydrolysate of polytheonamide B indicated the presence of Asp (7.9 mol), Thr (3.9 mol), Ser (1.5 mol), Glu (2 mol), Gly (10 mol), Ala (8.9 mol), Val (4.3 mol), and Ile (2.3 mol).[7] In addition to these amino acids, distinct peaks for 4 unusual components were also found: X1 (acidic amino acid with a shorter retention time than Asp), X2 (neutral amino acid with a similar retention time as Cys), X3 (neutral amino acid with a slightly longer retention time than Ile), and X4 (basic component with a similar retention time to Orn). The peaks for Thr, Ser, and Glu were broad, suggesting the presence of overlapping peaks for unusual amino acids.



OHAsp (X1) *t*-Leu (X2) βMeIle (X4) OHVal βMeGlu OH-*t*-Leu

In order to identify the unknown components, the acid hydrolysate was directly analyzed by 2D NMR including COSY, HOHAHA, HMQC, and HMBC spectra. Signals for the usual amino acids were readily assigned by comparing the NMR data with those of authentic samples. In addition to an A3MX system for Thr (δ 1.21, 3.82, and 4.28), there was another A3MX system with similar chemical shift values (δ 1.15, 3.94, and, 4.25), which was assigned as *a*Thr. An AB system at δ 4.75 and 4.28 indicated the presence of *threo*-β-hydroxyAsp, which gave peak X1 in the amino acid analysis. There was a huge methyl singlet at δ 0.96, which gave HMBC crosspeaks with carbons at δ 26.0, 33.0, and 62.5. The α-methine proton attached to a carbon at δ 62.5 resonated at δ 3.32, thereby indicating the presence of *t*-Leu (peak X2), which had been known in discodermins,[8] polydiscamide A,[9] and bottromycins.[10] This was confirmed by comparison with anthentic sample. There were two singlet methyls at δ 1.17 and 1.32, both of which were correlated with carbons at δ 62.9 and 70.6 in the HMBC spectrum. These data led to identification of β-hydroxyVal, which was found as a component of aureobasidin A isolated from the yeast *Aureobasidium pullulaus*.[11] A methine proton at δ 2.50 exhibited COSY crosspeaks with an α-methine signal at δ 3.98, a methylene at 2.39 and 2.53, and a methyl at 0.95, thus revealing the presence of β-methylGlu.[12] The peak X3 was identified as methylamine on the basis of NMR data (δ_H 2.44, δ_C 25.4). The last unusual amino acid found in the hydrolyzate was β-methylIle (X4). Since interpretation of the HMBC spectrum of the hydrolysate mixture was ambiguous due to overlapping of methyl signals, β-methylIle was isolated from the acid hydrolyzate, together with *t*-Leu, β-hydroxyVal, and other standard amino acids by ODS-HPLC.[13] Its ^{1}H NMR data [δ 0.78 (3H, t), 0.88 (3H, s), 0.89 (3H, s), 1.32 (2H, q), and 3.40 (1H, s)] were comparable with those reported in polydiscamide A.[9] In addition, there was a spin system assignable to an *n*-propyl group [δ 0.80 (3H), 1.57 (2H), and 2.92(2H)], which was due to tri(*n*-propyl)amine as identified by comparison with an authentic sample.

These amino acid units were easily assigned in the 2D NMR spectra of polytheonamide B, which contained one spin system absent in the spectra of the acid hydrolyzate. An isolated oxygenated methylene (δ_H 2.95 and 2.68; δ_C 63.0) was coupled to two tertiary methyls (δ_H 0.98 and 1.05; δ_C 23.7 and 23.9), a quaternary carbon (δ_C 37.7), and an α-methine carbon (δ_H 4.59; δ_C 57.8) in the HMBC spectrum, thereby indicating the presence of γ-hydroxy-*t*-Leu residue, which was, to the best of our knowledge, a new amino acid.

Similarly, polytheonamides A and C were analyzed to obtain almost identical results.[14] Polytheonamides were highly cytotoxic against L1210 with IC_{50} < 4 ng/mL.

Acknowledgment: We thank Professor P. J. Scheuer, the University of Hawaii, for editorial comments. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

References and Notes:

1. Bioactive Marine Metabolites. 55. Part 54: Nakao, Y.; Matsunaga, S.; Fusetani, N. *Tetrahedron*, in press.
2. Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93*, 1793.
3. Fusetani, N.; Matsunaga, S.; Matsumoto, H.; Takebayashi, Y. *J. Am. Chem. Soc.* **1990**, *112*, 7053.
4. Fusetani, N.; Sugawara, T.; Matsunaga, S.; Hirota, H. *J. Am. Chem. Soc.* **1991**, *113*, 7811.
5. Fusetani, N.; Nakao, Y.; Matsunaga, S. *Tetrahedron Lett.* **1991**, *32*, 7073.
6. Matsunaga, S.; Fusetani, N.; Nakao, Y. *Tetrahedron* **1992**, *48*, 8369.
7. The molar equivalents are incorrect due to peak overlapping with unusual amino acids.
8. Matsunaga, S.; Fusetani, N.; Konosu, S. *Tetrahedron Lett.* **1985**, *26*, 855.
9. Gulavita, N. K.; Gunasekera, S. P.; Pomponi, S. A.; Robinson, E. V. *J. Org. Chem.* **1992**, *57*, 1767.
10. Schipper, D. *J. Antibiot.* **1983**, *36*, 1076.
11. Ikai, K.; Takesako, K.; Shiomi, K.; Moriguchi, M.; Umeda, Y.; Yamamoto, J.; Kato, I. *J. Antibiot.* **1991**, *44*, 925.
12. Debono, M.; Barnhart, M.; Carrell, C. B.; Hoffmann, J. A.; Occolowitz, J. L.; Abbott, B. J.; Fukuda, D. S. *J. Antibiot.* **1987**, *40*, 761.
13. Stereochemistry of the amino acid residues is currently under investigation.
14. Polytheonamide A had an identical amino acid composition as polytheonamide B, whereas polytheonamide C had a second β-methylGlu residue instead of a Glu residue in polytheonamide B.

(*Received in Japan* 27 *September* 1993)