Crystal Structure and Molecular Modelling of the Antimicrobial Drug Pentamidine

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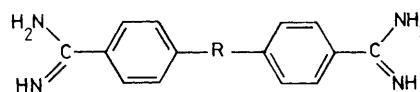
The solid-state conformation of pentamidine, a drug effective against *Pneumocystis carinii* pneumonia, is shown by X-ray diffraction to be flat and extended; molecular modelling shows that a more curved conformation could bind to the minor groove of DNA spanning four base pairs.

Pentamidine (1) is a member of a series of aromatic diamidines, many of which have antimicrobial properties.[1] The drug is used to treat *Pneumocystis carinii* pneumonia (PCP)[2] in patients with compromised immune systems resulting from infection with human immunodeficiency virus.[3] The mode of action of pentamidine against PCP is unknown although the drug forms insoluble precipitates with nucleotides and DNA,[4] and could bind to DNA in a similar manner to the related drugs berenil (2) and stilbamidine (3). We now report the crystal structure of pentamidine as its hydrated isethionate (2-hydroxyethanesulphonate) salt,† and present a study of its conformational and electronic properties using molecular modelling techniques leading to a conformation which is predicted to bind to the minor groove of DNA.

The molecules of (1) adopt a flat, extended, 'bow-shaped'

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† *Crystal data* for (1) = $C_{19}H_{26}N_4O_2^{2+} \cdot 2(C_2H_5SO_4)^- \cdot 3(H_2O)$, $M =$ 646.73, monoclinic, space group *C2/c*, $a = 25.959(6)$, $b = 14.105(3)$, $c = 8.781(6)$ Å, $\beta = 102.44(3)°$, $U = 3140(2)$ Å^3, $Z = 4$, $D_c = 1.37$ g cm^{-3}, $F(000) = 1376$, $\lambda(Mo-K_\alpha) = 0.71069$ Å, $\mu = 0.191$ mm^{-1}. A plate $0.5 \times 0.4 \times 0.1$ mm was mounted on an Enraf-Nonius four-circle diffractometer, 2043 unique reflections were collected by ω–2θ scans for $2° \leqslant \theta \leqslant 22.5°$ and phased by direct methods.[5] Refinement[5] was by full-matrix least-squares with anisotropic thermal parameters for non-hydrogen atoms, final weights $1/[\sigma^2(F) + 0.001135\ F^2]$, amidinium H atoms in fixed positions from difference Fourier syntheses, other H atoms calculated. At convergence $R = 0.059$, $R_w = 0.079$ for 1303 observed reflections $[F > 3\sigma(F)]$ with typical standard deviations of 0.007 Å and 0.5° for non-hydrogen bond distances and angles. Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

conformation (Figure 1) similar to that of berenil[6] (2) but with a larger radius of curvature. The asymmetric unit contains half a pentamidine molecule. A two-fold symmetry axis through the central methylene carbon generates the other half. All torsion angles of the central methylene chain are antiperiplanar, and all amidinium H atoms participate in hydrogen bonding to the highly charged O atoms of various anions, not to neutral solvent. One water molecule is associated with the isethionate hydroxy group, while two other partially occupied and mutually incompatible water locations provide an outer zone of hydration between ion-pairs. The C–O–C angle of 117.8° with no significant out-of-plane twist about either bond matches one of the two energy minima found by STO-3G quantum mechanics calculations for anisole.[7] The planes of the amidinium groups and the benzene rings intersect by 27° in (1), compared to 8° in (2).[6] *Ab initio* MO calculations on the benzamidinium cation with Gaussian 80,[8] using an STO-3G basis set, yielded minimum energy with this twist angle very close to the former value and little sensitivity of energy to twist angles up to 35°. A search of the Cambridge Crystallographic Database[9] gave a mean of 30 ± 12° for twist angles in aromatic amidinium compounds with no *ortho* substituents.

(1) R = $O(CH_2)_5O$

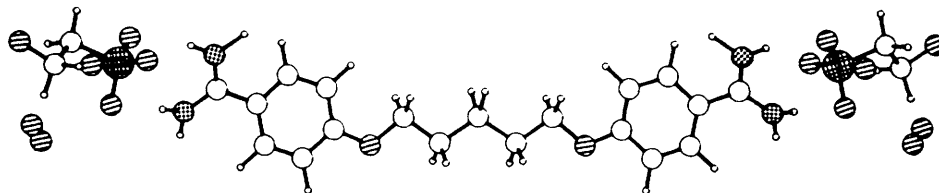(2) R = HN–N=N

(3) R = HC=CH

Figure 1. PLUTO drawing of one formula unit in the crystal structure of pentamidine isethionate trihydrate. Nitrogen atoms are stippled, oxygen atoms hatched, and sulphur atoms cross-hatched. Two mutually exclusive quarter-occupied water sites occur at each extremity.
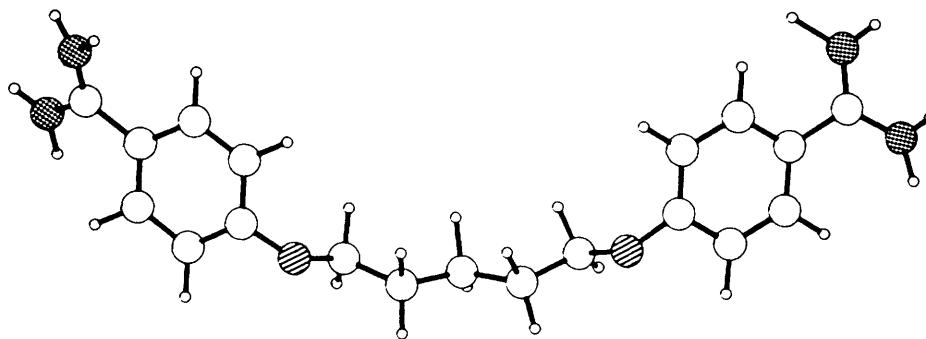

Figure 2. PLUTO drawing of the diprotonated pentamidinium cation with the central chain torsion angles distorted alternately by $+15$ and $-15°$, reducing the chain length to 9.24 Å. This conformation is predicted to be isohelical to DNA and to bind in the minor groove. The rings have been twisted so that the conformation resembles that of berenil bound to DNA.

Calculation of electrostatic potential with the VSS program[10] showed that the whole surfaces of protonated (1) and (2) are at a high positive potential which facilitates binding in the minor groove of DNA. The amidinium groups can form hydrogen bonds to the O atoms of thymine bases and those of phosphate groups;[6] the latter appear preferable because of salt-bridge formation and by analogy with the interactions observed with the isethionate anion.

The crystal structure of berenil is slightly distorted when it is bound to DNA so that it can span exactly three base pairs;[6] pentamidine could span one more. A chain repeat length of 4.61 Å is required for a polymer to have an isohelical fit with the minor groove of DNA at a distance of 5 Å from the helix axis.[11] The length of the central chain of pentamidine is 9.753 Å, compared to 4.63 Å in bound berenil.[12] Reduction of the pentamidine chain vector length to 9.24 Å would therefore be expected to optimise its isohelical fit to the minor groove. Such compression of an extended $(CH_2)_n$ chain can be accomplished either by altering the bond angles from their ideal values, or by the energetically preferred process of twisting the chain torsion angles away from 180°. Twisting alternate main-chain bonds by $+x°$ and $-x°$ where $x \approx 15°$ produced a regular 'isohelical' conformation for pentamidine with a chain length of 9.243 Å and no unacceptable steric contacts. Semi-empirical MO calculations with AMPAC[13] gave an upper limit of 29.5 kcal/mol for the increase in heat of formation resulting from this distortion. It was also necessary to twist the benzene rings of (1) away from their crystallographic positions by approximately 90° (close to the other low-energy conformation reported for anisole)[7] to enable them to fit the minor groove (Figure 2). A strong interaction with the minor groove of DNA would be able to counteract these gains in energy.

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