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

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***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.**


Induction of apoptosis is considered to be important for the clinical effect of anticancer agents. However, adriamycin[1)], cisplatin[2)], and vinblastine[3)], 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° (*c* 0.5, $CHCl_3$); UV: $\lambda_{max}^{MeOH}$ (ε) 202 nm (37000), $\lambda_{max}^{0.1N\ HCl/MeOH}$ (ε) 204 nm (37000), $\lambda_{max}^{0.1N\ NaOH/MeOH}$ (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 $C_{45}H_{76}N_8O_{15}Na$, 991.5328. The $^1H$ and $^{13}C$ NMR chemical shifts, measured in $CDCl_3$, 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.

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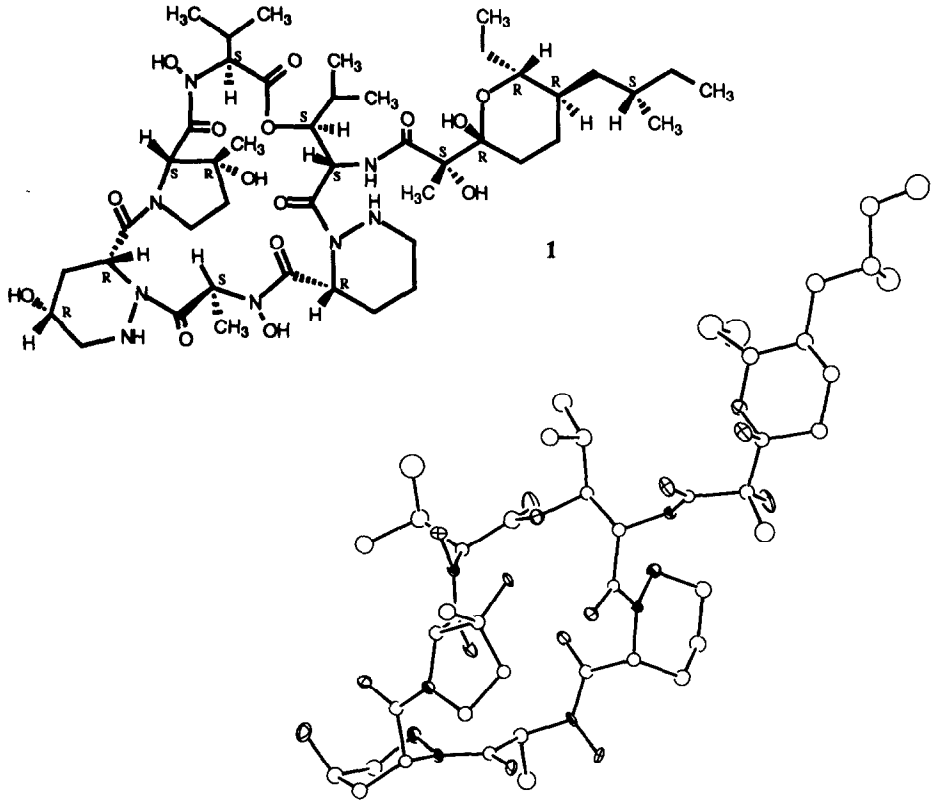
Table 1. $^{1}H$ and $^{13}C$ NMR data (δ) for polyoxypeptin

| Position | $^{13}C$ | $^{1}H$ | Position | $^{13}C$ | $^{1}H$ |
|---|---|---|---|---|---|
| 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 acid[5] (Pip), and 3-hydroxyleucine[6] (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 $^{1}H$-$^{1}H$ COSY, HMBC

and NOESY experiments. **1** is most similar to variapeptin[7] and L-156,602[8] among the known cyclic hexapeptides[9-11] produced by *Actinomycetes*, but is significantly different in having new three components, 3-OH-3-MePro, 5-OHPyr, and an acyl side chain.



**1**

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 electrophoresis[12] (3300 volts for 15 min at pH 1.8, relative mobility to alanine, Rm, was measured). A new amino acid (Rm 0.74), (2*S*,3*R*)-3-hydroxy-3-methylproline[13] 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, $H_2O$), $[\alpha]_D^{23}$ -5° (*c* 0.5, 5N HCl); FAB-MS (pos.): *m/z* 146 $(M+H)^+$; $^1H$ NMR ($D_2O$): δ 1.60 (3H, s, $CH_3$), 2.15 (2H, m, 4-$H_2$), 3.45 (1H, ddd, 5-Ha), 3.54 (1H, ddd, 5-Hb), 3.86 (1H, s, 2-H); $^{13}C$ NMR ($D_2O$): δ 171.2 (C-1), 78.8 (C-3), 70.1 (C-2), 43.7 (C-5), 39.9 (C-4), 24.3 ($CH_3$). L-Alanine (Rm 1.0) and L-valine (Rm 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-$H_2O$-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: $C_{45}H_{76}N_8O_{15}\cdot 1.5(H_2O)$, 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) Å$^3$, $Z$ = 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 reflections[15]. 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.

Polypxypeptin induced loss of viability in AsPC-1 cells with an $IC_{50}$ of 80 ng/mL in 24 h. It also induced nuclear fragmentation and internucleosomal DNA fragmentation, which are characteristic of apoptotic cell death.

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