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The Structure of the Dihydrofolate Reductase Inhibitor 2,4,6-Triamino-5-chloroquinazoline*

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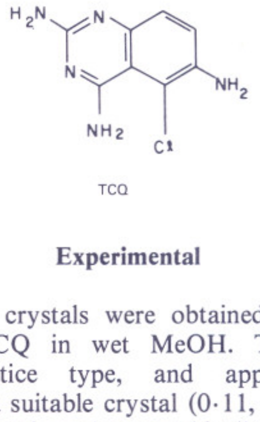
Abstract

Crystals of the title compound, $C_8H_8ClN_5.H_2O$, are monoclinic, $C2/c$, with $a = 29{\cdot}955$ (9), $b = 3{\cdot}853$ (1), $c = 17{\cdot}742$ (5) Å, $\beta = 109{\cdot}44$ (3)°, $Z = 8$. Intensity data were obtained using Cu $K\alpha$ radiation on an automatic diffractometer. The structure has been refined by full-matrix least squares against 1736 observed structure factors to yield a final R of 0·064. The molecule is planar with some evidence for a preferred canonical form of the quinazoline system. Partial sp^3 hybridization of two of the three exocyclic amino N atoms permits them to exhibit both donor and acceptor modes in a pair of intermolecular hydrogen bonds. The packing geometry indicates an infinite system of disordered H_2O molecules running parallel to **b**. Hydrogen-bonding interactions similar to those noted in the dihydrofolate reductase/methotrexate complex and in other antifolate crystal structures are also observed here.

Introduction

Several compounds which contain a 2,4-diaminopyrimidine structural feature are used as analogs of folic acid in the treatment of some neoplastic, protozoal, and bacterial diseases. They retard such growths by competitively, and effectively irreversibly, inhibiting the enzyme dihydrofolate reductase (DHFR) thereby preventing normal synthesis of tetrahydrofolate (THF). Because a sustaining supply of THF is required for several anabolic processes of the biochemical apparatus, a deficiency can lead to metabolic death of a swiftly developing infection. This point is exploited, as noted above, in situations where the deleterious growth has a proliferation rate significantly greater than that of the host organisms or when the inhibitor binds more strongly to the DHFR of the invading organism than it does to the host's enzyme.

Although almost all effective antifolates contain a 2,4-diaminopyrimidine fragment as part of their structure, several different molecular constitutions are exemplified by this functional class. The present compound (2,4,6-triamino-5-chloroquinazoline, TCQ) is a fused di-aryl heterocycle with no conformational flexibility and represents a structural type distinct from other antifolates that have been studied crystallographically. The crystallographic analysis of this material was undertaken to provide some additional insight into the hydrogen bonding, base stacking or other associative modes of DHFR inhibitors. [This compound should not be confused with tricycloquinazoline, a carcinogenic compound which has also been abbreviated as TCQ (Iball & Motherwell, 1969).]



TCQ

Experimental

Orange needle crystals were obtained by cooling a solution of TCQ in wet MeOH. The diffraction symmetry, lattice type, and approximate cell dimensions of a suitable crystal (0·11, 0·5, 0·16 mm) were determined photographically. Systematic absences (hkl: $h + k = 2n + 1$; $h0l$: $l = 2n + 1$) correspond to two possible space groups, $C2/c$ and Cc. The more symmetric group, which was indicated by the cell occupancy, and the E statistics (Karle, Dragonette & Brenner, 1965) was chosen for the structure analysis. This choice was confirmed by the successful determination of the structure.

Cell parameters (Table 1) and errors were derived from least-squares refinement of angular settings on an

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Table 1. *Crystallographic data for* 2,4,6-*triamino*-5-*chloroquinazoline*

$C_8H_8ClN_5.H_2O$, space group $C2/c$, $M_r = 227{\cdot}53$
$a = 29{\cdot}955\ (9)$ Å* $\rho_{obs} = 1{\cdot}557$ Mg m^{-3} (flotation; petroleum ether/$CHBr_3$/CCl_4)
$b = 3{\cdot}853\ (1)$ $\rho_{calc} = 1{\cdot}576$ ($Z = 8$)
$c = 17{\cdot}742\ (5)$ $\mu(\mathrm{Cu}\ K\alpha) = 3{\cdot}29\ \mathrm{mm}^{-1}$
$\beta = 109{\cdot}44\ (3)°$
$V = 1931{\cdot}3$ Å^3

* Estimated standard deviations in terms of the least significant figure quoted are given here, and throughout this paper, in parentheses.

Enraf–Nonius CAD-4 diffractometer (monochromated Cu $K\alpha$ radiation, $\lambda = 1{\cdot}54051$ Å) based upon 14 reflections with $2\theta > 60°$. The intensities of 2855 (including 522 zonal systematic absences) reflections were measured by the $\theta/2\theta$ scan method ($0° < 2\theta < 150°$). Periodic checks of four designated monitor reflections gave no indication of long-term experimental instability. After application of background, Lorentz, polarization (for an ideally mosaic monochromator), and Gaussian absorption correction (Busing & Levy, 1957) (range = 0·914 to 0·972) terms, followed by averaging, 1988 unique intensities remained.

The phase problem was solved without difficulty by *MULTAN* (Germain, Main & Woolfson, 1971).

The initial residual index was 0·44 for the trial structure. The model was refined by a full-matrix least-squares procedure based on F_o using the 1736 reflections with $|F_o| > 3\sigma_{F_o}$ (computer programs used in this laboratory are described by Berman, Bernstein, Bernstein, Koetzle, & Williams, 1976). Weighting terms were computed according to $w^{-1} = [\sigma_c^2 + (0{\cdot}02F_o^2)^2 + 0{\cdot}02(F_o^2 - AF_o^2)^2]/4F_o^2$, where A is the absorption correction and σ_c^2 represents the variance based upon counting statistics corrected by the A and Lp terms. Five cycles of refinement reduced R to 0·198. Two additional iterations with anisotropic vibrational factors descreased the residual to 0·170. A difference electron density map at this point revealed previously unrecognized scattering matter at two non-equivalent points in the asymmetric unit apparently related by a pseudo inversion center at $\frac{1}{4},\frac{1}{4},\frac{1}{4}$.

These regions were suspected to contain MeOH, H_2O, or both. Inclusion of the stronger peak as an isotropic O atom in the next two least-squares cycles diminished the reliability index to 0·122. A difference synthesis suggested that the second region of density was not an artifact of the pseudo center and when it was added as an isotropic C atom, the R value decreased to 0·089. H atoms were introduced after another difference Fourier analysis. At this time, the Cl atom was first treated as an anomalous scatterer ($f' = 0{\cdot}348$, $f'' = 0{\cdot}702$) (Cromer & Liberman, 1970). Non-hydrogen form factors were taken from Cromer & Mann (1968), and the scattering factor for H was drawn from Stewart, Davidson & Simpson (1965). At this stage, the residual was reduced to 0·067. Distance and angle computations eliminated the possibility of any C—O bonds connecting the two solvent atoms or their symmetry equivalents and thus the model was adjusted to accommodate each as an O atom with 0·5 occupancy in a disordered H_2O system. This revised model was refined for five further cycles resulting in large decreases in the vibrational parameters of the O atoms. Although electron densities of up to 0·76 e Å^{-3} between the disordered O atoms were located on a difference map, the definition of these peaks was not sufficient to allow placement of the H atoms of the H_2O molecules.

In order to test the postulate that the true space group is Cc and that the water chain is ordered over two non-equivalent (in Cc) sites, a variety of least-squares and Fourier analyses were performed. Least-squares refinement of two TCQ molecules and two water molecules chosen to form ordered chains resulted in a large increase in the residual and imaginary thermal-vibration terms. Fourier analysis of the ordered model yielded maps containing peaks related by false symmetry elements. We therefore conclude that the diffraction data are unable to distinguish the disordered state in $C2/c$ from the ordered model in Cc. The analysis was continued in $C2/c$.

Table 2. *Final fractional coordinates* (×10^4 *for* Cl, N *and* C, ×10^3 *for* O *and* H) *and isotropic temperature factors*

	x	*y*	*z*	*U* (Å^2)†
Cl	6910 (0·2)	5952 (2)	7165 (0·3)	0·0458 (2)
N(1)	6053 (1)	345 (6)	4364 (1)	0·0403 (7)
C(2)	6466 (1)	−49 (7)	4278 (2)	0·0390 (8)
N(2)	6486 (1)	−1550 (8)	3588 (2)	0·0523 (10)
N(3)	6889 (1)	1032 (6)	4792 (1)	0·0397 (7)
C(4)	6900 (1)	2543 (7)	5467 (1)	0·0358 (7)
N(4)	7332 (1)	3535 (8)	5943 (2)	0·0491 (9)
C(5)	6421 (1)	4533 (7)	6362 (1)	0·0342 (7)
C(6)	5988 (1)	4968 (7)	6469 (2)	0·0385 (8)
N(6)	5932 (1)	6328 (7)	7151 (2)	0·0460 (8)
C(7)	5582 (1)	3758 (8)	5849 (2)	0·0436 (9)
C(8)	5615 (1)	2277 (8)	5179 (2)	0·0436 (9)
C(9)	6054 (1)	1873 (7)	5063 (2)	0·0356 (7)
C(10)	6471 (1)	3033 (7)	5663 (1)	0·0329 (7)
O(1)	488 (0·2)	122 (2)	180 (0·4)	0·094 (3)
O(2)	479 (0·2)	−167 (2)	176 (0·5)	0·080 (3)
H1(N2)	626 (1)	−273 (11)	336 (2)	0·052 (10)
H2(N2)	677 (2)	−234 (11)	360 (3)	0·061 (10)
H1(N4)	735 (1)	490 (11)	644 (2)	0·054 (10)
H2(N4)	753 (2)	351 (11)	570 (3)	0·062 (11)
H1(N6)	561 (1)	747 (12)	707 (2)	0·068 (11)
H2(N6)	615 (1)	769 (8)	741 (2)	0·029 (6)
H(C7)	529 (1)	396 (9)	591 (2)	0·056 (10)
H(C8)	531 (2)	161 (11)	471 (3)	0·070 (11)

† For the anisotropic atoms, Cl–C(10), the equivalent isotropic U was computed from $U_{eq} = \frac{1}{3}\sum_i\sum_j U_{ij}a_i^*a_j^*a_ia_j\cos\alpha_{ij}$.

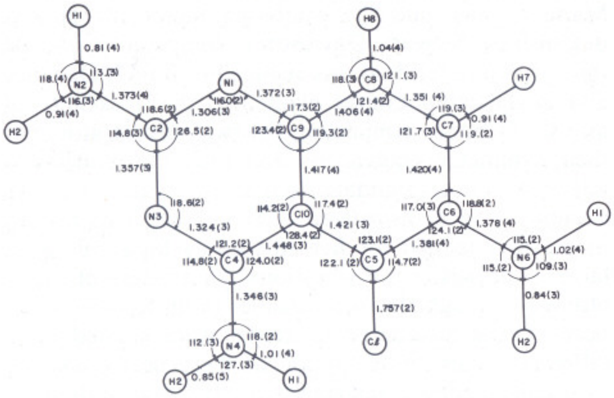

Fig. 1. Nomenclature for TCQ, including bond distances (Å) and interbond angles (°). A correction for rigid-body motion resulted in insignificant modifications in the structure and the corrected bond distances are not reported here.

A maximum $|\Delta/\sigma| = 0.4$ over all variables established that the 169 parameters had converged at $R = 0.064$, $R_{\text{weighted}} = 0.091$ and $S = [\sum w(F_o - k|F_c|)^2/(m - n)]^{1/2} = 1.80$, where S is the standard deviation of an observation of unit weight.* Final atom positions are indicated in Table 2. The molecular structure with bond distances and angles is illustrated in Fig. 1.

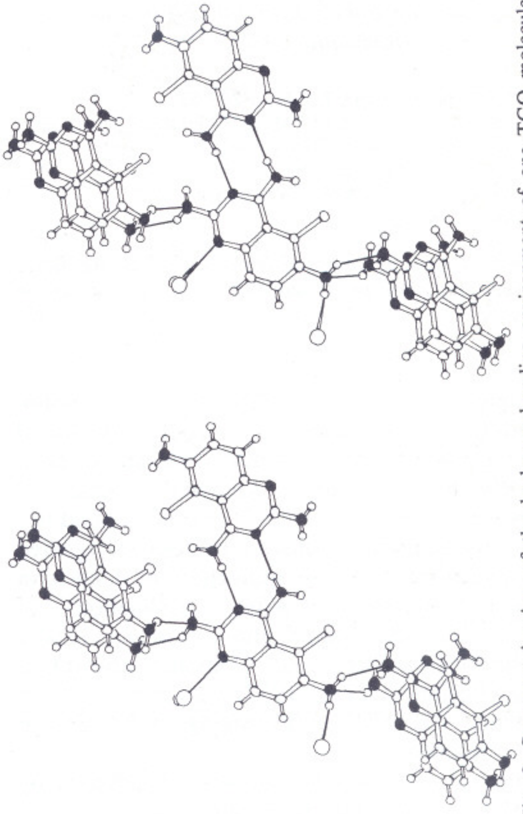

Fig. 2. Stereoscopic view of the hydrogen-bonding environment of one TCQ molecule. Atomic vibrational ellipsoids are scaled to the 30% enclosure level, except those for the H atoms, which are represented by spheres of a fixed arbitrary radius. N atoms are denoted by filled circles. This view is along the **b** direction with **a** horizontal and **c** approximately vertical.

Discussion

Table 3 details the displacements of atoms from three least-squares planes. As expected, the TCQ molecule is very nearly planar; no non-hydrogen atom of the quinazoline skeleton deviates by more than 0.024 Å from the best plane and the pyrimidine and benzenoid systems show even smaller deviations. C(2) exhibits the largest displacement of a ring atom – perhaps because it is bound by three N atoms, each active in intermolecular associations. The dihedral angle between the planes of the two six-membered rings is 1.2°.

In other structures containing the 2,4-diaminopyrimidine feature it is observed that the four cyclic C—N bonds are approximately equal in length (Koetzle & Williams, 1978, and references therein). In the present case, however, there are significant differences over these chemically similar linkages and, if the complete bicyclic system is considered (Fig. 2), some alternation of bond order may be noted. This observation suggests that the specific canonical form illustrated in Fig. 1 is preferred.

The expected Ar—NH_2 bond distance is *ca* 1.35 Å (Voet & Rich, 1970) and the C(4)—N(4) bond distance is therefore normal. The C(2)—N(2) and C(6)—N(6) distances, although both of this type, are significantly longer than the literature value. The hydrogen-bonding environments of N(2) and N(6) are particularly influential in determining the lengths of these C—N bonds. The observed C—N distances can be interpreted

Table 3. *Displacements* (Å) *of atoms from mean planes*

In each case, the asterisks denote the atoms used to define the plane. The equation of the best plane through the quinazoline system is $-1.0980X - 3.445Y + 7.565Z - 2.5205 = 0$, where X, Y and Z are fractional coordinates.

	Pyrimidine ring		Benzene ring		Quinazoline system	
	Δ (Å)	$\|\Delta/\sigma\|$	Δ (Å)	$\|\Delta/\sigma\|$	Δ (Å)	$\|\Delta/\sigma\|$
Cl	–	–	0.058	23.0	0.085	38.9
C(5)	–	–	0.008*	3.2	0.021*	9.0
C(6)	–	–	−0.006*	2.2	0.001*	0.5
N(6)	–	–	0.041	17.3	0.051	21.2
C(7)	–	–	−0.001*	0.4	−0.008*	2.4
C(8)	–	–	0.005*	1.6	−0.006*	2.6
C(9)	−0.005*	1.8	0.001*	0.2	−0.002*	1.8
C(10)	0.011*	4.6	−0.005*	1.9	0.005*	1.5
N(1)	−0.011*	4.4	–	–	−0.003*	0.7
C(2)	0.019*	7.0	–	–	0.023*	9.6
N(2)	0.002	0.7	–	–	0.017	4.7
N(3)	−0.004*	2.4	–	–	−0.009*	4.0
C(4)	−0.011*	4.2	–	–	−0.021*	8.6
N(4)	−0.033	13.9	–	–	−0.051	17.7

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* Lists of structure factors and thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 35350 (12 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

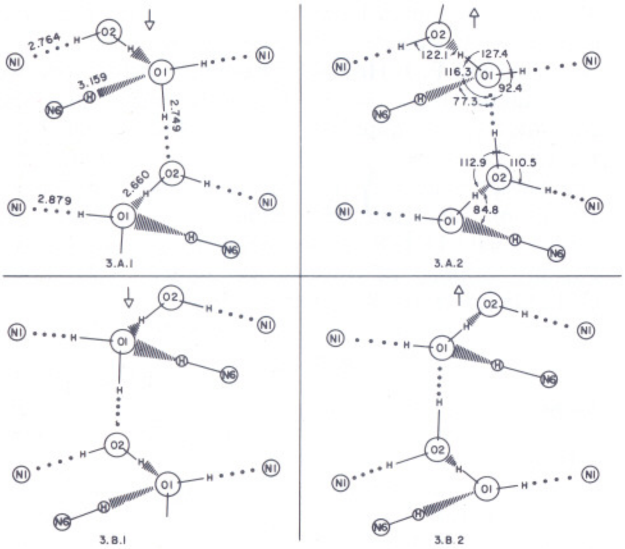

Fig. 3. The four possible hydrogen-bonding schemes involving disordered water molecules. The water molecules of part *A* are related to those of *B* by a vertical diad in the plane of this illustration. Only those atoms involved in any one scheme are indicated.

Table 4. *Hydrogen-bond lengths and angles*

		Angle at H atom
2,4,6-Triamino-5-chloroquinazoline		
H2(N4)–N(4)	0·85 (5) Å	
H2(N4)···N(3)	2·19 (5)	171 (2)°
N(4)···N(3)	3·030 (4)	
H1(N2)–N(2)	0·81 (4)	
H1(N2)···N(6)	2·47 (4)	139 (2)
N(2)···N(6)	3·182 (4)	
H1(N6)–N(6)	1·0 (4)	
H1(N6)···O(1)	2·15 (4)	160 (2)
N(6)···O(1)	3·159 (4)	
H2(N6)–N(6)	0·84 (3)	
H2(N6)···N(2)	2·49 (3)	149 (2)
N(6)···N(2)	3·235 (4)	
N(1)···O(1)	2·879 (4)	
N(1)···O(2)	2·764 (4)	
O(1)···O(2)	2·660 (4)	
O(2)···O(1)	2·749 (4)	
Thiosemicarbazide (Andreeti *et al.*, 1970)		
N(1)–H(1,2)	0·86 (3)	
N(3)···H(1,2)	2·39 (3)	140 (2)
N(1)···N(3)	3·09 (3)	

as resulting from the interaction of two opposing forces. There is a tendency for the lone-pair electrons on the N atoms to interact with the bicyclic π system, thereby increasing the bond order and decreasing the bond lengths. The hydrogen-bond-induced sp^3 character of these N atoms (see below) inhibits this interaction and results in longer Ar–N bonds and H–N–H angles which are nearer to the tetrahedral value than are usually observed.

The complex hydrogen-bonding network, which exerts a marked influence on the packing geometry, is illustrated in Fig. 3. Each N and O atom in the crystal structure participates in at least one such interaction.

The set of N(2)···N(6) associations is an unusual and interesting hydrogen-bonding pattern. The connectivity with regard to N(2) and N(6) discloses two distinct amino–amino hydrogen bonds, in which each N atom acts as both donor and acceptor. The N–H···N angles and the N···N distances (Table 4) are in accord with Hamilton & Ibers's (1968) criteria for the existence of a hydrogen bond. These factors, as well as the increased availability of the lone-pair electrons on the N atoms with partial tetrahedral character, reinforce the plausibility of this interaction.

A computerized literature search (Heller, Milne & Feldmann, 1977) of precisely determined structures exhibiting hydrogen bonds, with $-\mathrm{NH}_2$ groups acting as both donor and acceptor, identified only one such structure, that of thiosemicarbazide (Andreeti, Domiano, Gasparri, Nardelli & Sgarabotto, 1970). The properties of this unusual interaction are similar in both thiosemicarbazide and TCQ (Table 4): Distances and angles clearly show that the N–H···N(sp^3) hydrogen bond is weaker than the more usual N–H···N(sp^2) interaction which is also observed in this structure [N(4)–H···N(3)]. The diminished strength can be interpreted as a consequence of the difference in electronegativities associated with sp^2 and sp^3 hybrid orbitals, leading to a smaller electrostatic attraction across the N(sp^3)···H span (McWeeny, 1979).

In the crystalline state 2-aminopyridine moieties usually form hydrogen-bonded dimers across centers of inversion (Kvick, 1974). The N(4)–H···N(3) bond and its symmetry mate of the present structure furnish another example of this phenomenon.

The infinite ribbon of disordered water molecules running parallel to **b** is another notable characteristic in the present structure. Each O atom has two partially populated positions with occupancy factors for the reported positions for O(1) and O(2) of 0·57 (1) and 0·46 (1). The model predicts that each intra-oxygen H atom can occupy four sites while each H atom between water molecules and ring-N atoms can exist in either of two positions. For each arrangement of water molecules (Fig. 3, *A* and *B*), the O–H···O vectors may have either positive or negative sense with respect to the *b* axis. As can be seen from Fig. 3, the distances and angles defined by the donor and acceptor atoms are consistent with the normal geometries of hydrogen-bonded water molecules. The fact that N(1) is bound in a total of four strong and distinct linkages and N(6) in two implies that substantial stability is lent to the packing geometry by this feature.

The distance between the least-squares planes of two stacked TCQ molecules is 3·445 Å. This value compares favorably with the effective van der Waals

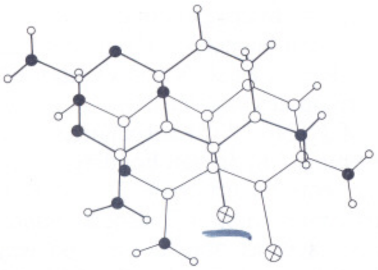

Fig. 4. TCQ vertical stacking interactions involving extensive overlap. N atoms are shaded and the Cl atoms are denoted with crosses.

thickness of aromatic ring systems, 3·40 Å. The stacked rings are related by a unit-cell translation, and are thus parallel rather than antiparallel to each other (Fig. 4). The observed base-overlap distance is typical of a dipole/induced-dipole interaction (Bugg, Thomas, Sundaralingam & Rao, 1971), but in this case it is difficult to discern any specific components of this association. The superposition of N and C atoms in several places is noteworthy, however, and it is possible that the juxtaposition of these elements is significant in this context. The halogen substituent, which was expected to have a prominent function in this contact (Bugg *et al.*, 1971) appears to be isolated from any neighboring π systems.

In this compound, in other antifolates, and in folic acid itself one side of the exocyclic substituent of C(4) is partially occluded by a bulky group attached to C(5). In TCQ the Cl atom inhibits the in-plane approach of a hydrogen-bond acceptor to H1(N4). Atoms N(4) [through H2(N4)] and N(3) participate in a 2-amino-pyridine-style dimerization (Kvick, 1974; Kvick, Thomas & Koetzle, 1976) which simultaneously occludes H2(N2) in a manner similar to that noted for H1(N4). The fact that these two H atoms do not participate in any hydrogen bonds is a recurrent aspect in the crystal structures of antifolates. The hydrogen bonds which are formed by N(2) and N(4) of this structure have their counterparts in all other known crystal structures of DHFR inhibitors. Similar interactions are noted in the crystal structures of the methotrexate/DHFR (*Escherichia coli*) (Matthews *et al.*, 1977) and methotrexate/DHFR (*Lactobacillus casei*) (Matthews *et al.*, 1978) binary complexes. Further discussion of these interactions will be presented elsewhere.

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References

ANDREETI, G. D., DOMIANO, P., GASPARRI, G. F., NARDELLI, M. & SGARABOTTO, P. (1970). *Acta Cryst.* B**26**, 1005–1009.

BERMAN, H. M., BERNSTEIN, F. C., BERNSTEIN, H. J., KOETZLE, T. F. & WILLIAMS, G. J. B. (1976). Brookhaven National Laboratory *CRYSNET* manual. Informal Report BNL-21714.

BUGG, C. E., THOMAS, J. M., SUNDARALINGAM, M. & RAO, S. (1971). *Biopolymers*, **10**, 175.

BUSING, W. R. & LEVY, H. A. (1957). *Acta Cryst.* **10**, 180–182.

CROMER, D. T. & LIBERMAN, D. (1970). *Acta Cryst.* A**24**, 1891–1898.

CROMER, D. T. & MANN, J. B. (1968). *Acta Cryst.* A**24**, 321–324.

GERMAIN, G., MAIN, P. & WOOLFSON, M. M. (1971). *Acta Cryst.* A**27**, 368–376.

HAMILTON, W. C. & IBERS, J. A. (1968). *Hydrogen Bonding in Solids*. New York: Benjamin.

HELLER, S. R., MILNE, G. W. A. & FELDMANN, R. J. (1977). *Science*, **195**, 253–259.

IBALL, J. & MOTHERWELL, W. D. S. (1969). *Acta Cryst.* B**25**, 882–888.

KARLE, I. L., DRAGONETTE, K. S. & BRENNER, S. A. (1965). *Acta Cryst.* **19**, 713–716.

KOETZLE, T. F. & WILLIAMS, G. J. B. (1978). *Acta Cryst.* B**34**, 323–326.

KVICK, Å. (1974). *Acta Univ. Ups.* **322**, 1–53.

KVICK, Å., THOMAS, R. & KOETZLE, T. F. (1976). *Acta Cryst.* B**32**, 224–231.

MCWEENY, R. (1979). *Coulson's Valence*. Oxford Univ. Press.

MATTHEWS, D., ALDEN, R., BOLIN, J., FILMAN, D., FREER, S., HAMLIN, R., HOL, W., KISLIUK, R., PASTORE, E., PLANTE, L., XUONG, N. & KRAUT, J. (1978). *J. Biol. Chem.* **253**, 6946–6954.

MATTHEWS, D. A., ALDEN, R. A., BOLIN, J. T., FREER, S. T., HAMLIN, R., XUONG, N., KRAUT, J., POE, M., WILLIAMS, M. & HOOGSTEEN, K. (1977). *Science*, **197**, 452–455.

STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175–3187.

VOET, D. & RICH, A. (1970). *Prog. Nucleic Acid Res. Mol. Biol.* **10**, 183–265.