Figure 2 shows the two pairs of hydrogen bonds that link adenine base to pyrophosphate oxygens.

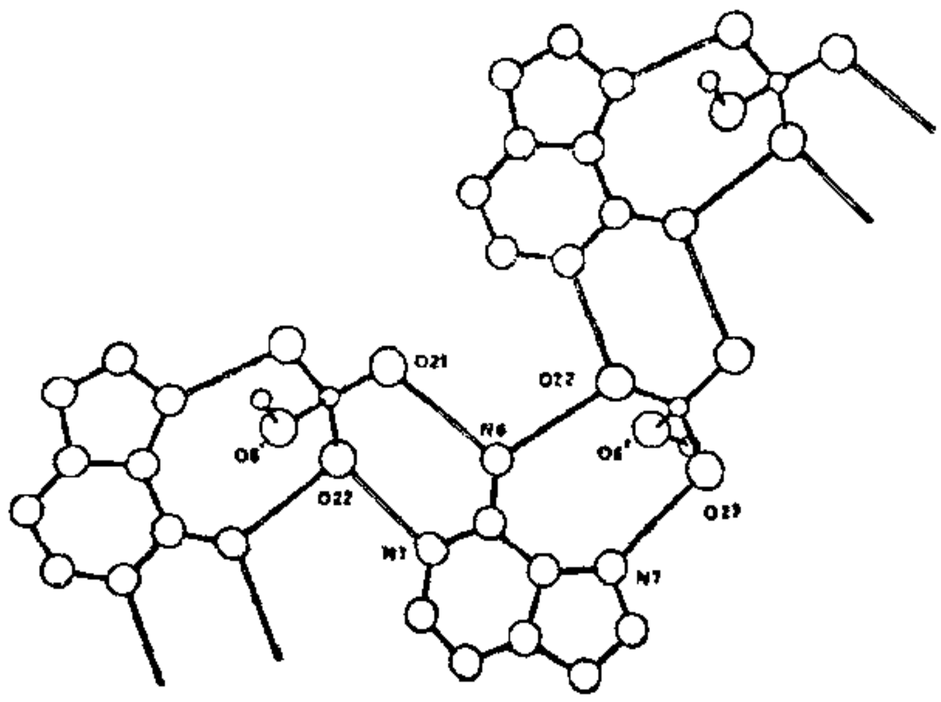

FIG. 2. The two pairs of hydrogen bonds between adenine base and phosphate oxygen.

Interestingly these pairs of hydrogen bonds are observed also in ADP–K and ADP–Rb structures. These interactions could be relevant to the recognition of the base and phosphate parts of ADP by polar side groups of specific amino acid residues in proteins[5]. The absence of the metal ion has apparently influenced the pyrophosphate bond lengths in ADP-free acid. While the two P–O (P) bonds are unequal in ADP–Rb, they are equal in ADP–free acid (P1–06′ = 1·60 Å = P2–06′). Such metal induced changes in P–O (P) lengths may influence chemical reactivity of the ADP molecule in a specific way.

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1. Mahler, H. R. and Cordes, E. H., ' *Basic Physiological Chemistry*, Harper and Row, New York.
2. Viswamitra, M. A., Hosur, M. V., Shakked, Z. and Kennard, O., *Cryst. Struct. Comm.*, 1976, **5**, 819.
3. —, —, — and —, *Nature*, 1976, **262**, 234.
4. Katti, S. K. and Viswamitra, M. A., *Curr. Sci.*, (Communicated).
5. Hosur, M. V., *Ph.D. Thesis*, I.I.Sc. 1979.

THE CRYSTAL AND MOLECULAR STRUCTURE OF THE RADIOPROTECTANT CYSTAMINE DIHYDROCHLORIDE

CYSTAMINE dihydrochloride is known to be an effective radioprotectant[1]. Here we report the results of the x-ray analysis of this compound carried out as part of a programme of x-ray investigations on radioprotectants[2–4].

Crystal data : Space group $P2_1/c$

$a = 18{\cdot}15\,(1)$, $b = 4{\cdot}98$ (1), $c = 20{\cdot}88\,(1)$ Å, $\beta = 143{\cdot}9$ (1·0)°, $D_c = 1{\cdot}35$ gm. cm^{-3}. As the compound reacted with most of the commonly available solvents, the density could not be measured.

Intensity data from three reciprocal levels *hkl*, $k = 0$ to 2, were recorded by the multiple film equi-inclination Weissenberg method using CuKα radiation. As the only available crystal disintegrated at this stage, further data could not be collected and repeated attempts to recrystallize the sample were not successful. The intensities were estimated visually and this data set contained 476 observable reflections. The incomplete nature of the data set leads to high standard deviations associated with bond lengths and angles. The analysis, however, provides an adequate and reliable description of molecular conformation, especially that about the disulphide bridge, which indeed is its major objective. The crystal structure was solved by a combination of direct methods and Fourier techniques and refined anisotropically to $R = 0{\cdot}122$. The final positional coordinates are given in Table I.

TABLE I

Final positional coordinates in fractional units ($\times 10^3$) *and thermal parameters; e.s.d's in parentheses*

	x	y	z	B (Å^2)
S (1)	587 (1)	231 (4)	157 (1)	5·0
S (2)	538 (1)	61 (5)	209 (1)	4·4
Cl (1)	858 (1)	350 (4)	105 (1)	4·7
Cl (2)	122 (2)	777 (5)	15 (1)	7·3
C (1)	650 (5)	− 48 (15)	154 (5)	6·1
C (2)	773 (6)	−171 (15)	267 (5)	6·6
C (3)	374 (5)	− 57 (15)	83 (4)	3·8
C (4)	302 (5)	226 (13)	23 (4)	3·8
N (1)	874 (4)	55 (12)	338 (3)	4·5
N (2)	158 (3)	132 (13)	−84 (3)	4·5

Figure 1 gives the atomic numbering scheme and a view of the structure along the *b*-axis. The molecular dimensions are listed in Table II. The dimensions

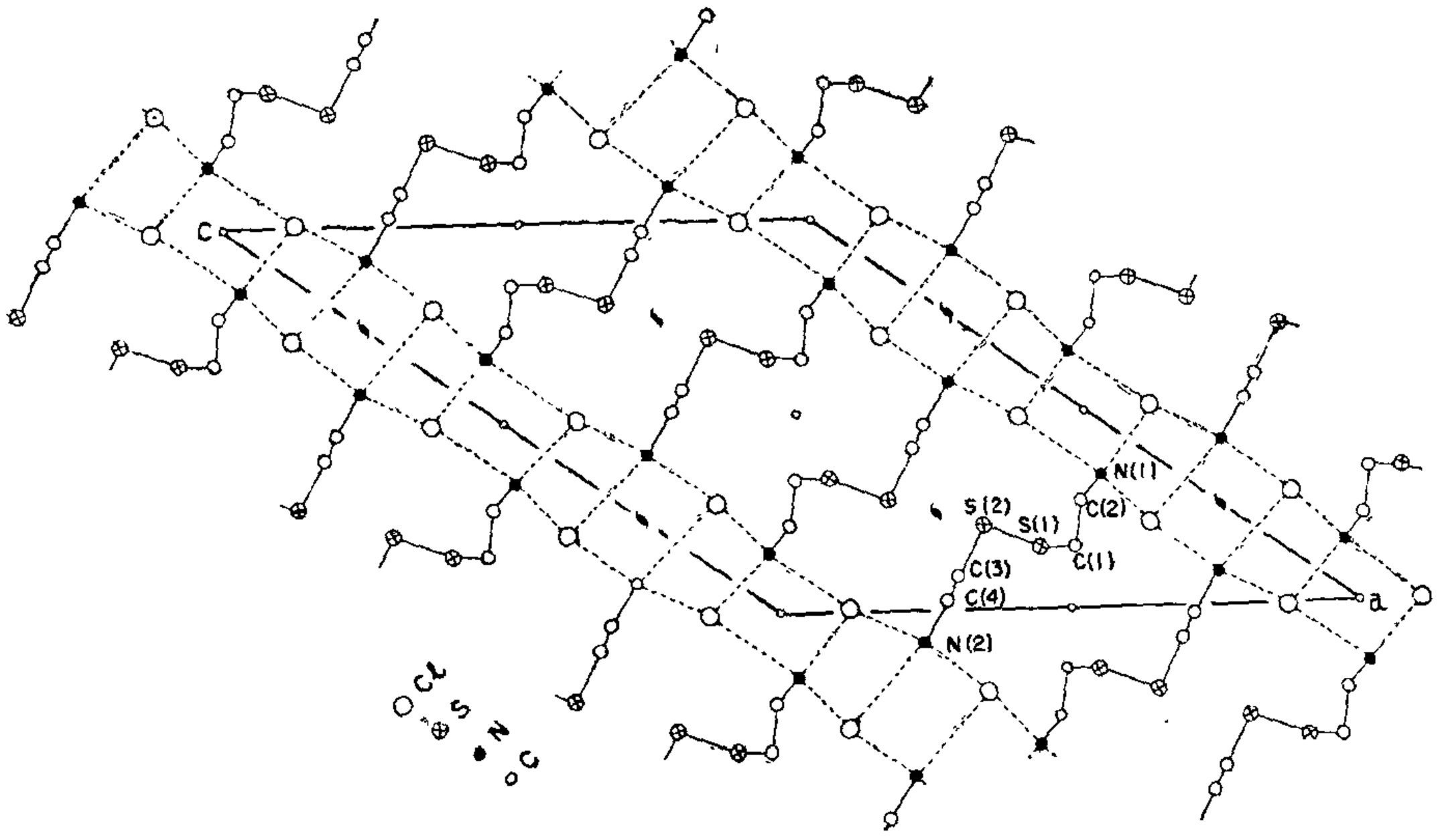

FIG. 1. View of the crystal structure as seen along the *b* axis.

TABLE II

Molecular dimensions with e.s.d.'s in parentheses

N (1) – C (2)	1·56 (9) Å
C (2) – C (1)	1·56 (7)
C (1) – S (1)	1·83 (10)
S (1) – S (2)	2·06 (4)
S (2) – C (3)	1·87 (5)
C (3) – C (4)	1·62 (9)
C (4) – N (2)	1·62 (6)
N (1) – C (2) – C (1) – S (1)	– 61 (8)°
C (2) – C (1) – S (1) – S (2)	– 65 (7)
C (1) – S (1) – S (2) – C (3)	– 89 (5)
S (1) – S (2) – C (3) – C (4)	– 66 (5)
S (2) – C (3) – C (4) – N (2)	– 178 (5)
N (1) – C (2) – C (1)	108 (7)°
C (2) – C (1) – S (1)	114 (6)
C (1) – S (1) – S (2)	105 (3)
S (1) – S (2) – C (3)	105 (3)
S (2) – C (3) – C (4)	101 (5)
C (3) – C (4) – N (2)	102 (6)

of the terminal regions of the molecule deviate substantially from standard values, though the deviations are not significant in view of the large standard deviations. Careful examination of Fourier and difference Fourier maps did not suggest any disorder or modification in the structure. Therefore, it is believed that the abnormal dimensions are due to the poor quality of the data which is well described by the high values of standard deviations.

In the cystamine molecule, the disulphide bond is flanked by linear chains and therefore, the conformation about this bond is unaffected by the steric effects caused by cyclisation or branching in adjacent chains as in most of the other compounds with S-S bond studied so far. Thus the arrangement observed in this structure is likely to represent the intrinsic conformational propensity of an unconstrained disulphide bridge. It, however, turns out that the dihedral angle about the S-S bond has a value in the neighbourhood of 90° as in the derivatives of L-cystine[5]. Even though, the two halves of the cystamine molecule are chemically identical, they have very different conformations. The atoms N (2), C (4), C (3) and S (2) form a planar group, whereas N (1), C (2), C (1) and S (1) do not. S (2) and N (2) in one half of the molecule are *trans* about the connecting C-C bond, whereas the corresponding atoms S (1) and N (1) in the other half are *gauche* about the C-C bond. The *gauche* conformation leads to an intramolecular

$NH_3^+ \cdots S$ interaction. Such an intramolecular sulphur-nitrogen interaction has been postulated in Doherty's theory of radioprotection[6] and has also been observed in the crystal structure of another radioprotectant β-mercapto ethylamine hydrochloride[7].

The crystal structure consists of layers of cystamine molecules stacked parallel to the *ab*-plane. Both sides of each layer are lined with positively charged amino groups and adjacent layers are connected through chloride ions. The interface between layers involves ionic interactions between amino groups and the chloride ions and NH $\cdots$ Cl hydrogen bonds. The nitrogen atom N(1) is surrounded by three chloride ions and the nitrogen atom N(2) by four chloride ions, two of which appear to be involved in a bifurcated hydrogen bond.

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1. Eldjarn, L. and Pihl, A., *J. Biol. Chem.*, 1956, **223**, 341.
2. Vijayan, K., Mani, A., Vedavathi, B. M. and Ramaseshan, S., *Acta Cryst.*, 1975, **A31**, 558.
3. Vijayan, K. and Mani, A., *Ibid.*, 1977, **B33**, 279.
4. Vedavathi, B. M. and Vijayan, K., *Ibid.*, 1977, **B33**, 946.
5. Vijayan, M., in *Handbook of Biochemistry and Molecular Biology*, 3rd edition, Proteins 2. Edited by G. D. Fosman, CRC Press, Cleveland, Ohio, 1976, p. 742.
6. Doherty, D. G., Burnett, Jr., W. T. and Shapira, R., *Radiat. Res.*, 1957, **7**, 13.
7. Jandacek, R. J. and Swartz, H. M., *Ibid.*, 1970, **44**, 523.

COLORIMETRIC DETERMINATION OF MICROGRAM QUANTITIES OF ISONIAZID

SIMPLE colorimetric method of estimation of isoniazid is reported. In alkaline medium isoniazid forms an intense coloured species with catechol, levo-3 (3, 4-dihydroxyphenyl)-2 methylalanine (methyldopa) and *p*-aminophenol (PAP). Beer's law is obeyed in the concentration range of 1 to 11 μg./ml. of isoniazid with catechol, 2·5 to 70 μg./ml. of isoniazid with methyldopa and 10 to 80 μg./ml of isoniazid with PAP.

Isonicotinic acid hydrazide commercially known as isoniazid is an antitubercular drug. The official iodometric method[1] suffers from interference of vitamin 'C'. The colorimetric methods are with *p*-dimethylaminobenzaldehyde[2–5], 1, 2-naphthoquinone-4-sulphonic acid[6–9], 1-chloro-2, 4-dinitrobenzene[2], Na-pentacyanamine ferrate[10], phosphomolybdic acid[5], 3, 4-dinitro-benzoic acid[11], Vanadium[12], etc. Many commercial samples of isoniazid are formulated with vitamin 'C' and hence a method for colorimetric estimation of isoniazid in presence of vitamin 'C' has been developed using catechol, methyldopa and PAP.

Experimental

Catechol (0·04% aqueous), 0·1% methyldopa in 0·1 M HCl, 0·04% PAP (ethanolic) were used as reagents.

Standard isoniazid solution of the concentration of 100 μg/ml was prepared in water.

Sample solution (100 μg/ml) was also prepared in water. The absorbance readings were noted on C.Z. colorimeter equipped with matched glass cells.

Assay Procedure

(1) Catechol : 0·5 ml. of each standard and sample were taken separately in 10 ml. graduated test-tubes, 5 ml. of 1·0 M ammonia and 0·4 ml. of the reagent were added. The volume was adjusted to the mark with water and after 5 minutes the absorbance was noted at 485 nm against the reagent blank.

(2) Methyldopa : 2 ml of each standard and sample were taken separately in 10 ml graduated test-tubes, 5 ml of 1·0 M ammonia and 0·5 ml of the reagent were added. The volume was adjusted to the mark with water and after 5 minutes the absorbance readings were noted at 485 nm against the reagent blank.

(3) PAP : 3 ml of each standard and sample were taken separately in 10 ml graduated test-tubes, 1·0 ml of 0·1 N NaOH and 0·2 ml. of the reagent were added. The volume was adjusted to the mark with water and the absorbance readings were taken after 15 to 20 minutes at 420 nm against the reagent blank.

Table I summarises the experimental conditions of the estimation of isoniazid using catechol, methyldopa and PAP as reagents.

Table II gives the comparative data of the application of the proposed methods in the estimation of isoniazid in the commercial products.

Discussion

The proposed methods are very simple and less time consuming. The results are reproducible and compare satisfactorily with those obtained from the official method. Interference due to common exci-