Crystallographic and Molecular-Orbital Studies on the Geometry of Antifolate Drugs

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In the common dihydrofolate reductase inhibitors an amino substituent replaces the pteridine carbonyl oxygen atom of folates, with altered hydrogen-bonding properties and size. Flexibility in the amino groups could facilitate enzyme binding. Studies of cycloguanil hydrochloride by neutron diffraction show both in-plane and out-of-plane deformation of amino groups. Molecular-orbital calculations *ab initio* on 2,4-diamino-5-methylpyrimidinium cation confirm that the 4-amino group is readily deformable. The 2,4-diaminoquinazoline structure is reported. Atomic co-ordinates, thermal parameters, bond distances and bond angles for cycloguanil and 2,4-diaminoquinazoline have been deposited as Supplementary Publication SUP 50108 (13 pages) at the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in *Biochem. J.* (1978) 169, 5.

Antifolate drugs are noteworthy for their strong binding of dihydrofolate reductase (tetrahydrofolate dehydrogenase, EC 1.5.1.3). Although these drugs differ in their chemical nature, they contain a common structural unit (I) replacing a portion (II) of the pteridine ring of folates:

4 3 2 1

(I)

4 3 2 1

(II)

Alteration merely of positions 3 and 4 of folate (II) to those of aminopterin (I) enhances binding by several orders of magnitude (Werkheiser, 1961). Explanations of this remarkable effect of substitution have invoked either the modified pattern of hydrogen-bond donors and acceptors (Zakrzewski, 1963) or else the change in electron density leading to increased basicity (Baker, 1959). The crystal structures of dihydrofolate reductase–methotrexate complexes (Matthews *et al.*, 1977, 1978) strongly suggest that the drugs bind when protonated at N₁₁ of structure (I); thus basicity is important. The evidence concerning extra hydrogen-bonding by dihydrofolate reductase inhibitors is ambiguous. An additional factor to be considered is the increased size of C–NH₂ in the inhibitors relative to C=O,

which one would expect to be inimical to inhibitor binding. We propose that the great stereochemical adaptability of the NH₂ group helps structure (I) to fit the enzyme so well. To examine its geometry we have carried out the following investigations on antifolate drugs: a neutron-diffraction study of

cycloguanil hydrochloride to extend our X-ray work (Schwalbe & Hunt, 1978), an X-ray-diffraction analysis of 2,4-diaminoquinazoline monohydrate to compare its hydrogen bonds with the unusual system (Rogan & Williams, 1980) found in 2,4,6-triamino-5-chloroquinazoline, and theoretical optimization of the geometry of 2,4-diamino-5-methylpyrimidine as a model for the important drug trimethoprim [2,4-diamino-5-(3',4',5'-trimethoxybenzyl)pyrimidine] (Koetzle & Williams, 1976).

Methods

Large crystals of cycloguanil hydrochloride, C₁₁H₁₅Cl₂N₃, could be grown by very slow evaporation of a saturated ethanol/water solution in

Cycloguanil hydrochloride

2,4-Diamino-5-methylpyrimidine

2,4-Diaminoquinazoline

2,4,6-Triamino-5-chloroquinazoline

which a seed crystal was suspended. Their space group is $P2_1/c$, and the unit cell of dimensions (estimated standard deviations in parentheses) $a = 0.887(2)\text{ nm}$ [$8.87(2)\text{ \AA}$], $b = 1.039(2)\text{ nm}$ [$10.39(2)\text{ \AA}$], $c = 1.714(3)\text{ nm}$ [$17.14(3)\text{ \AA}$] and $\beta = 115.2(1)^\circ$ contains four molecules, giving a calculated density 1.339 (observed 1.336) g/cm^3 . A crystal $2\text{ mm} \times 2\text{ mm} \times 1\text{ mm}$ in size was examined on a Grubb-Parsons mark VI neutron diffractometer at the Atomic Energy Research Establishment, Harwell, Berks., U.K. The resulting 289 independent reflexions were used to obtain precise positional and isotropic thermal parameters for hydrogen atoms by least-squares refinement. By accepting the co-ordinates and anisotropic thermal parameters for non-hydrogen atoms from our X-ray refinement (Schwalbe & Hunt, 1978), we were able to maintain a ratio of observations to refinable parameters of nearly 5:1. Refinement converged at discrepancy indices $R = 0.060$, $R_w = 0.038$. Atomic co-ordinates, thermal parameters, bond distances and bond angles for this and the following structure have been deposited as Supplementary Publication SUP 50108 (13 pages) at the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K.

Evaporation of an ethanol/water solution of 2,4-diaminoquinazoline yielded crystals of the monohydrate $\text{C}_8\text{H}_8\text{N}_4 \cdot \text{H}_2\text{O}$ as octagonal prisms. Their symmetry is tetragonal, space group $I4_1/a$, and unit-cell dimensions are $a = b = 2.159(1)\text{ nm}$ [$21.59(1)\text{ \AA}$] and $c = 0.755(2)\text{ nm}$ [$7.55(2)\text{ \AA}$]. With 16 formula units per cell the calculated density is 1.345 (observed 1.33) g/cm^3 . Data were collected on a Stoe two-circle diffractometer, phases were determined by MULTAN (Germain *et al.*, 1971), and least-squares refinement with anisotropic thermal parameters for non-hydrogen atoms reduced the discrepancy indices to $R = 0.130$, $R_w = 0.087$. In this procedure the geometry of the phenyl hydrogen

atoms was fixed, and the distances of other hydrogen atoms from their neighbour atoms were loosely constrained.

Molecular-orbital calculations *ab initio* were carried out on 2,4-diamino-5-methylpyrimidine and also on the species protonated at $\text{N}_{(1)}$. A minimal basis set (STO-3G) was used with the program GAUSSIAN 70 (Hehre *et al.*, 1971). Starting with the geometry found in trimethoprim (Koetzle & Williams, 1976), bond distances for structural unit (I) within the molecule were successively varied, the position of minimum energy being sought each time. After the optimum value of each distance had been found in turn, the cycle was repeated until no distance changed by more than 0.0004 nm ($0.004\text{ \AA}$). At this stage a routine (Schlegel, 1975) became available implementing the force-relaxation method (Pulay, 1969) of geometry optimization. The forces on all atoms of the protonated species were calculated. Atoms were then shifted in the direction of the force, and the process was repeated until no force exceeded 0.4 mdyn/nm ($0.04\text{ mdyn/\AA}$). Any remaining errors in the theoretical position should be less than 0.0004 nm ($0.004\text{ \AA}$). Energies were then obtained for the protonated molecule subjected to various distortions.

Results and Discussion

In these systems every bond distance between sp^2 hybridized atoms is intermediate between single-bond and double-bond lengths, showing extensive delocalization. The formally single exocyclic C-N bonds are not noticeably longer than some C-N bonds within the ring. A short exocyclic $\text{C}_{(4)}\text{-N}$ bond could mimic the rather long C=O bond found in 5,10-methenyltetrahydrofolic acid (Fontecilla-Camps *et al.*, 1979). Bond distances calculated theoretically by energy minimization and by force relaxation generally agree well and are reproducibly

Table 1. *Amino-group geometry of selected antifolate drugs as indicated by their bond angles at amino N atoms, their exocyclic N-C-N angles and the relationship between each amino group and the six-membered ring to which it is attached.*

Atoms are numbered as in moiety (I) (see the text). Abbreviations used: DAQ, 2,4-diaminoquinazoline; TCQ, 2,4,6-triamino-5-chloroquinazoline; TMP, trimethoprim; CGT, cycloguanil; DMP, 2,4-diamino-5-methylpyrimidine.

Compound	Position	Angle (°)					Deviation from 6-ring plane [nm (Å)]				Dihedral angle NH ₂ -ring		
		H-N-C	H'-N-C	H-N-C	H-N-H'	Sum at N	N-C-N ₍₃₎	N	H	H'			
Unprotonated	DAQ	2										17.7	
		4										7.8	
	TCQ	2	116	115	116	116	347	115	0.006 (0.06)	-0.019 (-0.19)	0.003 (0.03)	36.2	
		4	116	112	128	128	356	115	-0.007 (-0.07)	-0.016 (-0.16)	-0.010 (-0.10)	22.9	
	6	115	115	109	109	339		0.000 (0.00)	0.026 (0.26)	0.028 (0.28)			
								-0.003 (-0.03)	-0.013 (-0.13)	-0.024 (-0.24)			
TMP	2	119	121	118	118	357	118	Attached to a different ring	-0.002 (-0.02)	-0.018 (-0.18)	11.0		
	4	119	121	119	119	359	117		0.019 (0.19)	0.030 (0.30)	15.5		
	Protonated at N ₍₁₎	CGT*	2	115	118	124	357		120	0.003 (0.03)	0.025 (0.25)	0.010 (0.10)	19.1
		4	115	125	120	120	360		118	-0.023 (-0.23)	-0.027 (-0.27)	-0.023 (-0.23)	2.8
DMP†		2	121	119	119	119	360	119	0.000 (0.00)	0.000 (0.00)	0.001 (0.01)	0.5	
		4	122	119	119	119	360	117	0.001 (0.01)	0.001 (0.01)	0.002 (0.02)	0.8	

* Ring plane calculated without considering saturated C and substituted N.

† Geometry optimized by force relaxation.

longer than the accurate neutron-diffraction results (Koetzle & Williams, 1976).

In view of the extensive π delocalization one would expect all ring atoms and the amino groups to lie near a common plane. This is indeed the result of theoretical calculations on the unperturbed 2,4-diamino-5-methylpyrimidine molecule. Hydrogen-bonding and crystal packing may distort the amino groups in several ways: by in-plane bending to alter the H-N-C and N-C-N angles, and by various out-of-plane distortions including twisting about C-N, uniform translation of NH₂ perpendicular to the plane ('step') and out-of-plane bending of NH₂ ('ramp'). All of these modes are observed (Table 1). The most remarkable distortion affects N₍₂₎ and N₍₆₎ of 2,4,6-triamino-5-chloroquinazoline (Rogan & Williams, 1980), where the sum of bond angles at these nitrogen atoms suggests pyramidal geometry and partial sp^3 hybridization. These nitrogen atoms are acting as proton acceptors as well as proton donors for hydrogen-bonding. Comparison of 2,4,6-triamino-5-chloroquinazoline and 2,4-diaminoquinazoline shows that, apart from a modest lengthening of the C-N bond, rehybridization has little effect on the rings, any changes being attributable to steric effects of substitution, even though the 2,4-diaminoquinazoline amino groups do not accept protons.

Such rehybridization could be relevant to the binding of antifolate drugs. The complexes of methotrexate with *Escherichia coli* dihydrofolate reductase (Matthews *et al.*, 1977) and with *Lactobacillus casei* dihydrofolate reductase and NADPH (Matthews *et al.*, 1978) show considerable hydrogen-bonding between enzyme and inhibitor. The enzymes supply a carboxylate anion near the protonated N₍₁₎, a proton acceptor for the 2-amino group, and another proton acceptor near the 4-substituent. In addition, the *E. coli* enzyme, but not the *L. casei* enzyme, appears to furnish a proton donor in the vicinity of the 4-substituent. This interpretation of the map was queried (Matthews *et al.*, 1978) because it seems to pose a problem: the *E. coli* dihydrofolate reductase should offer equal hydrogen-bonding possibilities to inhibitor and substrate whereas the *L. casei* enzyme should form one less bond with substrate. If, however, the 4-amino group of the inhibitor is capable of rehybridization, the difficulty disappears. Acting both as proton donor and as acceptor, it can form two hydrogen bonds to the *E. coli* enzyme, compared with just one for the carbonyl oxygen atom of the substrate. Acting solely as a proton donor, it can form one to *L. casei* dihydrofolate reductase compared with none for the carbonyl oxygen atom. It has been suggested that the pteridine ring of bound folates may be turned over from its orientation in bound methotrexate (Matthews *et al.*, 1978). If so, a ring nitrogen atom capable of accepting but not donating a proton could

approach the carbonyl position, and the above reasoning would still hold.

This hypothesis implies that the energy penalty for rehybridization is small compared to the energy gain from an extra hydrogen bond. The assumption was tested by calculating the energy of protonated 2,4-diamino-5-methylpyrimidine after imposing on the 4-amino group the same distortion that was observed at the 2-amino position of 2,4,6-triamino-5-chloroquinazoline. The total energy increased by 7.25 kJ/mol. With other deformations of the 4-amino group the energy increase was as follows: 3.15 kJ/mol for a 5° in-plane bend of the hydrogen atoms, 3.60 kJ/mol for a 10° twist about C-N, and 8.25 kJ/mol for 0.025 nm (0.25 Å) step distortion. The low value of these energies suggests that any of these deformations could occur in order to accommodate the inhibitor to the enzyme.

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