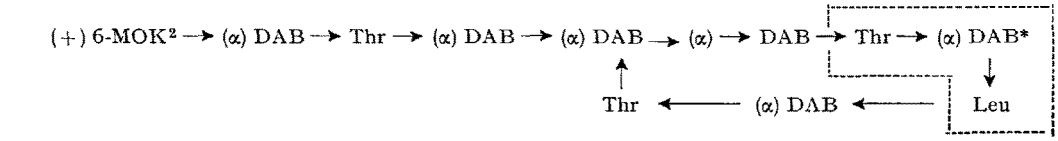
A Study of the Mechanism of Polymyxin M Inactivation

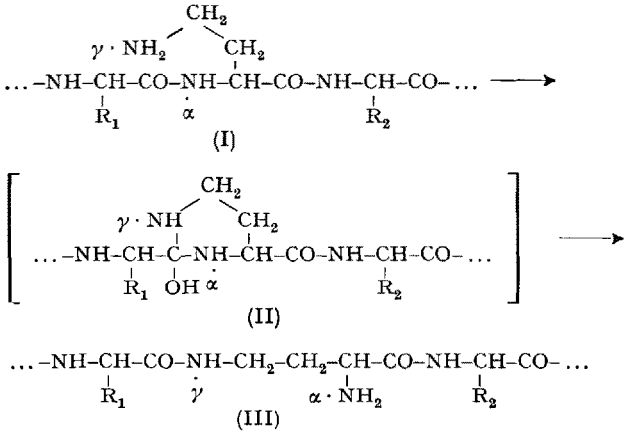
By studying the mechanism of inactivation of polymyxin M, we proceed from the supposed structural formula of antibiotics[1].



According to the data reported in the literature, salts of antibiotics of the polymyxin group are stable in weak acid and neutral solutions, but are inactivated in acid solutions and particulary in alkaline ones[3–7].

We found that antibacterial activity of polymyxin M quickly decreased during incubation of antibiotics in 0.1 *N* NH_4OH (pH 10.3) at 37 °C[8,9]. It disappeared completely and irreversibly in 7–8 days. It was shown by dinitrophenylation followed by analysis of acidic hydrolysate automatic amino acid analyser[10] that inactive form of antibiotics, unlike active one, contains about 1.5 moles of α-DNP-DAB, as well as an increased amount of free α,γ-DAB. Also in contrast to active form the ratio of leucine/threonine was not 1:3 and the total number of diaminobutyric acids (in form of α-DNP- and γ-DNP-DAB as well as α,γ-DAB) was not equal to 6. Thus some structural changes occur in the course of inactivation.

Previous suggestions that by inactivation of polymyxin M, $N^{\alpha} \rightarrow N^{\gamma}$-migration took place[11,12]



can explain only part of experimental facts – the appearance of α-amino groups and the increase of the free α,γ-DAB (after hydrolysis of intermediate substance II (see scheme).

However, recently we have shown that intramolecular aminolysis of peptide bond α,γ-DAB by distant γ-amino group followed by opening of cycle owing to formation of 3-acylamino-2-pyrrolidone and by appearance of free amino group of adjacent amino acid occurs together with $N^{\alpha} \rightarrow N^{\gamma}$-migration.

These amino groups were dinitrophenylated and owing to this, DNP-amino acids were not elucidated by amino-analyser. The studies on the model peptides confirmed this assumption[13–16].

In view of the fact that di-DNP-DAB and particularly DNP-Thr during hydrolysis were greatly destroyed[17,18], we determined them as DNS-derivatives[19]. The acid hydrolysats, DNS-derivatives, active and inactive forms of antibiotics, were studied by TLC in different solvents and on various carriers. DNS-Thr and di-DNS-DAB were indeed found in reaction mixture.

It should be noted that this mechanism of inactivation of polymyxin M requires some corrections in its structural formula which was proposed previously. Thus di-DNS-DAB can be obtained only if 2 residues of α,γ-DAB take

[1] A. B. SILAEV, E. P. YULIKOVA and L. A. BARATOVA, Zh. obshch. Khim. III, 198 (1965).
[2] Abbreviations: (+)6-MOK, (+)6-methyloctanoic acid; α,γ-DAB, α,γ-diaminobutyric acid; DNP-2,4-dinitrophenyl-; DNS-derivative 1-dimethylaminonaphthalene-5-sulpholyl-derivative; Thr, threonine; Leu, leucine; TLC, thin layer chromatography.
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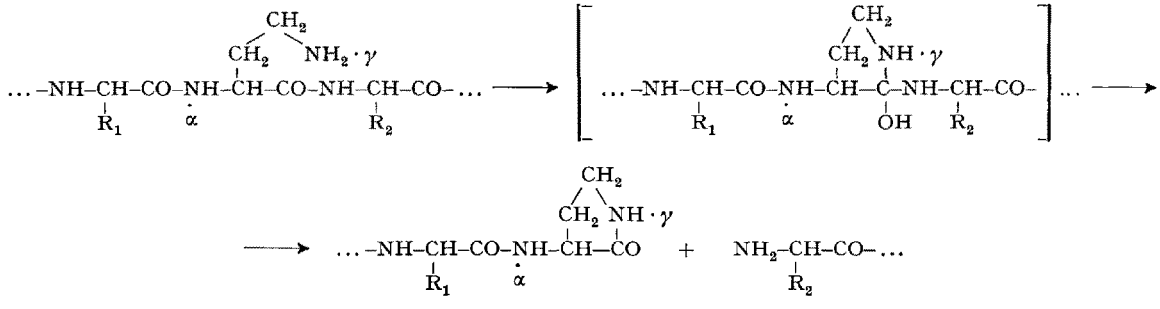
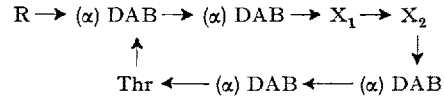


the neighbouring places on peptide chain. Therefore α,γ-DAB* should be in the place of leucine or threonine. However, comparison of structural formula of polymyxins[18,20–22], colistins[23] and circulin[24] shows that all these antibiotics have following sequence amino acid in cycle.



R is three-peptide, where α-amino group of α,γ-DAB is acylated with fatty acid; X_1 and X_2 are different amino acids depending on types of antibiotic. It is unlikely that polymyxin M which is a related antibiotic has a different type of structure. The problem of position of leucine and threonine requires further investigation.

Выводы. При изучении механизма инактивации Полимиксина М в О.1н.NH_4OH было обнаружено, что наряду с $N^{\alpha} \rightarrow N^{\gamma}$-ацильной миграцией имеет место и интрамолекулярный аминолиз пептидной связи γ-аминной группой α,γ-ДАМК. Авторы считают, что в ранее предложенную формулу полимиксина М следует внести некоторые коррективы.

G. S. Katrukha, L. A. Baratova and A. B. Silaev

Moscow State University, Laboratory of Bioorganic Chemistry, Moscow W-234 (USSR), 4 January 1968.

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Stéréochimie d'acides gras α-hydroxylés isolés d'une souche de *Streptomyces*[1]

Nous avons montré antérieurement[2] que l'analyse des lipides peut apporter une aide efficace à la classification de bactéries appartenant aux genres *Mycobacterium* et *Nocardia*. Récemment[3], nous avons étendu ces recherches au genre *Streptomyces*.

Nous avons en particulier isolé, à partir d'une souche de *Streptomyces* (No 5017, Collection de l'Istituto Superiore di Sanità, Rome), un acide α-hydroxylé, $C_{15}H_{30}O_3$, qui consiste en un mélange d'acides hydroxy-2 méthyl-13 tétradécanoïque et hydroxy-2 méthyl-12 tétradécanoïque[3]. Dans la présente Note, nous établissons la stéréochimie de ces acides α-hydroxylés, et décrivons la synthèse de termes de comparaison.

Stéréochimie de l'acide α-hydroxylé naturel. Centre asymétrique en 2: La variation positive de la rotation moléculaire de l'acide naturel, lorsqu'on passe du chloroforme à la pyridine comme solvant, est du même ordre de grandeur et du même sens que celles observées dans le cas de l'acide cérébronique (acide hydroxy-2D tétracosanoïque et homologues)[4] ou d'α-hydroxy-acides synthétiques[5] (voir Tableau). Le centre asymétrique en 2 appartient donc à la série D (configuration R). Ce résultat est confirmé par la variation du même ordre de grandeur, mais négative, de la rotation moléculaire, dans les mêmes conditions, présentée par la préparation d'acide synthétique, dont le centre en 2 appartient à la série L (voir Tableau).

Centre asymétrique en 12: L'oxydation chromique de l'acide naturel $C_{15}H_{30}O_3$ fournit un mélange d'acides $C_{14}H_{28}O_2$ et $C_{13}H_{26}O_2$: ce mélange d'acides possède une rotation moléculaire nettement dextrogyre (voir Tableau). Ces acides ont été identifiés, par chromatographie en phase gazeuse sur colonne capillaire de leurs esters méthyliques, aux acides méthyl-11 tridécanoïque (VIII) et méthyl-10 dodécanoïque (IX), contenant environ 25% des acides isomères de la série *iso* (acides méthyl-12 tridécanoïque et méthyl-11 dodécanoïque)[3].

Il a été établi[6] que les acides aliphatiques de la série *anteiso* ont la configuration L (ou S) lorsqu'ils sont dextrogyres: le centre asymétrique en 12 de l'acide hydroxy-2D méthyl-12 tétradécanoïque naturel est donc de configuration L.

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Rotations moléculaires d'α-hydroxy-acides et de quelques dérivés

	Ester méthylique ($CHCl_3$)	Acide libre chloroforme	pyridine	Δpyr-chlo	Acide de coupure oxydative ($CHCl_3$)
Acide α-hydroxylé de *Streptomyces*	− 1°	+ 0,4°	+ 18,7°	+ 18,3°	+ 8,3°
Acide α-hydroxylé synthétique[a]	+ 17,7°	+ 13,0°	− 8,5°	− 21,5°	+ 10,7°
Acide α-D-hydroxylé à chaîne droite (9)	− 10,3°	− 8,7°	+ 9,8°	+ 18,5°	
Acide cérébronique (série D) (8)		− 6,8°	+ 12,7°	+ 19,5°	

[a] Voir texte.