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THE STRUCTURES OF POLYOXINS A AND B*

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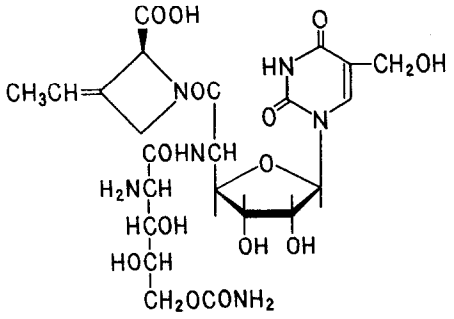
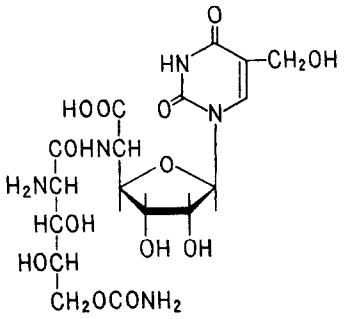
Polyoxins A and B, as have been reported[1], are main active components of agriculturally useful antifungal antibiotics, polyoxins. In the preceding paper [2], we proposed the structure of polyoxin C. This communication presents evidences which assign the structures I and II to polyoxins A and B, respectively. The structures are quite unique and can be designated "peptidic nucleoside", because they are nucleosides simultaneously being tri- or dipeptide of unusual all L-α-amino acids.



I



II

The schematic outline of hydrolyses of polyoxin A is shown in Fig. 1. Mild alkali hydrolysis[3] of polyoxin A (I), $C_{23}H_{32}N_6O_{14}$, $[\alpha]_D^{20}$ -30° (c 1.02, H_2O), gave four degradation products, 5-hydroxymethyluracil, polyoxin C (III), polyoximic acid (IV) and polyoxamic acid (V), evolving each one equiv of NH_3 and CO_2. On the other hand, acid hydrolysis of I gave mainly three products, III,

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* This paper comprises part VII of the series, "Studies on Polyoxins, Antifungal Antibiotics". Preceding paper, part VI, K. Isono and S. Suzuki, Tetrahedron Letters, No. 2 (1968) in press.

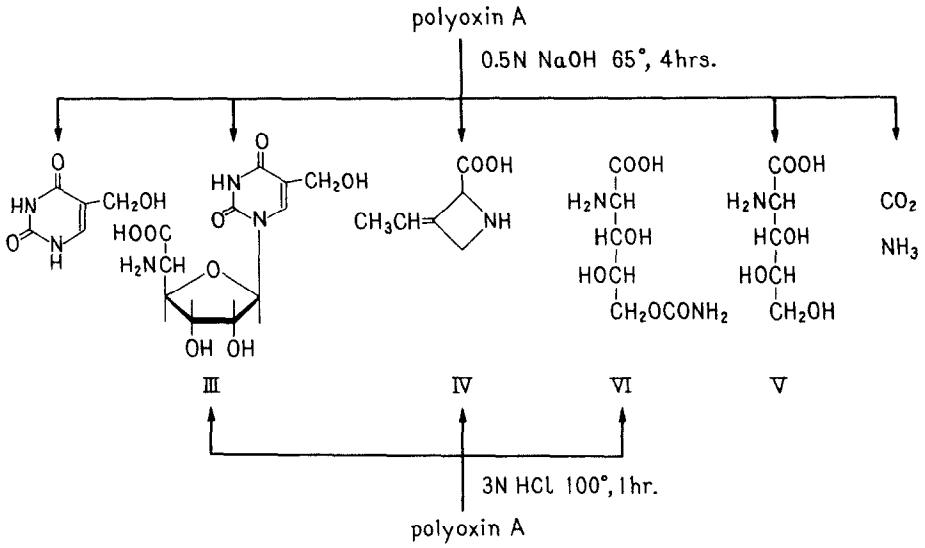


FIG. 1

IV and carbamylpolyoxamic acid (VI). 5-Hydroxymethyluracil was not appreciable and the yield of V was small.

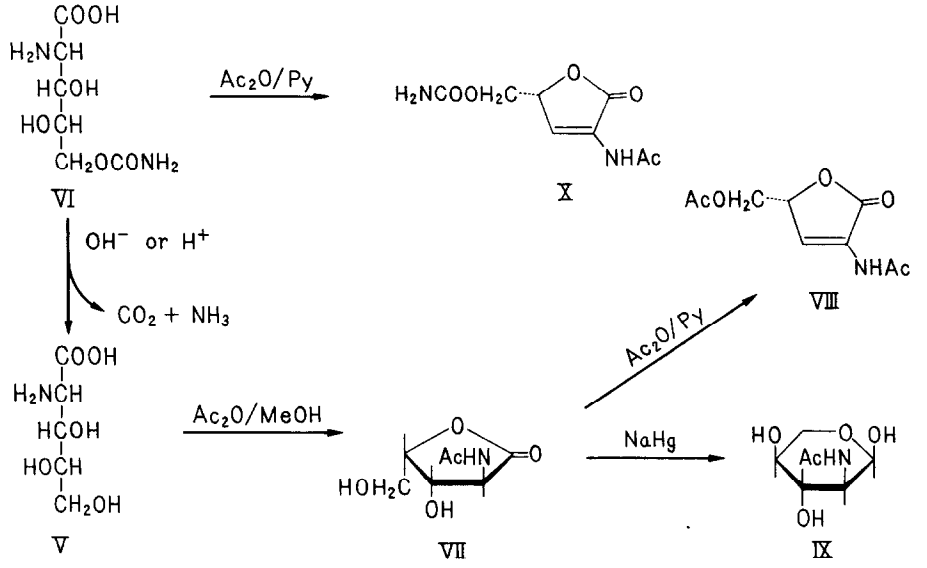
The structure of polyoxin C and polyoximic acid were already presented as 1-β-(5'-amino-5'-deoxy-D-allofuranuronosyl)-5-hydroxymethyuracil (III), 3-ethylidene-L-azetidine-2-carboxylic acid (IV), respectively, in the previous papers of this series [2)4)].

Polyoxamic acid (V), $C_5H_{11}NO_5 \cdot H_2O$, $[\alpha]_D^{23}$ +2.8° (c 1.02, H_2O), $[\Phi]_{213}$ +2,060pk (H_2O), $[\Phi]_{224}$ +3,960pk (0.5N HCl), pKa' 2.9, 8.7, which was obtained either by acid or alkali hydrolysis, was presumed to be a straight-chain trihydroxyamino acid, because on periodate oxidation it gave approximately each one equiv of CO_2, NH_3 and HCHO as well as 3 equiv of HCOOH consuming 4 equiv of periodate. The amino-position was suggested to be α by the paper-chromatographic detection of a tetrose on ninhydrin-oxidation[5)] and the insusceptibility to ninhydrin of the Cu^{++}-complex[6)]. The unambiguous proof was made by converting V to N-acetyl-γ-lactone (VII), ν^{CHCl_3} 1787, which was not oxidized with periodate, and the formation of a five-membered unsaturated lactone (VIII), λ_{max}^{MeOH} 243 mμ (ε 8,300), ν^{CCl_4} 1,777, 1,753, by subsequent exaustive acetylation accompanied

by β-elimination. The configuration was established by reduction of VII with sodium amalgam to give 2-acetamido-2-deoxy-β-L-xylose (IX)[7], mp. 190-3° (dec.), $[\alpha]_D^{20}$ -45°⟶ -6.4° (3 min.⟶ equi.)(c 1.08, H_2O). The idetity with the corresponding synthetic D-xylose derivative*[8] was proved by X-ray powder diffraction.

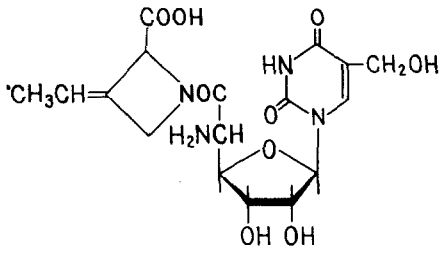
Carbamylpolyoxamic acid (VI), $C_6H_{12}N_2O_6$, dp. 226-232°, $[\alpha]_D^{23}$ +1.3° (c 1.04, H_2O), $[\phi]_{209}$ 2,410pk (H_2O), $[\phi]_{224}$ 3,380pk (0.5N HCl), ν^{KBr} 1,705, a predominant product of acid hydrolysis, gave V on mild alkali or prolonged acid hydrolysis; evolving each one equiv of NH_3 and CO_2. The carbamyl-position was presumed to be C5, because VI consumed 3 equiv of periodate. This was confirmed by obtaining a five-membered unsaturated lactone (X), λ_{max}^{MeOH} 243 mμ (ε 8,880), ν^{CHCl_3} 1,765, 1,740, 1,714, by acetylation. Cis-elimination is feasible because of the active nature of α-hydrogen. Thus, the structure, 5-O-carbamyl-2-amino-2-deoxy-L-xylonic acid (VI) was given to carbamylpolyoxamic acid.

The sequence of the three unusual all L-α-amino acids, III, IV and VI, was established by the deamination method. I was deaminated first with nitrous acid,



* We wish to thank Prof. M. L. Wolfrom, The Ohio State University, for a generous sample.

followed by alkali hydrolysis, affording III and IV, but no V, indicating V is N-terminal. The partial hydrolysis product with alkali (X), $C_{17}H_{22}N_4O_9$, van Slyke-NH_2 0.72 equiv, probably be a diastereomeric mixture, was reacted similarly, affording IV but no III, indicating III is N-terminal.



X

The structure I is well consistent with the physical and chemical properties of polyoxin A. The UV spectra, λ_{max}^{HCl} 262mμ (ε 8,700), λ_{max}^{NaOH} 264mμ (ε 6,400), are of the characteristics of the N-1-substituted uracil derivatives[9]. The pKa' values, 3.0, 7.3 and 9.6, correspond reasonably to -COOH, $-NH_2$ and uracil, respectively. The J value of an anomeric proton, 5.5 cps, is suggestive for the furanose structure. Moreover, I consumed rapidly 3 equiv of periodate, supporting strongly the structure I. Van Slyke-amino group determination gave 0.82 equiv in 10 min. and 1.49 equiv in 30 min., indicative of the presence of $-NH_2$ and $-OCONH_2$. Consumption of 4 equiv of periodate by N-acetylpolyoxin A is explained by the overoxidation of an active α-hydrogen of an aldehyde produced by the primary oxidation of the polyoxamic acid moiety. Only the remaining part to be clarified is cis or trans configuration about a double bond.

Polyoxin B (II), $C_{17}H_{25}N_5O_{13}$, $[\alpha]_D^{20}$ +34° (c 1.03, H_2O), λ_{max}^{HCl} 262mμ (ε 8,700), λ_{max}^{NaOH} 264mμ (ε 7,400), is also an amphoteric compound (pKa' 3.0, 6.9, 9.4), hydrolysis of which gave the similar products as in the case of I except affording no IV and very small amount of 5-hydroxymethyluracil. II consumed also 3 equiv of periodate rapidly and the sequence was determined by deamination followed by hydrolysis in the similar way.

The unusually labile nature of the nucleosidic bond of I to alkali hydrolysis is reasonably explained by the susceptibility of a peptidic α-hydrogen to hydroxide ion (Fig. 2). In the case of II or III, a carboxylate anion prevent the dissosiation of the α-hydrogen, thus yielding no significant 5-hydroxymethyluracil. The analogous mechanisms were postulated to the alkali hydrolysis of

adenosylmethionine[10] and the cyanide-catalysed decomposition of vitamine B_{12}[11]

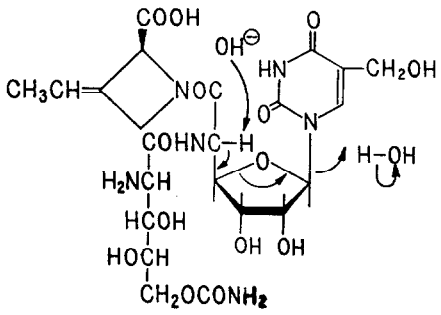


FIG. 2

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