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# THE STRUCTURAL STUDIES OF FORMYCIN AND FORMYCIN B

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As reported in previous papers[1,2], formycin and formycin B are antibiotics produced by Nocardia interforma and formycin inhibits growth of Yoshida rat sarcoma cells, Mycobacterium 607 and Xanthomonas oryzae, and formycin B inhibits growth of Xanthomonas oryzae. Both antibiotics exhibit a preventive effect on infection of Xanthomonas oryzae on rice plants. It has been also established[2,3] that formycin B is a deaminated product of formycin. In this paper, the molecular structure of formycin elucidated by X-ray analysis is presented.

Formycin hydrobromide monohydrate, $C_{10}H_{13}N_5O_4 \cdot HBr \cdot H_2O$, Anal.: calcd. C 32.80, H 4.40, N 19.13, O 21.85, Br 21.82; found C 32.91, H 4.52, N 18.75, O 21.38, Br 22.93, m.w. 366.18 (371 by X-ray method) melted at 180-180.5°C, and was recrystallized from an aqueous solution in the form of colorless prisms. The crystals are triclinic with the following cell dimensions:

$a = 6.66_0$ Å $\quad \alpha = 103.4°$

$b = 11.00_3$ Å $\quad \beta = 101.2°$

$c = 4.96_2$ Å $\quad \gamma = 96.0°$

which were determined from the precession photographs of $0kl$, $h0l$ and $hk0$ taken by CuK$\alpha$ radiation ($\lambda$=1.5418Å). The density measured by the flotation method was 1.80 g/cm$^3$ which indicated one molecule in the unit cell. The calculated value is 1.78 g/cm$^3$. Since the compound is optically active, the space group was determined to be P1. Three dimensional intensity data were collected from the c-axis equi-inclination Weissenberg photographs. The layers from $hk0$ to $hk3$ were taken by CuK$\alpha$ radiation using the multiple film technique. The intensities were estimated visually with the use of a calibrated intensity scale. Totally 1130 independent structure factors were taken for the analysis.

A three dimensional Fourier synthesis using the signs of Fourier coefficients calculated from the contributions of the bromine atom at the origin of the unit cell (R=0.266), revealed 40 well resolved electron density peaks, which corresponded just to the twice of the number of expected peaks. This electron density distribution must contain a false centre of symmetry. So we encountered the problem to distinguish the real molecule from a superimposed spurious one.

Trials of rejecting the ghost peaks in the unreasonable interatomic distances indicated a plausible molecular model on the Fourier map. At this step, it was impossible to distinguish carbon and nitrogen atoms in the molecule. After one cycle of the full-matrix least squares refinement in which all of the light atoms were assumed to be oxygen, the R factor dropped to 0.16. The nitrogen and carbon atoms were now able to be distinguished and assignments of these atoms were then confirmed by computing a difference Fourier synthesis. A superimposed contour sections of the three dimensional electron density function along the c-axis is illustrated in Fig. 1. Subsequent two cycles of the full-matrix least squares refinement using IBM-7090

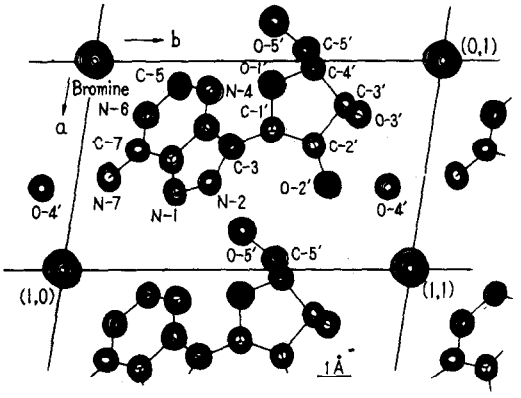
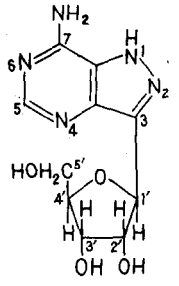
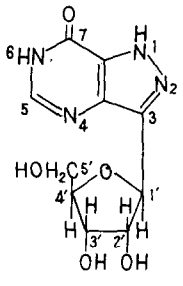



Fig. 1

computor reduced the R factor to 0.11. The absolute configuration of the molecule was determined by the use of the anomalous dispersion effect of the bromine atom and also presented in Fig. 1. The structure of formycin molecule found in the crystal of its hydrobromide monohydrate is now established as follows:



Formycin



Formycin B

Formycin : 7-amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidine

Formycin B : 3-(β-D-ribofuranosyl)pyrazolo[4,3-d]6(H)-7-pyrimidone

It is seen that the molecule consists of pyrazolo[4,3-d]pyrimidine base and ribofuranose residues comprising a nucleoside like

molecule. Here, however, the both residues are linked through a carbon-carbon single bond in place of the carbon-nitrogen glycosidic bond in the nucleosides. The bond distances and angles calculated in the molecule are normal. Crystal structure of formycin thus determined as mentioned above led us to the conclusion that formycin B has the structure as above described.

Pyrazolopyrimidine base and its exocyclic amino group are coplanar and the displacement of C(1') from this base plane is 0.14Å. The ribose ring puckers in such a way that C(2') is displaced from the plane of the three atoms C(1'), O(1') and C(4') by about 0.33Å in the direction of the same side as the C(4')-C(5') bond and C(3') is displaced by about 0.38Å in the opposite direction. These displacements of C(2') and C(3') indicate that the ribofuranose ring of formycin molecule in the crystal of its hydrobromide monohydrate exists in the C(2')-*endo*-C(3')-*exo* conformation. The plane of the sugar is at a dihedral angle of 64.2° to the base, a little smaller than the reported values for some nucleosides and -tides.[4)]

For the expression of the conformation about the glycosidic C-N bond in usual nucleosides, the torsion angle defined by Donohue and Trueblood[5)] has been utilized. This is defined as the angle formed by the trace of the plane of the base and the C(1')-O(1') bond projected along the glycosidic bond towards the plane. In the present case, though the glycosidic bond is carbon-carbon linkage, the value of this angle was found to be +148.33°. It indicates the normal *syn* orientation, in which this value is known to be +150°±45°, while in the *anti* orientation it is -30°±45°.

Although the location of the hydrogen atoms in formycin molecule is not yet determined directly from the electron density map, the protonated cite of formycin cation must be at N-6 from the data of bond distances,

electron deficiency at N-6 and hydrogen bonds in the crystal structure. Details will be published later.

The structure of formycin and formycin B here presented can explain the all physical and chemical data which have been obtained. Formycin and formycin B show similar ultraviolet spectra as 7-amino-pyrazolo [4,3-d] pyrimidine and 7-hydroxy-pyrazolo [4,3-d] pyr midine respectively which have been reported by R. K. Robins et al.[6]. As reported in another paper, these two pyrazolo [4,3-d] pyrimidines were obtained by periodate oxidation of formycin and formycin B followed by oxidation and decarboxylation. Phospholylated products of formycin and formycin B which have been reported[7] can be expressed as follows: FMP-(V) is Formycin 5'-monophosphate and FMBP-(V) is Formycin B 5'-monophosphate.

In n.m.r. spectrum of formycin measured in $D_2O$ using DSS as an internal standard, a sharp singlet at $\delta$8.02 (1H), a doublet at $\delta$5.24 (1H), a multiplet centered at $\delta$4.40 (3H) and a signal at $\delta$3.90 (2H) can be assigned to the single aromatic proton of the pyrimidine ring, the anomeric C(1') proton, the protons of C(2'), C(3') and C(4') and the methylenic protons of C(5') respectively. Formycin B in $D_2O$ shows the same n.m.r. spectrum as formycin. As found in pseudouridine[8], it is characteristic that the anomeric protons of formycin, formycin B and their derivatives appear at higher field than the reported values for usual nucleosides. This fact explains that the base and sugar moieties are linked through a C-C glycosidic bond in these antibiotics.

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