Tetrahedron Letters No.2, pp. 203-208, 1968. Pergamon Press Ltd. Printed in Great Britain.

THE STRUCTURE OF POLYOXIN C*

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(Received in Japan 20 September 1967)

Polyoxin complex, an antifungal antibiotics mixture produced by Streptomyces cacaoi var. asoensis, is in practical use as an agricultural fungicide in Japan. Nine components, polyoxins A, B, C, D, E, F, G, H and I were isolated successively[1)2)]. The biological activity is unique, because they, except polyoxins C and I, have selective high activities against phytopathogenic fungi, whereas virtually no activities to other organisms. Polyoxin C is the smallest molecule among them and, although it lacks biological activity, it is thought to be a key-compound to elucidate a series of the structures of polyoxins because it is also obtainable by the hydrolysis[2)3)] of polyoxins A, B, G and I. The corresponding acid, polyoxin C-acid[2)], is also obtained by the hydrolysis of polyoxins D, E and F.

We wish to present evidences which assign the structure, 1-β-(5'-amino-5'-deoxy-D-allofuranuronosyl)-5-hydroxymethyluracil (I) to polyoxin C, $C_{11}H_{15}N_3O_8 \cdot H_2O$ (anal. Found: C, 39.83; H, 4.78; N, 12.39;

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* This paper comprises part VI of the series, "Studies on Polyoxins, Antifungal Antibiotics". Preceding paper, part V, K. Isono, J. Nagatsu, K. Kobinata, S. Sasaki and S. Suzuki, Agri. Biol. Chem. (Japan), 31, 190 (1967).

Van Slyke-N, 4.38; titr. equiv., 310), d.p. 260–267°, $[\alpha]_D^{24}$ +11.2° (c 0.5, H_2O). It loses 1 mole of water on drying at 100°. It is an amphoteric compound [pKa'; 2.4(-COOH), 8.1(-NH_2), 9.5(uracil)] and gives positive ninhydrin and periodate-benzidine reactions.

Prolonged hydrolysis of I with 3N HCl gave 5-hydroxymethyluracil (II). I gave also II consuming 6 moles of periodate in 96 hours at room temperature. The UV spectra of I, λ_{max}^{HCl} 262 mμ (ε, 9,410), λ_{max}^{NaOH} 264 mμ (ε, 7,320), are closely similar to those of 5-hydroxymethyluridine, 5-methyluridine and thymidine[4] and the big difference in the UV spectra of 1-methyluracil from 3-methyluracil[5] strongly suggests I is N-1 substituted 5-hydroxymethyluracil.

Hydrogenolysis of N-acetylpolyoxin C (III), m.p. 220° (dec.), gave N-acetyldeoxypolyoxin C (IV), m.p. 223° (dec.), the NMR spectrum of which with assignments is given in Fig.1. The C5'proton (quartet) coupled with the AcNH-proton (J= 8.0 cps) becomes resolved <u>doublet</u> (J= 5.5

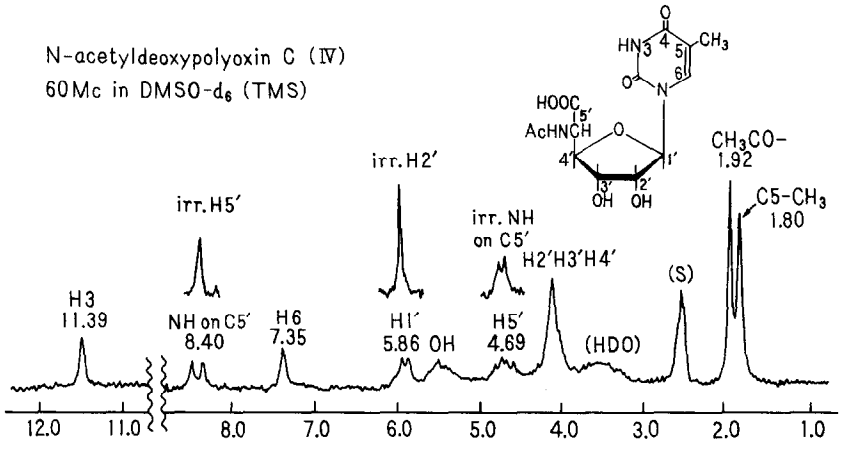
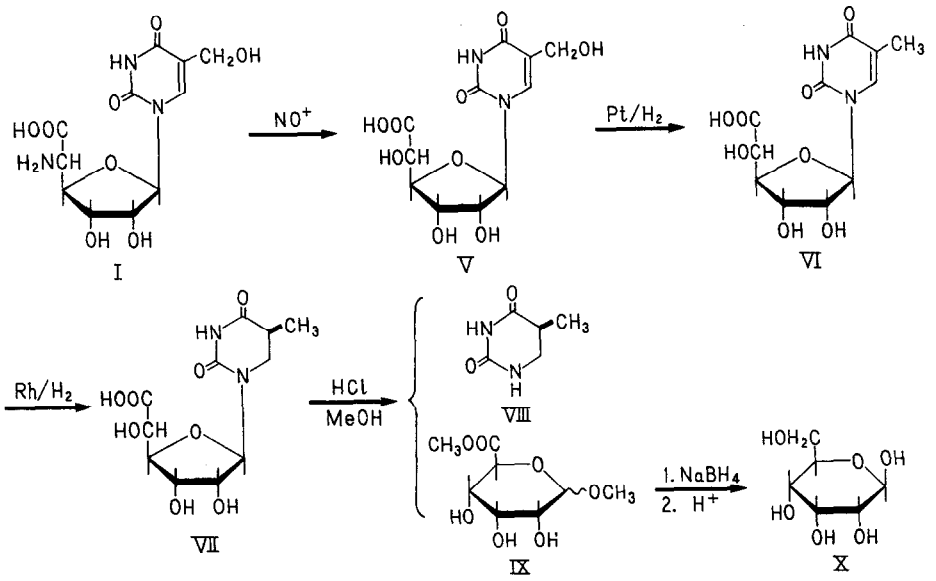


FIG. 1

cps)on a spin decoupling experiment indicating AcNH-position is C5'. Similar doublet was obtained on adding a drop of D_2O. The C5' amino position was complementally supported by the color reaction for α-amino acids[6]. Since I has a primary amino group, the furanose structure must be postulated. This is also supported by the J-value of the anomeric proton (5.0 cps) and the rapid consumption of 1 mole of periodate by III.

The aminouronic acid moiety was converted to the hexose in the following way. I was deaminated first with nitrous acid to give V, d.p. 250-



260°, which was hydrogenated over platinum followed by rhodium on alumina[7], affording VII. Methanolysis of VII gave (-)dihydrothymine[8] (VIII), m.p. 267-268°(dec.), $[\alpha]_D^{23}$ -8.0° (c 0.575, pyridine), as well as the methylglycoside of the ester (IX), which was without

isolating in pure form, reduced by sodium borohydride, followed by hydrolysis with Dowex 50W, yielding β-D-allose (X), m.p. 140-141°, $[\alpha]_D^{20}$ +4° (5 min.)→ +15°(equilibrium)(c 0.112, H_2O). The identity with the synthetic specimen* was performed by TLC and X-ray powder diffraction[9].

The configuration of the 5'-carbon was confirmed by the positive Cotton effect at 359mμ of N-dithiocarbethoxy**-2',3'-O-isopropylidene-deoxypolyoxin C (XI) (Fig. 2).

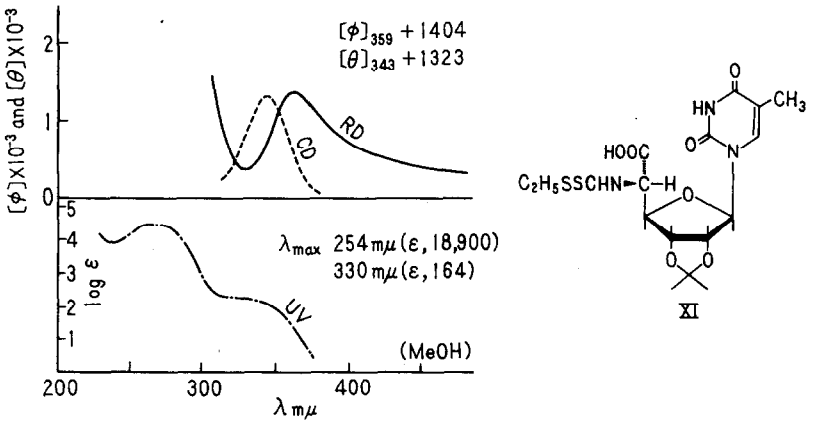


FIG. 2

Since polyoxin C has a normal uracil ring and a furanose ring, normal pyrimidine-nucleoside conformation of a uracil ring to a sugar ring would reasonably be expected, allowing the application of data compiled by Yang and Samejima[12] and Ulbricht et al[13)14] on the RD

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* We wish to thank Prof. M. Nakajima, Kyoto University for a generous sample.

** The ORD of dithiocarbamates of α-amino acids was extensively investigated (10,11).

of anomeric nucleosides and mononucleotides. Thus, the positive Cotton effects and CD maxima of polyoxin C ($[\phi]_{284}$=+1,980 pk, $[\phi]_{253}$ =-3,650 tr, $[\theta]_{268}$=+6,530) and desaminodesoxypolyoxin C (VI) ($[\phi]_{289}$ =+850 pk, $[\phi]_{258}$=-2,700 tr, $[\theta]_{266}$=+2,500) indicate the β-configuration of the nucleoside.

5-Amino sugar is very rare in nature, only the occurrence we know is nojirimycin, which structure was proposed as D-glucopiperidinose[15]. 5-Aminouronic acid is a compound of particular interest because it is a sugar with α-amino acid nature. This type of compound was unknown either synthetically or in nature.

We are indebted to Dr. Y. Sumiki for his warm encouragement and Dr. S. Emoto for his valuable discussion. We thank Mrs. K. Kobinata for her skillful assistance.

REFERENCES

1. K. Isono, J. Nagatsu, Y. Kawashima and S. Suzuki, Agri. Biol. Chem., 29, 848 (1965). [Short communication: S. Suzuki, K. Isono, J. Nagatsu, T. Mizutani, Y. Kawashima and T. Mizuno, J. Antibiotics, A18, 131 (1965).]
2. K. Isono, J. Nagatsu, Y. Kawashima, K. Yamagata, K. Sasaki and S. Suzuki, Agri. Biol. Chem., 31,190 (1967). [Short communication: S. Suzuki, K. Isono, J. Nagatsu, Y. Kawashima, K. Yamagata, K. Sasaki and K. Hashimoto, ibid., 30, 817 (1966).]
3. K. Isono and S. Suzuki, ibid., 30, 813 (1966).
4. E. Cline, R. M. Fink and K. Fink, J. Am. Chem. Soc., 81, 2521 (1959).
5. D. Shugar and J. J. Fox, Biochim. Biophy. Acta, 9, 199 (1952).

6. P. O. Larsen and A. Kjaer, ibid., 38, 148 (1960).

7. W. E. Cohn and D. G. Doherty, J. Am. Chem. Soc., 78, 2863 (1956).

8. K. Balenović and N. Bregant, Croat. Chim. Acta, 32, 193 (1960).

9. M. L. Wolfrom, J. N. Schumacher, H. S. Isbell and F. L. Humoller, J. Am. Chem. Soc., 76, 5816 (1954).

10. B. Sjöberg, A. Fredga and C. Djerassi, J. Am. Chem. Soc., 81, 5002 (1959).

11. C. Djerassi, K. Undheim, R. C. Sheppard, W. G. Terry and B. Sjöberg, Helv. Chem. Scand., 15, 903 (1961).

12. J. T. Yang and T. Samejima, J. Am. Chem. Soc., 85, 4039 (1963).

13. T. L. V. Ulbricht, J. P. Jennings, P. M. Scopes and W. Klyne, Tetrahedron Letters, No. 13, 695 (1964).

14. T. L. V. Ulbricht, T. R. Emerson and R. J. Swan, Biochem. Biophy. Res. Comm., 19, 643 (1965).

15. S. Inouye, T. Tsuruoka and T. Niida, J. Antibiotics, A19, 288 (1966).