PYRAZOMYCIN B: ISOLATION AND CHARACTERIZATION OF AN α-C-NUCLEOSIDE ANTIBIOTIC RELATED TO PYRAZOMYCIN

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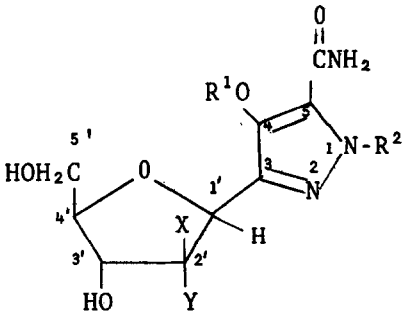
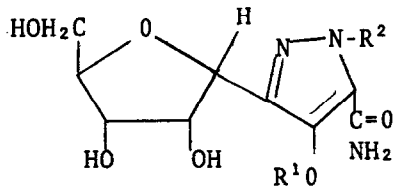
Summary: This communication reports the isolation and structure of pyrazomycin B, the α-anomer of the anti-tumor and anti-viral antibiotic pyrazomycin. Spectral, chemical and x-ray crystallographic evidence is provided.

The novel C-nucleoside antibiotic pyrazomycin[1] (1), holds considerable medicinal potential due to its antiviral[2] and anti-tumor[3] activity. We now report the isolation and characterization of a second active factor produced by the identical strain of *Streptomyces candidus*. Both were isolated from the broth filtrate by chromatography on IRA-400 (acetate) and separated on a Sephadex G-10 column.





1 $R^1=R^2=X=H$; Y=OH

2 $R^1=R^2=Y=H$; X=OH

3 $R^1=R^2=H$

4 $R^1=R^2=CH_3$; X=H; Y=OH

5 $R^1=R^2=CH_3$

Comparison of the ir, uv, and mass spectrum of the new component factor B, with those of pyrazomycin suggested their close similarity of structure. A peak at m/e 180 in the mass spectra of both was interpreted as arising from cleavage of the $C_{4'}$-$C_{5'}$ bond with loss of CH_2OH after prior loss of two moles of H_2O indicating a furanose form of the pentose moiety as opposed to an isomeric pyranose. Pmr (D_2O, 100 MHz) reveals a difference only for two protons. The $H_{1'}$ resonance, appearing at 5.42 δ (d, $J_{1',2'}$ = 6.5) in 1, is observed at 5.68 δ (d, $J_{1',2'}$ = 3.0) in factor B, while the well resolved $H_{3'}$ signal at 4.67 δ (d, d, $J_{2',3'}$ = 3.5) in 1 is noted in a position of partial overlap upon $H_{2'}$ at 4.83 δ in factor B. The return of the $H_{3'}$ signal in the latter to a position analogous to that which it held in 1 in TFA-d_1 as solvent suggested that a conformational effect in the pentose portion is responsible for the $H_{3'}$ chemical-shift variation between the two antibiotics. Spin decoupling confirmed all assignments. These observations imply a difference in the stereochemistry of either $C_{1'}$ or $C_{2'}$ in factor B relative to pyrazomycin, suggesting two possibilities for the former; i.e., a β-arabinofuranosyl nucleoside (2) or the α-anomer of pyrazomycin (3). The proximity of the resonances for $H_{2'}$ and $H_{3'}$ in the Pmr precludes the use of NOE to resolve the difference.

According to Rogers and Ulbricht[4], the sign and magnitude (Δε) of ORD/CD Cotton Effects should aid in distinguishing between these possibilities, providing the conformational relationship between sugar and base portions of the molecules is known. Pyrazomycin exists in the syn conformation as determined by x-ray[5]. The very strong intramolecular H-bond[5] in 1 between O_4 and O_5, argues for retention of a similar conformation in both solid state and solution. Based on the correlations of Rogers and Ulbricht[4], the

predicted negative B_{2u} Cotton effect in the CD of 1 ($\Delta\varepsilon$ = 1.125, 263 nm, H_2O) was verified. Molecular models suggest that a similarly strong H-bond probably exists between $O_{2'}$ and O_4 in factor B, regardless of whether its structure is 2 or 3. By comparison with results obtained with closely analogous structures[4], this assumption allows the prediction of a negative B_{2u} Cotton effect for 3 and an effect for 2 which is both positive and considerably greater in amplitude ($\Delta\varepsilon$) than that measured for 1. Factor B also exhibits a negative Cotton effect ($\Delta\varepsilon$ = 0.490, 263 nm, H_2O) for the same CD transition, supporting the α-ribofuranosyl structure 3.

Treatment of either 1 or factor B with diazomethane in methanol affords the crystalline dimethylated compounds* 4 and 5, respectively, as major products. Both 4 and 5 consume one molar equivalent of periodate at the same rate, giving rise to cleavage between $C_{2'}$ and $C_{3'}$ of the ribose moiety. The resulting bis-aldehydes displayed differing mobilities upon paper chromatography**. Mixed m.p. of their respective 2,4-DNP derivatives was depressed, further supporting the enantiomeric relationship of the two antibiotics at $C_{1'}$. The corresponding bis-aldehyde from the alternate possibility, 2, would have been identical to that from 1. ^{13}C-MR data in the following communication[6] provide additional evidence for 3 as the structure of factor B.

Crystallization from water affords colorless needles of factor B as a dihydrate (m.p. 69-70°), belonging to the orthorhombic space group $P2_12_12_1$. The unit cell contains four molecules and has the dimensions a = 7.216 ± 0.002 Å, b = 4.841 ± 0.001, and c = 37.243 ± 0.008. The density measured by flotation is 1.505 g cm^{-3}; the density calculated for $C_9H_{13}O_6N_3 \cdot 2H_2O$ (M = 295.3) is 1.507 g cm^{-3}.

*All new compounds have elemental analyses and spectra consistent with their proposed formulations.

**Solvent system: i-propanol/water/aq. ammonia (7/2/1).

X-ray intensity data were collected using an automated diffractometer with copper radiation. The structure was solved by direct phasing methods using the computer program MULTAN* and refined with anisotropic temperature factors by least-squares. All 17 hydrogen atoms were located from a difference synthesis and were included in the least-squares refinement with isotropic temperature factors. The final R factor was 0.053.

The conformation of the molecule is shown in Figure 1. The compound is confirmed as an α-ribofuranoside and differs from pyrazomycin only in the configuration (R) at $C_{1'}$. The pyrazole base is tilted such that the hydrogen atom on O_4 is in position to form an extremely strong hydrogen bond with $O_{2'}$, which lies exo to the ribose ring.** The O_4-$O_{2'}$ distance, 2.551 ± 0.004 Å, is extremely short; and this hydrogen bond probably holds the pyrazole ring in the same conformation in solution***. The dihedral angle between the ribose and the pyrazole rings is 71°. The pyrazole ring and its amide and hydroxyl substituents are nearly planar. The ribose ring assumes an envelope conformation with $C_{2'}$ displaced exo and the substituents staggered.

The asymmetric unit of the crystal structure shows a complex network of nine intermolecular hydrogen bonds involving adjacent molecules, including the water of hydration. The tightly bound

*P. Main, M. M. Woolfson and G. Germain, "MULTAN, A Computer Programme for the Automatic Solution of Crystal Structures", University of York Printing Unit, York, England, 1971.

**Following the convention of Sundaralingam and Jensen,[7] endo refers to the side of the ribose ring on which $C_{5'}$ lies and exo refers to the opposite side.

***In the crystal structure of the β-isomer (pyrazomycin) a similarly short hydrogen bond exists.[5] The pyrazole ring is tilted such that the hydroxyl O_4 lies endo to the ribose ring and is hydrogen-bonded to $O_{5'}$. The O_4-$O_{5'}$ distance is 2.61 Å. The two isomers of pyrazomycin are believed to be the first α-β nucleoside pair for which both crystal structures have been determined. A manuscript giving a detailed comparison of the two structures is in preparation.

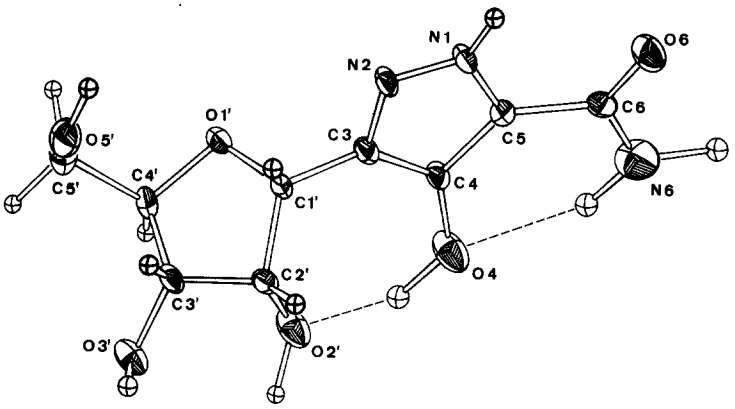


FIGURE I.

Conformation of the molecule in the crystal. Dotted lines indicate the two intramolecular hydrogen bonds. The anisotropic thermal ellipsoids are drawn to enclose 50% probability.

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nature of the structure manifests itself in the hardness and high density of the crystals. In addition to the intermolecular hydrogen bonds, there are two that are intramolecular, the O_4-H...O_2, bond mentioned above and the N_6-H....O_4 bond (2.867 ± 0.005 Å).

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