SYNTHESIS OF A LYSINE ANALOG OF THE ANTIBIOTIC POLYMYXIN M

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We have previously described the synthesis of a linear protected decapeptide forming a lysine analog of polymyxin M, the hydrazide of N^{α}-pel* -N^{ε}-Z-Lys-Thr-N^{ε}-Z-Lys-N^{ε}-(N^{α}-BOC-N^{ε}-Z-Lys-D-Leu-N^{ε}-Z-Lys-Thr)-Lys-N^{ε}-Z-Lys-Thr (I).

In the present paper we report the results of the cyclization of the decapeptide (I) by the azide method.

The lysine analog of polymixin M that has been synthesized, which was identified in the form of the pentahydrochloride (IV) possessed a high antibacterial activity in relation to Brucella bronchiseptica.

Hydrochloride of the Hydrazide of the Decapeptide N^{α}-pel-N^{ε}-Z-Lys-Thr-N^{ε}-Z-Lys-N^{ε}-(N^{ε}-Z-Lys-D-Leu-N^{ε}-Z-Lys-Thr-)-Lys-N^{ε}-Z-Lys-Thr (II). To 400 mg of (I) was added 25 ml of a 2 N solution of hydrogen chloride in dioxane. After the solution had been kept at 20°C for an hour and worked up subsequently in the usual way, 270 mg (69%) of (II) was obtained with mp 194-195°C, $[\alpha]_D^{20}$ −20.0 (c 0.8; methanol).

The following ratio of the amino acids was found: Leu 1.00 : Thr 2.92 : Lys 5.94. The substance was electrophoretically and chromatographically homogeneous.

N^{α}-pel-N^{ε}-Z-Lys-Thr-N^{ε}-Z-Lys-N^{α}-cyclo(-Lys-N^{ε}-Z-Lys-Thr-N^{ε}-Z-Lys-D-Leu-N^{ε}-Z-Lys-Thr-) (III). A solution of 200 mg of the hydrochloride of the hydrazide of the decapeptide (II) in 1.7 ml of CH_3COOH and 0.55 ml of 1 N hydrochloric acid was cooled to −5°C and, with stirring, a solution of 9.4 mg of $NaNO_2$ in 0.5 ml of water was added. The mixture was stirred for 20 min at −5°C and was diluted with 420 ml of 50% aqueous dimethylformamide. Then the reaction mixture was brought to pH 8.3 by the addition of 1 N NaOH solution and was left at +2°C for five days. The solution with the precipitate that had formed was acidified with 1 N hydrochloric acid to pH 6 and evaporated to dryness in vacuum. The residue was treated with 20 ml of 1 N hydrochloric acid. The insoluble fraction was filtered off and washed with water, 3% $NaHCO_3$ solution, and water again. The cyclopeptide was purified from contamination with the initial linear peptide on Dowex 50 × 2 ion-exchange resin in the H^+ form.

The yield of (III) was 83 mg (43%), mp 122-125°C, $[\alpha]_D^{20}$ −15.6 (c 1; methanol). The following amino-acid ratio was found: Leu 1.00: Thr 3.08: Lys 6.13. The substance was chromatographically and electrophoretically homogeneous. The molecular weight of the cyclopeptide (III) determined by isothermal distillation was 1957, the calculated molecular weight being 1994.

Pentahydrochloride of N^{α}-pel-Lys-Thr-Lys-N^{α}-cyclo-(Lys-Lys-Thr-D-Leu-Lys-Thr-) (IV). To 39 mg of the protected cyclopeptide (III) was added 1.5 ml of a 1 N solution of hydrogen chloride in methanol and 2 ml of absolute methanol. The solution was hydrogenated over Pd black with shaking for 20 h. After two reprecipitations from absolute methanol with absolute ether, (IV) was obtained: 13 mg (52%) with mp 210°C (decomp.), $[\alpha]_D^{20}$ −21.2° (c 0.48; methanol). The amino-acid ratio found was: Leu 1.00: Thr 3.01: Lys 5.87. The substance was chromatographically and electrophoretically homogeneous. By partial dinitrophenylation, five free amino groups were found. The absence of a free carboxy group was shown by hydrazinolysis.

*pel − Pelargonyl.

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Thus, a lysine analog of the antibiotic polymyxin M, the pentahydrochloride of N^{α}-pel-Lys-Thr-Lys-N^{α}-cyclo-(-Lys-Lys-Thr-D-Leu-Lys-Thr-), possessing a high antibacterial activity against Brucella bronchiseptica has been synthesized.

LITERATURE CITED

1. E. S. Oksenoit, E. Morozova, and E. N. Gorbacheva, Vestnik MGU, No. 6, 113 (1969).