THE STRUCTURE OF THE ANTIBIOTIC POLYMYXIN M

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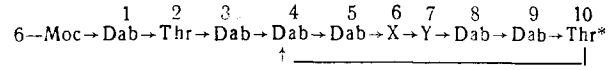
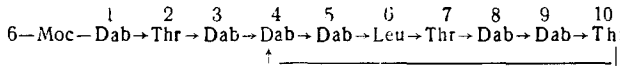
In the structural formula for polymyxin M proposed previously [1, 2],



the mutual positions of the leucine and threonine were not strictly proved, although they must be between the fifth and eighth [α, γ-diaminobutyric acid (α, γ-DABA)] residues. To determine the structure of polymyxin M definitively we have undertaken the specific cleavage of the antibiotic at the residues of the hydroxy amino acid threonine. According to an amino-acid analysis [1], polymyxin M contains only three threonine residues. Complete and specific cleavage should therefore give only three peptides, an analysis of which should accurately establish the position of the threonine and the leucine in the ring. We used two different methods for the specific cleavage of polymyxin M which enabled peptides to be obtained with the threonine only at the C end or only at the N end.

Method 1. Oxidation of the threonine residues in pentabenzyloxycarbonylpolymyxin M with a mixture of CrO_3, pyridine, and CH_3COOH (20°C, 8 h) to an oxo acid with subsequent treatment of the oxidation product with hydroxylamine (or phenylhydrazine) in 70% CH_3COOH (60°C, 1 h) leads to the specific cleavage of the molecule at the carboxy group of threonine [3]. Paper electrophoresis showed the presence of four products of the cleavage of the antibiotic, the dinitrophenylation of which gave only α- and γ-DNP derivatives of α, γ-DABA. There was no DNP-leucine in the hydrolyzate, which shows the cleavage of the peptide bonds between amino acids 2 →3, 7 →8, and 10 →4. Thus, the threonine must occupy position 7 in the molecule.

Method 2. For a stricter proof of the position of the leucine, penta-DNP-polymyxin M was cleaved at the threonine amino groups by means of a mixture of HCOOH and BF_3 (50°C 24 h) [4]. As a result of the N →O acyl migration taking place to the extent of 90-95% under these conditions, the NH_2 group of each of the three threonine residues of polymyxin M was liberated with the formation of an ester bond at the OH groups of the threonine residues. After the deamination of the three NH_2 groups, mild cleavage of the ester groupings with a 0.25 N solution of $NaHCO_3$ (30°C, 24 h), and subsequent hydrazinolysis, free α,γ-DABA and leucine were detected by electrophoresis and chromatography in a thin layer of cellulose. C-terminal leucine could be formed only if it occupied position 6. Consequently, polymyxin M has the following structure:



The cleavage of the antibiotic at the Thr residues by the methods of F. D'Angeli [3] and T. Kaneko [5] did not give satisfactory results because of the low degree of specificity of the cleavage of the molecule. The use of anhydrous HF [6] led to 90-95% N →O migration, but the reaction required a long time (24-30 days) which is extremely undesirable.

*Here and below, 6-Moc represents (+)-6-methyloctanoic acid, DAB α,γ-diaminobutyryl, Thr threonine, Leu leucine, and DNP 2,4-dinitrophenyl.

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