



Polyoxypeptin Isolated from *Streptomyces*: A Bioactive Cyclic Depsipeptide Containing the Novel Amino Acid 3-Hydroxy-3-methylproline

Kazuo Umezawa,^{*1} Kumi Nakazawa,¹ Toshio Uemura,¹ Yoko Ikeda,² Shinichi Kondo,² Hiroshi Naganawa,² Naoko Kinoshita,² Hideki Hashizume,¹ Masa Hamada,² Tomio Takeuchi,² and Shigeru Ohba³

¹Department of Applied Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223, Japan

² Institute of Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

³ Department of Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223, Japan

Received 6 October 1997; revised 15 December 1997; accepted 19 December 1997

Abstract: Polyoxypeptin, a potent inducer of apoptosis in human pancreatic carcinoma cells, was isolated from an ethyl acetate extract of a *Streptomyces* culture broth. Structural determination by 2D-NMR and X-ray crystallographic analysis revealed that it is a novel cyclic hexadepsipeptide containing five hydroxylated amino acids. The unusual and hitherto unreported amino acid 3-hydroxy-3-methylproline was one of them.

© 1998 Elsevier Science Ltd. All rights reserved.

Induction of apoptosis is considered to be important for the clinical effect of anticancer agents. However, adriamycin¹⁾, cisplatin²⁾, and vinblastine³⁾, all reported to induce apoptosis, did not induce apoptosis in human pancreatic carcinoma AsPC-1 cells. So, we screened microbial culture broths for apoptosis-inducing agents that would be effective in AsPC-1 cells. As a result, we isolated a novel cyclic depsipeptide, polyoxypeptin.

For isolation of polyoxypeptin, the culture broth (4 L) of *Streptomyces* sp. was extracted with EtOAc. The extract was evaporated, applied to a silica gel column, and eluted with hexane/EtOAc (1/3, 1/5). The active fraction was evaporated and washed with cold MeOH to give 330 mg of pure polyoxypeptin. Polyoxypeptin was dissolved in MeCN for crystallization.

Polyoxypeptin (1) was obtained as colorless crystals, mp 244–245 °C (uncorrected); $[\alpha]_D^{22} +162^\circ$ (c 0.5, CHCl₃); UV: $\lambda_{\max}^{\text{MeOH}}$ (ε) 202 nm (37000), $\lambda_{\max}^{0.1N \text{ HCl/MeOH}}$ (ε) 204 nm (37000), $\lambda_{\max}^{0.1N \text{ NaOH/MeOH}}$ (ε) 210 (101600), 243 nm (sh. 11800); IR $\nu_{\max}$ (KBr): 3381, 3265, 2964, 2935, 2877, 1745 (ester), 1670 (amide), 1639 (amide), 1610, 1506, 1444, 1410, 1302, 1263, 1095, 978, 914 cm⁻¹; FAB-MS (pos.): *m/z* 991 (M+Na)⁺; FAB-MS (neg.): *m/z* 967 (M-H)⁻; FAB-HRMS (pos.): *m/z* 991.5356 (M+Na)⁺, calcd for C₄₅H₇₆N₄O₁₅Na, 991.5328. The ¹H and ¹³C NMR chemical shifts, measured in CDCl₃, are shown in Table 1.

Fragmentation peaks commonly observed in MS for acyclic peptides were not noted. Therefore, a cyclic rather than linear oligopeptide was suggested for this compound. Polyoxypeptin also gave positive responses to the Rydon-Smith reaction, and negative responses to a ninhydrin test.

Table 1. ^1H and ^{13}C NMR data (δ) for polyoxypeptin

Position	^{13}C	^1H	Position	^{13}C	^1H
N-OHVal			β	24.9	1.78, 2.25
CO	169.4		γ	20.7	1.60
α	62.7	5.17	δ	46.6	2.82, 3.14
β	29.4	2.46	δ -NH		4.89
γ	19.4	1.04	3-OHLeu		
γ'	19.7	1.06	CO	171.5	
N-OH		8.32	α	55.9	4.88
3-OH-3-MePro			β	77.0	5.42
CO	166.0		γ	29.2	1.92
α	68.1	4.86	δ	15.6	0.96
β	78.4		δ'	19.9	0.91
β -CH ₃	27.3	1.47	NH		8.25
γ	37.3	1.86, 2.37	Acyl chain		
δ	45.9	3.24, 4.83	CO	177.4	
β -OH		5.92	α	76.8	
5-OHPyr			α -CH ₃	20.4	1.37
CO	170.7		β	99.0	
α	47.7	5.43	γ	27.8	1.67, 1.74
β	29.4	2.02, 2.20	δ	23.9	1.40, 1.76
γ	59.0	3.65	ϵ	35.8	1.27
δ	54.3	2.92, 3.06	ζ	38.1	1.02
γ -OH		6.90	η	31.0	1.40
δ -NH		4.33	η -CH ₃	18.6	0.81
N-OHAla			θ	31.0	1.18, 1.25
CO	179.6		ι	11.6	0.87
α	50.9	5.97	ζ'	75.8	3.61
β	14.6	1.46	η'	24.9	1.40, 1.55
N-OH		9.77	θ'	8.7	0.80
Pip			α -OH		3.03
CO	168.2		β -OH		6.51
α	50.0	5.06			

By the extensive NMR studies, the structure of **1** was proposed to be a novel cyclic hexapeptide consisting of *N*-hydroxyvaline (N-OHVal), 3-hydroxy-3-methylproline (3-OH-3-MePro), 5-hydroxy-hexahydropyridazine-3-carboxylic acid^{4,5)} (5-OHPyr), *N*-hydroxyalanine (N-OHAla), piperazic acid⁵⁾ (Pip), and 3-hydroxyleucine⁶⁾ (3-OHLeu). The amino acid sequence and acyl position were determined by 2D NMR (HMBC and NOESY). Two NOE's between *N*-OH (δ 9.77) in N-OHAla and α -CH (δ 5.06) in Pip, and between *N*-OH (δ 8.32) in N-OHVal and α -CH (δ 4.86) in 3-OH-3-MePro suggested both the *N*-hydroxyl positions. The structure of the new acyl side chain having 15 carbons was elucidated by ^1H - ^1H COSY, HMBC

and NOESY experiments. **1** is most similar to variapeptin⁷⁾ and L-156,602⁸⁾ among the known cyclic hexapeptides⁹⁻¹¹⁾ produced by *Actinomycetes*, but is significantly different in having new three components, 3-OH-3-MePro, 5-OHPyr, and an acyl side chain.

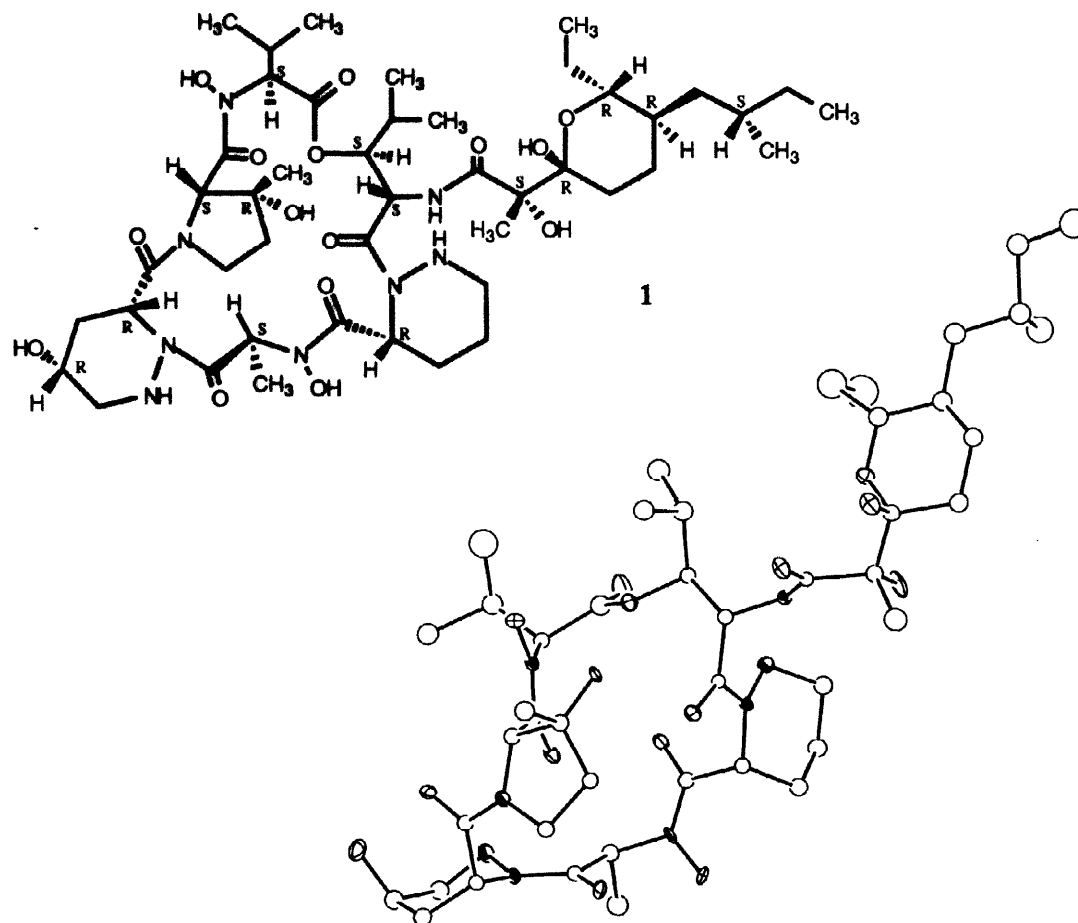


Fig. 1. Molecular structure of **1**.¹⁴⁾

Acid hydrolysis of **1** (133.4 mg) with 6N HCl in a sealed tube at 105 °C for 20 h gave several ninhydrin-positive compounds detected by high-voltage paper electrophoresis¹²⁾ (3300 volts for 15 min at pH 1.8, relative mobility to alanine, R_m , was measured). A new amino acid (R_m 0.74), (2*S*,3*R*)-3-hydroxy-3-methylproline¹³⁾ was isolated by column chromatography on Dowex 50W-X2 resin (Dow Chemical, Michigan), as a colorless solid (12.1 mg), $[\alpha]_D^{24}$ -33° (c 0.5, H₂O), $[\alpha]_D^{23}$ -5° (c 0.5, 5N HCl); FAB-MS (pos.): m/z 146 ($M+H$)⁺; ¹H NMR (D₂O): δ 1.60 (3H, s, CH₃), 2.15 (2H, m, 4-H₂), 3.45 (1H, ddd, 5-Ha), 3.54 (1H, ddd, 5-Hb), 3.86 (1H, s, 2-H); ¹³C NMR (D₂O): δ 171.2 (C-1), 78.8 (C-3), 70.1 (C-2), 43.7 (C-5), 39.9 (C-4), 24.3 (CH₃). L-Alanine (R_m 1.0) and L-valine (R_m 0.86) were also obtained by purification from the hydrolysate, and each chirality was determined by application onto HPTLC pre-coated plates CHIR (Merck Darmstadt, Germany) developed with MeOH-H₂O-MeCN, 1:1:4.

In order to confirm the proposed structure and to establish the overall stereostructure of **1**, X-ray crystallographic analysis was performed on a crystal obtained from the acetonitrile solution. Crystallographic data: C₄₅H₇₆N₈O₁₅·1.5(H₂O), MW 996.16, orthorhombic, $P2_12_12_1$, a = 17.615 (3) Å, b = 23.547 (3) Å, c =

13.313 (2) Å; $V = 5522.0$ (14) Å³, $Z = 4$, $D_x = 1.198$ Mg m⁻³, μ (Mo $K\alpha$) = 0.091 mm⁻¹. The X-ray intensities up to $2\theta = 50^\circ$ were measured on Rigaku AFC-5 four-circle diffractometer with graphite-monochromatized Mo $K\alpha$ radiation. Final R is 0.124 for 2934 reflections¹⁵. The absolute structure was not determined, with only relative stereochemistry being corroborated. Since the stereochemistry of L-valine was determined after hydrolysis of polyoxypeptin, the absolute configuration of the whole compound was elucidated as shown in **1**. Although 3-OH-3-MePro is a simple unusual amino acid, it has never been reported.

Polyoxypeptin induced loss of viability in AsPC-1 cells with an IC₅₀ of 80 ng/mL in 24 h. It also induced nuclear fragmentation and internucleosomal DNA fragmentation, which are characteristic of apoptotic cell death.

References and Notes

1. Ling, Y.-H.; Priebe, W.; Perez-Soler, R. *Cancer Res.* **1993**, *53*, 1845-1852.
2. Ormerod, M. G.; O'Neill, C. F.; Robertson, D.; Harrap, K. R. *Exp. Cell Res.* **1994**, *211*, 231-237.
3. Martin, S. J.; Cotter, T. G. *Cell Tissue Kinet.* **1990**, *23*, 545-559.
4. (3*S*,5*S*)-5-Hydroxyhexahydropyridazine-3-carboxylic acid was isolated as a component of antibiotic monamycin,⁵ but this compound from **1** was assigned to be (3*R*,5*R*) by the X-ray analysis of **1**.
5. Bevan, K.; Davies, J. S.; Hassall, C. H.; Morton, R. B.; Phillips, D. A. S. *J. Chem. Soc. (C)* **1971**, 514-522.
6. Sheehan, J. C.; Maeda, K.; Sen, A. K.; Stock, J. A. *J. Am. Chem. Soc.* **1962**, *84*, 1303-1305.
7. Nakagawa, M.; Hayakawa, Y.; Furihata, K.; Seto, H. *J. Antibiot.* **1990**, *43*, 477-484.
8. Hensens, O. D.; Borris, R. P.; Koupal, L. R.; Caldwell, C. G.; Currie, S. A.; Haidri, A. A.; Homnick, C. F.; Honeycutt, S. S.; Lindenmayer, S. M.; Schwartz, C. D.; Weissberger, B. A.; Woodruff, H. B.; Zink, D. L.; Zitano, L.; Fieldhouse, J. M.; Rollins, T.; Springer, M. S.; Springer, J. P. *J. Antibiot.* **1991**, *44*, 249-254.
9. Maehr, H.; Liu, C.; Palleroni, N. J.; Smallheer, J.; Todaro, L.; Williams, T. H.; Blount, J. F. *J. Antibiot.* **1986**, *39*, 17-25.
10. Smitka, T. A.; Deeter, J. B.; Hunt, A. H.; Mertz, F. P.; Ellis, R. M.; Boeck, L. D.; Yao, R. C. *J. Antibiot.* **1988**, *41*, 726-733.
11. Sugawara, K.; Toda, S.; Moriyama, T.; Konishi, M.; Oki, T. *J. Antibiot.* **1993**, *46*, 928-935.
12. Umezawa, H.; Kondo, S. In *Methods in Enzymology*, *43*, Antibiotics; Hash, J. H. Ed.; Academic Press: New York, 1975; pp. 279-290.
13. The stereochemistry was assigned from the X-ray analysis of **1**.
14. Edward, C.; Gilmore, C. J.; Mackay, S.; Stewart, N. *CRYSTAN-GM*. Version 6.3. **1996**, Mac Science, Japan.
15. Atomic coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.