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X-Ray Structural Studies: Model Compounds for NADH Coenzymes, the Anomeric Effect in the System C-O-C-N, and Intramolecular Hydrogen Bonding in the Maleate Monoanion.

by

© MASAAKI MATSUSHIMA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BIOCHEMISTRY

EDMONTON, ALBERTA

FALL, 1975

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled X-Ray Structural Studies: Model Compounds for NADH Coenzymes, the Anomeric Effect in the System C-O-C-N, and Intramolecular Hydrogen Bonding in the Maleate Monoanion.

submitted by MASAAKI MATSUSHIMA

in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry.

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Date July 21, 1975

Abstract

Chapter I Model compounds for NADH coenzyme.

Two model compounds, N-(β-D-ribofuranosyl) imidazole and N-(tri-D-acetyl-β-ribofuranosyl) imidazole, were studied. The conformations of these ribose moieties are almost identical, ^{3}E and $^{3}T_{2}$, in the two different crystalline environments. Although it was not possible to crystallize the analogous compounds which had a positive charge on their aglycons, several published reports show that the torsion angle C(4')-O(1')-C(1')-N is qualitatively smaller in the unprotonated cytidine than in the protonated derivatives. The anomeric effect and the reverse anomeric effect can be applied to the ribose system. It is highly probable that the stable conformation is $^{3}T_{2}$ or ^{3}E in the ribose system bonded to an uncharged aglycon and $^{2}T_{3}$ in the ribose system bonded to a positively charged aglycon.

Chapter II The anomeric effect in the system C-O-C-N.

The synthetic molecule, 1-methyl-5-fluoro-6-methoxy-5,6-dihydrouracil, was studied. The primary question was the configuration at carbon 5 and 6 of this molecule. The molecular structure shows that the addition of CH_3OF on the double bonded carbon atoms 5 and 6 has been cis-reaction. In addition the molecule has an interesting chemical bond system C(7)-O(6)-C(6)-N(1). Carbon atom C(6) is bonded to an oxygen and a nitrogen atom. The nitrogen atom is trigonal. In this system the observed torsion angle C-O-C-N was 70.6°,

and carbon atom C(7) was gauche with respect to the nitrogen atom N(1). This molecular conformation supports the fact that the anomeric effect can also be applied to such systems as well as to the system C-O-C-O found in pyranose and furanose rings.

Chapter III The intramolecular hydrogen bond in the maleate monoanion.

The crystal structure of imidazolium maleate was investigated. The space group of the crystals is $P2_1/c$. Neither mirror nor two-fold symmetry is across the molecules. Therefore the parameters of all the atoms were independently determined. The positional parameters of the hydrogen atom in the intramolecular hydrogen bond were in doubt, because of the weak X-ray scattering ability of hydrogen and the large anisotropic thermal vibration along the hydrogen bond. However the molecular symmetry on both sides of the carboxyl groups were found in this crystal structure. It can be concluded that the short intramolecular hydrogen bond is symmetric in this symmetric environment.

The accurate structure determination of the maleate monoanion in an asymmetric environment had been attempted in the crystal of pyridinium maleate. But the intramolecular hydrogen bond in the maleate monoanion had been destroyed by an unexpected chemical reaction. The actual molecule in the crystals was N-succino-pyridine. The molecular geometry is very close to that of aspartic acid rather than that of succinic acid derivatives. The molecular

packing shows that the carbonyl and half negatively charged oxygen atoms of neighbouring carboxyl groups approach closely to the two ortho and para positions with respect to the positively charged nitrogen atom in the pyridine ring.

Acknowledgement

I should like to thank all those people in the Province of Alberta who have allowed me to study in the stimulating circumstance, especially my supervisor, Dr. M.N.G. James, for his germane advices during my research works and for his great support in the production of this thesis.

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Chapter I part 1 N-(β-D-ribofuranosyl) imidazole

DEFINITIONS

The following terms are used throughout the X-ray studies in this thesis.

a, b, c	lengths of unit-cell edges
α, β, γ	α: angle between b and c β: angle between a and c γ: angle between a and b
a^*, b^*, c^*	lengths of the reciprocal lattice unit-cell edges
θ	Bragg angle of a reflexion
D_o	observed density of the crystals
D_c	calculated density of the crystals
h, k, ℓ	indices of the reflexion from a set of parallel planes
F_o or $F_{hk\ell}$	observed structure factor amplitude which is calculated by the following equation $\lvert F_o \rvert^2 = (1/k^2 . L . p) \times A \times I_o$ where k is scale factor, L is Lorentz factor, p is polarization factor, A is absorption factor and I_o observed intensity of the reflexion. The polarization factor is expressed by $p = (1 + \cos^2 2\theta_m \times \cos^2 2\theta)/2$ where θ_m is Bragg angle of monochrometer.
F_c	calculated structure factor $F_c = \sum_j f_j \times T \times \exp 2\pi i (hx_j + ky_j + \ell z_j)$ where f_js are form factors specified by atomic species at the observed value of

(sin θ)/λ (λ: wave length) and x_j, y_j, z_j is a set of fractional atomic coordinates, T is a thermal factor

$T = \exp(-8\pi^2 U_{iso} \sin^2\theta/\lambda^2)$ in an isotropic refinement mode, where U_{iso} is a mean square amplitude of vibration in $\AA^2$

$T = \exp\{-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}\ell^2c^{*2} + 2U_{12}hka^*b^* + 2U_{13}h\ell a^*c^* + 2U_{23}k\ell b^*c^*)\}$ in an anisotropic refinement mode, where U_{mn}s are mean square amplitudes of vibration in $\AA^2$, f_js are referred to those of Cromer & Mann (1968), except those for hydrogen atom are referred to those of Stewart, Davidson & Simpson (1965). The scattering curves were corrected for the real part of anomalous dispersion (Cromer & Liberman, 1969). The scattering factors for half negatively charged oxygen atoms are averaged values of those for neutral and negatively charged oxygen atom.

R $\quad \Sigma \, ||F_o| - k|F_c|| / \Sigma |F_o|$

R_w $\quad \Sigma \, w(|F_o| - k|F_c|)^2 / \Sigma \, w|F_o|^2$
where w is weighting value.

the goodness of fit $\quad \{\Sigma \, w(|F_o| - k|F_c|)^2/(m - n)\}^{\frac{1}{2}}$
where m is the number of observations and n is the number of variables.

$|E_{hk\ell}|$ or $|E_o|$ $\quad \dfrac{|F_{hk\ell}|}{(\varepsilon \sum_j^n f_j^2)^{\frac{1}{2}}} \times DK$

where ε is a correction factor allowing for space group extinctions and converts quase- into normalized structure factor and DK is the F relative scale factor correction to

insure that the average value of $|E_o|^2 = 1.0$ (Karle & Karle, 1966)

$|E_c|$ $\quad \sigma_2^{3/2}\sigma_3^{-1} \langle E_k \cdot E_{h-k} \rangle_k$

where $\sigma_n = \sum_j^N Z_j^n \quad (n = 3,2)$

R_k $\quad \dfrac{\Sigma\big||E_o| - K|E_c|\big|}{\Sigma|E_o|}$, $K = \dfrac{\sum_h |E_h|}{\sum_h |\langle E_k \cdot E_{h-k} \rangle_k|}$

$P_\pm$ $\quad \frac{1}{2} + \frac{1}{2}\tanh \sigma_3\sigma_2^{-3/2}|E_h|\Sigma(E_h \cdot E_{h-k})$

e. s. d. The estimated standard deviation will be shown in parentheses after the observed value.

Chapter I

Model Compounds for the Anomeric Effect in the Ribose System

Part 1

The Molecular and Crystal Structure of N-(β-D-ribofuranosyl) Imidazole

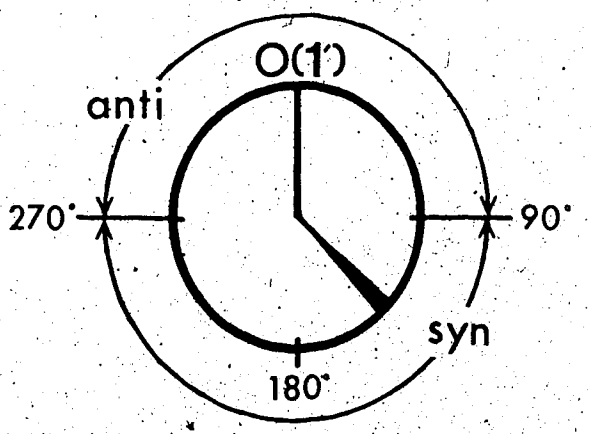
1-1 Introduction

X-ray crystallography can be applied to conformational studies of molecules if a conformation is carefully examined in many crystalline modifications of a molecule or in many crystal structures of closely related molecules. If common conformational tendencies are found for these molecules in different environments, it may be concluded that the common conformational tendency is determined by certain internal forces of the molecule(s). In this chapter the conformations of two ribose moieties are studied by the X-ray crystallographic method.

Before considering conformational parameters that describe five - and six-membered rings, several definitions introduced below as they will be used throughout this thesis.

Torsion angle: an angle about the bond B-C in a system comprising a sequence of atoms A-B-C-D. This angle is defined as the angle through which the far bond C-D is rotated relative to the near bond A-B from the eclipsed position of the bond A-B and C-D. The angle is considered positive for a right handed rotation, the far bond C-D rotates clockwise relative to the near bond A-B.

Torsion angle 'χ' around the glycosidic bonds in nucleosides and nucleotide: the angle is defined by the torsion angle of the system of C(8)-N(9)-C(1')-O(1') in purines, C(6)-N(1)-C(1')-O(1') in pyrimidines. The term 'anti' means the torsion angle = 270° to 90° via 0° and the term 'syn' means the torsion angle = 90° to 270°.



Conformational nomenclatures of monosaccharides and their derivatives:

These in this thesis are same as those which have been approved by the British Carbohydrate Nomenclature Committee and by the U.S. Carbohydrate Nomenclature Committee. The letters describe the approximate shape of the molecules. The superscript and the subscript mean that the numbered atoms are above and below a reference plane, respectively. Some of them are:

in six-membered rings

Fig. I 1-1 Chair conformations

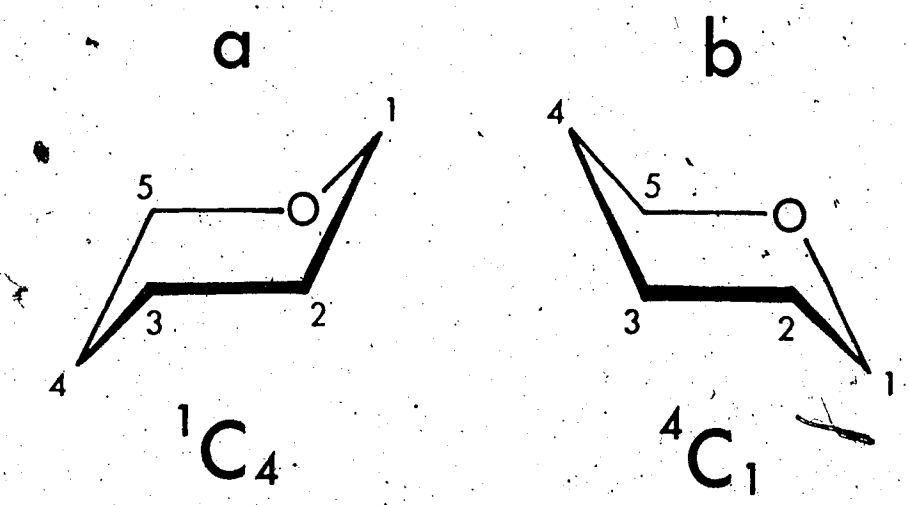
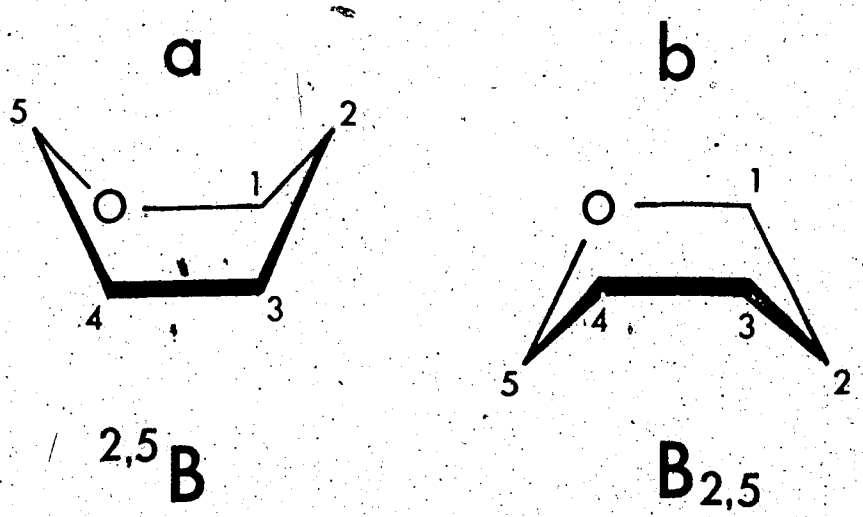


Fig. I 1-2 Boat conformations



in five-membered rings

Fig. I 1-3 Envelope conformations

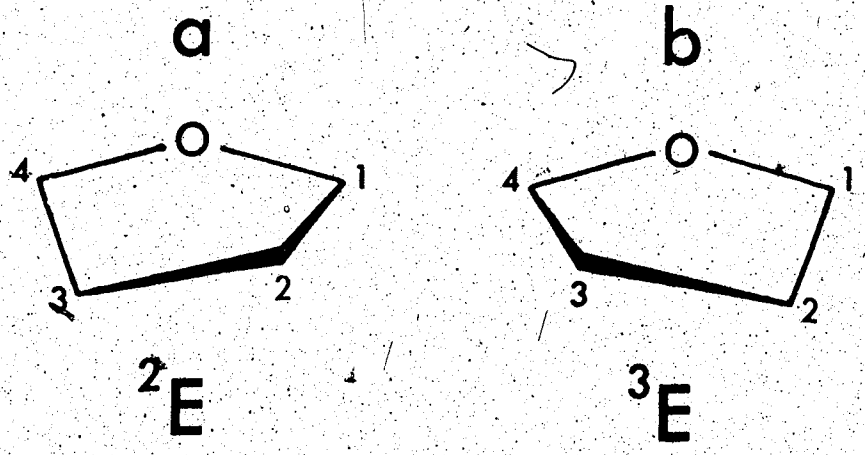
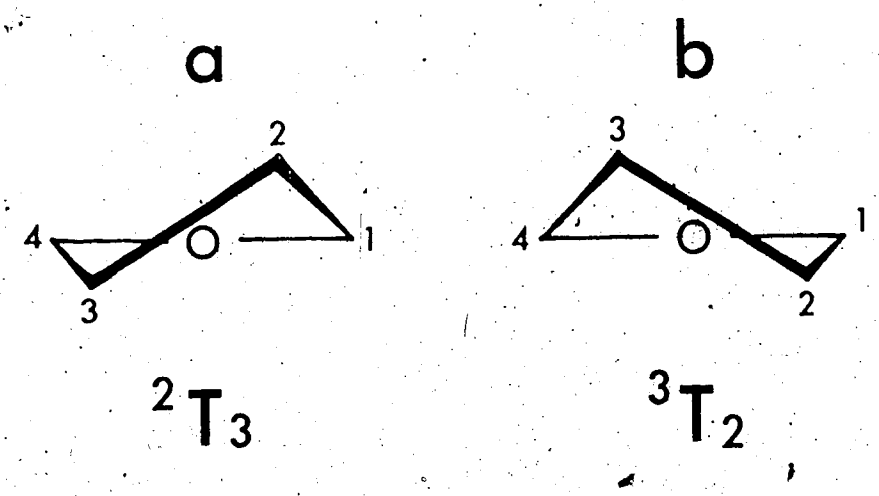
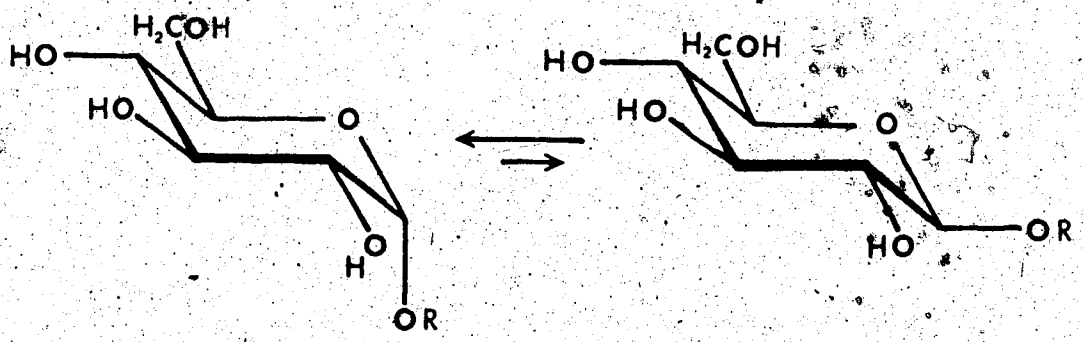


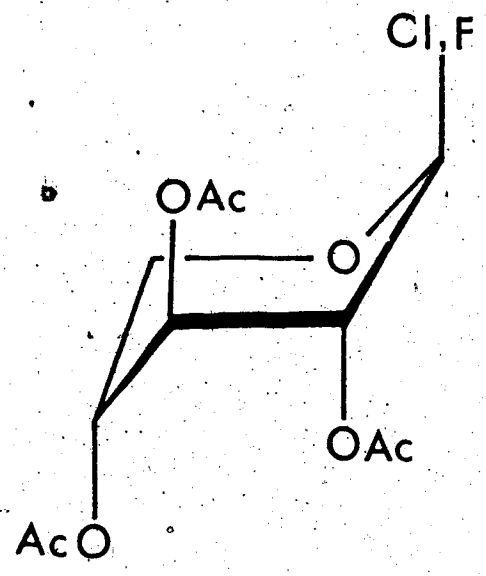
Fig. I 1-4 Twist conformations



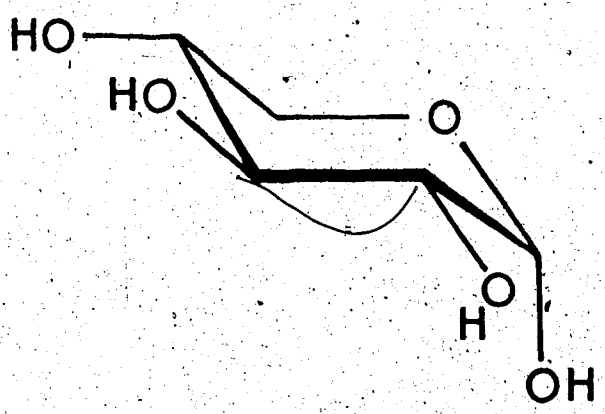
Conformational analyses in solution have been achieved by carbohydrate chemists. Generally a large substituent on a cyclohexane favours the equatorial orientation. This generality, however, does not appear to be applied to adjacent polar substituents on hetero oxygen six-membered rings. The polar substituents are alkoxy, acyloxy and halogens. The configurational stability was found in the α-glyco-pyranosides, because β-glyco-pyranosides are hydrolysed more rapidly than the α-anomer (Pigman and Goepp, 1948).



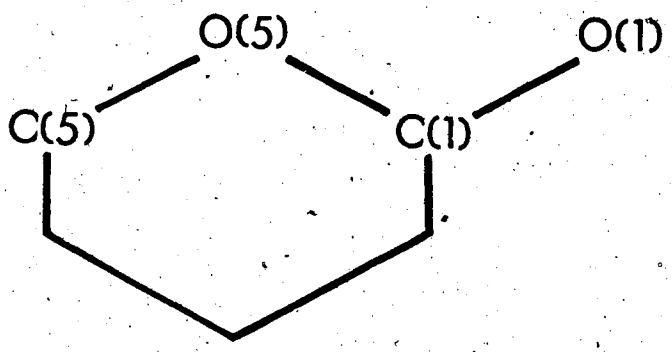
The α-anomer which is substituted in the axial position is more stable by about 2Kcal/mole than the β-anomer which is substituted in the equatorial position. This phenomenon is named the anomeric effect (Lemieux and Chu, 1958 and a review, Zefirov and Sekhtman, 1971). It is the most impressive information from the results of NMR experiments that both tri-O-acetyl-β-D-xylopyranosyl chloride and fluoride exist in the all axial conformer of $^1C_4$ (Holland, Horton and Jewell, 1967 and Hall and Manville, 1967).



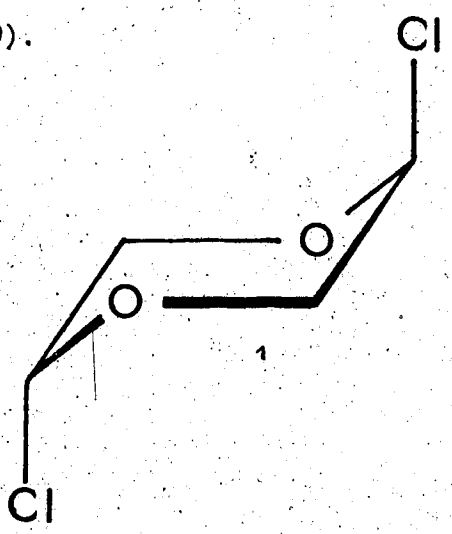
The crystal structure of α-xylose showed that the conformation of the molecule was $^4C_1$ and the α-monomer was in the axial position (Hordvik, 1971)



The X-ray structure analyses of pyranoses and pyranosides confirmed that α-anomers oriented the substituents in the axial position and revealed a difference in the ring C-O bond lengths (Berman, Chu and Jeffrey , 1967).



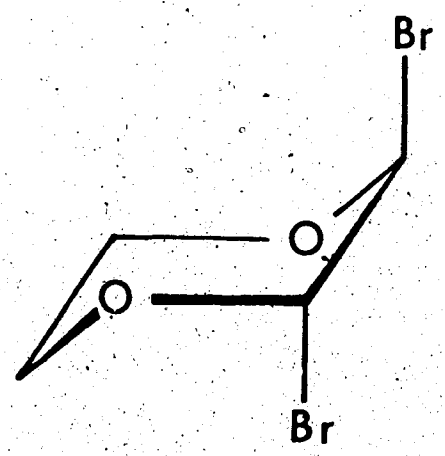
The bond length of C(1)-O(5) is shorter than that of C(5)-O(5). The bond length of C(1)-O(1) is shorter than the average bond length of C-O in the structures. Altona et al showed that the trans-2,5-dichloro and trans-2,3-dibromodioxanes exist in the chair conformation with both halogens in the axial orientation (Altona, Romer & Havinga, 1959).



C-Cl = 1.845(6)Å

C_{anom}-O = 1.388(7)Å

C-O = 1.428(7)Å

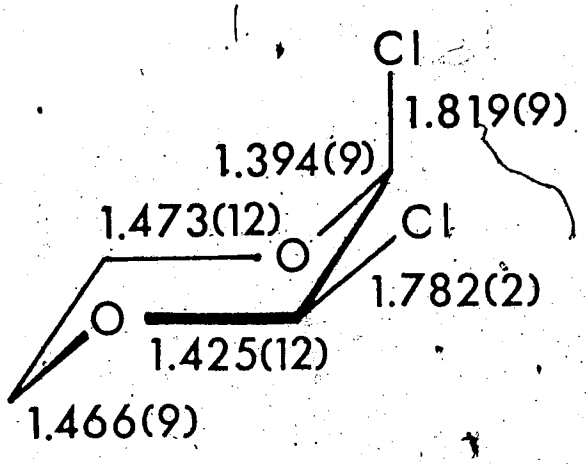


C-Br = 2.025(25)Å

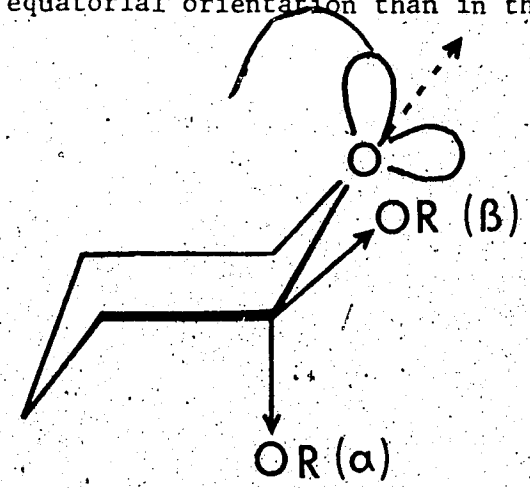
C_{anom}-O = 1.375(30)Å

C-O = 1.47(3)Å

They found that the bond C_{anom}-O were shortened in these structures, and that the bond lengths between the halogen atoms and the anomeric carbon were elongated, compared with accepted parafinic bond lengths, C-Cl = 1.767(2)Å, C-Br = 1.937(3)Å (International tables for X-ray crystallography, Vol. III). In the cis-2,3-dichlorodioxan there were one elongated C-Cl bond length in the axial orientation and a normal C-Cl bond length in the equatorial position. The difference of the C-O bond lengths was reduced in the side of the chlorine atom in the equatorial position.

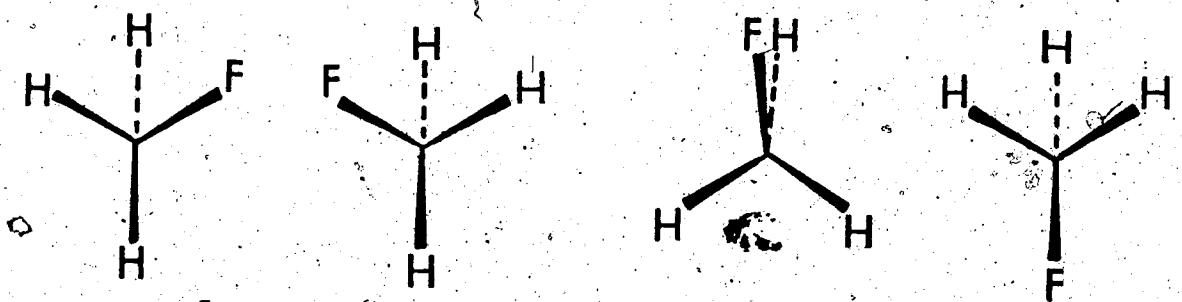


Edward (1955) proposed the first explanation of the anomeric effect. In his theory there exists a greater repulsion between the unshared electron pairs of the ring oxygen and an electronegative substituent in the equatorial orientation than in the axial orientation.



Romers et al. suggested that the non-bonding electrons on the ring oxygen were delocalized over the C-O bond by quantum mechanical mixing of the oxygen p-orbital with the suitably oriented anti-bonding σ bond orbital of the C-Halogen grouping (Romers, Altona, Buys and Havinga, 1969). This suggestion could well explain the observation that the interatomic distances of the C_{anom}-O bonds were shortened, and that the distance of the C_{anom}-Halogen bonds were elongated in the axial orientation and that the mixing of the electronic orbitals directed the suitable orientation of the substituents. That is the anomeric effect.

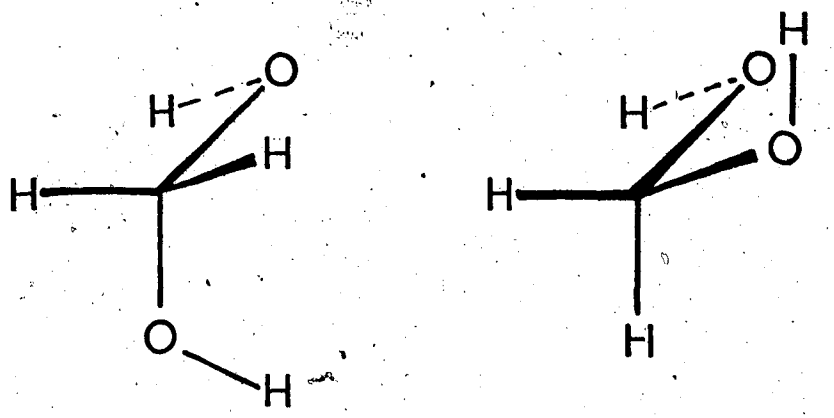
The recent molecular orbital theory has developed to such a stage that it can be quantitatively applied to explain in part the stereochemistry of simple molecules. Wolfe et al. examined the potential function for the internal rotation about the C-O bond in fluoromethanol, FCH_2OH (Wolfe, Rank, Tel & Csizmadia, 1971). They calculated the ab initio SCF-MO on the standard geometry of the molecule. All bond angles were taken to be tetrahedral and the bond lengths were O-H, 0.96Å; C-F, 1.375Å; C-H, 1.091Å and C-O, 1.428Å. The total energy curve of fluoromethanol showed that there were two minima and two barriers which were associated with the maxima at the torsion angles of H-O.C-F = 0° and 180°. The minimum was located at the angle of about $\pm 60^{\circ}$.



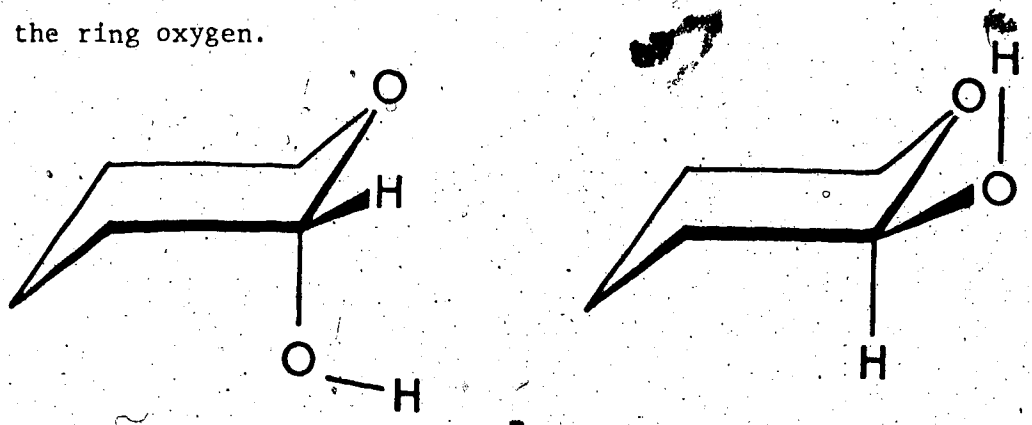
Favorable Unfavorable

This means that the gauche conformations are more stable than the eclipsed and trans conformations.

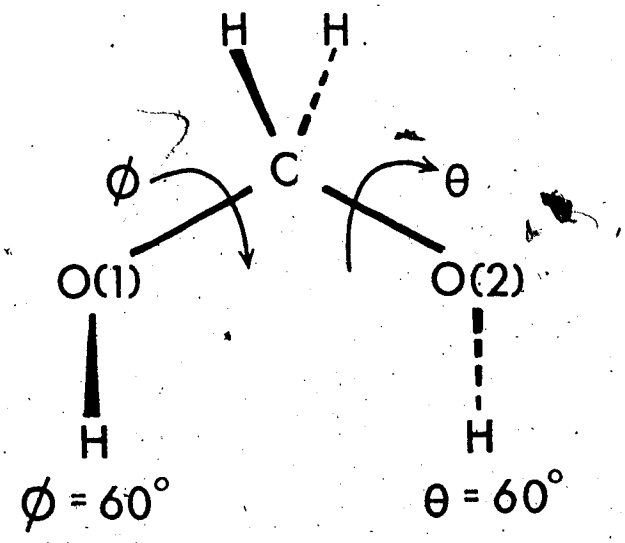
Moreover, Radom et al. applied the ab initio molecular orbital theory to methanediol, $HOCH_2OH$, which was more closely related with the saccharides (Radom, Hahre and Pople, 1971). The minimum potential was found at the conformation H-O, C-O gauche and O-C, O-H gauche. The minimum energy was 4.72 Kcal/mole less than the energy of the molecule with gauche-trans conformation.



If this energy profile is applied to the six-membered ring molecule, for example 2-hydroxy tetrahydro pyrane, the energy minimum could be located at the axial position for the alcoholic oxygen and the hydrogen atom of the hydroxyl group would be oriented gauche with respect to the ring oxygen.



Jeffrey et al. further calculated the total energy of methanediol. They kept the bond lengths and the angles fixed, and varied two geometrical parameters which were the dihedral angles around the C-O bonds and calculated the relative potential energy profile over the two dimensional parameters (Jeffrey , Pople and Radom, 1972).



C—O = 1.43Å
C—H = 1.09Å
O—H = 0.96Å

Only two energy minima which were equivalent with symmetry were found on the energy surface. These conformations were close to $\phi=60^{\circ}$, $\theta=60^{\circ}$ and the symmetry equivalent position. This result was the same as that of the gauche-gauche conformation by Radom et al. (Radom, et al, 1971).

When ϕ was fixed at 60°, the energy clearly had a sharp minimum at $\theta \sim + 60^{\circ}$. On the other hand when ϕ was fixed at 180°, there were two equivalent minima at $\theta \sim \pm 35^{\circ}$. The difference between the minima at $\phi=60^{\circ}$ and 180° was about 4 Kcal/mole and the conformation of $\phi=60^{\circ}$, $\theta \sim 60^{\circ}$ was more stable than any of the other conformations.

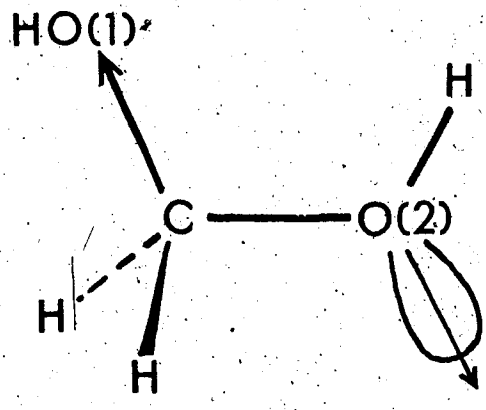
Once the energy curve was obtained, the interpretation of rotational potential functions was facilitated by decomposition of the

total function into Fourier components. By this way one can easily visualize certain factors that stabilize the molecular conformation. Supposing that the total energy is decomposed into three terms of cosine functions, the equation can be represented as:

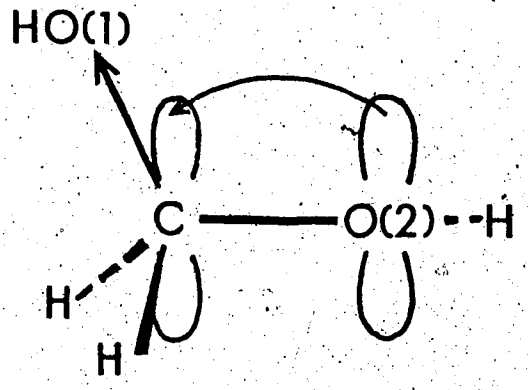
$$V(\theta) = \tfrac{1}{2} \sum_{n=1}^{3} Vn(1-\cos n\theta) \qquad \phi = 180^{o}$$

where $V(\theta)$: potential energy to be varied with the torsion angle, θ.

V_1: a component between the dipole moments which represent the lone pair electrons on the oxygen O(2) and the electronegative substituent O(1)



V_2: a component of the interaction between the p_z-orbital on the oxygen O(2) and the carbon electron orbital in which the partial electron is withdrawn by the electronegativity of the oxygen O(1).

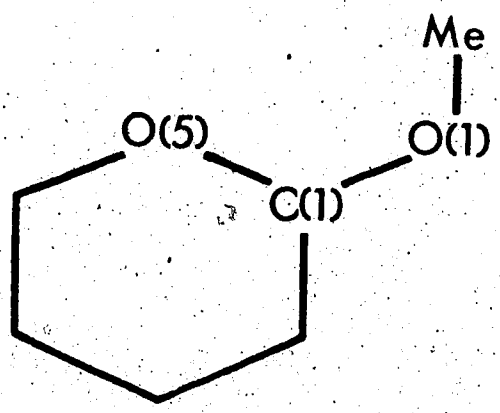


V_3 : bonding electron interactions between O(2)-H bond and three bonds on the carbon atom.

These potential constants, $V_{1,2,3}$, were obtained by inserting calculated total energies for the appropriate angles, θ. At $\phi=180^{o}$, $V_1=7.65$, $V_2=-0.97$, $V_3=-0.88$ Kcal/mole in methanediol. A positive value of V_1 means a stable conformation at the anti parallel dipole orientation which is illustrated under the V_1 explanation. The negative V_2 term showed that the orthogonal conformation was favored, (the illustration was given in the V_2 explanation). This result can be rationalized in terms of stabilization due to electron delocalization, where the p-orbital on the carbon was partially emptied by the oxygen O(1) and this facilitated the delocalization of the p_z-type lone pair electron on the adjacent oxygen, O(2), (David, Eisenstein, Hehre, Salem & Hoffmann; 1973; Van-Catledge, 1974). The negative V_3 term showed the familiar tendency towards a staggered conformation. A similar profile was shown in the potential function of fluoromethanol (Radom, Hahre and Pople, 1972).

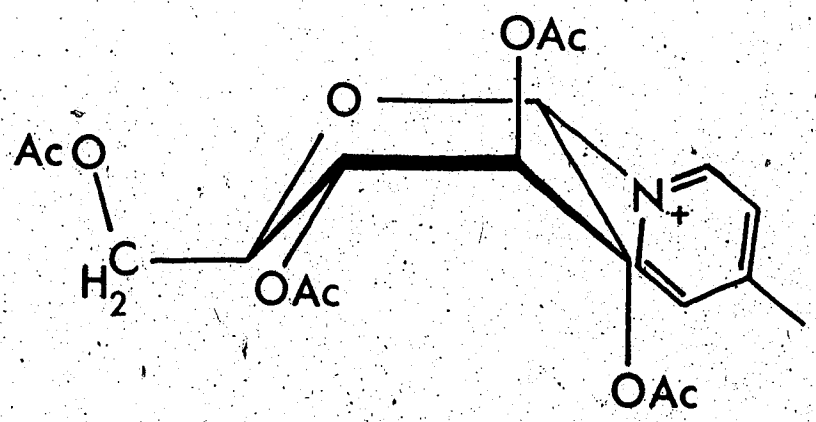
After these conformational analyses were carried out with standard bond lengths and angles, the bond lengths C-O were allowed to vary in four chosen conformations until the energy was minimized. In the calculations the authors found that at the energy minimum the C-O bond lengths were shortened in methanediol relative to the C-O bond length in methanol and that there was a strong conformational dependence of the calculated C-O bond lengths. The two C-O bond lengths were equivalent and 1.421Å in the conformation of $\phi=60^{o}$, $\theta=60^{o}$. Whereas in the conformation $\phi=180^{o}$; $\theta\pm60^{o}$ these two bonds were unequal and of length

1.428Å for C-O(1) and 1.396Å for C-O(2). The structure of the methanediol molecule has not been solved yet, but electron diffraction analysis of dimethoxymethane confirmed the gauche gauche conformation with the C-O, C-O torsion angle of 66.3°, (Astrup, 1971). Furthermore, in the methyl pyranosides, where the methyl group is not restricted in conformation about the C(1)-O(1) bond the torsion angles of O(5)-C(1), O(1)-Me were very close to 60° in α-anomers and close to -70° in β-anomers.

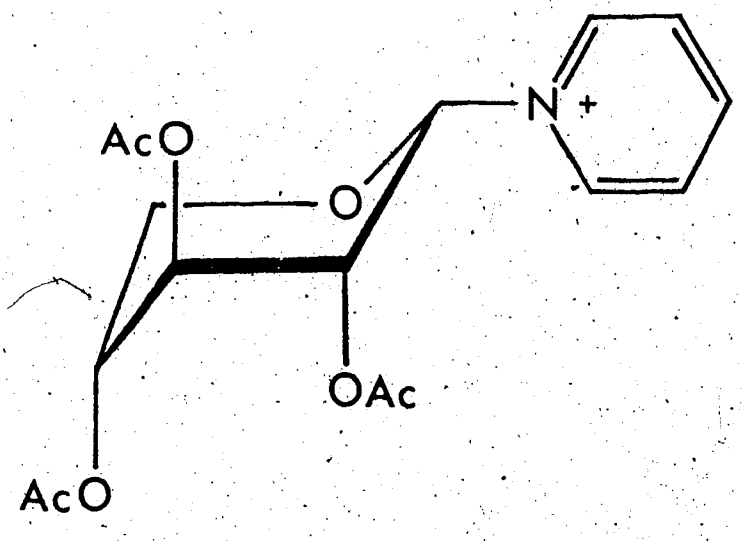


These conformational angles could be explained by a larger V_3 value for a methyl group than that for a hydrogen atom. The larger V_3 term can be expected not to change the torsion angle with the minimum energy, but to deepen the energy well in the α-anomers, because the α-anomers have already had the energy minimum at the torsion angle near 60° at which the term, $\frac{1}{2}V_3(1-\cos 3\theta)$, also has a minimum. On the other hand the larger V_3 value in the β-anomers would affect the torsion angles which were close to 35°, and would result in a more pronounced minimum in the region of $\theta \simeq 60°$ for the β-anomers.

So far, the substituents on the anomeric carbon have been neutral or electronegative. When a pyridinium group was substituted on the anomeric carbon of tetra-O-acetyl-α-D-glucose, a conformational change of the molecule was detected by consideration of the coupling constants of the PMR spectrum (Lemieux and Morgan, 1965). The crystal structure of the related compound, N-(tetra-O-acetyl-α-D-glucopyranosyl)-4-methyl pyridinium bromide monohydrate showed that the compound existed in an unusual conformation of the boat, B_{25}, (Fig. I 1-2(b)) with the acetyl groups on C(2) and C(3) in a quasiaxial orientation and the 4-methyl pyridinium moiety in quasi-equatorial position (James, 1969).

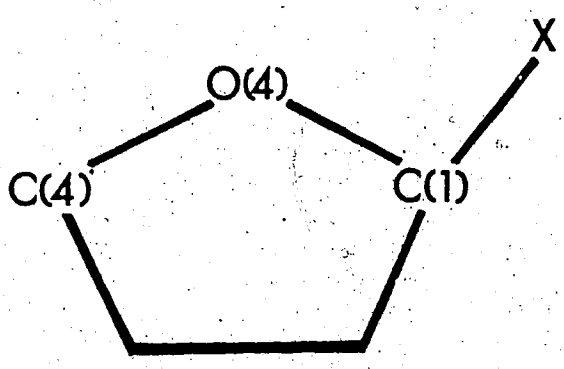


Similar aglycon orientation has been observed in the crystal structure of N-(tri-O-acetyl-α-D-xylopyranosyl)-pyridinium chloride, (James & C. Delbaere, personal communication). The molecular conformation was $^1C_4$ (Fig. I 1-1(a)) in the structure. All three acetyl groups were in the axial orientation and the aglycon which was positively charged was in the equatorial orientation.



These phenomena were named the reverse anomeric effect (Lemieux and Morgan, 1965), because the positively charged substituents on the anomeric carbon favored the equatorial orientation.

We have examined theoretically and experimentally that in the six-membered hetero oxygen ring system the electronegative and neutral substituents on the anomeric carbon favored the axial orientation, but the positively charged substituents on the anomeric carbon favored the equatorial orientation. In order to obtain the equatorial orientation in α-anomers gross conformational changes of the whole pyranose ring are required. In the five-membered ring the bond lengths and angles are more strained to make the ring than those are in the six-membered ring. But the same kind of the tendency can be found about the C(1)-O(4) bond lengths in the structures of furanose sugars, nucleosides and nucleotides.

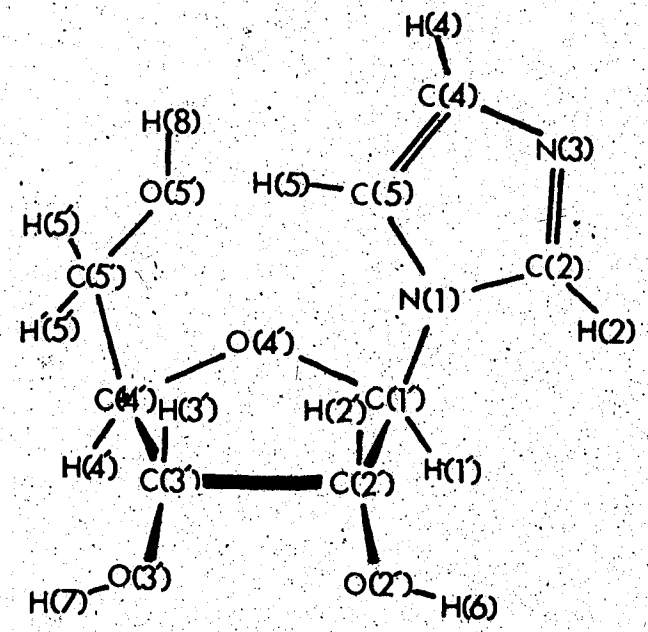


X = nitrogen atom in nucleosides or
oxygen atom in saccharides

These are significantly shorter than the C(4)-O(4) bond lengths (Sundaralingam and Jensen, 1965, reviews in Sundaralingam, 1969 and Arnott, 1970). The nitrogen atoms on the anomeric carbon could similarly affect the five-membered ring, as the alkoxy, acyloxy and halogens on the anomeric carbon of the six-membered rings do. Although the five-membered hetero oxygen rings, for example ribose, are less flexible about the conformation than the six-membered rings, the torsion angles of C(4)-O(4)-C(1)-X can be varied between about 110° and 175°. The angle can be 110° in $^{3}T_{2}$ conformation (Fig. I 1-4(b)), 145° in $^{2}T_{3}$ conformation (Fig. I 1-4(a)) and 170° in E_{1} conformation. (These angles were measured on a model with suitable bond lengths, the tetrahedral orientations on the carbon atoms and 110° for the bond angle at the ring oxygen atom). Compared with the torsion angles in six-membered rings, these are restricted in a much smaller range in furanose rings. The crystal structures of the nucleosides and nucleotides which consist of ribose moiety and bases showed that there are a variety of conformations of the ribose moiety (Sundaralingam, 1969, Arnott, 1970). The $^{2}T_{3}$ and $^{3}T_{2}$ conformations

are usually observed. Although what factor(s) determines the conformation is not known, there are the possibilities of the anomeric and reverse anomeric effects.

In order to investigate these effects, the crystal structure of N-(β-D-ribofuranosyl) imidazole (herinafter referred to as IMR) was studied. The anomeric effect may affect the conformation of the ribose moiety in the neutral molecule and the reverse anomeric effect may affect that in the positively charged molecule which can be made by protonation or methylation of the aglycon. The aromaticity of the aglycon delocalizes the positive charge on the nitrogen atom which is bonded to the anomeric carbon atom. IMR was kindly supplied by Dr. R. U. Lemieux of the Chemistry Department, University of Alberta. The molecular form and atomic numbering of IMR are shown in Fig. I 1-5.



FigI 1-5 The atomic numbering of N-(β-D-ribofuranosyl) imidazole.

1-2 Experimental

The purified compound was kindly supplied by Dr. R. U. Lemieux of the Chemistry Department, University of Alberta. It was subsequently recrystallized by slow vapour diffusion of diethyl ether into an ethanolic solution. Crystals of an approximately equidimensional habit resulted from this recrystallization. Preliminary oscillation, Weissenberg and precession diffraction photographs taken with Cu Kα radiation showed that the crystals were monoclinic with the only systematic absences being $0k0 = 2n + 1$. An experimental density was not obtained because of a paucity of crystals. The unit-cell dimensions were determined from the 2θ values of twelve reflexions in the range $35 < 2\theta < 46^{\circ}$ ($\lambda = 0.70926$ Å) using the least-squares procedure described by Busing & Levy (1967). These values and other crystal data are included in Table I. 1-1. The intensity data were collected at room temperature from a single crystal of dimensions 0.28 X 0.27 X 0.21 mm using graphite monochromated $M_oK\alpha$ radiation and a Picker FACS-1 diffractometer. The reflexions were scanned in the θ-2θ mode with a scan speed of 1° per minute and a basic peak width of 1.5°. Background counts were taken for 10 sec at either end of the 2θ scan for each reflexion. Crystal decomposition and instrumental instability were monitored by measuring three reflexions, 007, 039 and 023, after every 30 reflexions of the data run. At the start and finish of the complete data collection the intensities of these monitor reflexions agreed to within 2%, indicating only minor experimental instability. The total number of independent reflexions in the range $3 < 2\theta < 60^{\circ}$ was 1414, slightly more than available in the Cu limiting

Molecular formula	$C_8H_{12}N_2O_4$
Molecular weight	200.2 Dalton
System	Monoclinic
a	5.190(1) Å
b	7.775(1) Å
c	11.198(2) Å
β	92.49(2)°
V	451.4 Å^3
D_c	1.473 g/cm^3
Crystal size	0.28X0.27X0.21 mm
μ(Mo *kα*)	1.28 cm^{-1}
Systematic absences	0*k*0, *k* odd
Space group	$P2_1$
No. reflexions scanned	1414
No. reflexions included in refinement	1280 (90.7% of total)
2θ range explored	3<2θ<60°
Temperature during data collection	20-24°C

Table I 1-1 Crystal data for N-(β-D-ribofuranosyl)-imidazole

sphere. Using the criterion that $(\Delta I)/I = (T+(0.04I)^2+t^2B)^{\frac{1}{2}}/(T-tB)$, where I is the net peak count, T the total peak count, B the total background count and t is the ratio of total background time to peak scan time, should be less than 0.333 or the net intensity greater than a threshold of 69 counts, a total of 1280 reflexions were considered observed. This represents 90.7% of the total examined data.

The intensities were reduced to structure factors by the application of the appropriate Lorentz and polarization correction factors for normal-beam equatorial geometry. No absorption corrections were applied to these data.

I-3 Structure Solution and Refinement

An overall isotropic temperature factor, B, of 2.43 $\mathring{A}^2$ and scale factor 0.130 were computed from the intensity data using the program of Huber & Brisse (1970). Trial atomic positional parameters for all carbon-like atoms of IMR were determined from a sharpened three-demensional Patterson map computed with $Fo^2.1/LP$ as coefficients but the reliability of some positions was initially in doubt. The other non-hydrogen atom positions were obtained in two ways: (i) from a symmetry minimum function map (Simpson, Dobrott & Lipscomb, 1965) and (ii) from a difference Fourier map based on a partial molecular structure of eight atoms. The initial R value for the first 14 atom structure was 0.34. The y coordinate of atom N(1) was fixed at 0.0 in all subsequent least-squares computations. A difference Fourier map computed following two initial block-diagonal least-squares cycles on the complete molecular structure, contained reasonable peaks for 10 of the 12 hydrogen atoms; the two which did not appear on this map were hydroxyl hydrogen atoms. Six cycles of block-diagonal least-squares with unit weights for all reflexions refined the non-hydrogen atomic parameters. A second difference Fourier map clearly showed the other two hydrogen atoms and resolved the N(3) position of the imidazole ring on the basis of bond lengths C(2)-N(3) 1.34 $\mathring{A}$ compared with the C(4)-C(5) distance of 1.37 $\mathring{A}$. Four further least-squares cycles using observational weights ($\sqrt{w} = 2Fo/[T+(0.04I)^2+t^2B]^{\frac{1}{2}}$ and refinement of all atomic positional parameters, anisotropic thermal parameters for nonhydrogen atoms and isotropic B values for the hydrogen atoms reduced the residual to 0.034.

A close examination of the data at this stage showed that five reflexions were probably suffering from secondary extinction or counter coincidence loss. These reflexions, 020, 011, 003, 110 and 102, were excluded from the data set and further least-squares cycles. Three final cycles of refinement of the 174 parameters were computed using the full-matrix least-squares program ORFLS (Busing, Martin & Levy, 1962). These cycles were based on minimizing the quantity $\Sigma w(|Fo|-|Fc|)$ using an artificial weighting scheme $w = a/[a^2+(Fo-b)^2]^{\frac{1}{2}}$, with a=2.3e and b=3.7e. The following refinement parameters resulted from these computations; R=0.030, Rw=0.040 and the goodness of fit=1.11. From the final cycle of full-matrix least squares, all parameter shifts for the non-hydrogen atoms were less than 1/3 of the corresponding e.s.d. and those for the hydrogen atoms were all less than 1/2 of the corresponding e.s.d. In addition to the program ORFLS the initial block-diagonal refinement was carried out with the NRC set of programs (Ahmed, Hall, Pippy & Huber, 1966).

The structure amplitudes ($|Fo|$ and $|Fc|$ 10x absolute scale) are given in Table I 1-2. The final positional and thermal parameters for all atoms are listed in Table I 1-3, (a), (b) and (c). The absolute configuration of the IMR molecule has not been determined in the present study, but the atomic coordinates do conform to the configuration accepted for β-D-ribose.

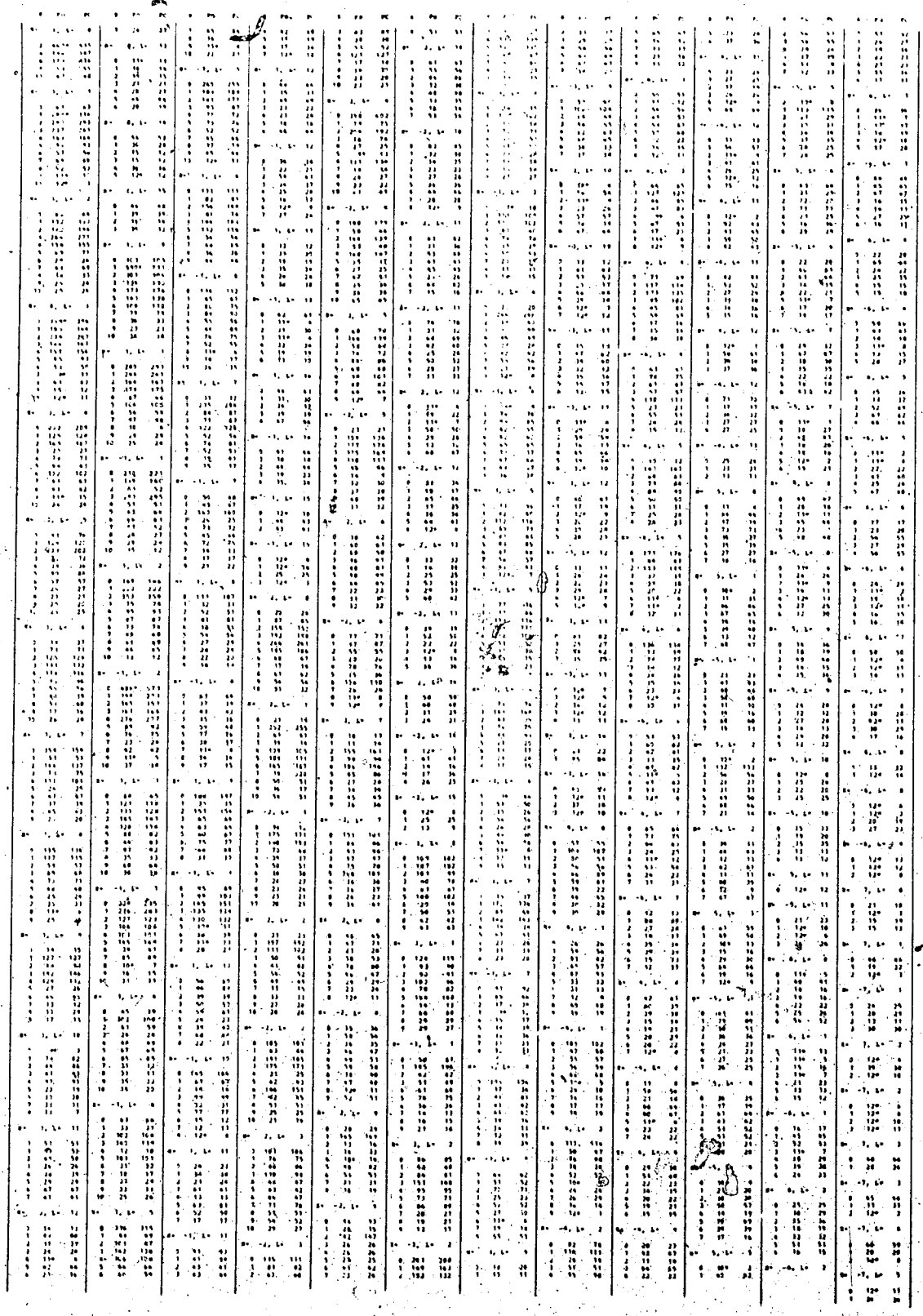


Table I 1-2 The observed and calculated structure factor amplitudes X 10 for N-(β-D-ribofuranosyl)-imidazole. Reflexions marked with an asterisk were excluded from the least-squares computations

Atom	x/a	y/b	z/c
N(1)	2644(3)	0(0)	4118(1)
C(2)	3731(3)	-515(3)	5177(1)
N(3)	2449(4)	52(3)	6086(1)
C(4)	421(4)	982(4)	5580(2)
C(5)	514(4)	968(4)	4368(2)
C(1')	13712(3)	-322(3)	2960(1)
C(2')	1691(3)	-717(3)	1946(1)
C(3')	1440(3)	1023(3)	1301(1)
C(4')	4173(3)	1740(3)	1448(1)
C(5')	4297(3)	3673(3)	1400(2)
O(1')	5079(2)	1173(3)	2617(1)
O(2')	2826(3)	-1965(3)	1223(1)
O(3')	462(2)	924(3)	110(1)
O(5')	6878(3)	4284(3)	1533(1)

Table I 1-3(a) Final positional parameters ($x10^4$) of the non-hydrogen atoms of N-(β-D-ribofuranosyl) imidazole

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N(1)	600(12)	597(13)	447(11)	25(10)	35(10)	-60(9)
C(2)	724(16)	885(20)	464(13)	122(15)	85(13)	-108(11)
N(3)	968(17)	1078(21)	497(13)	146(16)	12(13)	-64(12)
C(4)	969(19)	925(20)	648(15)	213(18)	-105(16)	14(14)
C(5)	790(16)	804(18)	633(14)	256(16)	14(15)	-77(13)
C(1')	526(12)	581(15)	468(12)	-34(11)	81(11)	-57(10)
C(2')	529(13)	538(14)	483(12)	-45(11)	-15(11)	-69(10)
C(3')	469(11)	553(13)	386(10)	36(11)	-32(10)	-38(9)
C(4')	485(13)	528(13)	397(11)	-1(10)	21(10)	-48(10)
C(5')	619(16)	563(14)	642(14)	-22(12)	30(12)	-30(12)
O(1')	689(11)	881(16)	577(11)	-313(11)	258(10)	-271(8)
O(2')	815(14)	582(12)	783(13)	74(10)	-192(10)	-181(11)
O(3')	611(11)	758(12)	429(9)	148(10)	-59(9)	-137(8)
O(5')	786(12)	779(14)	454(10)	-249(11)	-6(9)	30(8)

Table I 1-3(b) Final thermal parameters ($x10^4$) of the non-hydrogen atoms of N-(β-D-ribofuranosyl) imidazole

Atom	x/a	y/b	z/c	U_{iso}
H(2)	529(5)	-120(4)	526(2)	44(7)
H(4)	-102(7)	151(6)	605(3)	81(11)
H(5)	-55(5)	149(4)	372(2)	46(7)
H(1')	488(4)	-127(3)	310(2)	25(5)
H(2')	8(4)	-114(4)	222(2)	32(6)
H(3')	33(5)	179(4)	170(2)	32(6)
H(4')	529(4)	129(3)	88(2)	23(5)
H(5')	366(6)	407(5)	69(3)	51(8)
H'(5')	323(5)	416(4)	200(2)	41(6)
H(6)	177(7)	-245(6)	80(3)	60(9)
H(7)	152(5)	39(4)	-34(2)	36(6)
H(8)	710(5)	451(5)	223(3)	47(7)

Table I 1-3(c) Final hydrogen atom parameters ($x10^3$) for N-(β-D-ribofuranosyl) imidazole

1-4 Results and Discussion

The molecular conformation of IMR is shown in Fig. I 1-6. The molecular geometry is given in Fig. I 1-7 (a) and (b) which contain the bond lengths with the estimated standard deviations in parentheses and the bond angles in degrees. The average standard deviation for bond angles between non-hydrogen atoms is 0.2° whereas that for angles involving hydrogen atoms is 2.0°. All e.s.d.'s in atomic positions were determined from the diagonal elements of the inverse matrix in the least-squares calculation.

(i) Imidazole ring

The bond lengths and angles in the imidazole moiety agree well with the corresponding parameters from the structure determination of imidazole at $-150^{\circ}C$ (Martinez-Carrera, 1966). In this latter structure the imidazole molecules were linked together into ribbons by an intermolecular hydrogen bond >N(1)-H...N(3)<. A similar situation is observed in the present structure with the formation of an intermolecular hydrogen bond [-O(5')-H...N(3)<] having the nitrogen atom accepting a proton from the 5' hydroxyl of a 2_1 screw axis related molecule. Within the ring all of the distances correspond to bonds of intermediate order and there is a significant difference between bonds which one would expect to exhibit similar lengths. This fact coupled with the elongated appearance of the bonding electron-density peaks on the final difference map indicates the considerable aromatic nature of imidazole.

Least squares planes for the atoms of the imidazole ring are

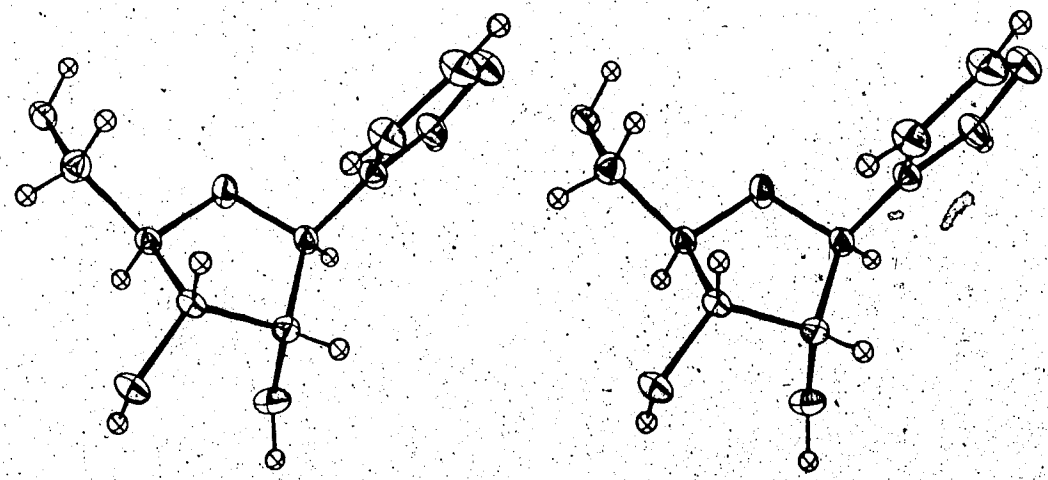


Fig. I 1-6 Stereoscopic diagram of the molecule of IMR. The thermal ellipsoids include 30% probability and the hydrogen atoms were drawn with artificial temperature factors of 1.0 $Å^2$ (Johnson, 1965)

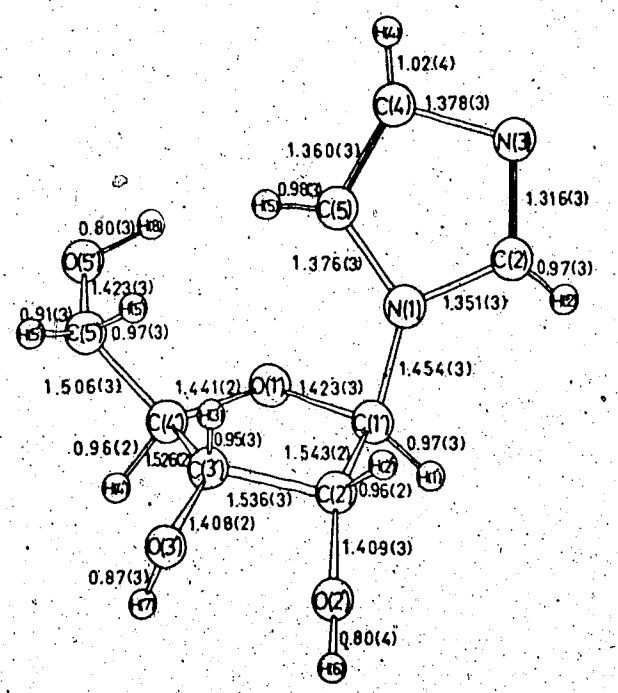


Fig. I 1-7(a) Schematic diagram showing interatomic bond lengths (Å)

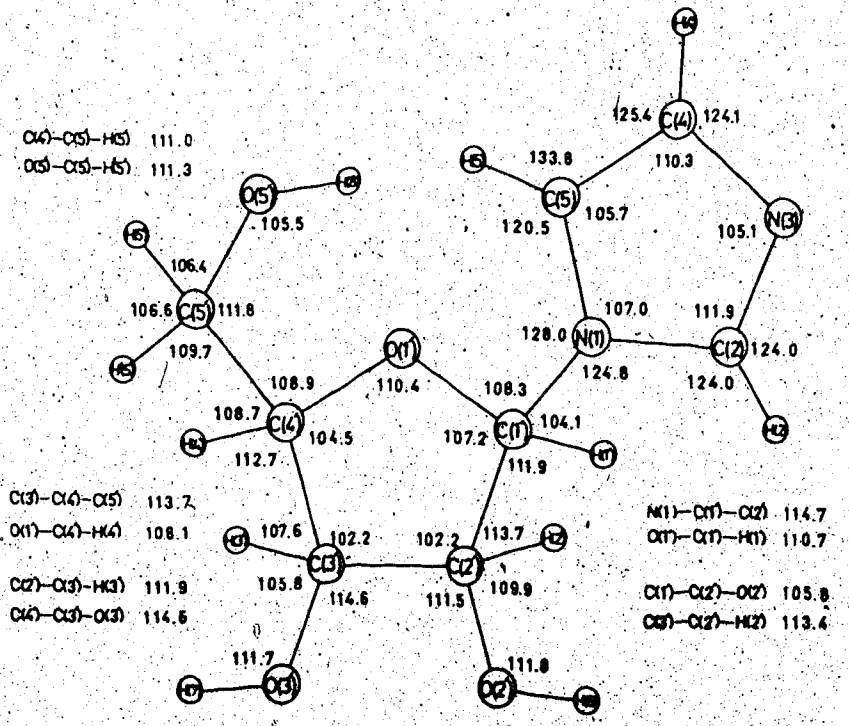


Fig. I 1-7(b) Schematic diagram showing the interbond angles in degrees. The averaged standard deviation for bond angles between non-hydrogen atoms is 0.2° and that for angles involving hydrogen atoms is 2°

Distances (Å) of atoms from least-squares planes.

Atoms	Plane A	Plane B	atoms	Plane C	Plane D
N(1)	0.001(1)*	0.032(1)*	C(1')	0.011(2)*	0.0*
C(2)	0.000(2)*	0.014(2)*	C(2')	-0.007(2)*	-0.055(2)
N(3)	-0.001(2)*	-0.015(2)*	C(3')	-0.562(2)	-0.604(2)
C(4)	0.002(3)*	-0.014(3)*	C(4')	0.007(2)*	0.0*
C(5)	-0.001(3)	0.011(3)*	O(1')	-0.012(2)*	0.0*
C(1')	-0.090(2)	-0.028(3)*	C(5')	-0.809(2)	-0.797(2)
C(2')	0.767(2)				
O(1')	-1.444(2)				
H(2)	-0.01				
H(4)	0.07				
H(5)	-0.02				

* These atomic positions were used to define the least-squares plane.

Equations of the least-squares planes.

Plane	p q r s	χ_2
A	-0.5631x - 0.8256y - 0.0360z + 0.8365 = 0	1.84
B	-0.5544x - 0.8304y - 0.0555z + 0.9363 = 0	1919.7
C	0.6852x - 0.5887y - 0.4289z + 0.0622 = 0	124.3
D	0.7036x - 0.5762y - 0.4160z - 0.0212 = 0	———

x,y,z refer to the a,b, and c* axes respectively, p,q,r are the firect cosines, S is in Å.

Table I 1-4 Least-squares planes for N-(β-D-ribofuranosyl) imidazole

included in Table I 1-4. This structural feature is exceptionally planar with all atoms less than one standard deviation from the plane (The χ^2 value is 1.84 with four degrees of freedom). Atom C(1') is, however, 0.090 Å from the plane of the imidazole ring (0.093 Å from the plane through the atoms N(1),C(2) and C(5)) and on the same side of ring as is atom O(1'). Atom N(1) is therefore definitely not trigonal but it has considerable tetrahedral character. The resulting partial lone-pair electrons on N(1) apparently reside on the opposite side of the imidazole plane as are O(1') and C(1').

(ii) Ribose ring

The dimensions of the ribose ring are similar to those found in other nucleosides and nucleotides [for reviews, see Arnott (1970); Sundaralingam (1969)]. The carbon-oxygen hydroxyl bond lengths are C(2') - O(2'), 1.409(3) Å; C(3') - O(3'), 1.408(2) Å and C(5') - O(5'), 1.423(3) Å. Although the first two are considerably shorter than the last it appears that these former are normal lengths in ribose-containing nucleosides (Lai & Marsh, 1972; Viswamitra, Swaminatha Reddy, James & Williams, 1972; Thewalt, Bugg & Marsh, 1970; Rao & Sundaralingam, 1970). The C(4') - C(5') length of 1.506(3) Å which at first sight appears short for a $C(sp^3) - C(sp^3)$ bond is not unusual but lies within the range found for other nucleosides (Sundaralingam & Jensen, 1965; Saenger & Scheit, 1970; Thewalt, Bugg & Marsh, 1970; Rao & Sundaralingam, 1970; Lai & Marsh, 1972; Viswamitra et al., 1972). As found in these other structures there is a large discrepancy between C(1') - O(1'), 1.423(3) Å and C(4') - O(1'), 1.441(2) Å. This distinction between the two ring C - O bond lengths most likely

has its source in the anomeric effect (Berman, Chu & Jeffrey, 1967).

The average bond length for the carbon-hydrogen bonds is 0.97(3) Å and that for the oxygen-hydrogen bonds is 0.82(3) Å. Both of these values are approximately 0.10-0.15 Å shorter than expected for the internuclear separation but this has been a common observation in other X-ray diffraction analyses (Hanson, Sieker & Jensen, 1973).

The bond angles in the ribose moiety are also close to those found in other nucleosides and nucleotides (Arnott, 1970). There are some differences in the exocyclic angles however, the most notable those angles at C(2'). Whereas the angles C(2') - C(3') - O(3'), 114.6(2)° and C(4') - C(3'), 114.6(2)° agree with the values observed for the corresponding angles in adenosine (Lai & Marsh, 1972), the angles C(1') - C(2') - O(2'), 105.8(2)° and C(3') - C(2') -O(2'), 11.5(2)° differ by almost 4° from the corresponding angles, 109.5(2)° and 107.9(2)° observed in adenosine.

The conformation of the ribose ring as determined here is ^{3}E (Fig. I 1-3(b) and Fig. I 1-8).

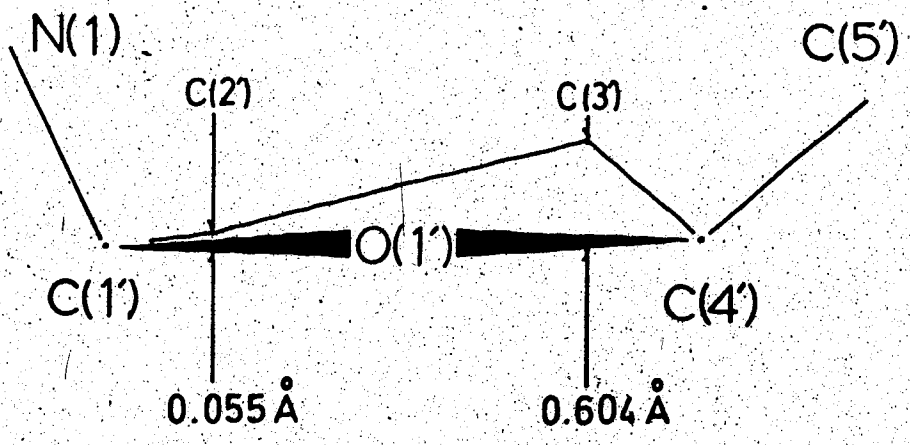


Fig. I 1-8 Schematic drawing showing the displacements of atoms C(2') and C(3') from the plane defined by atoms C(1'), O(1') and C(4') with the equation given in Table I 1-5 for plane D

The displacements of the carbon atoms C(2') and C(3') are almost the same as the corresponding displacements in the structure of adenosine (Lai & Marsh, 1972) and adenosine -3'-phosphate dihydrate (Sundaralingam, 1966). In considering the detail of the ribose conformation as expressed by the torsion angles around the ring (Fig. I 1-9), the conformation which the present study most closely resembles is adenosine -3'-phosphate.

In light of the fact that the aglycon is imidazole and not a pyrimidine or purine base, the definition of glycosidic torsion angle, X, and arguments restricting its possible values are not enforced. The imidazole ring resembles the five-membered ring of the purines and comparison of the bond lengths and angles of this portion of the adenine ring (Lai & Marsh, 1972, and references quoted therein) with those in the present structure shows that this resemblance is indeed close. The glycosidic torsion angle , C(2) - N(1) - C(1') - O(1'), is -97.8° and thus falls in the syn range. Saenger & Scheit (1970) point out the multitudinous definitions of the glycosidic torsion angle. For an attempt to overcome the confusion, the complete angles are given in the form of Newman projections in Fig. I 1-9.

The torsion angles about the bond C(4') - C(5') are also shown in Fig. I 1-9. The conformation is gauche-trans referred to O(1') and C(3') respectively with the angles O(5') - C(5') - C(4') - O(1'), $+ 64.3^{\circ}$ and O(5') - C(5') - C(4') - C(3'), $+ 180.4^{\circ}$. This latter value is very close to the 177.0° found by Lai & Marsh for adenosine. This is not the most commonly observed conformation of the C(4') - C(5') bond, but Wilson & Rahman (1971) have shown that for the $^{3}T_{2}$

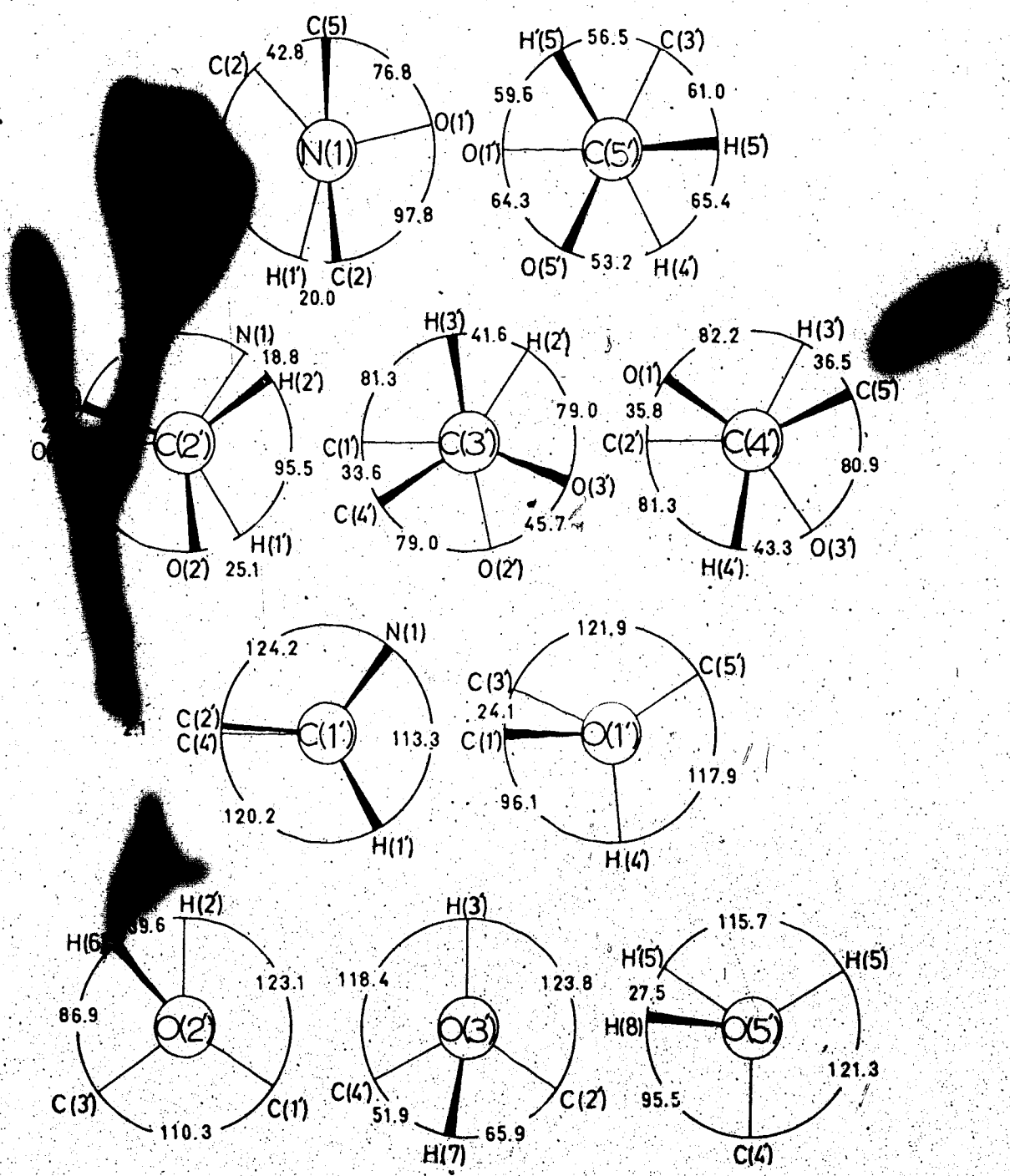


Fig. I 1-9 Conformational angles for the ribose in the form of Newman projection

ribose conformation, the variation in non-bonded interaction energy was not sufficiently large to exhibit a preference of one conformation over another of the three allowed, gauche-gauche, gauche-trans or trans-gauche. In the present crystal structure the O(5') gauche-trans conformation is adopted apparently because of the advantageous hydrogen bonding attainable for O(5').

The torsion angle N(1) - C(1') - O(1') - C(4') is 126.3°. This angle is smaller than the angle expected in ^2E or $^2T_3$ conformation. The electronegative and uncharged nitrogen atom N(1) favors the smaller angle about the carbon-oxygen bond, C(1') - O(1') by the anomeric effect (Lemieux & Chü, 1958). This effect seems to dominate other forces which affect the ribose conformation and determine the torsion angle about C(1') - O(1') bond.

(iii) Molecular packing

Fig. I 1-10 shows a stereoscopic view of the packing of IMR in the crystal as viewed almost parallel to the a axis of the crystal. The molecular packing is clearly dominated by the formation of hydrogen bonds. Each nucleoside is hydrogen bonded to six neighbouring molecules; all nitrogen and oxygen atoms except N(1) and O(1') are involved in this bonding scheme. The distances between the oxygen atoms and the hydrogen bond acceptors and the angles O-H.....X are listed in Table I 1-5. All three of these are normal hydrogen bonds (cf. Donohue, 1968).

O - H ... X		O...X	H...X	<O-H...X
O(2')-H(6)...O(3')	(-x,y-½,-z)	2.759	1.970	167.7
O(3')-H(7)...O(5')	(1-x,y-½,-z)	2.672	1.818	167.3
O(5')-H(8)...N(3)	(1-x,y+½,1-z)	2.740	1.938	178.8

Table I 1-5 Hydrogen bond distances (Å) and angles (°).

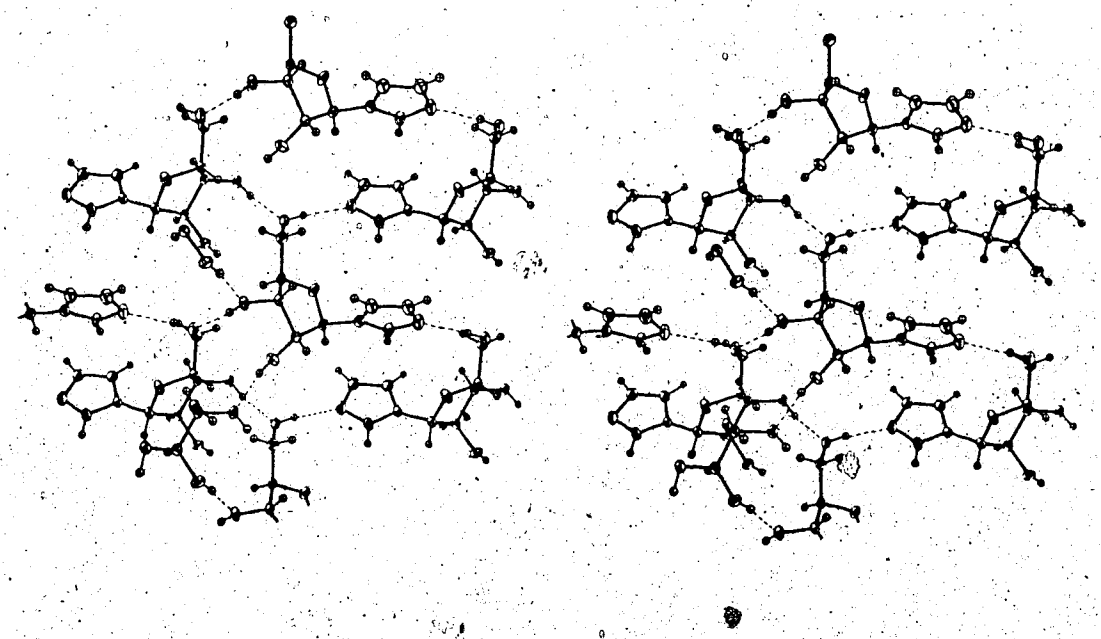


Fig. I 1-10 Stereoscopic drawing of the unit cell contents projected along the a axis. The intermolecular hydrogen bonds are identified by broken lines (Johnson, 1965)

From Fig. I 1-10 it may be seen that there is a hydrophilic surface parallel to the ab plane. The imidazole rings are stacked by the 2_1 screw axis through ½, y, ½ but there is no base stacking per se as occurs with other nucleosides (Bugg, Thomas, Rao & Sundaralingam, 1971) because the imidazole rings are not coplanar in this structure. The angle between adjacent imidazole planes is 68.7°. Oxygen atoms O(3') and O(5') act both as hydrogen-bond acceptors and donors whereas O(2') acts only in a donor role, donating its proton H(6) to oxygen o(3') at $\bar{x}$, y-½, $\bar{z}$. Oxygen O(3') donates its proton H(7) to oxygen O(5') at 1-x, y-½, -z and oxygen O(5') donates its proton to nitrogen atom N(3) in the imidazole ring of the molecule at 1-x, ½+y, 1-z.

(iv) Final difference electron density

A residual electron-density map shown in Fig. I 1-11 was computed from the final ΔF values. The overall standard deviation in electron density computed form the formula given by Cruickshank (1967) is ± 0.04 e Å^{-3}. The largest peak, 0.21 e Å^{-3}, on this map occurs along the bond mid-way between atoms C(1') - C(2'). The other large peaks shown on Fig.I 1-11 occur between σ-bonded carbon atoms in the ribose moiety and all have peak heights greater than + 0.15 e Å^{-3}. The peaks of the residual density around the imidazole ring were extended perpendicular to the plane of the ring whereas the bonding density in the ribose was more spherical. It is of interest to note that there was little if any bonding electron density in the C-O bonds. The fact that the bonding electron density was less along C-O bonds than along the C-C bonds was also

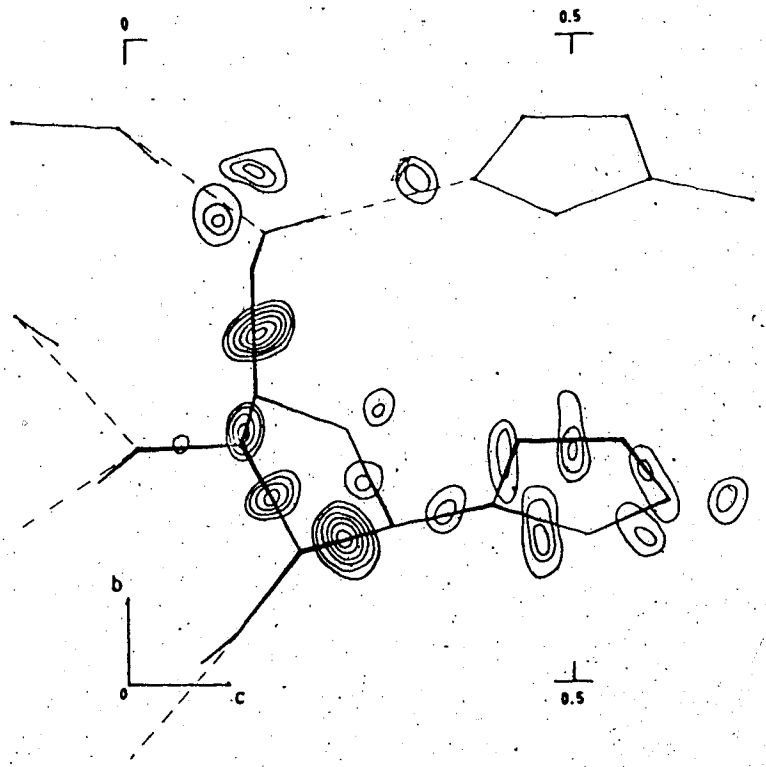


Fig. I 1-11 Composite drawing of the final difference electron density map showing those features greater than twice the estimated standard deviation of the electron density (0.037 e/Å^{-3}). The first contour is at 0.075 e/Å^{-3} and the contour interval is 0.025 e/Å^{-3}. The negative contours have been omitted from this drawing.

noted in a comparison of the X-ray and neutron structural studies of sucrose by Hanson, Sieker & Jensen (1973).

There is an interesting region occuring around oxygen atom O(5') which indicates that hydrogen bond formation may be causing a distortion in the electron density. The density in the bond C(5')-O(5') is less than 0.075 e Å^{-3} but there are two peaks of + 0.13 e Å^{-3} in the region where one might expect lone-pair electron-cloud density for O(5'). There is also a peak of + 0.10 e Å^{-3} in the

region neighboring atom N(3) of the imidazole ring along the line joining O(5')-H...N(3). One of the peaks (peak A in Fig. I 1-12) near O(5') is along the line O(3')-H(7)...O(5'). The geometry of the atomic grouping is such that atoms C(5'), O(5'), N(3) and O(3') lie approximately in a single plane (maximum displacement 0.23 Å). The distance of the second residual density peak (peak B in Fig. I 1-12) from this plane is 0.55 Å and it lies almost directly above the oxygen atom O(5'). The situation is shown diagramatically in Fig. I 1-12.

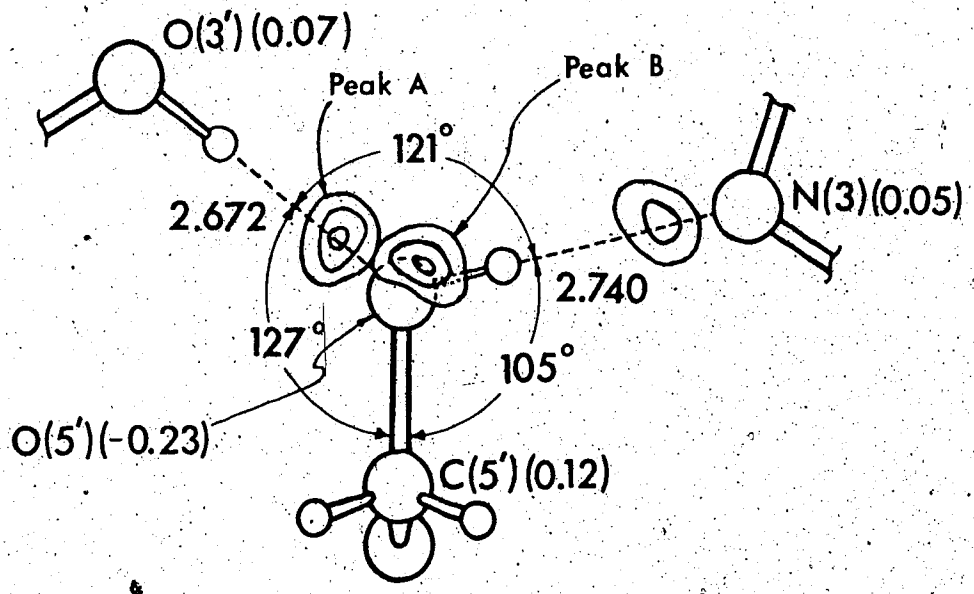


Fig. I 1-12 Diagrammatic sketch of the region around the atom O(5') showing the hydrogen bond distances and coordination angles. The numbers in parentheses after the atom symbols refer to the distances those atoms are from the plane with equation $-0.5555x + 0.8310y - 0.0297z - 1.0107 = 0$. Peak A is -0.20 Å from this plane, peak B is + 0.56 Å from this plane

The approximate trigonal coordination of O(5') has thus shifted the lone-pair electrons on this atom away from the expected sp^3 tetrahedral position.

Clearly further experimental work will have to be carried out before the significance of this apparent distortion can be assessed. From the work on sucrose it is obviously very important to use more than one crystal, more than one temperature, extinction and absorption corrections and a comparison of X-ray and neutron diffraction data if details of bonding density are to be evaluated. The present compound would be ideal for such a study as large crystals are easily grown by the vapour-diffusion technique.

Part 2

The Crystal and Molecular Structure of N-(tri-O-acetyl-β-D-ribofuranosyl) imidazole

2-1 Introduction

The compound N-(β-D-ribofuranosyl) imidazole was studied in part 1 of this chapter. This compound was intended as a model for illustrating the anomeric effect. The crystal structure showed that the ribose conformation was ^{3}E in which the anomeric effect could play a predominant role in the molecule, because the torsion angle C(4') - O(1') - C(1') - N(1) was less than that expected if the molecule had a conformation of ^{2}E or $^{2}T_{3}$. However a close examination of the crystal structure could lead to the possibility that the ribose conformation might be determined or severely affected by the six hydrogen bonds which extended to neighbouring molecules in the cyrstal of IMR.

To clear this possibility, the crystal structure of N-(tri-O-acetyl-β-D-ribofuranosyl) imidazole (herinafter referred to AIMR) was studied. Since the compound was acetylated on all three hydroxyl groups of IMR, there was no possibility of hydrogen bonding in the crystal structure. It was expected that there would be less disturbance of the ribose conformation by the packing of the molecules in these crystals. The compound was kindly provided by Dr. R.U. Lemieux of the Chemistry Department, University of Alberta. The chemical formula and the atomic numbering are shown in Fig. I 2-1.

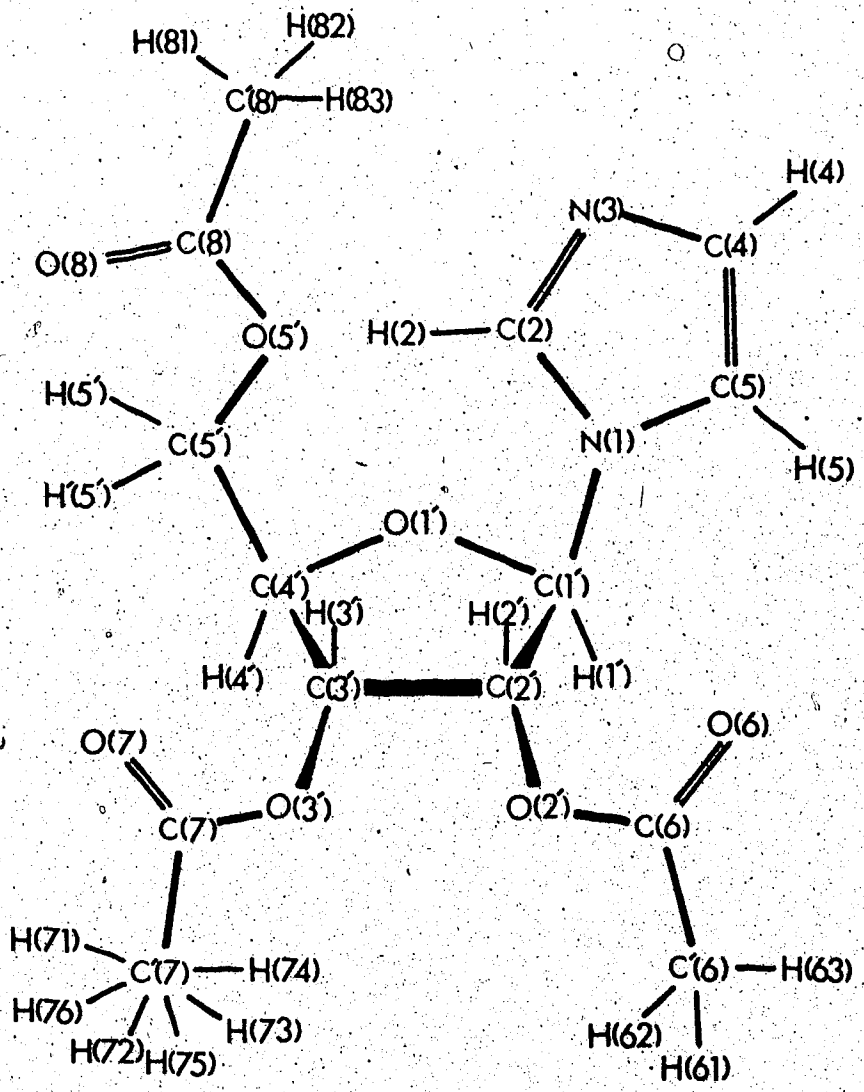


Fig. I 2-1 The atomic numbering for N-(tri-O-acetyl-β-D-ribofuranosyl) imidazole

2-2 Experimental

The compound AIMR was recrystallized by slow evaporation of a diethyl ether solution at room temperature (22 ± 2°C). Preliminary oscillation and Weissenberg photographs showed that the crystals belonged to the orthorhombic system with systematic absences, h00 h=2n+1; 0k0 k=2n+1, 00ℓ ℓ=2n+1. The space groups of these crystals was $P2_12_12_1$. The unit cell dimensions were determined from the Bragg angles of twelve accurately centered reflexions on the Picker FACS-1 diffractometer in the range $36^\circ \leq 2\theta \leq 54^\circ$ (λ=0.70926 Å). The density of the crystals, 1.412g/cm^3, was determined by the flotation method using a mixture of carbontetrachloride and n-hexane. Therefore, four molecules are in a unit cell or one molecule in an asymmetric unit. The crystal data are listed in Table I 2-1. A single crystal, 0.58 X 0.49 X 0.52 mm, for the intensity data collection was cleaved from a larger rod shaped crystal. The intensities were collected at room temperature, 22 ± 2°C, on a Picker FACS-1 diffractometer with graphite monochromatized MoKα radiation. The reflexions were scanned in the θ-2θ mode with a scan speed of 2°/minute. The background counts were measured for 10 seconds at either ends of peak scanning. Three standard reflexions, 0, 8, 0; 3, 7, 0 and 0, 5, 1, were measured every 50 reflexions. No indication of crystal decomposition nor of instrumental instability could be detected during the data collection, except that the crystal took on a slight yellow color at the end. The intensities for each reflexion were measured from the planes, hkl and $\bar{h}kl$, and the average of I_{hkl} and $I_{\bar{h}kl}$ was used to derive the value for

Molecular formula	$C_{14}H_{18}N_2O_7$
Molecular weight	326.3 Daltons
Crystal system	Orthorhombic
a	12.643(2) Å
b	15.802(2) Å
c	7.642(1) Å
V	1527 $Å^3$
D_c	1.419 g/cm^3
D_o	1.412 g/cm^3
Crystal size	0.58x0.49x0.52mm
μ(Mo Kα)	1.24 cm^{-1}
Systematic absences	h,0,0; h=2n+1 0,k,0; k=2n+1 0,0,ℓ: ℓ=2n+1
Space group	$P2_12_12_1$
2θ range searched	$2.5^\circ \leq 2\theta \leq 60^\circ$ (MoKα)
no. reflexions measured	2540
no. reflexions in refinement	2302 (90.6%)
Temperature during data collection	22 ± 2°C

Table I 2-1 Crystal data for N-(tri-O-acetyl-β-D-ribofuranosyl) imidazole

F_{hkl}. Twenty nine reflexions suffered from coincidence loss (count rate greater than 20,000 c.p.s.) and were remeasured with an attenuator in position (attenuator factor = 23.71). The total number of independent reflexions was 2540 within the Bragg angle range, $2.5^{o} \leq 2\theta \leq 60^{o}$. The number of the reflexions included in the refinement was 2302 on the basis of the same criteria in the experimental section 1-2 of the chapter I. No absorption corrections were applied because of the small absorption coefficient ($\mu R=0.072$) and of the almost spherical shape of the crystal. The intensities were reduced to structure factors by the application of the Lorentz and polarization corrections.

2-3 Structure Solution and Refinement

An overall isotropic temperature factor, B=3.22 $\AA^2$, and scale factor, 0.1433, were calculated by Wilson's method (Wilson, 1942). The structure factors were reduced to |E| values (Karle & Karle, 1966). The statistical averages of the |E| values were $\langle E \rangle = 0.857$, $\langle E^2 \rangle = 0.993$ and $\langle (E^2-1) \rangle = 0.812$ (cf theoretical values for non-centric, $\langle E \rangle = 0.886$, $\langle E^2 \rangle = 1.00$, $\langle (E^2-1) \rangle = 0.736$). A total of 245 reflexions had |E| values greater than 1.54. One of the combinations of the starting phases were 0,3,1; 0,19,4 and 3,7,0 for origin reflexions and 0,12,4 = 180° and 3,1,1 = 270° for the symbol reflexions. This combination showed R_k=0.21 after the tangent formula refinement (Karle & Hauptman, 1956). An E map from the refined phases showed all of non-hydrogen atoms in the molecule with reference to the chemically known stereoisomer of the molecule. The initial R value for the 23 non-hydrogen atoms was 0.34. After several cycles of block-diagonal least-squares refinement with isotropic temperature factors, the R value was reduced to 0.13 and a difference map showed 6 out of 18 hydrogen atoms. The atomic species in the acetyl groups were defined from the bond distances and the temperature factors. The difference map at R = 0.056 showed all hydrogen atoms, except those on the carbon atom C'(7) and the atomic species at the positions N(3) and C(4) were determined by the hydrogen position on the carbon atom C(4) and the bond lengths.

Because a total of 11 atoms, including the acetyl groups on O(2') and O(5'), plus C(2'), C(4') and C(5'), had almost the same

x-coordinates, one cycle of the full-matrix least-squares program (ORFLS), (Busing, Martin & Levy, 1962) was computed. But a difference map showed only ring electron densities with two maxima which were on the plane of the acetyl group. It was decided that six hydrogen atom positions with half occupancies should be placed around the carbon atom C'(7). The final full-matrix least-squares calculations reduced the R value to 0.039, R_w value to 0.055 and the goodness of fit to 2.10. The weighting scheme was expressed as $w = 2Fo/[T+(0.04I)^2 + B]^{\frac{1}{2}}$ where T is the total peak count, I is the net peak counts and B is the peak background (Peterson & Levy, 1957). The structure amplitudes are listed in Table I 2-2. The final positional and thermal parameters are listed in Table I 2-3(a), (b) and (c). The thermal parameters for all hydrogen atoms were fixed at $U_{iso} = 0.057$ $\AA^2$.

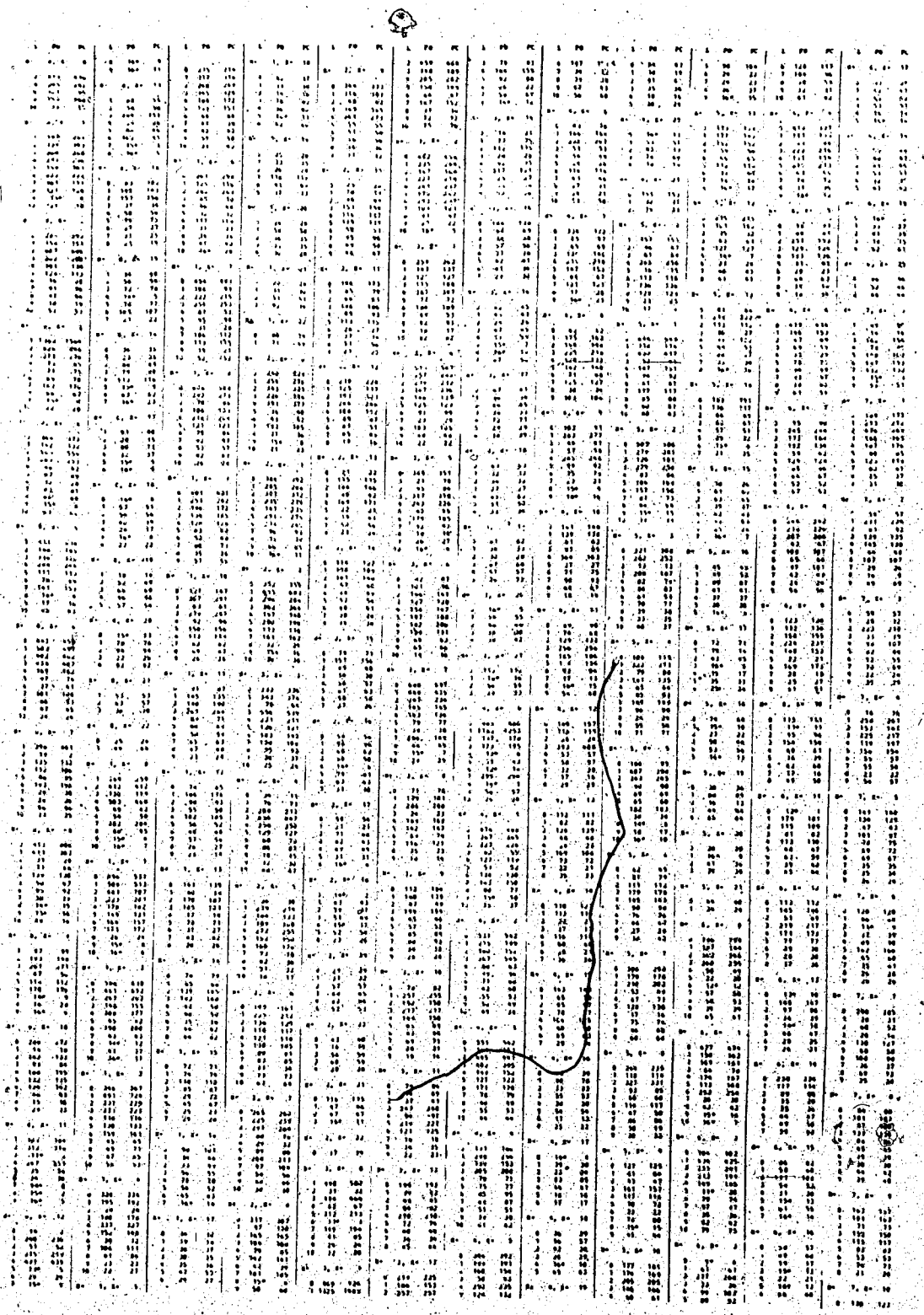


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Table I 2-2 Observed and calculated structure amplitudes (X10) of N-(tri-O-acetyl-β-D-ribofuranosyl) imidazole

Atom	x/a	y/b	z/c
N(1)	2234(1)	1594(1)	7060(2)
C(2)	2038(2)	2363(1)	6330(3)
N(3)	2421(2)	2979(1)	7280(3)
C(4)	2884(2)	2596(2)	8684(4)
C(5)	2778(2)	1747(1)	8588(3)
C(1')	1907(1)	760(1)	6444(2)
C(2')	802(2)	496(1)	7052(2)
C(3')	123(1)	789(1)	5522(2)
C(4')	826(1)	591(1)	3966(2)
C(5')	616(2)	1058(1)	2299(2)
O(1')	1880(1)	760(1)	4580(2)
O(2')	801(1)	-417(1)	7057(2)
O(3')	-850(1)	339(1)	5300(2)
O(5')	688(1)	1953(1)	2616(2)
C(6)	697(1)	-817(1)	8606(2)
O(6)	597(2)	-457(1)	9944(2)
C'(6)	790(2)	-1752(1)	8390(3)
C(7)	-1696(1)	628(1)	6203(3)
O(7)	-1638(1)	1214(1)	7183(3)
C'(7)	-2657(2)	116(2)	5810(4)
C(8)	554(1)	2429(1)	1156(2)
O(8)	346(1)	2122(1)	-235(2)
C'(8)	712(2)	3345(1)	1531(3)

Table I 2-3(a) The positional parameters of the non-hydrogen atoms. ($X10^4$) for N-(tri-O-acetyl-ribofuranosyl) imidazole

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N(1)	72(1)	70(1)	75(2)	3(1)	-7(1)	-8(1)
C(2)	122(2)	67(2)	104(2)	-7(2)	-18(2)	-2(2)
N(3)	130(2)	81(2)	130(3)	-17(2)	-9(2)	-15(2)
C(4)	82(2)	109(2)	132(3)	-8(2)	-19(2)	-42(2)
C(5)	79(2)	107(2)	95(2)	6(2)	-21(2)	-23(2)
C(1')	82(2)	60(1)	63(2)	12(1)	-7(1)	-5(1)
C(2')	101(2)	47(1)	54(1)	0(1)	7(1)	-4(1)
C(3')	73(1)	45(1)	67(2)	-1(1)	7(1)	-6(1)
C(4')	82(2)	48(1)	56(1)	0(1)	2(1)	-4(1)
C(5')	123(2)	56(1)	59(2)	-9(2)	-9(2)	-4(1)
O(1')	76(1)	88(1)	63(1)	2(1)	9(1)	-8(1)
O(2')	155(2)	50(1)	54(1)	-1(1)	-1(1)	-1(1)
O(3')	74(1)	66(1)	103(2)	-7(1)	11(1)	-24(1)
O(5')	132(2)	54(1)	57(1)	-6(1)	-15(1)	2(1)
C(6)	92(2)	58(1)	61(5)	-11(1)	-1(1)	4(1)
O(6)	244(3)	83(2)	67(1)	4(2)	35(2)	1(1)
C'(6)	162(3)	55(1)	85(2)	-18(2)	-25(2)	8(2)
C(7)	79(2)	77(2)	84(2)	-4(1)	13(2)	-9(2)
O(7)	113(2)	135(2)	156(3)	-29(2)	49(2)	-79(2)
C'(7)	84(2)	128(3)	136(3)	-22(2)	14(2)	-37(3)
C(8)	67(1)	66(1)	69(2)	2(1)	-3(1)	10(1)
O(8)	112(2)	89(1)	65(1)	5(1)	-10(1)	9(1)
C'(8)	117(2)	59(2)	109(2)	-2(2)	-4(2)	8(2)

Table I 2-3(b) The thermal parameters ($X10^3$) of non-hydrogen atoms for N-(tri-O-acetyl-β-D-ribofuramosyl) imidazole.

	x/a	y/b	z/c
H(2)	169(2)	245(2)	525(4)
H(4)	314(2)	282(2)	975(4)
H(5)	303(2)	133(2)	948(4)
H(1')	238(2)	33(2)	679(4)
H(2')	58(2)	74(2)	813(4)
H(3')	-5(2)	138(2)	561(4)
H(4')	79(2)	4(2)	370(4)
H(5')	110(2)	90(2)	139(4)
H'(5')	-6(2)	98(2)	183(4)
H(61)	26(2)	-184(2)	784(4)
H(62)	156(2)	-194(2)	815(4)
H(63)	80(2)	-200(2)	945(4)
H(71)*	-258(0)	-48(0)	621(0)
H(72)*	-297(0)	-21(0)	691(0)
H(73)*	-281(0)	21(0)	461(0)
H(74)*	-312(0)	48(0)	553(0)
H(75)*	-324(0)	30(0)	649(0)
H(76)*	-257(0)	-31(0)	484(0)
H(81)	53(2)	378(2)	62(5)
H(82)	141(2)	338(2)	187(5)
H(83)	36(2)	350(2)	257(4)

Tha atoms with asterisks have a half-occupancy.

Table I 2-3(c) The positional parameters ($X10^3$) of the hydrogen atoms for N-(tri-O-acetyl-β-D-ribofuranosyl) imidazole. The U_{iso} value for all hydrogens was set to 0.057 $Å^2$.

2-4 Results and Discussion

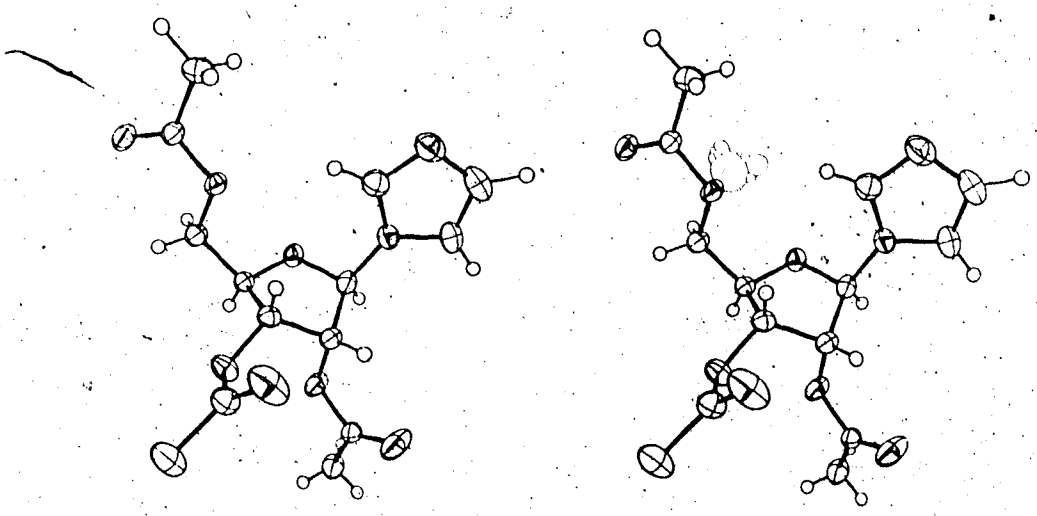
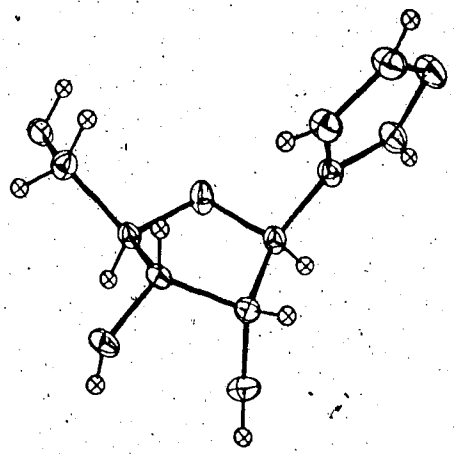


Fig. I 2-2 Stereoscopic view of N-(tri-O-acetyl-β-D-ribofuranosyl) imidazole (Johnson, 1965)



The molecule of IMR

Fig. I 2-2 shows the stereoscopic view of the molecule without the hydrogen atoms on the carbon atom C'(7). The puckering of the ribose ring is $^{3}T_{2}'$ which is close to that of IMR structure. This glycosidic torsion angle is different from that of IMR. This imidazole ring is in anti range regarded as the angle in purine nucleosides and nucleotides. The carbon-oxygen double bonds in the three acetyl groups are synplanar with respect to the C-O(ester) bonds. The molecular geometry of AIMR is given in Fig. I 2-3(a) and (b). The estimated standard deviations for bond angles between the non-hydrogen atoms are 0.1^{o} and those for angles involving the hydrogen atoms are 1^{o}.

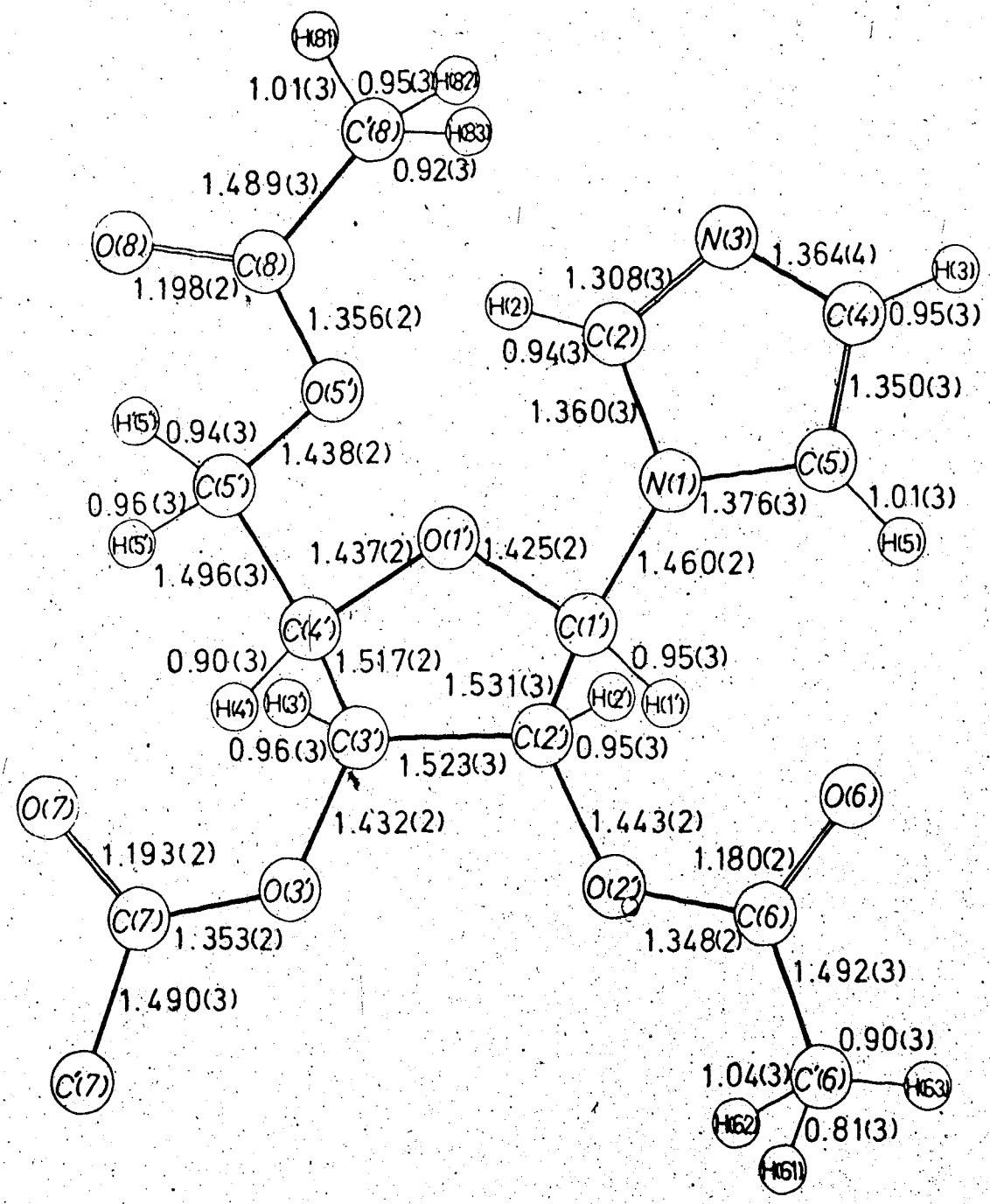
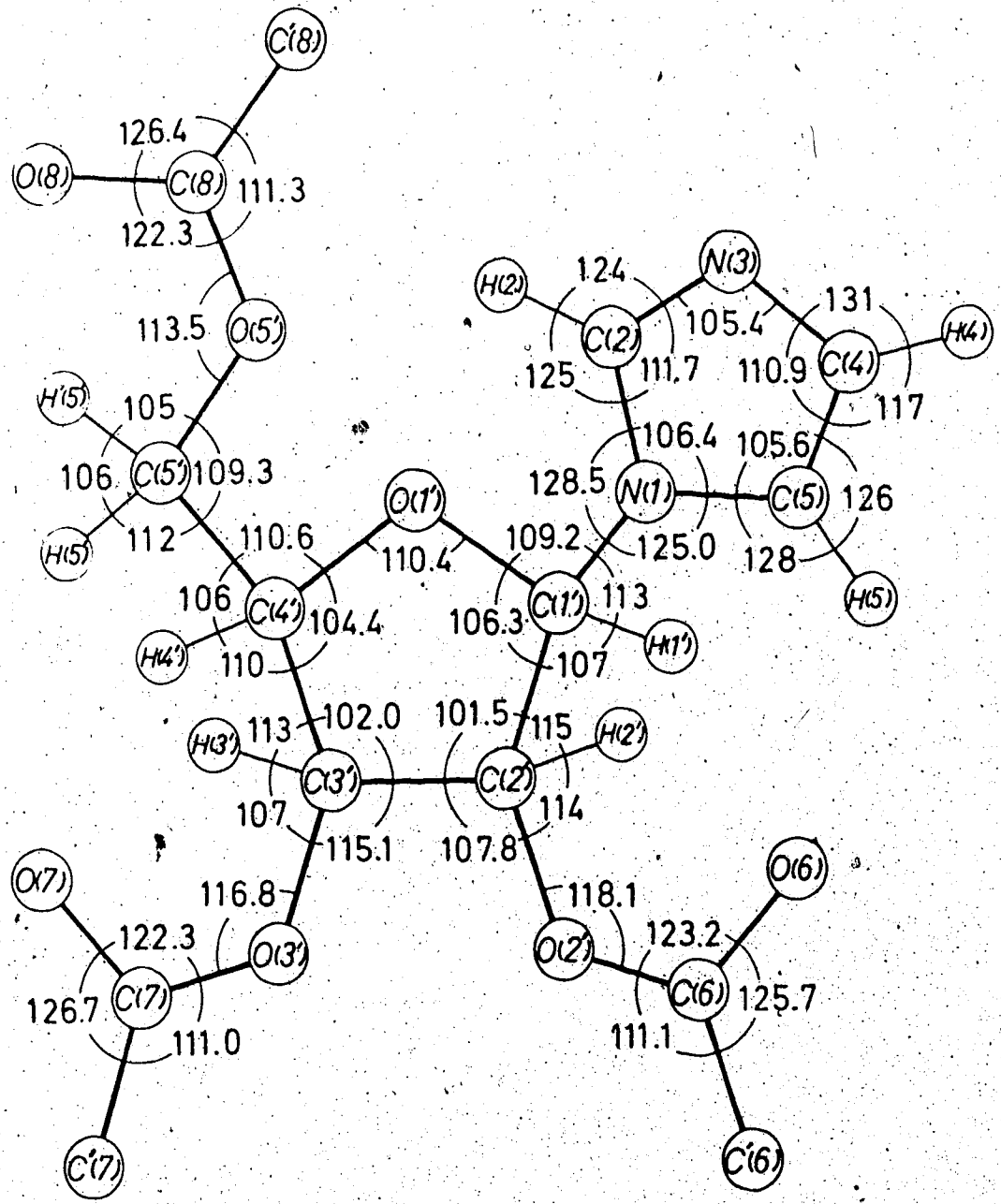


Fig. I 2-3(a) Bond lengths of N-(tri -O-acetyl-β-D-ribofuranosyl) imidazole



$N(1)-C(1')-C(2')$	114.0	$C(3')-C(4')-C(5')$	117.5
$O(1')-C(1')-H(1')$	107.	$O(1)-C(4)-H(4)$	107.
$C(1')-C(2')-O(2')$	105.9	$C(4')-C(5)-H(5')$	115.
$C(3')-C(2')-H(2')$	112.	$O(5')-C(5')-H(5')$	110.
$C(2')-C(3')-H(3')$	112.		
$C(4')-C(3')-O(3')$	108.9		

Fig. I 2-3(b) Bond angles of N-(tri-O-acetyl-β-ribofuranosyl) imidazole.

i) Imidazole ring

The bond lengths C(2) - N(3) and N(3) - C(4) in this molecule are 0.01 Å shorter than the corresponding lengths of the imidazole ring in IMR (Fig. I 1-7(a)) and of imidazole at -150°C (Martinez-Carrera, 1966). It is possible that these differences depend upon whether or not the nitrogen atom N(3) accepts an intermolecular hydrogen bond. The nitrogen atom N(3) in this structure does not contribute to hydrogen bonding, but those in the IMR and imidazole structures do. Such an effect was observed on carbon oxygen double bond lengths of various barbital structures (Craven, Cusatis, Cartland & Vizzini, 1969). On the other hand the bond lengths around the nitrogen atom N(1) are longer than those of IMR and imidazole.

The bond angles in the imidazole ring agree well with those of IMR, whereas the angle C(1') - N(1) - C(2) and C(1') - N(1) - C(5) are 128.5° and 125.0° in AIMR, respectively and the corresponding angles were 124.8° and 128.0° in IMR. As Rao and Sundaralingam (1970) pointed out the wider angle is always associated with the cis-conformation with respect to the ribose ring. This angle is affected by the steric interactions between the imidazole portion and the ribose moiety.

A description of the least-squares plane of the imidazole ring is given in Table I 2-4. The imidazole ring is as planar as that in IMR. The displacements of the atoms of the ring are insignificant, but the displacement of the carbon atom C(1') from the plane is 0.045(2)Å is on the opposite side of that of the oxygen atom O(1').

Atoms	Plane I	Atoms	Plane II
N(1) *	0.002(2) Å	C(1') *	0.00 Å
C(2) *	-0.001(2)	C(2')	0.130(2)
N(3) *	0.000(2)	C(3')	-0.489(2)
C(4) *	0.002(2)	C(4') *	0.00
C(5) *	0.002(2)	O(1') *	0.00
C(1')	-0.045(2)	C(5')	-0.769(2)
O(1')	0.638(1)		
H(2)	0.03(3)		
H(4)	-0.15(3)		
H(5)	0.03(3)		
O(5')	-0.018(2)		
O(8)(a)	-3.142(2)		
χ^2	2.80		

The atoms with * were included in the least-squares calculations.

(a) The oxygen atom O(8) was operated by a symmetry (x,y,1+z)

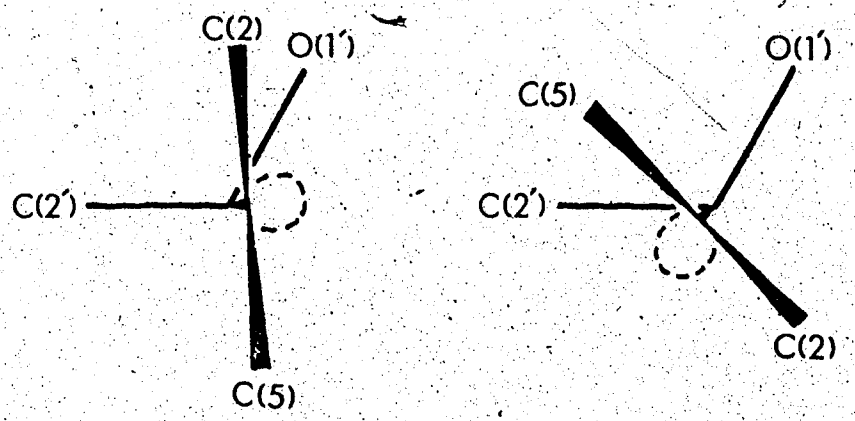
Equations of the least-squares planes

Plane I $0.8637x - 0.0560y - 0.5009z + 0.4059 = 0$

Plane II $0.1979x - 0.9802y - 0.0053z + 0.7255 = 0$

Table I 2-4 The least-squares planes and the atomic displacements of N-(tri-O-acetyl-β-D-ribofuranosyl) imidazole

(0.053 Å from the plane through the atoms N(1),C(2) and C(5)). The nitrogen atom N(1) has a tendency towards tetrahedral coordination, instead of the true trigonal coordination. This tendency was also observed in the structure of IMR. Therefore there is a partial lone pair electron density which extends to the same side of the plane as the oxygen atom O(1'). It is different from the direction of the partial lone pair electron on the nitrogen atom N(1) in IMR which extended to the opposite side of the imidazole plane with respect to the oxygen atom O(1').



This difference can be explained by the molecular packing in the crystals of AIMR (see molecular packing section).

i) ribose

The bond length O(1') - C(1') is shorter than bond length O(1') - C(4'). This difference has been observed in many ribose moieties (Sundaralingam, 1969, Arnott, 1970). The carbon-carbon bond lengths are on the average 0.01 Å shorter than those in IMR.

The bond lengths C(2') - O(2'), C(3') - O(3') and C(5') - O(5') are similar even though the first two carbon atoms are secondary and the last one is primary. The average carbon oxygen bond distance is 1.438(1) Å.

The angles in the ribose ring agree with those in IMR, but some angles of the exocyclic bonds are considerably different from those in IMR. The angles C(3') - C(2') - O(2') and C(4') - C(3') -O(3') are reduced from 115.5(2)° and 114.6(2)° in IMR to 107.8(1)° and 108.9(1)° in AIMR, respectively. The angle C(3') - C(4') - C(5') is 117.5(1)° in AIMR and 113.7(2)° in IMR. Sundaralingam (1966) pointed out that this bond angle is dependent upon the conformation around the C(4') - C(5') bond. The angle is 114° in the case where the oxygen atom O(5') is in the gauche-trans conformation with respect to the oxygen atom O(1') and the carbon atom C(3') respectively; and the angle is 117° in the case where O(5') is in the guache-gauche conformation. The torsion angle around the C(4') - C(5') bond is shown in Fig. I 2-4. The conformation of the oxygen atom O(5') is gauche-gauche in AIMR, but the conformation was gauche-trans in IMR. The angle C(3') - C(4') - C(5') agree well with Sundaralingam's result and the similar relation ragarding this angle is seen in the structures of 5-Iodouridine (Rahman & Wilson, 1970) and 6-Methyluridine (Suck & Saenger, 1972). Both structures contain two independent molecules in an asymmetric unit, and one of these molecules has the gauche-gauche conformation around the C(4') - C(5') bond and the other molecule has the gauche-trans conformation.

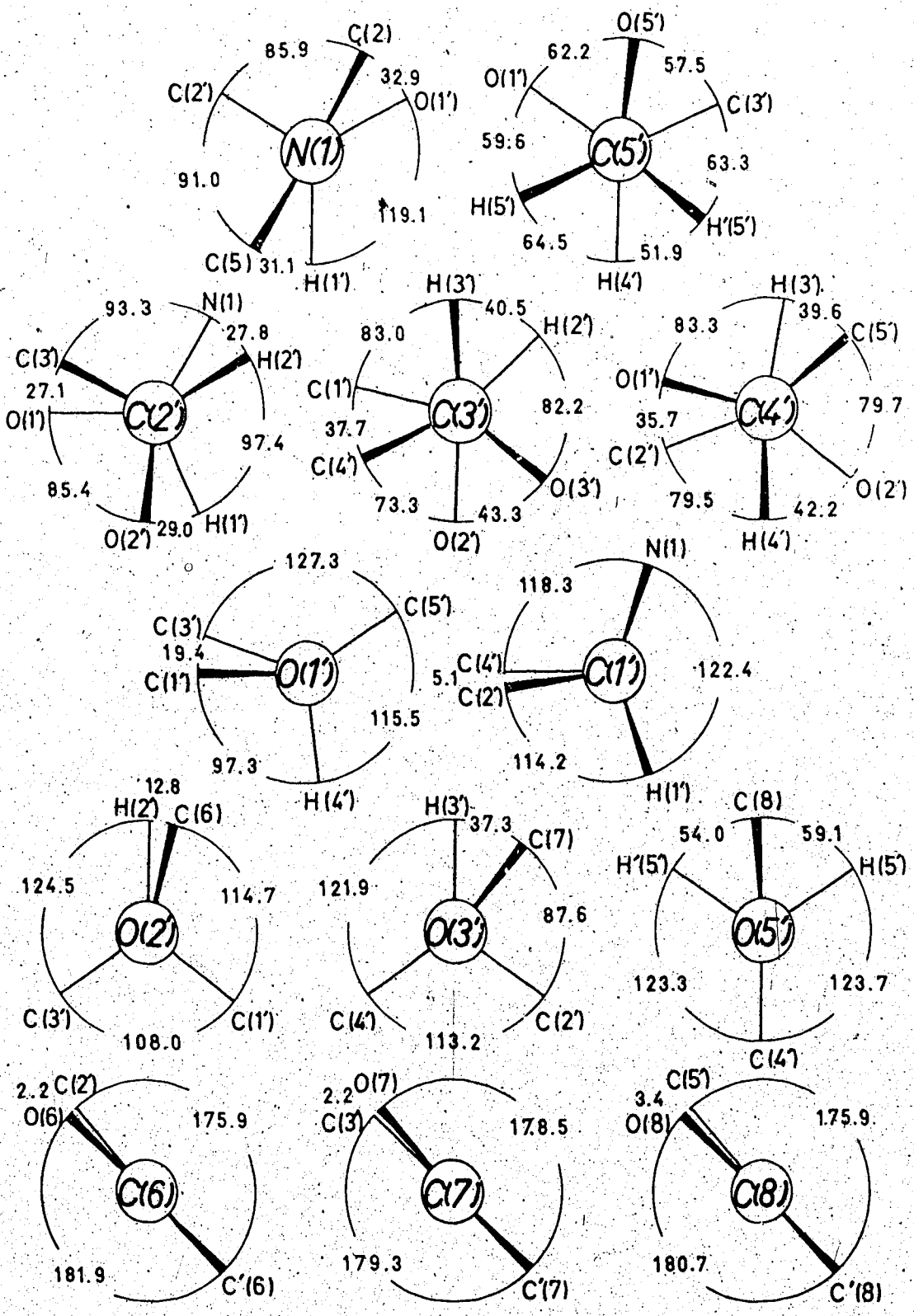


Fig. I 2-4 The torsion angles on the Newman projections for N-(tri-O-acetyl-β-D-ribofuranosyl) imidazole

The glycosidic torsion angle, χ, is 32.9°. Compared with that of -97.8° in IMR, the imidazole ring thus rotates by 130.7° around the glycosidic bond C(1') - N(1). This conformation can be regarded as the anti conformation. Although the aglycon is imidazole in this molecule, the glycosidic angle can be compared with those of the purine nucleosides and nucleotides, because the ring does not directly interact with the ribose ring in anti conformations. In fact the glycosidic angle in AIMR is that which is usually found in the structures of the purine nucleosides and nucleotides (Sundaralingam, 1969; Arnott, 1970).

Rubin et al., pointed out that the oxygen atom O(5') attracts the active H-C group in the bases in the gauche-gauche conformation around the bond C(4') - C(5'), (Rubin, Brennan & Sundaralingam, 1972). The carbon atom C(2) is bonded to two nitrogen atoms N(1) and N(3) as is the carbon atom C(8) in purine ring systems. In the structure of AIMR the interatomic distances are 2.51(3) Å between the oxygen atom O(5') and the hydrogen atom H(2), and 3.374(3) Å between the oxygen atom O(5') and the carbon atom C(2). These distances are not significantly short, but the displacement of the oxygen atom O(5') is only 0.018(2) Å from the least-squares plane through the imidazole ring (Table I 2-4). There may be a weak interaction between the oxygen atom O(5') and C(2) - H(2) group.

The displacements of the ribose atoms from the plane defined by the atoms C(1'), O(1') and C(4') are shown in Table I 2-4 and Fig. I 2-5. The carbon atoms C(2') and C(3') are by 0.130(2) Å and 0.489(2) Å from this plane respectively.

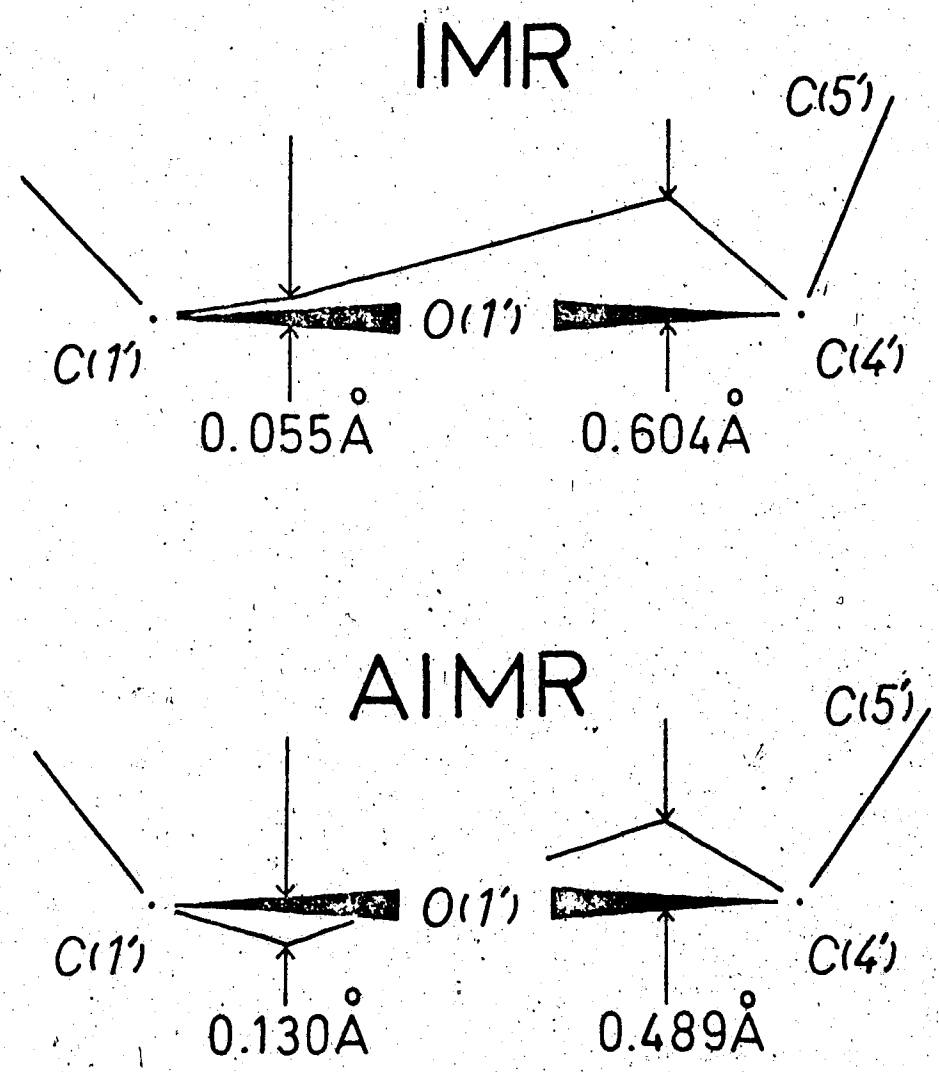


Fig. I 2-5 The displacements of the carbon atoms C(2') and C(3') from the plane defined by the atoms C(1), O(1') and C(4') in the structures of IMR and AIMR.

This shows the ribose conformation is $^3T_2$. Whereas the ribose conformation of IMR was ^3E. The torsion angles are shown in Fig. I 2-4 and some torsion angles of AIMR and IMR are listed in Table I 2-5. The largest difference of the angles between the two ribose rings is seen around O(1') - C(1') bonds. The torsion angle C(4') - O(1') - C(1') - C(2') is 5.1° in AIMR and -2.1° in IMR. The

torsion angle C(4') - O(1') - C(1') - N(1) which is important for the anomeric effect is -118.3° in AIMR and -126.3° in IMR. These differences can be considered small. Therefore both conformations of the ribose ring in the structures of AIMR and IMR are essentially the same and the hydrogen bonds in the crystal structure of IMR have not appreciably affected the ribose conformation of IMR.

	AIMR	IMR
O(1') - C(1') - N(1) - C(2)	32.9°	-97.8°
O(1') - C(1') - C(2') - C(3')	-27.1	-20.4
C(1') - C(2') - C(3') - C(4')	37.7	33.6
C(2') - C(3') - C(4') - O(1')	-35.7	-35.8
C(3') - C(4') - O(1') - C(1')	19.4	24.1
C(4') - O(1') - C(1') - C(2')	5.1	-2.1
C(4') - O(1') - C(1') - N(1)	-118.3	-126.3

Table I 2-5 Comparison of the torsion angles in AIMR and IMR.

(iii) Acetyl groups.

Both the bond lengths and angles in the three acetyl groups agree well with those in 3'-O-acetyl adenosine (Rao & Sundaralingam, 1970). The bond angles at the ester oxygen atoms are similar for the oxygen atoms O(2') and O(3'), but the corresponding angle at the oxygen atom O(5') is significantly smaller than these. One

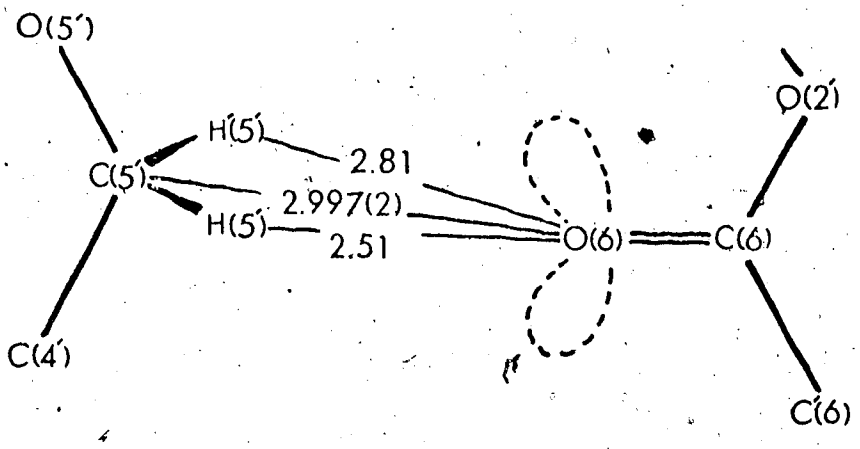
reason seems to be that the former two oxygen atoms are bonded to secondary carbon atoms and the oxygen atom O(5') is bonded to a primary carbon atom, C(5'). The interatomic distances between the carbonyl oxygen atom and the hydrogen atom on the secondary carbon atom are 2.34(3) Å and 2.36(3) Å. The distances between the oxygen atom O(8) and the hydrogen atoms H(5') and H'(5') are 2.49(3) Å and 2.45(3) Å. These distances are longer than the former two distances even though the bond angle at the oxygen atom O(5') is smaller.

The conformations of acetyl groups were discussed by Dunitz and Strickler (1968). A synplanar conformation is more stable than the anti planar conformation around the bond between the carboxyl carbon atom and the ester oxygen atom. All three acetyl groups have synplanar conformation in this crystal. The torsion angles are less than 3.4°.

(iv) Molecular packing

A short contact is found between the acetyl oxygen atom O(6) (x,y,z-1) and the carbon atom C(5'). The distance is 2.997(2) Å. This is nearly the same as the summation of Van der Waals radii of carbon and oxygen atoms, 1.6 Å and 1.4 Å.

The distances between the oxygen atom O(6) and the hydrogen atoms H(5') and H'(5') are 2.51 Å and 2.81 Å respectively. Supposing that the oxygen atom has sp^2 lone pair electrons, the plane through the atoms, H(5'), C(5') and H'(5') is almost perpendicular to the sp^2 plane on the oxygen atom.



This intermolecular distance shows the direct contact between the Van der Waals radii of the carbon and the oxygen atom.

Another relatively short contact is found between the imidazole ring and an acetyl oxygen O(8) (x,y,1+z). The displacement of the oxygen atom from the plane of the imidazolium ring is 3.142(2) Å. The distance from the oxygen atom to the nitrogen atom N(1) is 3.266(2) Å on the same side of the imidazole ring as the carbon atom C(1') is. This molecular packing suggests that the electronegative oxygen atom O(8) may affect the electrons on the nitrogen atom N(1) and drive the partial lone pair electron on it to the opposite side of the imidazole plane and the direction of the displacement of the carbon atom C(1') from the imidazole plane can be determined by the direction of the partial lone pair electrons.

2-5 Conclusions

As presented in the introductory chapter, the primary aim of this research project was to devise a model system for the anomeric effect and the reverse anomeric effect in the ribose ring. The compounds IMR and AIMR were considered to the system for the anomeric effect, whereas protonation or methylation of the imidazole ring in either compound would result in a positive charge on the aglycon which would then represent a model for the reverse anomeric effect. A number of attempts to crystallized the N(3) methylated IMR with a variety of anions from a variety of solvent systems were not successful and no further experimental work would be considered on this system without suitable crystalline specimens.

There are in the literature a number of other compounds which have a positively charged aglycon attached to the β-D- ribose ring which would satisfy these requirements and the following discussion is based in part on the results of these published studies. Table I. 2-6 shows the conformations of the unprotonated and protonated cytidine derivatives, IMR, AIMR and 1,7-dimethyl guanosine. The torsion angle C(4')-O(1')-C(1')-N was 122.1° in the unprotonated cytidine, but this torsion angle was increased to 140.8° in the protonated cytidine(orthorhombic form) and 138.4° in the protonated cytidine (monoclinic form). In addition there is a short communication about the conformation of 1,7- dimethyl guanosine iodide. The methylation on the nitrogen atom N(7) of the guanine base gave a positive charge to the imidazole ring. The conformation of the ribose ring in 1,7-dimethyl guanosine iodide was $^{2}T_{3}$. In this conformation the torsion angle C(4')-O(1')-C(1')-N

should be wider than that in the conformation ^3E or $^3T_2$.

	ref.	aglycon charge	ribose puckering	torsion angle C(4')-O(1')-C(1')-N
cytidine	1	0	$^3T_2$	122.1°
cytidine-3'-p (o)	2	+	$^2T_3$	140.8°
(m)	3	+	$^2T_3$	138.4°
IMR		0	^3E	126.3°
AIMR		0	$^3T_2$	118.3°
1,7-dimethyl guanosine	4	+	$^2T_3$	---.-

references

1 Furberg, Peterson & Rømming, 1965.

2 Sundaralingam & Jensen, 1965.

3 Bugg & Marsh, 1967.

4 Shefter, Singh & Sackman, 1974.

Table I 2-6 The conformation of the ribose rings bonded to the uncharged and positively charged aglycon.

These values in Table I 2-6 suggest that a positive charge on the nitrogen aton may affect the torsion angle in the system C-O-C-N. From these results and arguments presented earlier in this work, it is highly probable that the conformation of the ribose ring is affected by the anomeric effect and the reverse anomeric effect.

Of cource, an accurate molecular orbital calculation has to be applied to the reverse anomeric effect. But if the theoretical explanations of the anomeric effect are permitted to extend to an interpretation of the reverse anomeric effect, the dipole-dipole

interaction could affect the conformation of the furanosides and pyranosides. The dipole-dipole interaction term V_1 was the large positive value in the anomeric effect. This term would be negative, possibly large negative value in the reverse anomeric effect, because of the actual positive charge on the aglycon. As far as the substituent on the anomeric carbon atom is electronegative (that is, the substituent has the ability to attract the electron cloud on the anomeric carbon atom) and the anomeric carbon atom has the tetrahedral bonding orientation, the V_2 and V_3 terms might be the similar negative values in both effects. These expectations give that the energy minimum would be close to $\theta=180^{\circ}$ in the reverse anomeric effect, instead of $\theta=60^{\circ}$ in the anomeric effect. These agree with the observations of the conformations in the furanoses and pyranoses with the system C-O-C-N^+ which have a wider torsion angle about the ring oxygen-anomeric carbon bond.

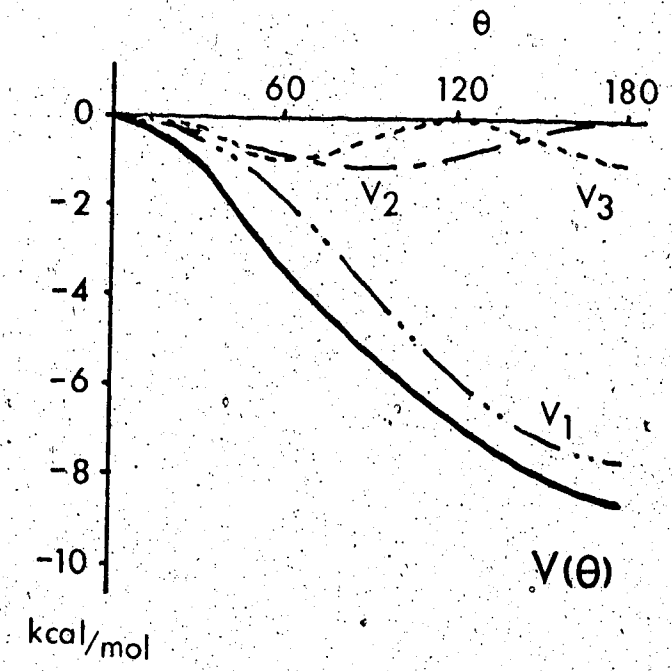


Fig. I 2-6 Possible energy curve for the reverse anomeric effect.
V_1= -7.65, V_2= -0.97, V_3= -0.88 Kcal/mole

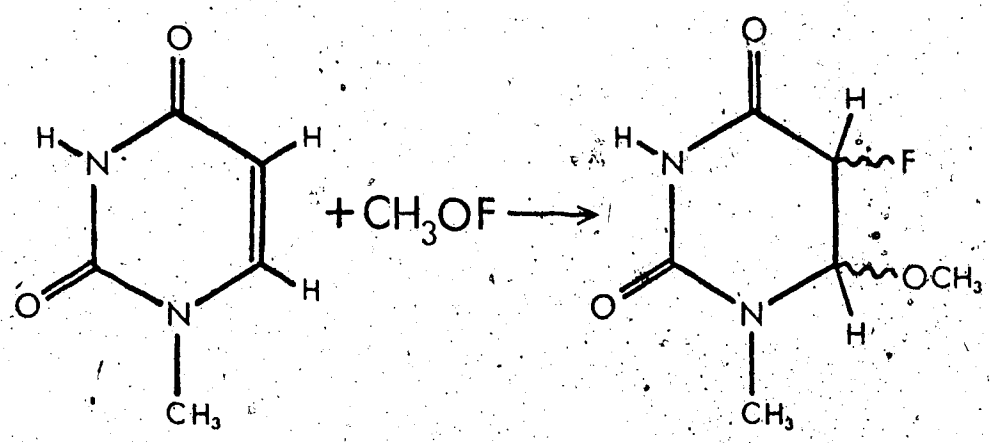
CHAPTER II

The Conformation of the Methoxy Group of 1-methyl-5-fluoro-6-methoxy-5,6-dihydrouracil.

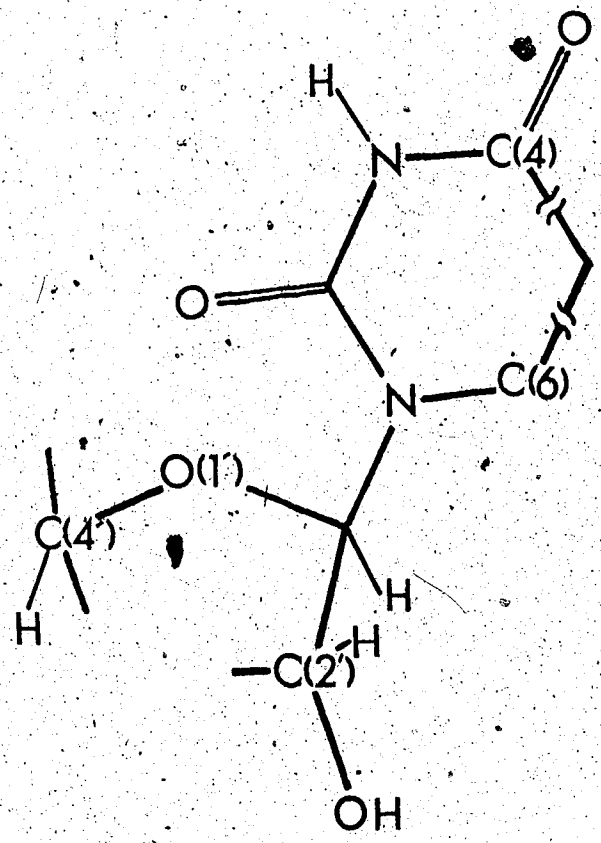
1-1 Introduction

5-fluorouracil and 5-fluoro-2'-deoxyuridine are employed as standard clinical drugs for treatment of certain solid tumors and viral infection (Heidelberger, 1970). The methods (Heidelberger, 1965) for preparation of 5-fluoropyrimidine and their nucleosides had depended on de novo construction of the 5-fluoropyrimidine ring beginning ultimately with the highly toxic ethyl fluoroacetate. Robins and Naik (1971) found a direct route applicable to uracil bases using trifluoromethyl hypofluorite in trichlorofluoromethane.

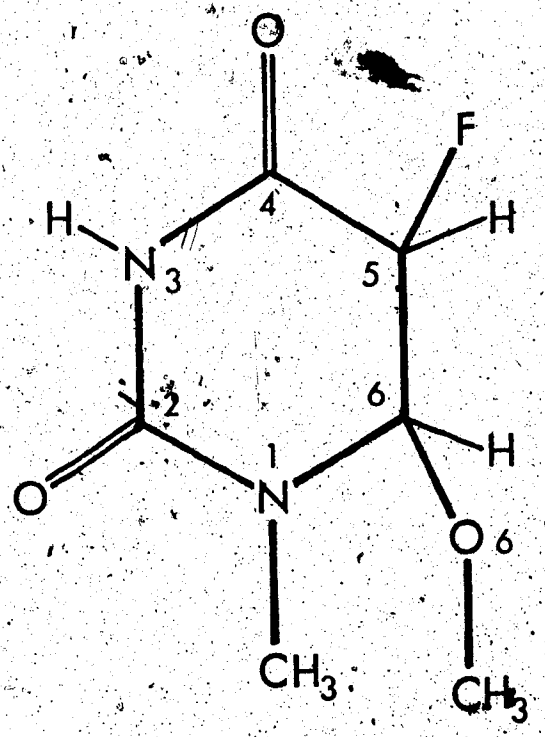
1-methyl-5-fluoro-6-methoxy-5,6-dihydrouracil which is studied in this chapter is an intermediate in the synthetic pathway from 1-methyl uracil to 1-methyl-5-fluoro uracil. The primary problem in this analysis concerned whether the configuration of the addition reaction was cis or trans with respect to the substituents on the double bonded carbon atoms C(5) and C(6).



In addition this molecule has an interesting molecular system which is very similar to the pyrimidine nucleosides, such as dihydrouridine (compare I and II below).



(I)



(II)

The similarity of the pyrimidine ring portion between I and II is clear. However, it is possible to look at II in another fashion. The portion of the pyridine ring that has the C(5) C(6) substituents is chemically similar to the portion of the ribose ring drawn in I as follows. A hypothetical two-fold rotation about the vector N(1) → C(2) would interchange C(6) in I with the methyl group on N(1) in II. Also C(1') in I would occupy the C(6) position and the system C(1') - O(1') - C(4') has its counter part in II with atoms C(6) - O(6) - CH_3. Similarly the C(5)-F grouping in II would correspond to atoms C(2') - OH in I. As Jeffrey, Pople and Radom (1972) pointed out the conformations of methyl groups of methyl glycosides in the crystals were similar to those predicted by the ab initio molecular orbital calculations in a simple molecular system. Therefore, the conformation of the methoxy group in the compound II could be a good subject to study the energy minimum conformation in the pyrimidine nucleosides and nucleotides.

In addition the space group of this compound in the crystals had tetragonal symmetry. It is worthwhile to apply the direct methods (Karle & Karle, 1966) to solve the crystal structure on a complex space group symmetry.

1-2 Experimental

The compound was crystallized in methanol and supplied by Dr. M.J. Robins, Chemistry Department, University of Alberta. Preliminary oscillation and Weissenberg photographs revealed that the crystal belonged to the space group $I4_1/a$ in the tetragonal system, since the same intensities of the reflexions were repeated every 90° rotation around c^* axis, and the following condition applied to possible reflexions for 0,0,ℓ ℓ=4n; h,k,0 h or k = 2n and h,k,ℓ h+k+ℓ=2n.

The cell dimensions were determined by the least-squares program from the Bragg angles of twelve reflexions which were in the range of $35^\circ<2\theta<40^\circ$ using Mo Kα radiation. The density of the crystals was measured by the flotation method. The number of the molecules in unit cell was 16, that is, one molecule in the asymmetric unit. These crystal values are summarized in Table II 1-1.

Intensity data were collected up to $2\theta=55^\circ$ by θ-2θ scan mode on a Picker FACS-1 diffractometer with graphite monochromatized Mo Kα radiation. The background was measured for 10 seconds at the either sides of the reflexion scan. Three reflexions as standards were measured every 30 reflexions. No decay of the crystal could be detected during the data collection. The strongest 24 intensities were remeasured with an attenuator in position. The reflexions of which intensities were stronger than three times the estimated standard deviations ($\sigma(I)=(\text{total count})^{\frac{1}{2}}$) were included in the refinement. The number of these reflexions was 1298 out of 1841 reflexions

Molecular formula	$C_6H_9FN_2O_3$
Molecular weight	176.15 dalton
System	Tetragonal
Possible reflexions	h,k,ℓ ; h+k+ℓ=2n
	h,k,0 ; h(k)=2n
	0,0,ℓ ; ℓ=4n
Space group	$I4_1/a$
a = b	21.102(3) Å
c	7.141(1) Å
D_o	1.49 $g.cm^{-3}$
D_c	1.47 $g.cm^{-3}$
Molecules in unit cell	16(1 in asymmetric unit)
Crystal size	0.3x0.4x0.4 mm
μ (Mo Kα)	1.43 cm^{-1}
No. total reflexions	1841
No. reflexion included in refinement	1298 (70.5% of total)
2θ range explored	$3^o \leq 2\theta \leq 55^o$ (Mo Kα)

Table II 1-1 Physical values for 1-methyl-5-fluoro-6-methoxy-5,6-dihyrouracil

scanned. The data were corrected for the variation of the intensities of the stardard reflexions and usual Lorentz and polarization factors, but no absorption corrections were done, because of the small linear absorption coefficient, 0.06 and the almost spherical shape of the crystal.

1-3 Structure Solving and Refinement

There are two alternative origin choices in space group $I4_1/a$. The origin for this structure was chosen at the center of symmetry 0,0,0 at 0,1/4,1/8 from the other possible origin on the $\bar{4}$ axis. An overall isotropic temperature factor, B_{iso}, of 3.75 $\AA^2$ and a scale factor, 0.478, were computed by Wilson's method (Wilson, 1942).

A Patterson distribution of interatomic vectors was computed from the $|Fo|^2$ values but interpretation of this map proved difficult. The molecule has relatively large conformational flexibility and as the configuration of the ring substituents was in doubt it seemed that the most promising phasing technique would be that offered by direct methods. The intensities were converted to a set of relative $|E|$ values. The $|E|$ statistics showed a good correlation with the theoretical values for centrosymmetric crystals.

	theoretical		experimental
	centric	noncentric	
$\|E\|$	0.798	0.886	0.773
$\|E^2\|$	1.000	1.000	1.000
$\|(E^2-1)\|$	0.968	0.736	1.004

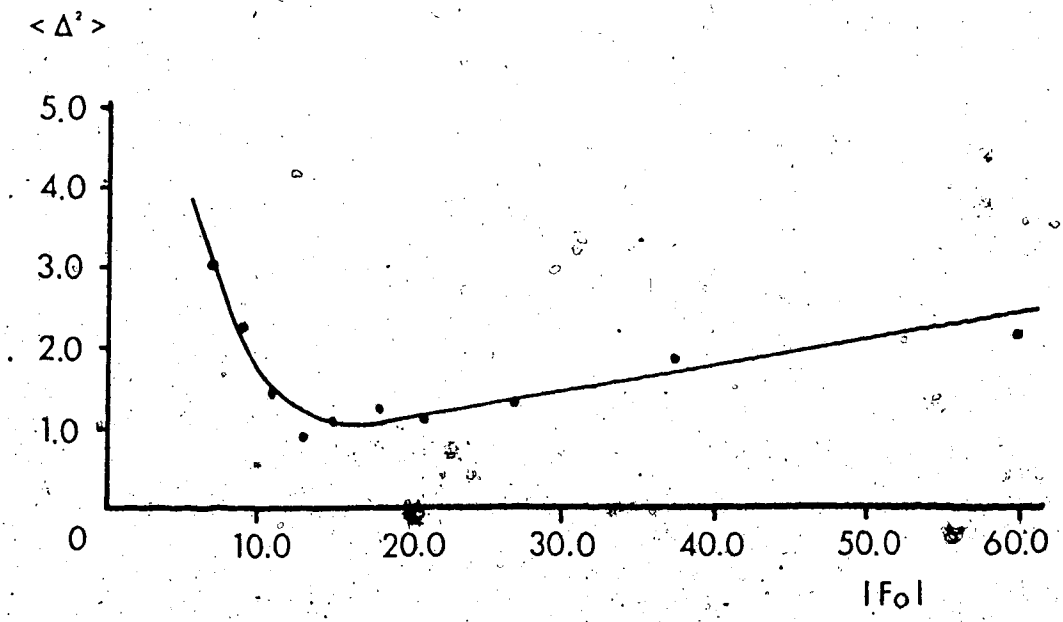
A SIGMA2 program which was modified by the author in the XRAY70 program system determined the Σ_2 relations and sign probabilities within a set of 147 reflexions of which the $|E|$ values were larger than 1.80. Only one reflexion is required to define the origin. The

reflexion 18,9,1 had the largest |E| value of 2.93 among those reflexions possible for the origin definition. The program PHASE produced a set of phases for 141 of the 147 reflexions with |E| values greater than 1.80. The cut-off in accepting a phase indication to be the correct value was chosen at P± = 0.85. Those Σ_2 relations involving three reflexions from the same zone, ie $h_1k_1 0, h_2k_2 0, h_3k_3 0$ etc. were excluded and no symbol was introduced during the phase determination. The resulting E map based on these 141 phased reflexions clearly showed all of the non-hydrogen atoms of the molecule and one additional spurious peak.

Atomic coordinates were measured from the E map and the chemical identity of the atoms based on the expected chemical formula was chosen. Using these atomic coordinates and an overall isotropic temperature factor, $B_{iso} = 3.75$ A^{o2} for all the atoms led to an R factor of 0.21. Two cycles of full-matrix least-squares refinement with unit weight for the reflexions and one cycle with the anisotropic thermal model reduced R to 0.08. A difference map showed five of the nine hydrogen atom positions. Two other doubtful electron positive peaks for other hydrogen atoms could be found around the either methyl carbon atoms, C(1) and C(7). The positions for the remaining four hydrogen atoms were calculated on the assumption that the methyl hydrogen atoms would be at the tetrahedral orientations with respect to the known atomic coordinates and their bond distances from the carbon atoms would be 0.95 A^o. One refinement cycle including all hydrogen coordinates with isotropic temperature factors was computed, but the parameters of the four hydrogen atoms positioned were

fixed. After this refinement a difference map at R = 0.048 showed that the four hydrogen atoms were at almost correct positions, but the temperature factors which had been set to the isotropic temperature factors of the bonded carbon atoms were too small. All the parameters, which were the atomic coordinates with the anisotropic temperature factors for the non-hydrogen atoms and with the isotropic temperature factors for the hydrogen atoms, were refined in the final full-matrix least-squares calculations. The final R index was 0.042. Although unit weights were not appropriate in the least-squares refinement, further refinement was not considered important as the main chemical problems about the configuration and the conformation were solved satisfactorily (Fig. II 1-1).

The final atomic parameters are given in Table II 1-2 (a), (b) and (c) and the observed and calculated structure factors are listed in Table II 1-3.



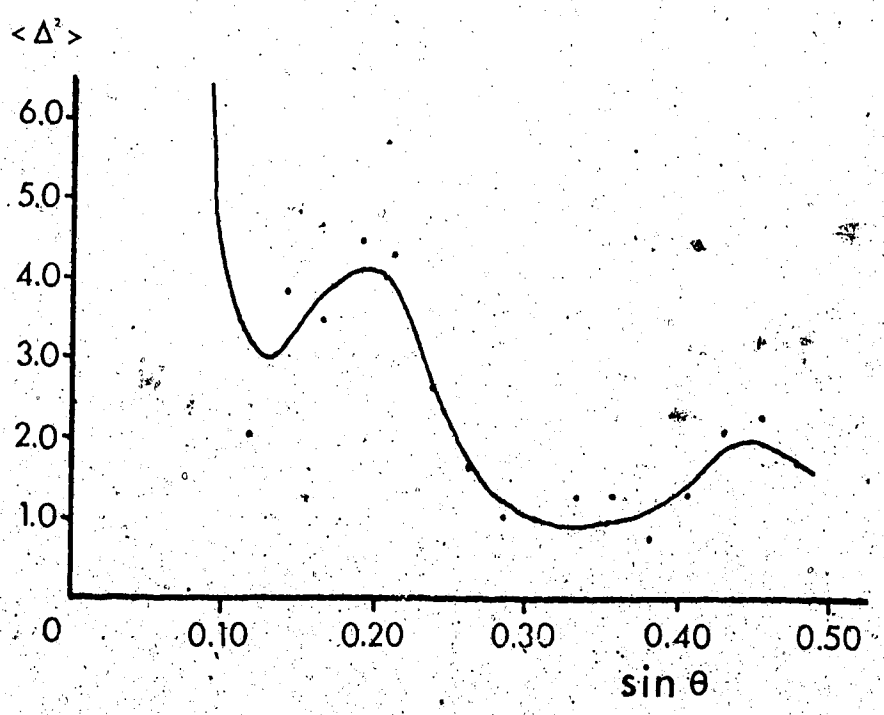


Fig. II 1-1 Statistics of the average ($|Fo|$ [redacted] vs. $|Fo|$ and sin θ for 1-methyl-5-fluoro-6-[redacted]ethoxy-5,6-dihydrouracil

Atom	x/a	y/b	z/c
N1	1551(1)	2215(1)	4372(3)
C2	937(1)	2364(1)	4118(3)
N3	547(1)	1887(1)	3396(3)
C4	756(1)	1351(1)	2542(3)
C5	1462(1)	1364(1)	2197(3)
C6	1787(1)	1580(1)	3967(3)
C1	1974(1)	2712(1)	5043(5)
O2	713(1)	2884(1)	4490(3)
O4	408(1)	921(1)	2077(3)
F5	1665(1)	766(1)	1678(2)
O6	1629(1)	1132(1)	5336(2)
C7	1954(2)	1206(2)	7072(5)

Table III 1-2(a) The positional parameters ($X10^4$) of the non-hydrogen atoms of 1-methyl-5-fluoro-6-methoxy-5,6-dihydrouracil

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N1	289(9)	363(9)	421(11)	-18(7)	-11(8)	-63(8)
C2	320(10)	385(11)	396(12)	-22(8)	-2(9)	-32(10)
N3	297(9)	372(10)	527(12)	-22(7)	-43(9)	-57(9)
C4	478(12)	345(11)	342(11)	-20(9)	-78(10)	22(9)
C5	504(13)	351(11)	359(12)	-46(10)	53(10)	-44(10)
C6	315(11)	405(11)	406(12)	22(9)	28(9)	6(10)
C1	339(13)	519(16)	765(22)	-48(11)	-85(14)	-176(15)
O2	328(8)	406(9)	724(13)	26(7)	-26(8)	-149(9)
O4	607(11)	390(9)	614(12)	-101(8)	-157(9)	-35(8)
F5	723(10)	482(8)	595(10)	108(7)	72(8)	-155(8)
O6	445(9)	499(10)	403(9)	-12(7)	-50(7)	69(8)
C7	647(21)	1056(30)	439(17)	-32(20)	-134(16)	97(19)

Table II 1-2(b) The thermal parameters ($X10^4$) of the non-hydrogen atoms of 1-methyl-5-fluoro-6-methoxy-5,6-dihydrouracil

Atom	x/a	y/b	z/c	U_{iso}
H3	14(1)	194(1)	361(4)	56(8)
H5	158(1)	166(1)	112(4)	47(7)
H6	226(1)	161(1)	384(3)	35(6)
H11	193(2)	307(2)	432(6)	118(15)
H12	239(2)	256(2)	510(5)	83(11)
H13	185(2)	285(2)	630(6)	116(15)
H71	199(1)	66(2)	773(5)	91(11)
H72	178(2)	153(2)	776(7)	156(20)
H73	241(2)	124(2)	696(6)	119(15)

Table II 1-2(c) The positional and isotropic thermal parameters ($X10^3$) of the hydrogen atoms of 1-methyl-5-fluoro-6,-methoxy-5,6-dihydrouracil

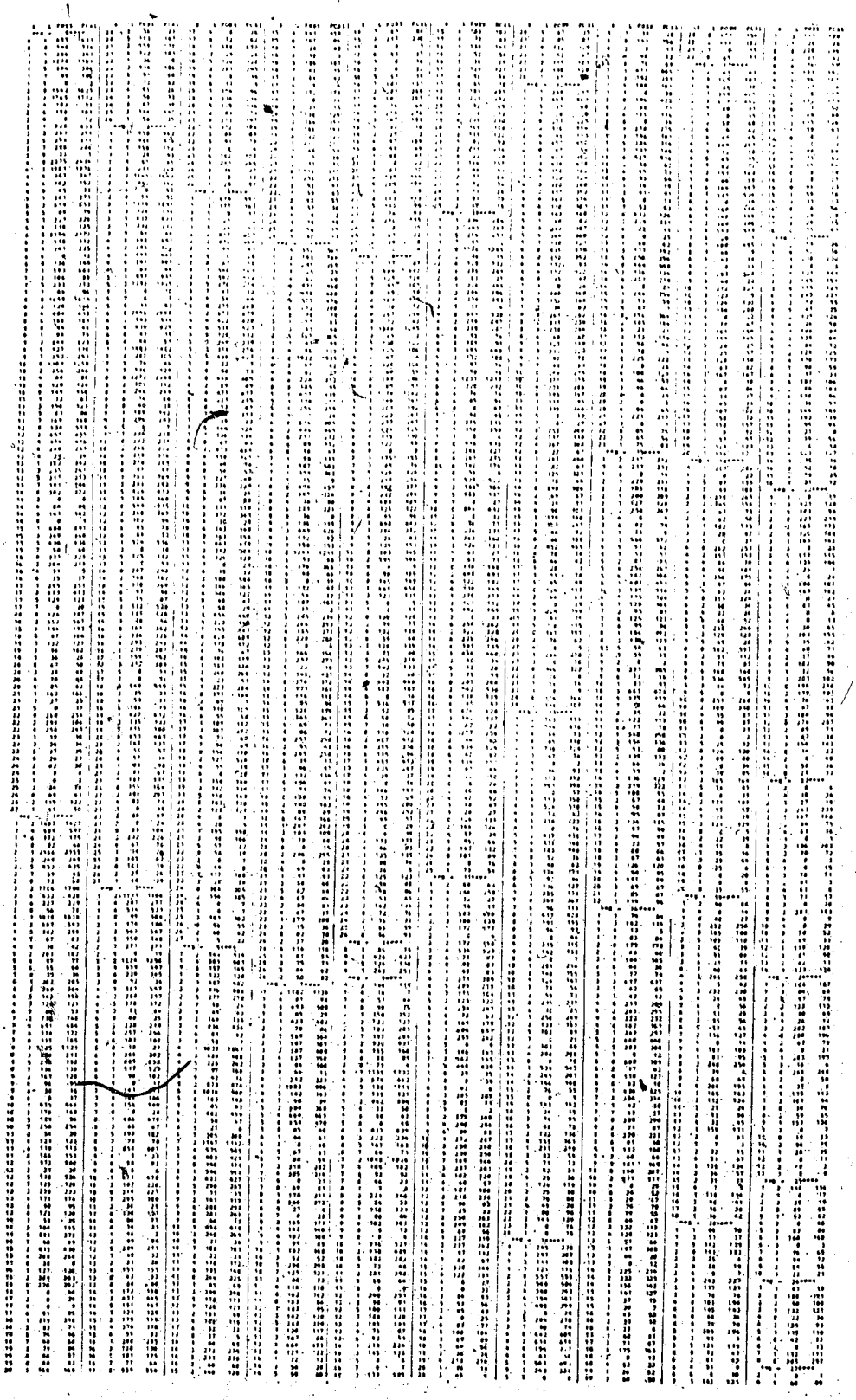
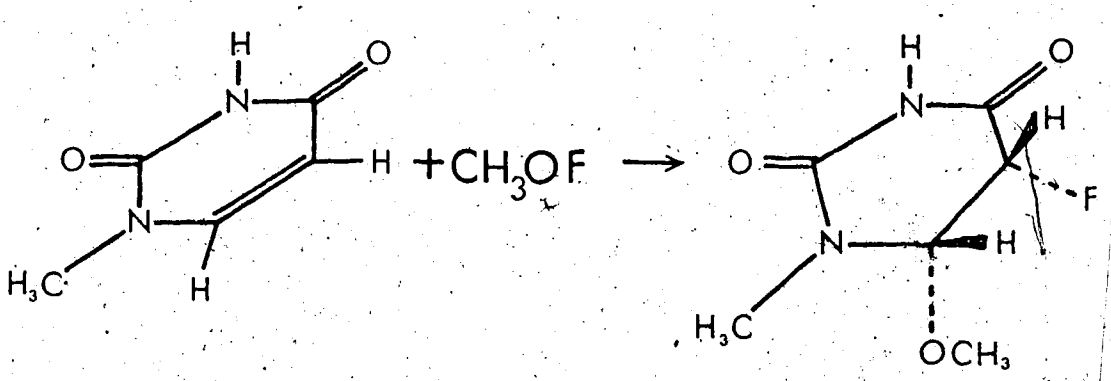


Table II 1-3 The observed and calculated structure factors (X10) of 1-methyl-5-fluoro-6-methoxy-5,6-dihydrouracil

1-4 Results and Discussion

The stereoscopic view of the molecule is shown in Fig. II 1-2. The configuration of the addition of the substituents, the fluorine atom and the methoxy group is definitly cis; that is, both of the substituents attack from the same side of the carbon-carbon double bond. The fluorine atom is added to the carbon atom C(5) and the methoxy group to the carbon atomC(6).



Although the temperature factors of this structure were high, there was no trace of the disorder, such as was observed in the related molecular structure, dihydrothymine (Furberg & Jensen, 1968) or of the decomposition to a uracil derivative, like that of the dihydrothymidine to thymidine (Konnert & Karle, 1970).

The bond lengths and angles with associated standard deviations are shown in Fig. II 1-3(a) and (b), respectively.

The bond angles are similar to those in the dihydrouracil derivatives except for the three internal ring angles at carbon atoms, C(4), C(5) and C(6). These angles are smaller than those observed in dihydrouracil (Rohrer & Sundaralingam, 1970) and dihydrouridine (Suck, Saenger & Zechmeister, 1972), but agree with those observed

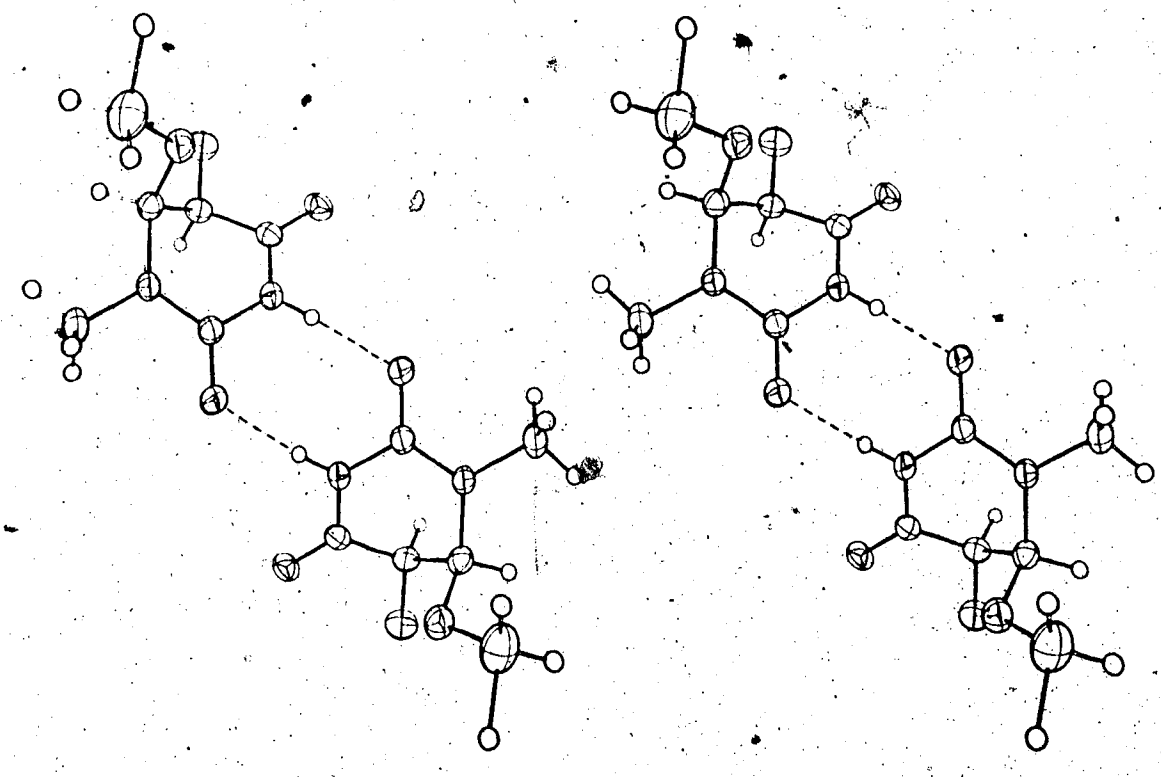


Fig. II 1-2 The stereoscopic view for the hydrogen bonded molecules of 1-methyl-5-fluoro-6-methoxy-5,6-dihydrouracil (Johnson, 1965)

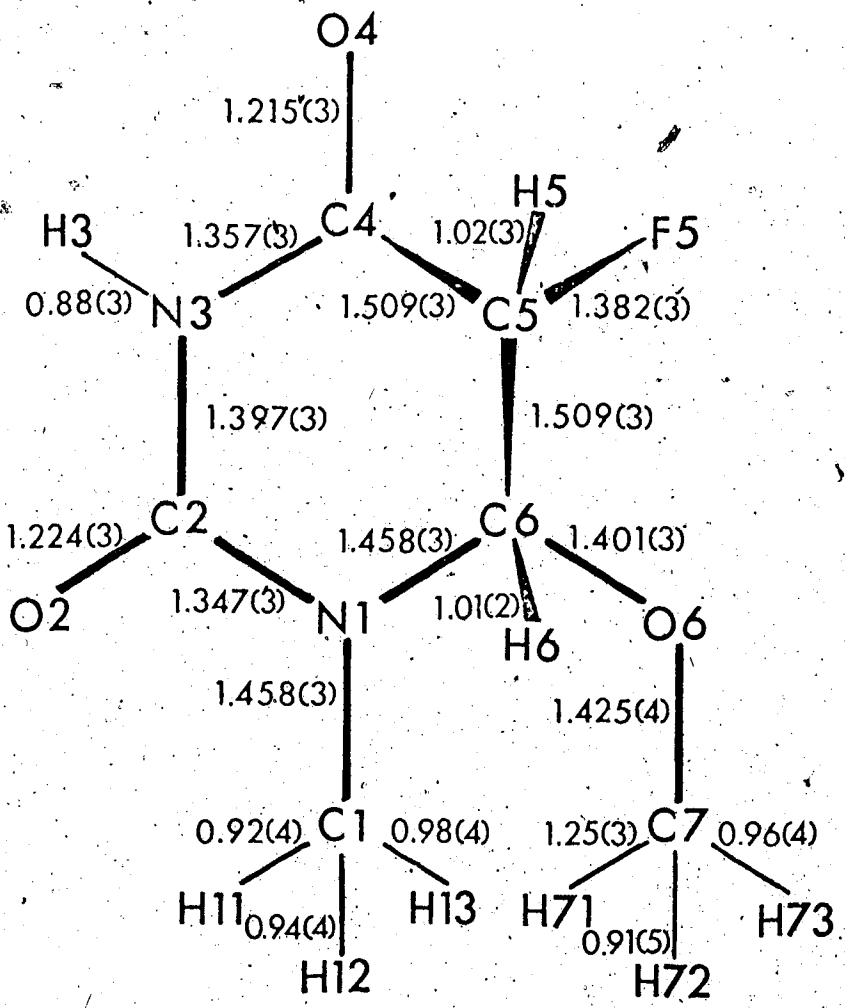


Fig. II 1-3(a) - The bond lengths of 1-methyl-5-fluoro-6-methoxy-5,6-dihydrouracil

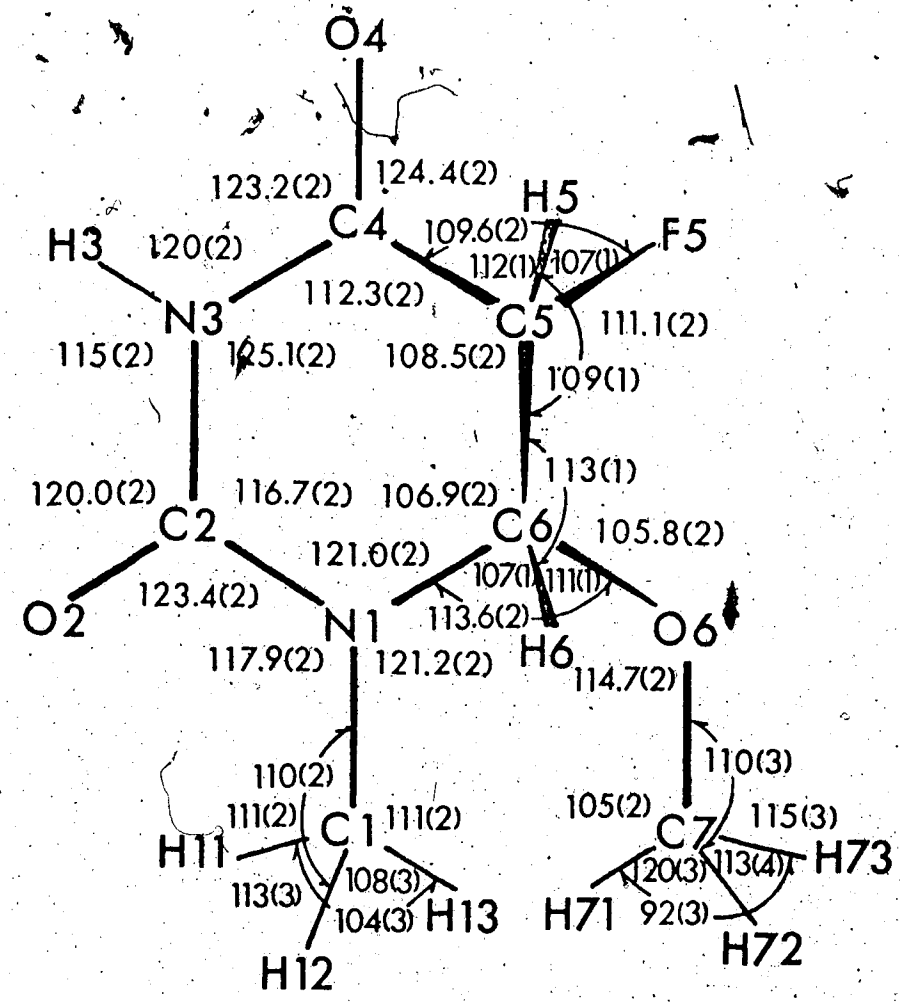


Table II 1-3(b) The bond angles of 1-methyl-5-fluoro-6-methoxy-5,6-dihydrouracil

in the dihydrothymine (Furberg & Jensen, 1968) and the dihydrothymidine (Konnert, Karle & Karle, 1970) structures.

		angles N3-C4-C5	C4-C5-C6	C5-C6-N1
dihydrouracil		115.1(3)	112.3(3)	110.3(3)
dihydrouridine	A	115.9(5)	111.0(5)	110.8(3)
	B	115.9(5)	111.0(5)	109.4(5)
dihydrothymine		113.4(3)	108.1(15)	108.8(-)
dihydrothymidine		114.8(10)	109.6(10)	110.6(10)
this compound		112.3(2)	108.5(2)	106.9(2)

The most likely reason for this particular similarity in geometry is due to the substitution on atom C(5) of the ring by methyl in the two dihydrothymine structures compared with the substitution by fluorine in the present compound.

Taking account of the estimated standard deviations in the related molecular structures, there were no disagreements in the bond distances. In spite of the introduction of the substituents, the bond lengths agree with those of dihydrouracil.

There is significant difference in the two carbonyl bond lengths, C(2) - O(2) = 1.224(3) Å and C(4) - O(4) = 1.215(3) Å (3σ). This same difference was observed in the structure of dihydrouracil, where C(2) - O(2) = 1.222(5) Å and C(4) - O(4) = 1.211(5) Å. A likely reason for these differences is that the oxygen atoms O(2) accept hydrogen bond(s), whereas the oxygen atom O(4) has no hydrogen bonding interactions. A similar situation was discussed in the

structures of barbital (Craven, Casatis, Cartland & Vizzini, 1969), and these observations support their arguments.

The bond length C(5) - C(6) seems to be shorter than those of the ordinary $C(sp^2) - C(sp^3)$ bonds, 1.541(3) Å (International Tables for X-ray Crystallography, Vol III), but the average bond length of the related dihydrogenated compounds is the same, 1.509(3) Å. The bond length C(5) - F(5), 1.382(2) Å, agrees with that of the simple parafinic mono-fluorine compounds, such as CH_3F, CH_3CH_2F, etc. The average value for these lengths in these compounds is 1.381(5) Å (International Tables for X-ray Crystallography, Vol III), but the bond length in monofluoroacetamide, CH_2FCONH_2, which has similar fragment to this compound was 1.406(5) Å (Hughes & Small, 1962). The average value of the bond lengths in 5-fluorouracil and its derivatives is 1.346(4) Å (Voet & Rich, 1969; Kim & Rich, 1968; Mazza, Sobell & Kartha, 1969; Harris & Macintyre, 1964). Therefore, it appears that if carbon atom C(5) is an sp^3 carbon then the C - F bond length increases by 0.04 Å over that observed in 5-fluorouracil derivatives where C(5) is an sp^2 hybridized carbon.

At first sight it appears that the atoms N(1), C(2), O(2), N(3), C(4) and O(4) might be involved in an extensive delocalized π-electron system similar to that observed in uracil (Stewart & Jensen, 1967). In that structure and other uracil derivatives this delocalization is exhibited in the equality of the bond lengths N(1) - C(2), C(2) - N(3) and N(3) - C(4) viz. 1.371(2), 1.376(2) and 1.371(2) Å respectively. A similar situation regarding this delocalization was observed in 1-methyl thymine (Hoogsteen, 1963). In uracil and 1-methyl thymine all

of the above atoms were co-planar (maximum deviation from the molecular plane was 0.007 Å, atoms N(3) and O(4) in uracil). However, this deviation is not significant.

On the other hand in the present compound these three bonds are decidedly unequal with bonds N(1) - C(2), 1.347(3) Å and N(3) - C(4), 1.357(3) Å significantly shorter than bond C(2) - N(3), 1.397(3) Å. This trend is also exhibited in the other compounds which have sp^3 carbon atoms at positions 5 and 6 in the pyrimidine ring (see Table II 1-4). Also presented in this table are the torsion angles about the bond C(2) - N(3). From these values it is apparent that there is no systematic variation of this torsion angle for these several compounds. In addition the conformations of the dihydrogenated rings exhibit no systematic trends. However, when one considers the displacements of the atoms C(5) and C(6) from the best planes computed from the coordinates of the atoms N(1), C(2), N(3), C(4) (Table II 1-4) for these several compounds, it can be seen that in all cases, except where the substitution on C(5) is a particularly electronegative atom (fluorine in this compound), the atom furthest from this plane N(1), C(2), N(3), C(4) is C(6). In addition the present compound is substituted on C(6) by a methoxy group which is also a major difference among these several ring systems. The apparent anomaly, that of dihydrothymine in which both C(5) and C(6) have almost equal displacements from this plane, is explained by the disorder of the atoms C(5) and C(6) in the crystals of this compound.

Table II 1-4 gives the bond lengths, N(1) - C(2), C(2) - N(3) and N(3) - C(4), for these several compounds in all cases the bond C(2) -

		torsion angle N(1)-C(2)-N(3)-C(4)	displacement from best plane N(1),C(2),N(3),C(4) C(5)	C(6)	bond lengths N(1)-C(2)	C(2)-N(3)	N(3)-C(4)
di H uracil		11.1°	-0.141 Å	0.449 Å	1.335(5) Å	1.395(5) Å	1.364(5)
di H uradine	A	10.2°	0.166	-0.466	1.365(5)	1.389(5)	1.364(5)
	B	-17.4°	-0.001	-0.632	1.351(5)	1.393(5)	1.371(5)
di H thymine		-1.8°	-0.396	0.325	1.326(2)	1.383(2)	1.358(3)
di H thymidine		12.6°	0.266	-0.411	1.347(10)	1.387(10)	1.370(10)
this compound		-16.0°	0.499	-0.298	1.347(3)	1.397(3)	1.357(3)

The signs of the displacements are arbitrary in each structure.

Table II 1-4 The geometries of the dihydrouracil derivatives

N(3) is considerably longer than the other two bonds. The fact that C(5) and C(6) are sp^3 carbon atoms may lead to the redistribution of the delocalized electron cloud over these atomic centers. It does appear from the bond lengths that there are two major regions for the delocalization of the atom groupings N(1), C(2), O(2) and N(3), C(4), O(4). The attempt to correlate the torsion angle variation with this delocalization is not convincing due to the variation of the torsion angle about C(2) - N(3) (Table II 1-4). Thus the bond length differences involving the three C - N bonds can not be explained by a simple torsion angle dependence of the overlapping π electron systems. The details of the least squares planes for 1-methyl-5-fluoro-6-methoxy-5,6-dihydrouracil are given in Table II 1-5. The equation of the best plane through the atoms N(1), C(2), N(3), C(4) has not been included as these atoms are not coplanar (χ^2=2312). Rather the two planar sections have been considered, and the angle between these planes (plane I N(1), C(2), O(2), N(3) and plane II N(3), C(4), O(4), C(5)) is 21.5°. It is clear that there is a large torsion angle about the bond C(2) - N(3) (see Fig. II 1-4). This angle probably results from the fact that the two atoms C(5) and C(6) are saturated sp^3 carbon atoms. In addition the fact that the bond C(2) - N(3) has more single bond character than the bonds N(1) - C(2) and N(3) - C(4) allows for a relatively large torsion angle here.

Atom	Plane I	Plane II	Plane III
N1	-0.0004(7)*Å	-0.631 Å	
C1	0.0063	-0.735	
C2	0.0011(7)*	-0.142	——— *
O2	-0.0004(7)*	0.165	——— *
N3	-0.0003(7)*	0.003(6)*	——— *
C4	0.304	-0.009(6)*	
O4	0.180	0.003(6)*	
C5	0.844	0.002(6)*	
C6	-0.047	-1.025	
H3 i)			-0.36
N3 i)			-0.339
χ^2	0.41	21.58	———

i) x, ½-y, z

equations

Plane I $4.30520x + 6.56347y - 6.62860z = -0.77618$

II $-3.10972x + 9.12247y - 6.35270z = -0.60873$

III $4.25929x + 6.57433y - 6.63076z = -0.77771$

Table II 1-5 Least-squares planes for 1-methyl-5-fluoro-6-methoxy-5,6-dihydrouracil

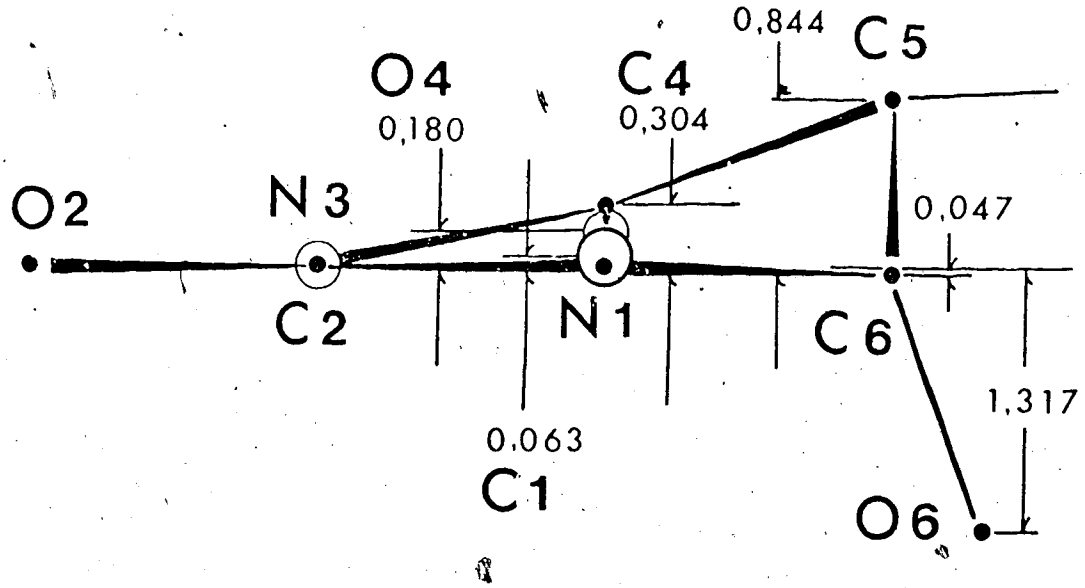


Fig. II 1-4 The displacements of the atoms from the least-squares plane defined by the atoms N(1), C(2), O(2) and N(3)

Fig. II 1-4 shows that the six membered ring closely approximates a half chair conformation with atom C(5) furthest from the plane of the ring. In this conformation the fluorine atom is in a quasi-equatorial orientation and as seen in Fig. II 1-5 the torsion angle about the C(4) - C(5) bond (i.e. F(5) - C(5) - C(4) - O(4)) is very small, 13.5°. This is rather unexpected since one might conclude that because there are two adjacent electronegative substituents the torsion angle C(4) - C(5) would be large ca 90°. A conformation in which the fluorine atom F(5) was in an axial orientation would afford this large angle.

Another unexpected result is seen in the torsion angles about the bond C(5) - C(6). Here all angles are very close to the perfect staggered conformation of 60° and in fact are closer to this value

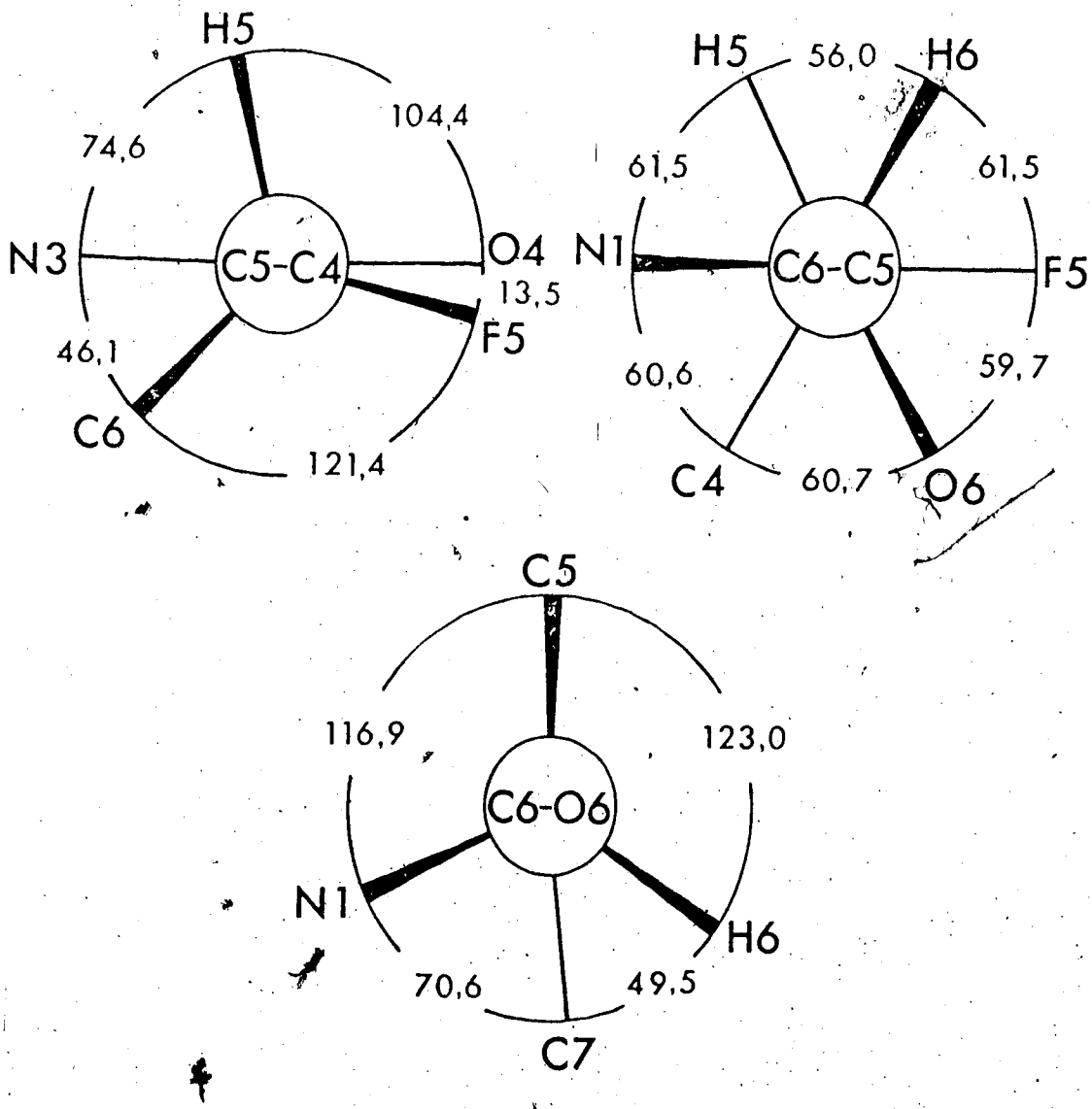


Fig. II 1-5 Newman projections of the torsion angles of 1-methyl-5-fluoro-6-methoxy-5,6-dihydrouracil

than the torsion angles exhibited by pyranose rings, notwithstanding the fact that the present compound has part of the six-membered ring involved in a conjugated system.

Consider that the atom system in this compound C(7) - O(6) - C(6) - N(1) is analogous to the atom system in ribose rings C(4') - O(1') - C(1') - N. In this situation atom O(6) is analogous to the ring oxygen atom O(1'), and atom C(6) is analogous to the anomeric carbon atom C(1'). That the anomeric effect influences this atomic system can be seen from the fact that the two bond lengths C(6) - O(6), 1.401(3) $\mathring{A}$ and C(7) - O(6), 1.425(4) $\mathring{A}$ are unequal. This same phenomenon is observed in the ribose ring systems of purine and pyrimidine nucleosides and nucleotides at the ring oxygen atom. Frequently in X-ray work variations of conformation are glossed over by referring to influences of molecular packing on the conformation observed in single crystal structures. However, an alternative explanation may be considered in the present case and the following argument, based on the C - O bond length dependence and conformation observed in sugar molecules, may offer at least a qualitative explanation for the torsion angle around the bond C(6) - O(6).

As discussed in Chapter I, Jeffrey et al., found a potential energy minimum at the gauche-gauche conformation around the carbon-oxygen bonds of the methanediol system by ab initio molecular orbital calculations (Jeffrey et al., 1972). They drew attention to the similarity of this system with that of the α and β methyl glycosides in single crystal structures. As the theoretical calculations predicted, the methyl groups were gauche with respect to the ring oxygen

and trans with respect to C(2). In addition, the molecular orbital calculations have been applied to the system HO - CH_2 - NH_2 which has an oxygen and a nitrogen atom bonded to the same carbon atom instead of two oxygen atoms on both sides of the carbon atom, (Pople & Radom, 1973). The energy minimum was calculated at the eclipsed position with respect to the bonds O - H and C - N. It is no doubt partially stabilized by a distorted hydrogen bond from the hydroxyl hydrogen to the nitrogen lone pair of electrons. Whereas the present molecule does not have a hydroxyl hydrogen atom and also the nitrogen lone pair electrons is delocalized by the adjacent carboxyl group the results of Pople and Radom, (1973) can be applied with caution. Therefore, this molecule is more close to the system of nucleosides, where the nitrogen lone pair of electrons are delocalized and the oxygen atom is one of the atoms in ribose ring. The torsion angle N(1) - C(6) - O(6) - C(7) would show the favorable torsion angle in the system N - C - O - C in which the nitrogen lone pair of electrons is delocalized. The methyl carbon atom C(7) is trans with respect to the carbon atom C(5), 172.5° and gauche with respect to the nitrogen atom N(1), 70.6°. This observed angle is close to the 60° angle in the methanediol system. The nitrogen atom can also affect the torsion angle around the carbon C(1') oxygen O(1') bond as does an exocyclic oxygen atom in monosaccharide molecules.

Two molecules of 1-methyl-5-fluoro-6-methoxy-5,6-dihydrouracil are joined together by two hydrogen bonds (Fig. II 1-2) to form dimers. The molecules are related by the two fold symmetry of the inversion tetrad axis. Each molecule donated the hydrogen atom H(3)

to the carbonyl oxygen atom O(2) of the other molecule. The interatomic distances are 1.95(3) Å between the hydrogen atom and the oxygen atom, and 2.814(2) Å between the nitrogen atom and the oxygen atom. The angles are 170(3)° for N - H...O and 117.3(8)° for C - O...H. The angle between the planes through the atoms C(2), O(2), N(3) in one molecule and in the other molecule is 43.6°. Such a large angle suggests large deviations of the hydrogen and nitrogen atom positions from the best plane of this system in the other molecule. However, they are not displaced far from the plane; 0.36 Å for the hydrogen atom and 0.339 Å for the nitrogen atom.

The stereoscopic molecular packing diagram is shown in Fig. II 1-6. The dimers which are hydrogen bonded are stacked around inversion tetrad axis. There is no short contact between the stacked columns. Some relatively short contacts are bound within a single column (Table II 1-6).

The distances are 3.177(3) Å and 3.180(3) Å from the oxygen atom O(4) to the nitrogen atom N(1) and the carbon atom C(2) of the molecule (-1/4+y, 1/4-x, 1/4-z), respectively. In addition, the carbon atom C(2) and the nitrogen atom N(3) make a contact with the oxygen atom O(2) in the neighbouring molecule (-1/4+y, 1/4-x, 5/4-z). These distances are 3.250(3) Å and 3.320(3) Å, respectively.

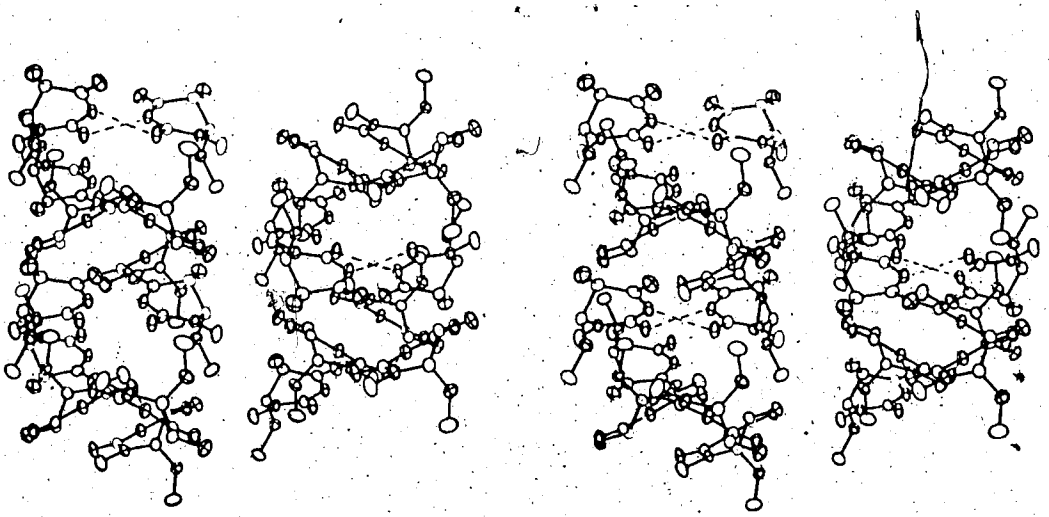


Fig. II 1-6 Molecular packing in the crystals of 1-methyl-5-fluoro-6-methoxy-5,6-dihydrouracil (Johnson, 1965)

Atom A	Atom B		Distances (Å) A....B
O4	N1	(-1/4+y, 1/4-x, 1/4-z)	3.177(3)
O4	C2		3.180(3)
C2	O2	(-1/4+y, 1/4-x, 5/4-z)	3.250(3)
N3	O2		3.320(3)

Table II 1-5 Contacts of the molecules in the crystals of 1-methyl-5-fluoro-6-methoxy-5,6-dihydrouracil

Chapter III

The Short Intramolecular Hydrogen Bond of Hydrogen Maleate Monoanion.

Part 1

The Crystal and Molecular Structure of Imidazolium Maleate

1-1 Introduction

The nature of hydrogen bond has been studied for a long time (a review, Hamilton & Ibers, 1968). The name 'hydrogen bond' refers to the group of three or more atoms which are in a configuration A-H...B. Atoms A and B are electronegative atoms which have a strong affinity for the electron cloud on the hydrogen atom. The common atomic species for A and B are oxygen, nitrogen and halogens. The distance H···B is less than the sum of the Van der Waals radii of H and B minus 0.2Å (Hamilton, 1968). The preferred orientation of the atoms is linear, but the maximum allowable angle H-A ···B is near 30° (Donohue, 1968). The energy of the ordinary hydrogen bonds is about 5 to 10 Kcal/mole (Coulson, 1961). This amount of energy is far below covalent single bonds of about 100 Kcal/mole (Kerr & Trotman-Dickenson, 1967). Some hydrogen bonds have 30~60 Kcal/mole. For example, the energy of F...H...F hydrogen bond is 58 ± 5 Kcal/mole (Waddington, 1958). These are called strong hydrogen bonds, and the distance between the two atoms on both sides of the proton are shorter than those in weak hydrogen bonds. Such short hydrogen bonds have been found in inter- and intra-molecular systems. The well known systems are the $(FHF)^-$ bonds and the intramolecular hydrogen bonds of maleic acid and its derivatives.

The carboxyl groups are forced to approach each other by the planar cis-configuration of the double bond of the ethylene system. Accordingly as the hydrogen bonds are strong and the distances between atoms A and B are short, the potential energy well profiles for the proton are changed from a double minimum to a single minimum. If the atom A and atom B are the same species and the hydrogen bond system is isolated, the potential energy levels at the double minima are equal. It is believed that the proton in the crystal structures of potassium dihydrogen phosphate (Bacon & Pease, 1953 and 1955) and the deuterium in the crystal structure of $DCrO_2$ (Hamilton & Ibers, 1963) are in one of the double minima potential energy well and the proton in the bifluoride ion are in a single minimum potential energy well (Peterson & Levy, 1952; Ibers, 1964; McGraw & Ibers, 1963). Between these two cases the potential energy function is often described as a broad well. Kollman and Allen calculated the energetic problems in the strong hydrogen bonds (Kollman & Allen, 1970). They found that in the bifluoride ion system the potential energy profile was a single minimum at an F...F distance of 2.249Å and a double minima with a very low energy barrier at an F...F distance of 2.381Å. In addition in the $H_5O_2^+$ system the potential energy profile was a sharp single minimum at an O...O distance of 2.302Å, a double minima at an O...O distance of 2.487Å and at the intermediate distance of 2.381Å the energy profile became an extremely shallow single minimum with the lowest energy level. These may be regarded as a fundamental description of strong hydrogen bonding character. But the calculations that determined the potential energy profiles were normally carried out without consideration of the environment of the hydrogen bond atomic grouping.

One may look in detail at strong and short hydrogen bonds to examine the effects of environment in crystal structures where the environment has been expressed by the positional parameters.

The distances of the hydrogen bonds of bifluorides were about 2.27Å for the F...F separations and the hydrogen atoms in the structures were located in the middle of the bonds (Peterson & Levy, 1952; Ibers, 1964; McGraw & Ibers, 1963; McDonald, 1960). But these studies have been carried out mainly on the metal salts of the bifluoride anion and the anions were located in symmetric environments in these structures.

Another example of short symmetric hydrogen bonds is found in potassium hydrogen or deuterium bis (trifluoroacetate) (Macdonald & Speakman, 1972). The O...O separations were 2.437(4)Å for the hydrogen derivative and 2.437(3)Å for the deuterium derivative. It showed that the hydrogen or the deuterium atom in the hydrogen bonds was on a center of symmetry by which the oxygen atoms of these bonds were related with each other. Thus the functional oxygen atoms were surrounded by identical environments.

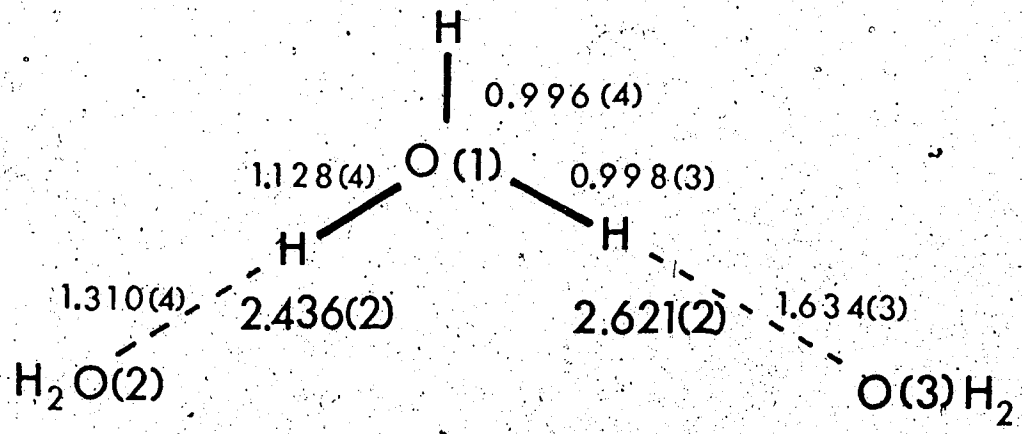
In these structure discussed above we have examined hydrogen bonds which occur between the same elements in equivalent states. In these cases some of the parameters from the environments need not be taken into account. On the other hand some strong hydrogen bonds were observed to be asymmetric in crystal structures. Here the same electronegative atoms in a hydrogen bond were in different environments.

At first, let us check some short hydrogen bonds between the same atomic species in different kinds of molecules. The atoms might be in different electronic states and energy levels in the structures. The

structure determinations by neutron diffraction studies were chosen to consider the accurate parameters of the hydrogen atoms, because the hydrogen atom parameters are believed to be more precise from neutron diffraction data than X-ray data.

The character of the oxygen atoms of the hydrogen bond may be apparently different in the complex of urea: phosphoric acid, since one of the oxygen atoms belongs to urea and the other to phosphoric acid (Kostansek & Busing, 1972). The distance between them was 2.421(3)Å and the distances from the hydrogen atom were 1.223(6)Å to the oxygen atom of the phosphoric acid and 1.207(6)Å to the uronic oxygen atom. The angle of oxygen-hydrogen-oxygen was 169.9(4)°. These oxygen-hydrogen distances are different but only at the 3σ level of confidence.

The solvated hydronium ion, $H_7O_3^+$, in the structure of picrylsulfonic acid trihydrate, $C_6H_3(NO_2)_3SO_3H.3H_2O$, showed that one of the hydrogen bonds had a distance of 2.436(2)Å between the oxygen atoms O(1) and O(2) (Lundgren & Tellgren, 1974).



The hydrogen atom H(2) was located 1.128(4)Å from the oxygen O(1) of the oxonium ion. The oxygen atom O(1) was coordinated by three hydrogen atoms and the oxygen atom O(2) which accepted the hydrogen bond had covalent bonds with two hydrogen atoms. Although the hydrogen bond distance was short, it could be distinguished that the oxygen atoms were in different electronic states.

These two hydrogen bonds in the urea: phosphoric acid complex and the hydronium ion were strong enough to have a single and broad energy well because of the short distances but they were observed to be asymmetric.

In the next case, we have the same elements on both sides of a hydrogen bond but in different ionic environments. In the structure of p-toluidinium bifluoride, $CH_3C_6H_4NH_3^+,(FHF)^-$, the authors found that the distance of one side of the F...H bond in the bifluoride ion was significantly shorter than that of the other side, F(1)-H=1.235(6)Å, F(2)-H=1.025(6)Å and F(1)...F(2)=2.260(4)Å (Williams & Schneemeyer, 1973). They pointed out that this difference came from the environmental differences around the fluorine atoms. The near-neighbors around F(1) were two hydrogen atoms of two $-NH_3^+$ groups. The distances of F...H were 1.608Å and 1.675Å. Also the near-neighbors around F(2) were two hydrogen atoms of two other $-NH_3^+$ groups. But the distances of F...H were longer than those around F(1). These distances were 1.777Å and 2.518Å.

A similar observation was found in the structure of a nickel chelate molecule with an intramolecular asymmetric hydrogen bond with an O(1)...O(2) distance of 2.420(3)Å (Schlemper, Hamilton & LaPlaca, 1971). The authors discussed the asymmetry of the hydrogen bond. The distances

of O(1)...H and H...O(2) were 1.242(5)Å and 1.187(5)Å respectively. The shorter O...H distance was associated with the longer N-O(2) covalent bond distance of 1.346(2)Å. On the other hand, the longer O...H distance was associated with the shorter N-O(1) distance of 1.335(2)Å. Although the analysis of the thermal motion led to a conclusion that the hydrogen atom moved in a broad and single minimum potential well, they pointed out that the surroundings of the two oxygens were not identical and that the small change in intermolecular environment could lead to significant asymmetry in the hydrogen bond.

Maleic acid has a short hydrogen bond between the carboxyl groups joined by a ethylene double bond in cis-configuration. The hydrogen bond distance between the oxygens was 2.502(1)Å (James & Williams, 1974a). The monoanionic form of this molecule maintains the bond which is believed to be symmetrical. The hydrogen atom may lie in a broad single energy well. To estimate the electronic states on the oxygens and the negative charge distribution over the molecule, one has to have observable values which are to be influenced by the environmental factors. For these purposes the bond lengths and angles are good indicators in the system of the maleate monoanion. Dunitz and Strickler summarized the geometry of carboxyl groups (Dunitz & Strickler, 1968). The average bond lengths are 1.229Å for carbon oxygen double bond and 1.309Å for carbon oxygen single bond. The average bond angles are 122.7° for C-C=O and 115.0° for C-C-OH. When the carboxyl group dissociates the acidic hydrogen, the negative charge is usually distributed to both oxygens and they are charged half negatively. The bond lengths between the carbon and the oxygen atoms in such ionized carboxyl groups have an intermediate value of the double and single bond lengths. Those have

been observed in the range of 1.250Å to 1.293Å in the structures of disodium maleate (James & Williams, 1974b) and dilithium maleate (Town & Small, 1973). The differences of the bond lengths and angles are fortunately significant in accurate structure determinations by X-ray diffraction analyses at present, in spite of the underestimated standard deviations associated with them. Of course, the charge would not equally be distributed over the oxygen atoms, but the distribution should make the total energy lower. This freedom may allow that the environmental effects would appear on the molecular dimensions and the electronic state of the atoms.

In the maleate monoanion system the observable values of the bond lengths and angles and the hydrogen position should hopefully show the small differences in the dimensions of the carboxyl group in a crystal if the carboxyl groups were in different environments. In contrast with this, there should be no difference if the carboxyl groups are in similar environments.

The structure determination of potassium hydrogen maleate has been reported by Darlow and Cochran (1961) and by Peterson and Levy (1968). It was shown that the crystals belonged to the space group of Pbcm and that a mirror plane in the crystal structure was right across the maleate moiety. This means that the two carboxyl groups are related by mirror symmetry and there are two possible positions for the hydrogen atom. In one case the proton sits exactly on the mirror plane, and in the other the proton is disordered on either side of the mirror plane corresponding to either a double minima well or statistical disorder in the molecules of the crystal. An independent X-ray study (P. Codding, personal communication) could not distinguish between these two cases

nor could a model which did not include the mirror symmetry be deduced. One of the ways to overcome this difficulty was the substitution of a chlorine atom on the double bond. The structure determination of potassium hydrogen chloromaleate was carried out by the neutron diffraction method (Ellison and Levy, 1965). It clearly showed that the hydrogen in the intramolecular hydrogen bond lay at the middle of the oxygen-oxygen separation. It seems that the introduction of the chlorine atom and the differences of the arrangement of the potassium cations around the halves of the molecule does not effect the hydrogen atom position. But some differences in the carboxyl group bond lengths were detected. It is not certain that the discriminations come from the substitution of the chlorine atom or from the environmental differences.

Three crystal structures, so far, have been reported in which the maleate monoanion was present with organic cations in the crystals. These were the crystals of dl-Bromopheniramine maleate (James & Williams 1971), (+)-Chloropheniramine maleate (James & Williams, 1974c), Methoxypromazine (Marsau & Gauthier, 1973). The mirror symmetry was broken in them. One of the carboxyl groups was bound with a hydrogen bond to an amino group of the cationic molecules and the other carboxyl group was free from an intermolecular hydrogen bond. The atomic parameters determined by X-ray diffraction studies suggested the offset of the hydrogen electron density in the intramolecular hydrogen bond in the three structures.

The molecular geometries of the maleate molecules showed that the oxygen atoms with the longer C-O bond lengths were closer to the hydrogen atom than those with the shorter C-O bonds in the hydrogen bonds and that the differences of the C-O bond lengths in a carboxyl group which was

bound by an intermolecular hydrogen bond were less than those in the other free carboxyl group. That is, there was a clear difference in the bond characters of the C-O bonds.

The crystal structure of imidazolium maleate was hoped to get clear the effect of the environments on the short hydrogen bond and the molecular geometries.

Since the dissociation constants of the maleic acid are pK_{a1}= 1.83 and pK_{a2}=6.07 at 25°C and the dissociation constant of imidazole is pK_a=6.953 at 25°C (Weast et al., 1968). It can be expected that the maleic acid becomes maleate monoanion in the crystal in a 1:1 ratio.

1-2 Experimental

Equal molar amounts of imidazole and maleic acid were mixed together in methanol. The principal salt compound was crystallized by evaporating the solution. The preliminally oscillation and Weissenberg photographs showed that the crystals belonged to the space group, $P2_1/c$, in the monoclinic system. Systematic absences were noted as $0k0$; $k=2n+1$; $h0\ell$; $\ell=2n+1$. It was noticed that there was a small amount of crystals with a different habit in the bottom of the crystallization container. They belong to the space group of $P2_12_12_1$ and their cell dimensions were $a=7.90\text{Å}$, $b=14.27\text{Å}$, $c=7.24\text{Å}$ measured from the diffraction photographs. Since the cell dimensions were similar with those of N-succinopyridine (see later) and the sizes of the pyridine and the imidazole molecules are similar, the compound in this modification was considered to be the covalently bonded one of imidazole and maleic acid in the presence of the water as moisture or impurity in the solvent.

	a	b	c	space group
imidazole:maleic acid	7.91Å	14.29Å	7.24Å	$P2_12_12_1$
N-succinyl pyridine	7.775(2)	14.974(3)	7.730(1)	$P2_12_12_1$

A single crystal of monoclinic form was recrystallized from methanol solution for the data collection. A crystal of the size 0.3X0.5 X0.15 mm was mounted along the C^* axis on a Picker FACS-1 diffractometer with graphite monochromatized MoKα radiation. The 2θ of the monochrometer was 12.09°. The cell dimensions were deduced from the indices and Bragg angles of 12 reflexions by least squares calculations. The

density of the crystals was measured by flotation method in a mixture of carbon tetrachloride (ρ = 1.58) and chlorobenzene (ρ = 1.10). The crystal data are summarized in Table III 1-1. The intensities were collected out to $2\theta_{max} = 60^{o}$ (λ = 0.7093Å) in a room where the temperature was controlled at 16 ± 1°C. The θ-2θ mode was used at the scan rate of 2.0° a minute over the basic width of 1.50°. The backgrounds were counted at both extreme ends of the 2θ driving for 20 seconds each. Three standard reflexions were measured every 50 reflexions. No crystal decay or moving of the crystal was observed throughout the data collection. The variation of the standards was ±2%.

Since sixteen reflexions were considered to be suffering from the effects of coincidence loss, they were remeasured with an attenuator in position. The attenuator factor of 8.335 was experimentally determined by 10 reflexions measured twice of each with and without the attenuator. These reflexions were estimated not to be suffering from the effect. The intensities were between 10,000 and 15,000 counts per second at the maximum rate without the attenuator.

Although the linear absorption coefficient is 1.29 cm^{-1}, and the size of the crystal was small, the absorption correction curves were empirically obtained around the phi axis for the reflexions 0,0,2; 2θ=5.72° and 0,0,8; 2θ=23.02° (North, Phillips and Mathews, 1968). The correction values were averaged on the values which were 180° apart, for that the translational minor mis-aligment of the crystal is overcome. These absorption curves are shown in Fig. III 1-1.

molecular formula	$C_3H_5N_2^+ \cdot C_4H_3O_4^-$
molecular weight	184.15 Daltons
space group	$P2_1/c$
a	10.869(1)Å
b	5.523(1)Å
c	14.614(3)Å
β	102.85(2)°
V	855.29Å^3
Z	4
D_o	1.426 g/cm^3
D_c	1.426 g/cm^3
$\bar{\mu}$	1.29 cm^{-1}
2θ range explored	$3^\circ \sim 60^\circ$ h k l and $\bar{h}$ k l
no. unique reflexions	2495
no. reflexions for refinement	1621 (65.0%)
final R	0.038
final R_w	0.029

Table III 1-1

Physical constants and other data for imidazolium maleate.

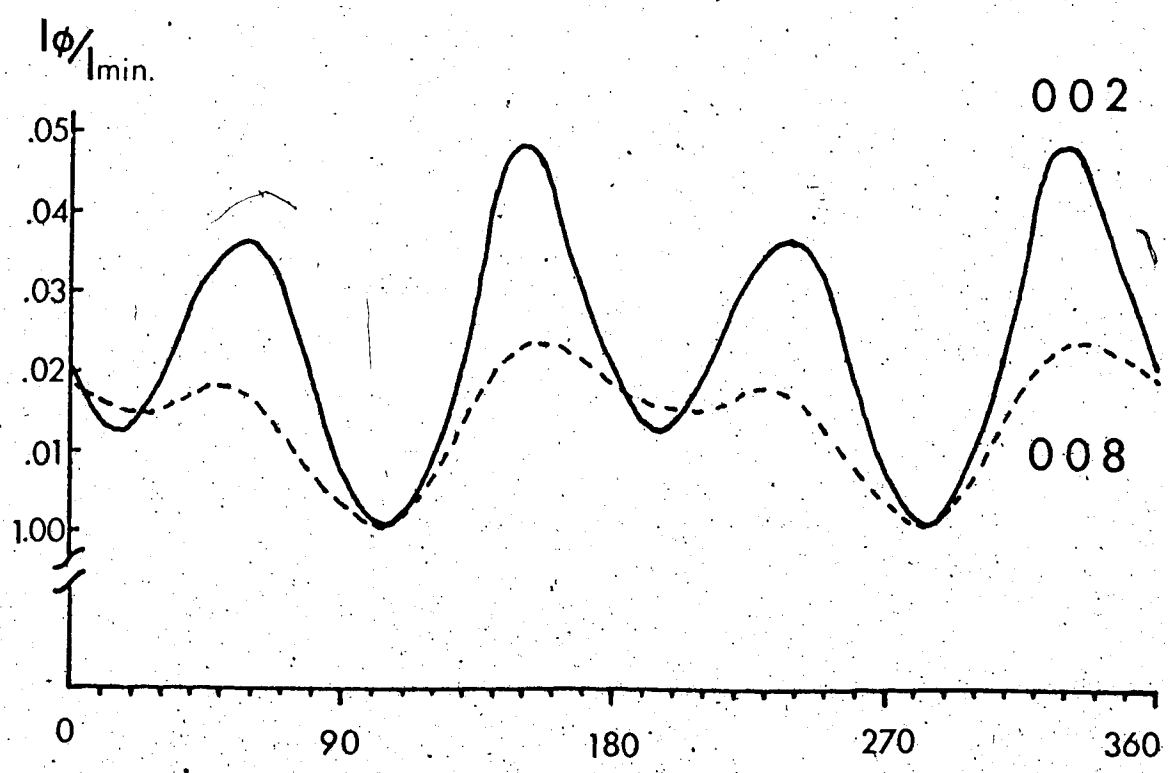


Fig. III 1-1. A plot of relative intensity versus ϕ for the imidazolium maleate crystal.

The transmission curve values from the reflexion 0,0,2 were applied on the data reflexions of which 2θ were less than 10.0°. The data reflexions of which 2θ more than 10.0° were multiplied by the values from the curve for 0,0,8. The intensities were corrected for the appropriate Lorentz and polarization factors and reduced to the structure factors. The standard deviation, $\sigma(I)$, of the intensities were estimated by the following equation

$$\sigma(I) = \{P+C^2P^2+t^2(B_1+B_2+C^2B_1^2+C^2B_2^2)\}^{\frac{1}{2}}$$

where P: total counts of peak

c: instrumental instability coefficient chosen equal to 0.01

t: the ratio of the back ground counting time to the peak counting time.

B_1 and B_2: back ground counts.

The intensities of 2495 unique reflexions with indices h,k,l and $\bar{h}$,k,l were surveyed. A total of 1621 reflexions whose net intensities were greater than $3\sigma(I)$ were considered for the structure solution and refinement. These represented 65.0% of the total reflexions. The molecular forms and atomic numbering of imidazolium maleate are shown in Fig. III 1-2.

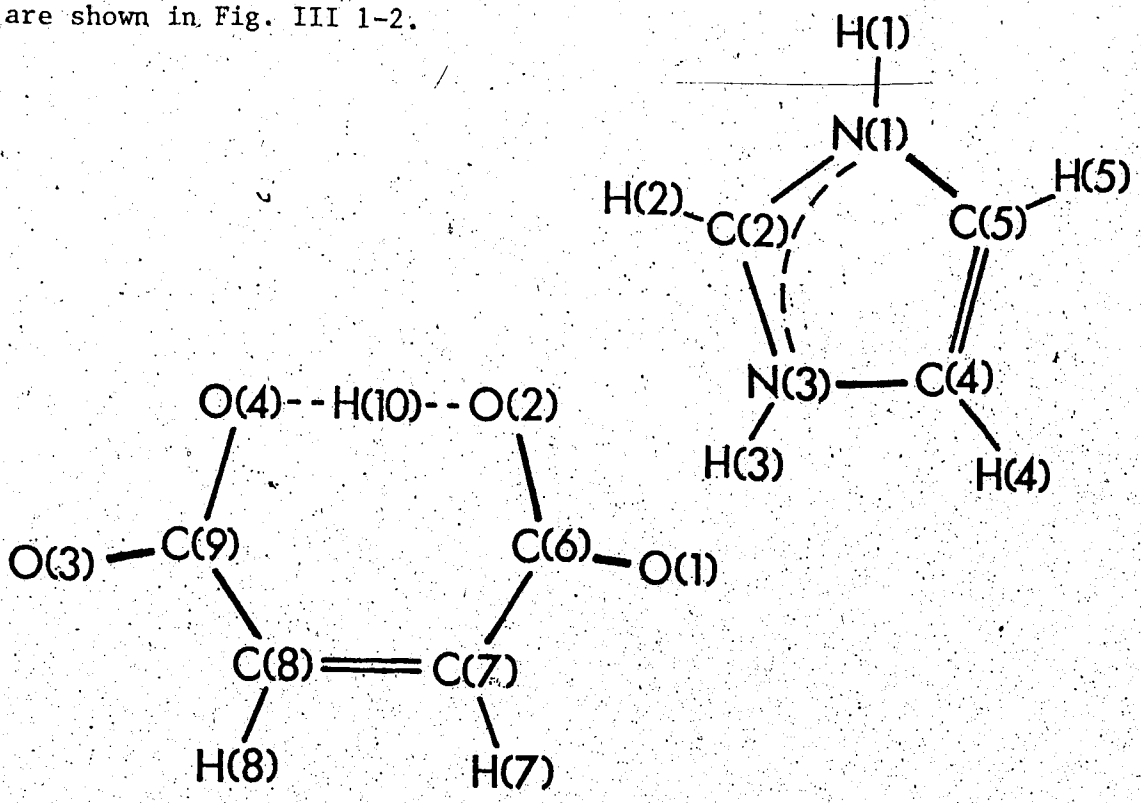


Fig. III 1-2. Molecular forms and atomic numbering of imidazolium maleate

1-3 Solution and Refinement

The overall isotropic temperature factor $B=3.81e/A^2$ and the scale factor K=0.178 were calculated by least squares fit to a Wilson plot (Wilson, 1942) (Fig. III 1-3).

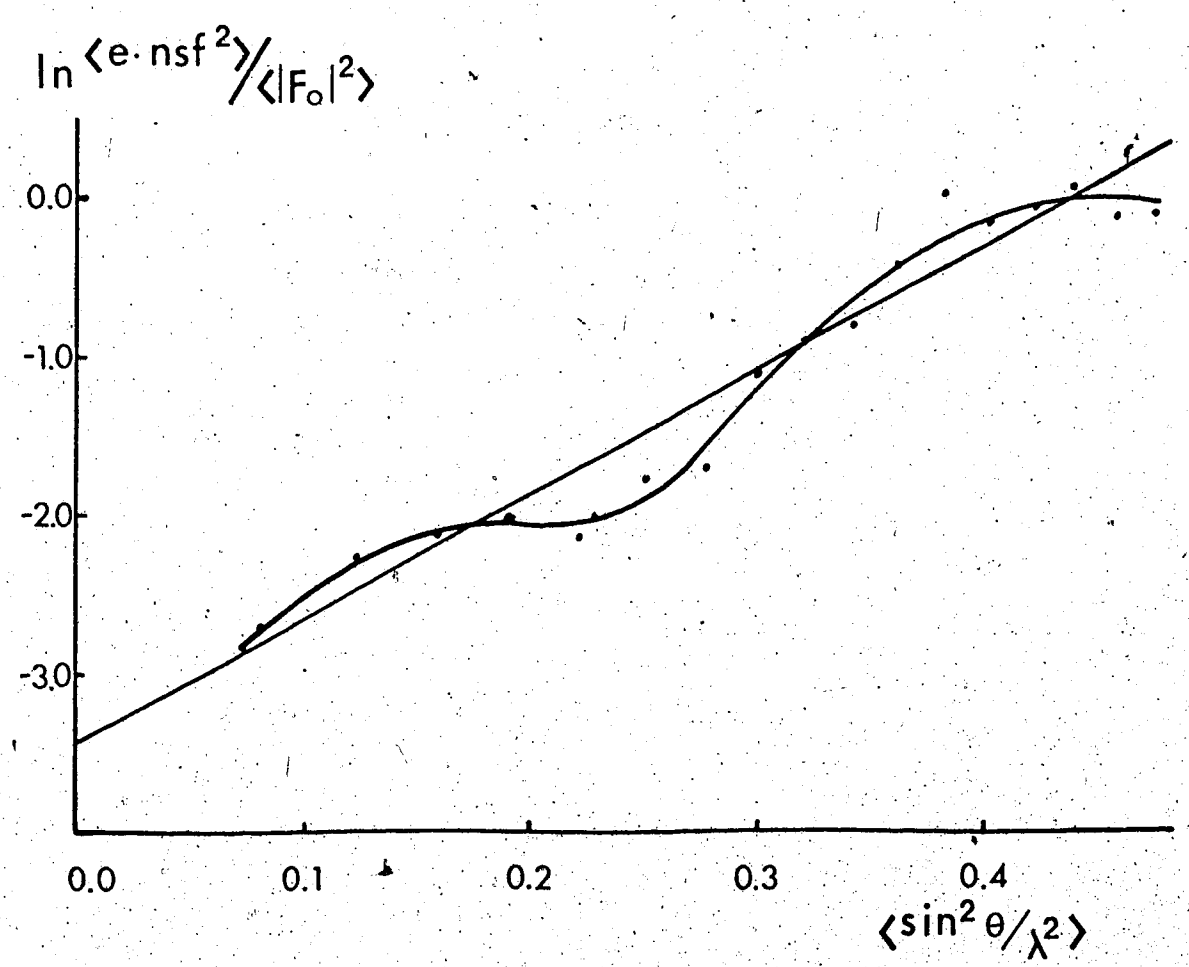


Fig. III 1-3. Wilson plot of imidazolium maleate.

The structure factors were reduced to |E| values (Karle and Karle, 1966). The statistics and the distribution of the |E| values are listed in Table III 1-2

	theoretical		experimental
	centric	acentric	
average value of $\|E\|$	0.798	0.886	0.773
average value of $\|E^2\|$	1.000	1.000	1.000
average value of $\|E^2-1\|$	0.968	0.736	1.008

	theoretical		experimental	
	centric %	acentric %	%	no. of reflexion
$\|E\|>3.0$	0.27	0.01	0.34	8
2.5	1.23	0.19	1.60	39
2.0	4.55	1.83	5.04	127
1.8	7.19	3.92	7.53	187
1.6	10.96	7.73	11.34	281
1.4	16.15	14.09	16.19	400
1.2	23.01	23.69	22.79	564
1.0	31.73	36.79	31.75	788
0.0	100.00	100.00	100.00	2495

Table III 1-2. The theoretical and experimental statistics of the $|E|$ values of imidazolium maleate.

After a failure of the structure determination by the statistical method with 127|E| values which were greater than 2.0, the number of |E| values used was expanded to 187. These |E| values were greater than 1.8. The solution was continued by MULTAN and three origin reflexions were assigned and four other reflexions given symbols. A total of 16 sets of starting phase combinations were examined. The computation by the program PHASE in the XRAY70 system determined 147 to 159 reflexion phases in these 16 sets. It was difficult to pick up the most probable phase combinations, because none of the results from these 16 phase sets was better than any of the others by any criteria.

In addition to the automated phasing by MULTAN hand-phasing (Karle & Karle, 1966) was also undertaken. Three different origin reflexions were chosen and only one additional reflexion assigned a symbol. The summary of the results from the combinations is listed on Table III 1-3.

h	k	l	|E|	combination I	combination II
-5	2	16 *	3.30	+	+
-1	1	3 *	2.84	+	+
-1	1	4 *	2.70	+	+
-9	5	11	2.89	+	-
cycle(s) to refine the generators				1	1
descrepancies in extended phases				2	6
total number of phases determined				167	164
no. positive phases				91	87
no. negative phases				76	77

(* origin defining reflexions.)

Table III 1-3. Comparison between the two sets of the starting phase combinations.

An E map was computed with the phases from the starting phase combination I. It showed the clear figure of the molecular structures with reasonable molecular packing in the unit cell.

The atom species were assigned to the peaks on the E map and the imidazole ring was assumed to be hydrogen bonded to an oxygen of the maleate anion. The structure factors within $2\theta=40^{o}$ were computed. The initial R value was 0.32 and it was reduced to 0.21 after two cycles of full matrix least squares refinement with unit weights for the reflexions and isotropic temperature factors for all non-hydrogen atoms. The R value was tremendously reduced to 0.087 by one cycle of least squares calculations with unit weighting of the 1621 reflexion data and anisotropic temperature factors.

A difference Fourier map showed electron density peaks for the hydrogen atoms, except that of H(10), the intramolecular proton, and [redacted]itive and negative peaks around some non-hydrogen atoms. [redacted]drogen atoms with isotropic temperature factors were in-c[redacted]he next two cycles of the least squares computations with the [redacted]vational weighting scheme $[\sqrt{w} = (2|Fo|)^{\frac{1}{2}}/\sigma(I)]$. Another dif[redacted]ce map was calculated aroung the oxygen atoms O(2) and O(4). A se[redacted]n of the map is shown in Fig. III 1-4.

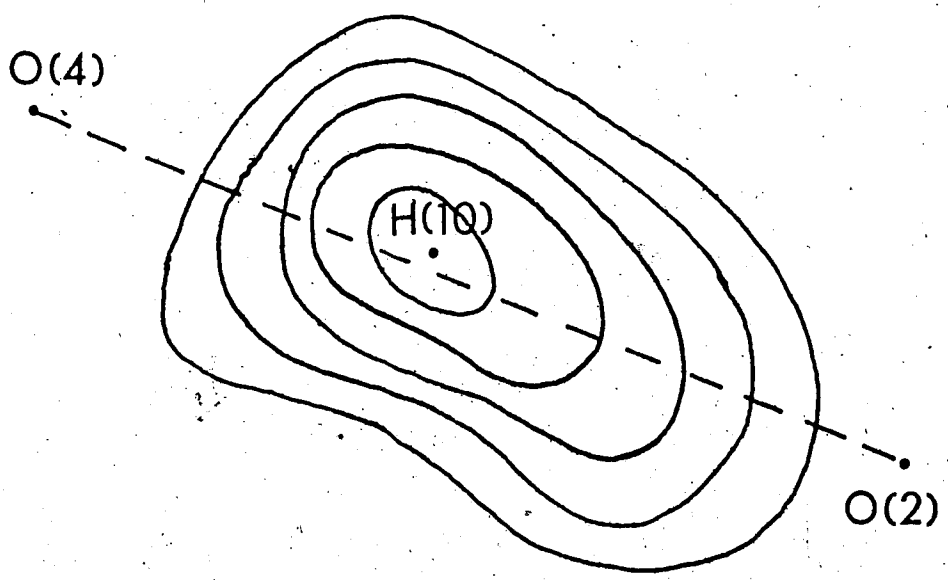


Fig. III 1-4: A section around H(10) in the difference map of imidazolium malèate. The first contour line is drawn at 0.15e/Å^3 and the increment is 0.05e/Å^3.

It revealed a clear but broad electron density peak which was elongated in the direction of the oxygen atoms. It was decided that the temperature parameters of this hydrogen atom should be refined anisotropically.

The full matrix least squares refinement converged at R = 0.036, Rw = 0.071. But it was found that several reflexions with strong intensities and low Bragg angles seemed to suffer from extinction. This factor was taken into account in the further refinement (Zachariasen, 1963). The atomic scattering factors for the oxygen atoms O(2) and O(4) were changed to those of a half negatively charged oxygen atom, because the bond lengths between the carbon and the oxygen atoms were longer than C = O bonds and shorter than C - O bonds. This final refinement procedure affected the hydrogen temperature factors and posi-

tions as well as the non-hydrogen atomic positional parameters. The maximum shift of the interatomic bond distances for non-hydrogen atoms was as large as 0.013(3)Å.

The final secondary extinction coefficient was $0.191(7)X10^{-6}$. The ratio of parameter shifts to those of the parameter error were all less than 0.3. The R, Rw and the goodness of fit were 0.038, 0.029 and 3.021, respectively. The Rw value was greatly improved by the extinction refinement. The structure factors with the phases are given in Table III 1-4. The final positional and thermal parameters for all the atoms are listed in Table III 1-5 (a), (b) and (c). The ORTEP drawing (Johnson, 1965) of two units of imidazole maleate molecules is shown in Fig. III 1-5. The imidazolium maleate is herinafter referred to IMIMAL.

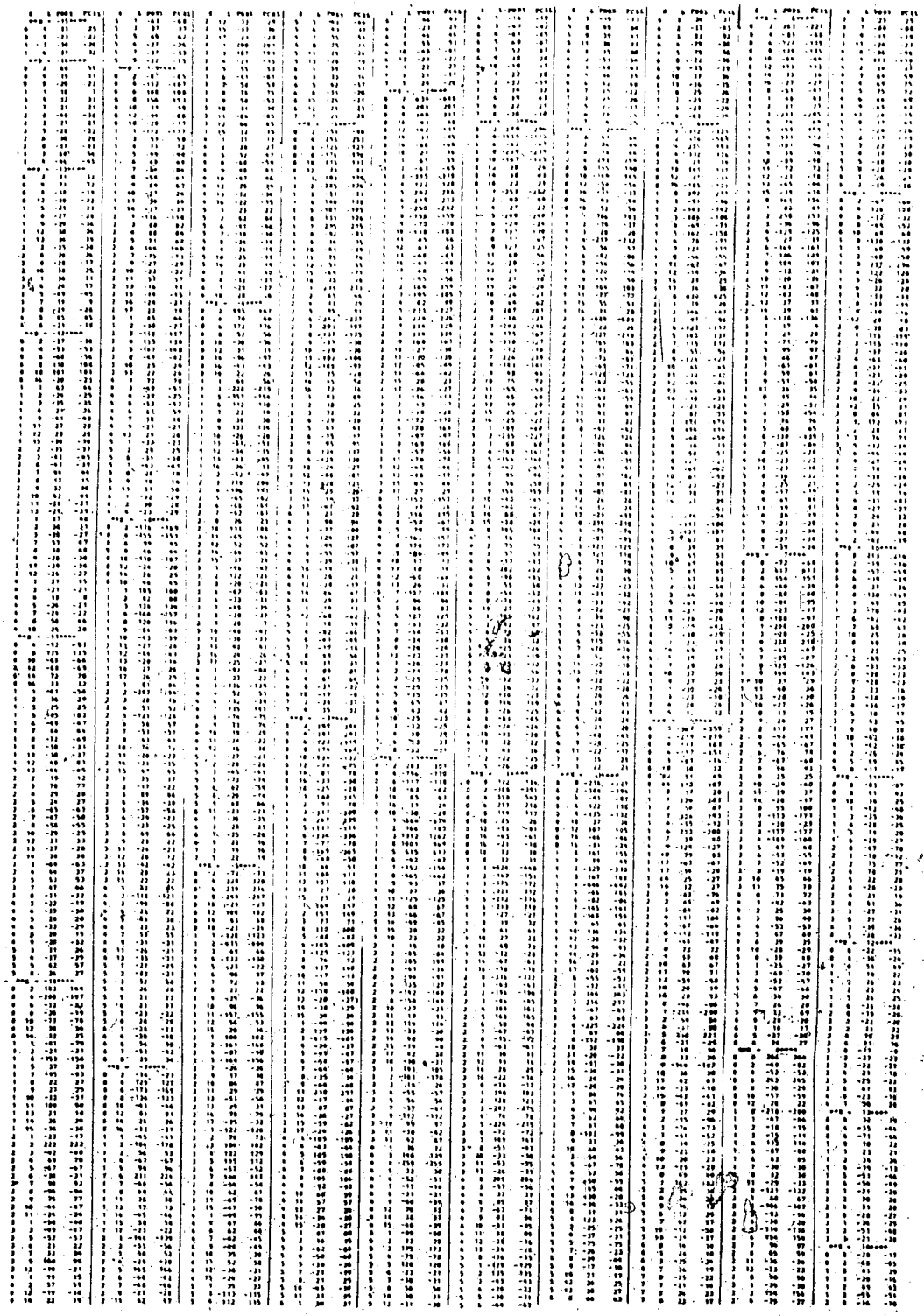


Table III 1-4. The observed and calculated structure factors (absolute scale X 10) which were included in the refinements in the crystal structure of imidazolium maleate.

	x/a	y/b	z/c
N(1)	3970(1)	11518(3)	709(1)
C(2)	3158(1)	9975(3)	920(1)
N(3)	3768(1)	8324(2)	1492(1)
C(4)	5039(1)	8838(3)	1652(1)
C(5)	5162(1)	10826(3)	1166(1)
C(6)	1212(1)	5036(3)	1590(1)
C(7)	234(1)	3154(3)	1605(1)
C(8)	-1005(1)	3185(3)	1264(1)
C(9)	-1838(1)	5106(3)	751(1)
O(1)	2312(1)	4586(2)	1980(1)
O(2)	891(1)	7053(2)	1171(1)
O(3)	-2977(1)	4700(2)	505(1)
O(4)	-1357(1)	7121(3)	563(1)

Table III 1-5 (a) Positional parameters ($X10^4$) of non-hydrogen atoms of imidazolium maleate.

	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N(1)	435(7)	455(8)	511(9)	-45(6)	119(6)	43(7)
C(2)	353(8)	468(9)	552(10)	-39(7)	58(7)	28(8)
N(3)	373(7)	456(8)	526(8)	-72(6)	47(6)	44(7)
C(4)	353(8)	601(11)	540(10)	-23(8)	36(7)	3(9)
C(5)	368(8)	612(11)	532(10)	-102(8)	118(7)	-44(9)
C(6)	405(8)	407(9)	457(9)	-31(7)	80(7)	17(8)
C(7)	473(9)	370(9)	541(10)	-25(7)	89(7)	104(8)
C(8)	446(9)	401(9)	568(10)	-99(7)	98(7)	93(8)
C(9)	411(8)	404(9)	522(9)	-79(7)	91(7)	24(8)
O(1)	370(5)	579(7)	728(8)	-9(5)	-7(5)	134(6)
O(2)	359(5)	441(7)	832(9)	-79(5)	36(6)	162(6)
O(3)	352(5)	593(7)	811(8)	-113(5)	58(5)	138(6)
O(4)	382(6)	454(7)	928(10)	-66(6)	-13(7)	203(7)
H(10)	37 (4)	2 (1)	17 (3)	4 (3)	19 (3)	5 (z)

Table III 1-5 (b) Thermal parameters ($X10^4$) of non-hydrogen atoms and the hydrogen atom, H(10) of imidazolium maleate.

	x/a	y/b	z/c	U_{iso}
H(1)	374(1)	1278(3)	33(1)	52(5)
H(2)	235(1)	1004(3)	73(1)	46(4)
H(3)	337(1)	705(3)	169(1)	71(6)
H(4)	565(1)	787(3)	205(1)	66(5)
H(5)	594(1)	1172(3)	112(1)	66(5)
H(7)	58(1)	173(3)	191(1)	55(4)
H(8)	-150(1)	180(3)	134(1)	56(5)
H(10)	-37(3)	713(4)	83(2)	

Table III 1-5 (c) Positional and thermal parameters ($X10^3$) of the hydrogen atoms of imidazolium maleate.

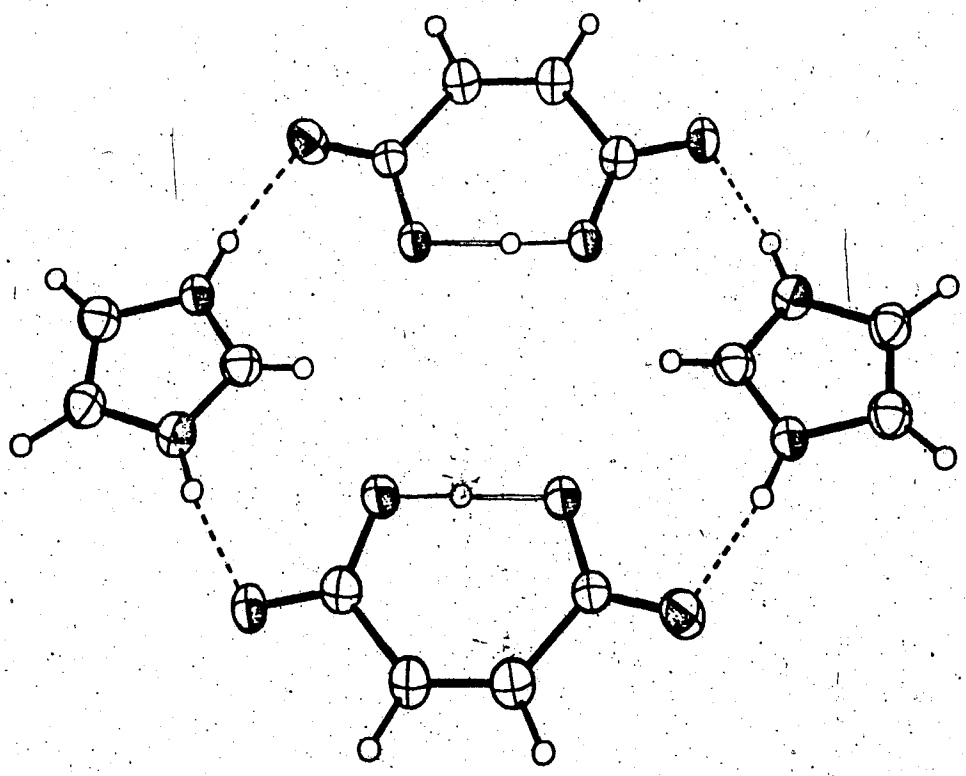


Fig. III 1-5 Two units of imidazole maleate related by a center of symmetry

1-4 Results and Discussion

All of the atomic parameters in this structure have been independently determined, because the space group symmetry does not require mirror or two fold symmetry on either the maleate or imidazole ions.

(i) imidazolium moiety

The bond lengths and angles of the imidazolium moiety are illustrated in Fig. III 1-6. Protonation on one of the nitrogen atoms of the imidazole ring would create a mirror symmetry in the molecule along the bond joining atoms C(2) and H(2). The observed values of the bond lengths and angles agree well with this postulate and the values of the bond lengths and angles on one half of the molecule are very close to the corresponding values on the other half.

Table III 1-6 shows the bond lengths of the imidazole moieties in the structures of imidazole itself, histidines and histamines which were unprotonated and protonated and the structures of two N-ribofuranosyl imidazoles. Most of the bond lengths in this structure agree reasonably well with those of the imidazolium phosphate structure with the exception of C(4) - C(5). The bond length C(4) - C(5) is much shorter than the equivalent distance in the related compounds. The extinction correction in the refinement reduced this length from 1.343(3)Å. However thermal vibration of the atoms in crystals results in apparently short bond length (Busing & Levy, 1964). The bond length C(4) - C(5) closely corresponds to a double bond rather than a delocalized aromatic C-C bond length. The bond lengths are 1.337(6)Å for simple carbon-carbon double bond and 1.395(3)Å in

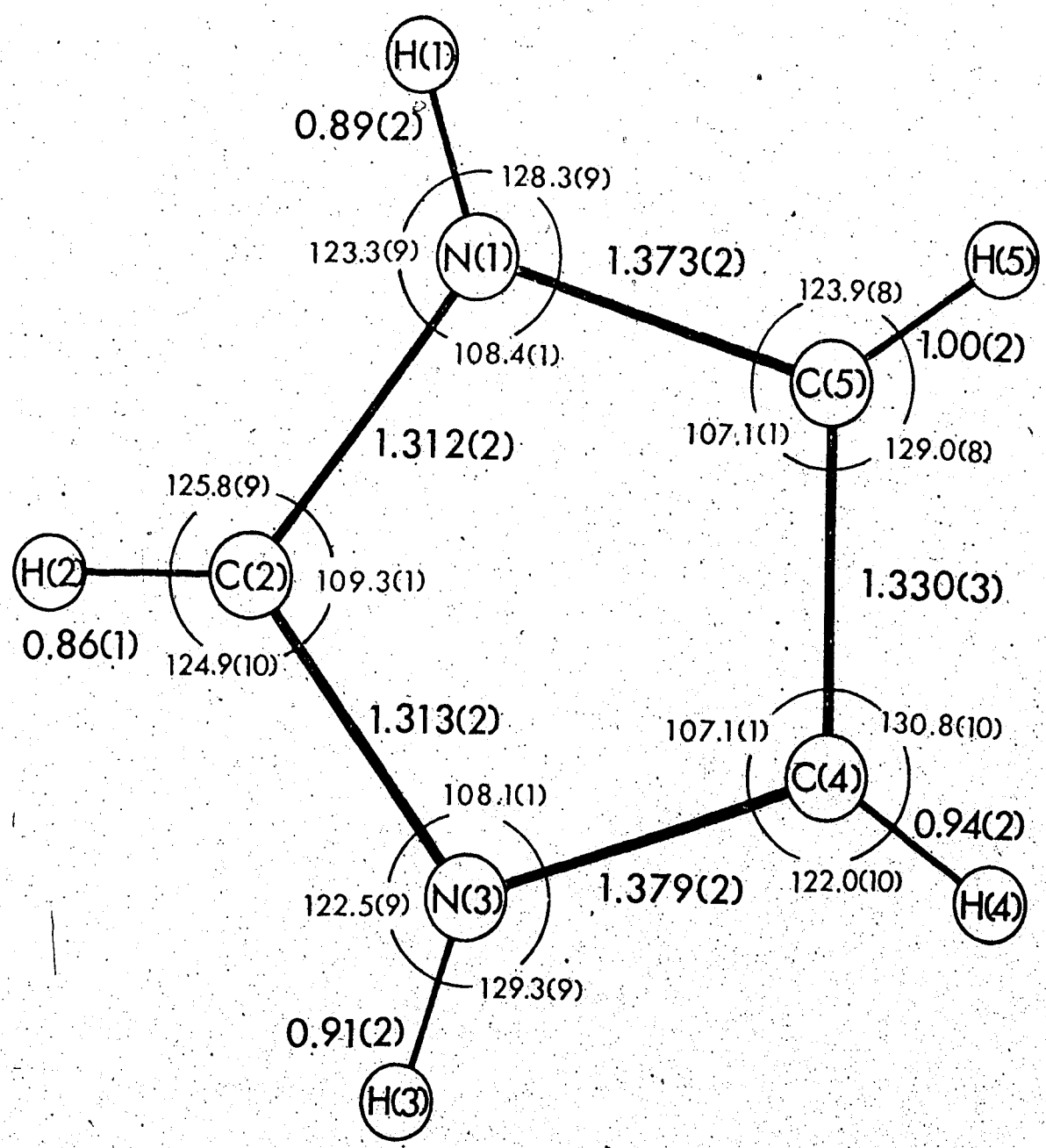
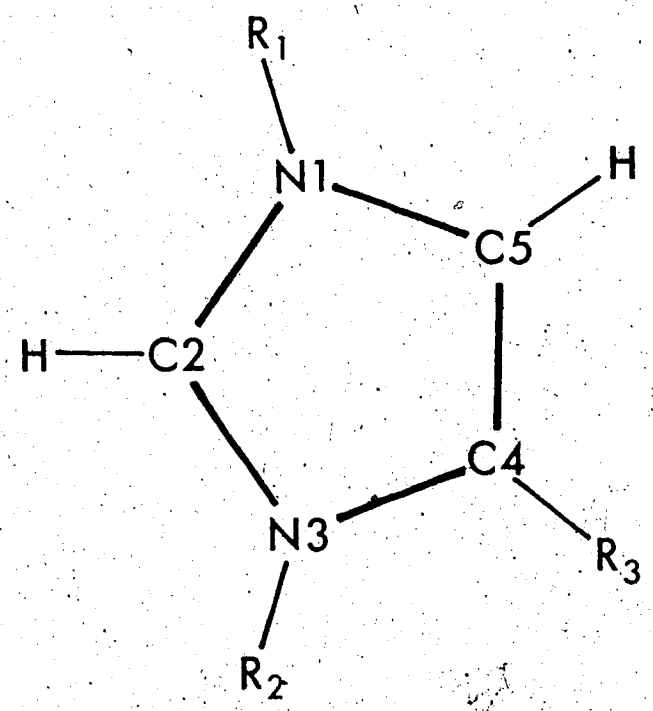


Fig. III 1-6 The bond lengths and angles of the imidazolium moiety.



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continued

Table III 1-6 The bond lengths and angles of the imidazolium derivatives

	Ref.	N1-C2	C2-N3	N3-C4	C4-C5	C5-N1
neutral imidazole derivatives						
Imidazole (R_2,R_3=H)	1	1.311(5)	1.337(5)	1.372(5)	1.311(5)	1.381(5)
L-Histidine (R_1=H,R_3=$-CH_2-$)	2	1.339(3)	1.327(3)	1.382(5)	1.361(3)	1.374(3)
Histamine (R_2=H,R_3=$-CH_2-$)	3	1.303(4)	1.349(3)	1.365(3)	1.356(4)	1.380(4)
IMR (R_2=$-CH<$,R_3=H)	4	1.316(3)	1.351(3)	1.376(3)	1.360(3)	1.378(3)
AIMR (R_2=$-CH<$,R_3=H)	5	1.308(3)	1.360(3)	1.376(3)	1.350(3)	1.364(4)
weighted average		1.318(1)	1.346(1)	1.376(1)	1.352(1)	1.375(2)
imidazolium derivatives						
Imidazolium phosphate (R_1,R_2,R_3=H)	6	1.320(4)	1.320(4)	1.357(12)	1.345(7)	1.375(12)
L-N-acetyl histidine (R_1,R_2=H,R_3=$-CH_2-$)	7	A 1.314(5) B 1.315(5)	1.323(5) 1.326(5)	1.371(5) 1.374(5)	1.356(5) 1.356(5)	1.370(5) 1.370(5)
L-histidine HCl (R_1,R_2=H,R_3=$-CH_2-$)	8	1.313(4)	1.340(4)	1.384(4)	1.355(4)	1.377(4)
L-histidine 2HCl (R_1,R_2=H,R_3=$-CH_2-$)	9	1.310(3)	1.325(3)	1.379(3)	1.351(3)	1.380(3)
DL-histidine (R_1,R_2=H,R_3=$-CH_2-$)	10	1.359(7)	1.314(6)	1.385(6)	1.374(6)	1.377(6)
histamine diphosphate (R_1,R_2=H,R_3=$-CH_2-$)	11	1.314(4)	1.332(4)	1.379(4)	1.349(4)	1.373(4)
pilocalpine (R_1=H,R_2=CH_3,R_3=$-CH_2-$)	12	1.327(2)	1.328(2)	1.383(2)	1.362(2)	1.383(2)
Weighted average		1.320(1)	1.327(1)	1.380(1)	1.357(1)	1.378(1)
Imidazolium maleate (R_1,R_2,R_3=H)	This work	1.312(2)	1.313(2)	1.379(2)	1.330(3)	1.373(2)

continued...

(a) bond lengths of imidazole derivatives

	Ref.	C5-N1-C2	N1-C2-N3	C2-N3-C4	N3-C4-C5	C4-C5-N1
neutral imidazole derivative						
Imidazole	1	102.6(5)	114.2(5)	104.4(5)	107.5(5)	111.2(5)
Histidine	2	106.9(2)	112.2(2)	104.9(2)	109.6(2)	106.4(2)
Histamine	3	104.5(2)	112.3(2)	110.8(2)	105.1(2)	110.8(2)
IMR	4	105.1(2)	111.9(2)	107.9(2)	105.7(2)	110.3(2)
AIMR	5	105.4(2)	111.7(2)	106.4(2)	105.6(2)	110.9(2)
weighted average		105.4(1)	112.1(1)	107.1(1)	106.5(1)	109.6(1)
imidazolium derivatives						
Imidazolium phosphate	6	108.6(6)	108.7(3)	108.6(6)	107.3(3)	107.0(3)
L-N-acetyl histidine	7	A 108.7(2)	108.7(2)	109.2(2)	106.0(2)	107.4(2)
		B 108.8(2)	108.8(2)	108.9(2)	106.2(2)	107.3(2)
L-Histidine.HCl	8	109.6(2)	108.1(2)	108.7(2)	106.4(2)	106.9(2)
L-Histidine-2HCl	9	109.2(2)	108.2(2)	109.5(2)	105.9(2)	107.1(2)
DL-Histidine	10	107.4(4)	111.6(4)	105.7(4)	109.7(4)	105.6(4)
Histamine diphosphate	11	109.1(2)	108.5(2)	108.6(2)	106.5(2)	107.2(2)
pilocalpine	12	108.9(1)	108.5(1)	109.2(1)	106.3(1)	107.0(1)
weighted average		109.0(1)	108.6(1)	109.0(1)	106.4(1)	107.1(1)
Imidazolium maleate	This work	108.4(1)	109.3(1)	108.1(1)	107.1(1)	107.1(1)

(b) bond angles of imidazole derivatives

aromatic compounds. The bond lengths are referred to those in "International Tables for X-ray Crystallography" vol III, p 276. In addi[redacted] lengths N(1) - C(5) and N(3)- C(4) are longer than th[redacted]-N length, 1.340(1)Å of pyridine ring (Bak, Hansen-[redacted]strup-Anderson, 1958), although they are much s[redacted]er t[redacted]ffinic C-N bond length, 1.472(5)Å. The lengths N[redacted] C([redacted].312(2)Å and N(3) - C(2), 1.313(2) were almost equa[redacted] and [redacted]re [redacted]rter than aromatic C-N length. These facts indicate delo[redacted]ion of π-electron of the carbon C(2) over these three atoms [redacted]ly with less involvement of the other ring atoms. The positi[redacted]charge is equally distributed on both nitrogen atoms.

T[redacted]ond angles agree more closely with other protonated imidazo[redacted]oieties than bond lengths. The protonation mainly affects the angles at the nitrogen atoms and the angle N(1) - C(2) - N(3). The angle C - N - C are widened from 105.4(1)° at the unprotonated nitrogen a[redacted] to 109.0(1)° by the protonation. The angle N - C - N is simul[redacted]usly reduced from 112.1(1)° to 108.6(1)° by the protonation. The weighted averaged values agree with the observed values in the structure of IMIMAL.

These factors of the similarity of the geometries in the molecule and the tendency of the angles could be a good indicator of protonation on the imidazole moieties in the solid state, in addition to a direct search for the hydrogen position in a difference map. The bond angles which include hydrogen atoms are usually less reliable than those between non-hydrogen atoms only. One of the reasons is that the X-ray scattering power from hydrogen atoms affects reflexions

with low Bragg angles and those reflexions systematically suffer from the secondary extinction. The refinement including the extinction factors should improve the hydrogen parameters. In fact the angles including the hydrogen atoms were closer to those between the non-hydrogen atoms which were substituted on the imidazole ring.

The carbon and nitrogen atoms of the imidazolium moiety lie in a plane within the experimental errors. The equation of the best plane through them and the displacements of the hydrogen atoms from the plane are listed in Table III 1-7. The largest displaced hydrogen atom was atom H(3) on nitrogen atom N(3) which was 0.04Å from the plane, whereas the displacement of the oxygen, O(1), which is hydrogen-bonded to N(3) was 0.154Å on the same side of this plane. The hydrogen atom, H(1), on the nitrogen, N(1), and the hydrogen bonded oxygen, O(3), were almost in the imidazolium ring plane. Although the magnitide of the displacement of the hydrogen atom H(3) may not be significant in this structure determination, it may be worth while to look for this tendency in other similar structures.

Atoms	Plane I	Atoms	Plane II	Plane III	Plane IV	Plane V
N(1)	0.000*	C(6)	0.000*	0.002*	0.0001*	
C(2)	-0.001*	C(7)	00.015*	-0.001*	-0.0001*	
N(3)	0.001*	C(8)	-0.012*	0.032	0.0002*	-0.002*
C(4)	-0.001*	C(9)	0.008*		-0.0001*	0.005*
C(5)	0.000*	O(1)	0.026*	-0.001*	0.0329	
H(1)	0.001	O(2)	-0.021*	-0.001*	-0.0410	
H(2)	0.015	O(3)	0.010*		0.0029	-0.002*
H(3)	-0.041	O(4)	0.003*		-0.0235	-0.002*
H(4)	0.000	H(7)	-0.038		-0.0084	
H(5)	0.010	H(8)	-0.026		-0.0021	
O(1)	-0.154					
O(3)(a)	-0.001					
standard deviation	0.001		0.015	0.001	0.0001	0.004
χ^2	1.51		800	2.61	0.02	16.76
degree of freedom	4		7	3	3	3

* the atoms included in the least squares plane calculations.

(a) the atom which was operated by the symmetry (-x, 2.0-y, -z).

Table III 1-7 Displacements of the atoms from the least-squares planes of imidazolium maleate

The equations of the least squares planes.

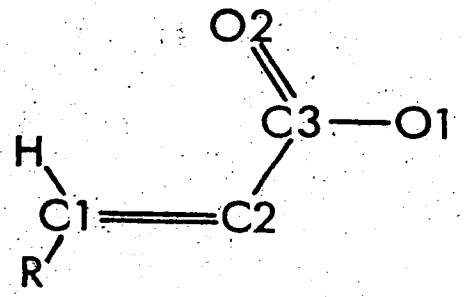
Pi + Qj + Rk = S in orthogronal angstrom space.

	P	Q	R	S
Plane I	-0.25720	0.56427	0.78450	3,33118
II	-0.33654	0.37907	0.86200	2.73735
III	-0.35576	0.38595	0.85116	2.71484
IV	-0.33652	0.36431	0.86835	2.71090
V	-0.32929	0.37478	0.86666	2.71709

(ii) maleate ion

The least squares planes of the non-hydrogen atoms of the maleate moiety are shown in Table III 1-7. The maleate moiety is less planar than the imidazolium moiety. The atomic groups of the two C-COO and C-C = C-C are relatively planar. The angle between the least squares planes III and IV is 1.9° and the angle between the planes IV and V is 0.7°. The angle between the least squares planes of imidazolium and maleate moieties is 12.4°.

α, β-unsaturated carboxyl systems have been studied in many structures. The conformation of the systems appears to prefer co-planarity of the carboxyl group and the sp^2 planes of the carbon-carbon double bond (Dunitz & Strickler, 1968). There are two conformations of the carboxyl group with respect to the double bond. These are the synplanar and antiplanar conformations of the carbon-oxygen double bond with respect to the α,β carbon double bond round the carbon-carbon single bond. Table III 1-8 shows the inter-atomic dimensions of such systems. The bond distances agree in both confor-



geometries of the α, β-unsaturated carboxyl groups with synplanar conformation.

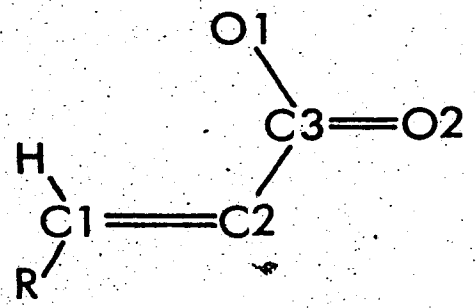
	C1-C2	C2-C3	C3-O1	C3-O2	∠C1-C2-C3	∠C2-C3-O1	∠C2-C3-O2	∠O1-C3-O2
fumaramic acid	1.320(2)	1.497(2)	1.310(2)	1.209(2)	118.3(1)	113.6(1)	122.4(1)	124.0(1)
dimethyl muconate	1.334(2)	1.476(2)	1.338(2)	1.203(2)	120.9(2)	111.0(2)	125.7(2)	123.0(2)
NaH fumarate	1.327(9)	1.489(9)	1.338(8)	1.211(11)	118.6(7)	114.2(6)	125.5(6)	120.0(6)
Weighted mean	1.327(1)	1.487(1)	1.324(1)	1.206(1)	118.8(1)	113.1(1)	123.1(1)	123.7(1)

fumaric acid: Benghiat et al., (1972)

dimethyl muconate: Filippakis et al., (1967)

NaH fumarate: Gupta et al., (1970)

continued...



geometries of the α, β-unsaturated carboxyl groups with antiplanar conformation.

		C1-C2	C2-C3	C3-O1	C3-O2	∠C1-C2-C3	∠C2-C3-O1	∠C2-C3-O2	∠O1-C3-O2
KH fumaric	A	1.305(5)	1.531(10)	1.294(4)	1.190(10)	122.7(6)	113.2(6)	121.7(6)	125.0(8)
	B	1.333(17)	1.495(10)	1.295(9)	1.215(12)	121.8(6)	115.9(7)	119.6(6)	122.9(6)
dimethyl dichloromuconate		1.329(5)	1.495(5)	1.219(5)	1.190(5)	124.0(2)	111.2(2)	123.6(2)	125.2(2)
Itaconic acid		1.323(3)	1.478(3)	1.317(3)	1.216(3)	121.2(2)	114.7(2)	121.9(2)	123.5(2)
Weighted mean		1.321(2)	1.486(2)	1.313(2)	1.208(2)	122.6(1)	113.1(1)	122.5(1)	124.3(1)

KH fumarate : Gupta et al., (1972)

dimethyl diCl muconate: Einspahr et al., (1973)

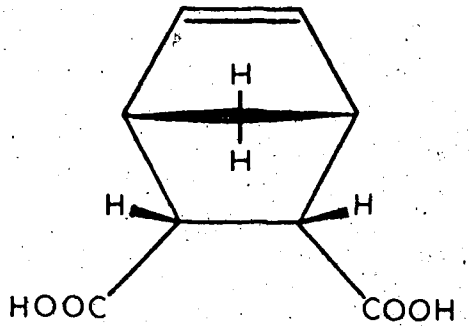
Itaconic acid : Harlow et al., (1973)

Table III 1-8 Geometries of α, β-unsaturated carboxyl groups

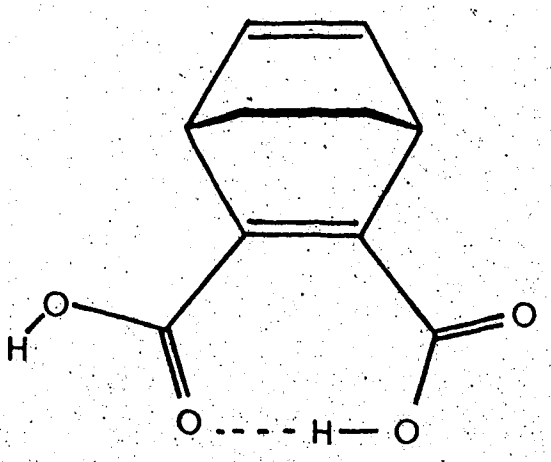
mations, 1.327(2) Å for C = C bond, 1.486(2) Å for C - C bond, 1.208(2) Å for C = O bond and 1.313(2) Å for C - O bond. The bond angles in the carboxyl groups were 113.1(1)° for C - C - O angle, 122.8(1)° for C - C = O angle and 124.0(1)° for O - C = O angle. These bond lengths and angles were almost uniform in the synplanar and antiplanar conformations and were comparable with the angles in α, β - saturated carboxyl compounds (Dunitz & Strickler, 1968). A variation can be found in a bond angle between C = C and C - C bonds. The angle in the synplanar conformation is 118.8(1)° and that in the antiplanar conformation is 122.6(1)°.

It is suggested that the difference of the angle comes from the difference between the C - C - O and C - C = O angles, that is, the narrower angle of C - C - O may demand a wider angle of C = C - C, so that the wide angle might compensate for the non-bonded distance between the cis oxygen atom and the β carbon or its hydrogen atom from that which would have become short in the anti conformation.

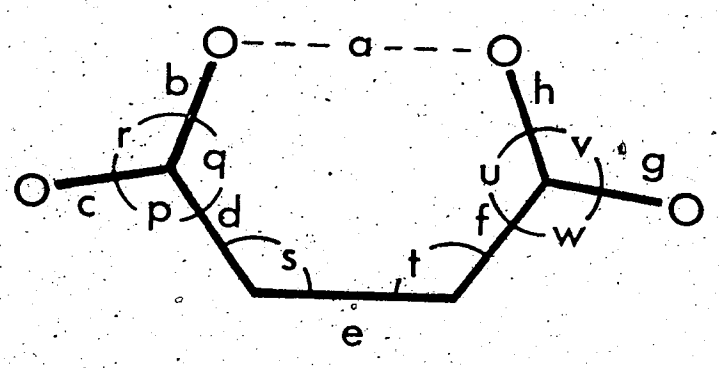
As well as in the α,β-unsaturated carboxyl derivatives, the double bond in cis-dicarboxyl ethylene derivatives prefers the coincidence of the planes of double bond and carboxyl groups in the neutral and monoanion forms, making a short intramolecular hydrogen bond between the carboxyl groups. On the other hand, two carboxyl groups in the bicyclo [2,2,1] hept-5-ene-2,3-endo-dicarboxylic acid (Pfluger, Harlow and Simonsen,1973) do not maintain the intramolecular hydrogen bond, even though the carboxyl groups are preferably oriented in the rigid molecule.



Each one of the carboxyl groups donates and accepts intermolecular hydrogen bonds with a carboxyl group of a neighboring molecule. The difference is that the two carboxyl groups are separated by a carbon-carbon single bond in this molecule, on the other hand, by a carbon-carbon double bond in maleic acid derivatives and the tricyclic alkene (Hechtfisher, Steigemann and Hoppe, 1970).



The weighted mean geometries of the cis-dicarboxyl ethylene and the closely related compounds were listed in Table III 1-9 and the bond lengths and angles of the maleate moiety in IMIMAL are illustrated in Figure III 1-7.



	a	b	c	d	e	f	g	h
Maleic acid derivatives	2.500(1)	1.304(1)	1.219(1)	1.492(1)	1.340(2)	1.481(1)	1.304(1)	1.222(1)
Maleate monoanion derivatives	2.422(1)	1.287(1)	1.230(1)	1.501(1)	1.343(1)	1.506(1)	1.236(1)	1.283(1)
IMIMAL	2.408(1)	1.284(2)	1.231(2)	1.485(2)	1.328(2)	1.491(2)	1.230(2)	1.281(2)
Maleate dianions		1.289(1)	1.267(1)	1.506(1)	1.335(1)	1.495(1)	1.250(1)	1/267(1)

Table III 1-9 geometries of the cis-dicarboxyl ethylene (neutral, monoanion and dianion molecules)

	p	q	r	s	t	u	v	w
Maleic acid derivatives	119.6(1)	121.0(1)	119.7(1)	131.4(1)	127.9(1)	124.7(1)	122.4(1)	113.0(1)
Maleate monoanion derivatives	117.8(1)	119.6(1)	122.4(1)	130.9(1)	128.7(1)	119.9(1)	122.4(1)	118.0(1)
IMIMAL	118.6(1)	119.7(1)	121.7(1)	130.7(2)	130.7(1)	119.6(1)	122.0(1)	118.4(1)
Maleate dianions	113.9(1)	120.3(1)	125.7(1)	129.1(1)	126.2(1)	118.7(1)	124.9(1)	116.4(1)

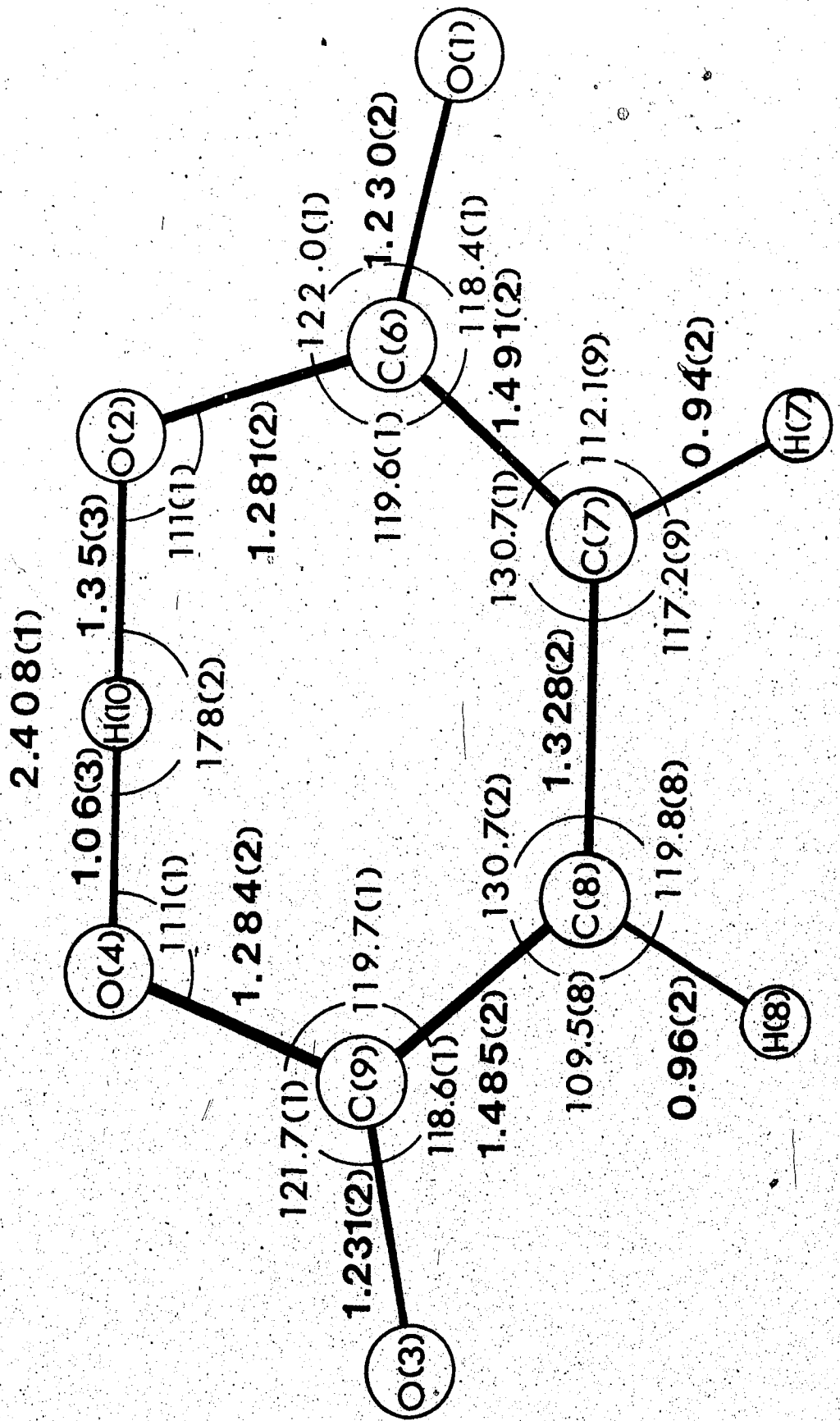


Fig. III 1-7 The bond lengths and angles of the maleate monoanion moiety

The values of IMIMAL agree well with the weighted mean values of six maleate monoanion derivatives: potassium hydrogen maleate (Parlow, 1961), potassium hydrogen chloromaleate (Ellison & Levy, 1965), copper hydrogen maleate (Prout, Carruthers & Rossotti, 1971), cis-aconitate (Glusker, Orehowsky, Casciato & Carrell, 1972). Brompheniramine hydrogen maleate (James & Williams, 1971), Chlorpheniramine hydrogen maleate (James & Williams, 1974c) and Methoxypromazine (Marsau & Gauthier, 1973). The bond lengths 1.287(1) in 'b' and 1.283(1) in 'h' of maleate monoanion derivatives are intermediate between the bond lengths of carbon-oxygen double and single bond of the α, β-unsaturated carboxyl groups in Table III 1-8, and these lengths are comparable with those in the maleate dianion molecules, disodium maleate (James & Williams, 1974b) and dilithium maleate (Town & Small, 1973). The bond lengths 'd' 1.485(2) Å and 'e' 1.328(2) Å in IMIMAL are shorter than the weighted average bond lengths of maleate monoamion derivatives but the bond lengths are close to those of the α, β-unsaturated carboxyl groups (Table III 1-8).

The carboxyl compounds in Table III 1-8 have been chosen to be trans configuration with respect to the substituents on the β carbon. It is hardly expected that there would be direct steric hinderance from the substituents to the carboxyl groups. The maleic acid derivatives, of course, hold strong steric interactions between the cis related carboxyl groups in a plane. To compare with the values in Table III 1-8 and Table III 1-9 differences are observed in the bond angles. The angles of the cis dicarboxyl derivatives indicate uniform tendencies. The angles which are inside of the hydrogen-bonded seven-

membered ring are widened, while those on the outside of the ring are made narrow. The most affected angles are those of C = C - C and the least effected are those of O - C = O. The angles of C = C - C in IMIMAL 130.7(2) ^{o} are expanded by 8.1^{o} from that of the antiplanar conformational α, β-unsaturated carboxyl acid. The angles of C - C - O in IMIMAL, 119.7(1)^{o} and 119.6(1)^{o}, also are widened from 113.1(1)^{o} of unstrained compounds but are comparable with the angle in maleate dianion molecules.

The distances between the intramolecular hydrogen-bonded oxygens are presented in the column 'a' of Table III 1-9. The distance, 2.408(1)Å, of IMIMAL is one of the shortest hydrogen bonds in the maleate monoanions observed. The distance was not corrected for thermal motion. Since the bond lengths of IMIMAL in the columns 'b' and 'h' are comparable with those of the maleate dianion compounds and the charges in the dianions appear to be distributed over the four oxygen atoms, the oxygen atoms on both sides of the hydrogen bond in the monoanions seemed to have a half negative charge. The negative charge distribution clearly strengthens the intramolecular hydrogen bond, since the distance in the monoanions is shorter than that in the neutral maleic acid derivatives.

The distances from the hydrogen atom H(10) are 1.35(3)Å to the oxygen atom O(2) and 1.06(3)Å to the oxygen atom O(4). The angle of O(2)...H(10...O(4) is 178(2)^{o}. These values show that this strong intramolecular hydrogen bond seems to be asymmetric. But a little doubt to this conclusion can be allowed, because of the reliability of the positional parameters of the hydrogen atom.

Firstly since atomic scattering factors of X-ray are proportional to atomic number, the X-ray scattering from hydrogen atom is only one sixth of that from carbon atom. Therefore hydrogen atoms in a crystal contribute less to structure factors than carbon, nitrogen or oxygen atoms do, and the least squares refinement is less sensitive to the parameters of the hydrogen atoms. Secondly the hydrogen atom H(10) has been refined with anisotropic temperature factors. The anisotropy became very large along the direction of the hydrogen bond. It may be worthwhile to note that the contribution of the hydrogen atom is shown in a difference map which was computed with the coefficient (Fo-kFc), but in Fc there was no contribution from the hydrogen atom H(10). The map is shown in Fig. III 1-8.

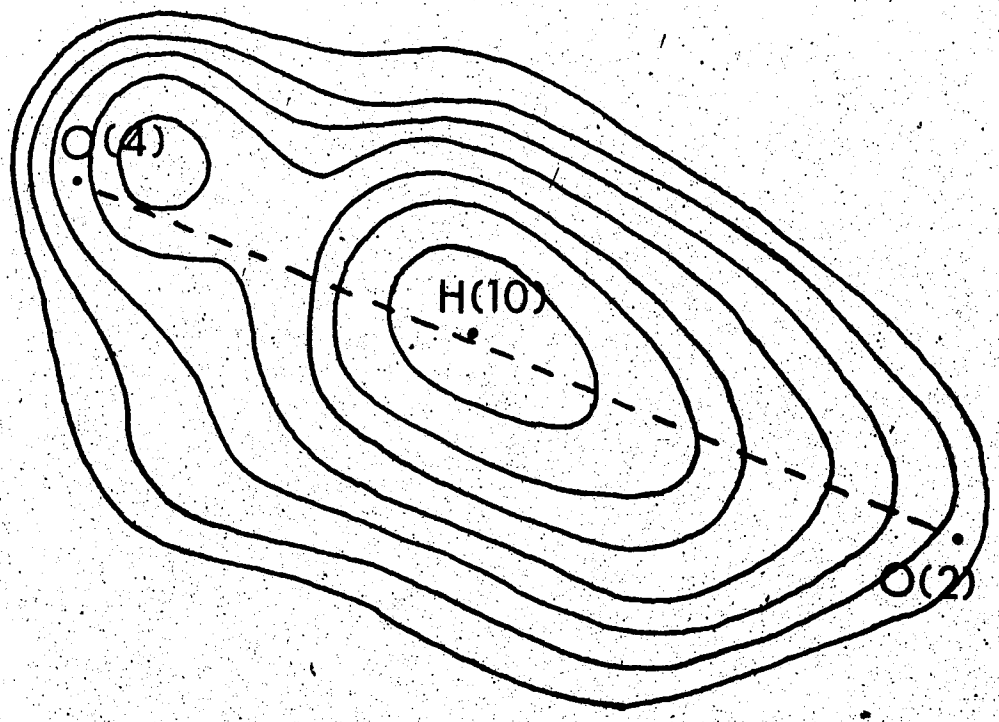


Fig. III 1-8. The final difference map around the intramolecular hydrogen bond of imidazolium maleate.

The section was at y/b = 0.710 which was between the y coordinates of the hydrogen bonded oxygen atoms, 0.03 Å above O(2) and 0.01 Å below O(4). The map had one large peak elongated along the line between the oxygen atoms centered nearly at the mid point between them, and another peak which was smaller and closer to oxygen O(4). This electron density peak had appeared after the extinction refinement and it could not be interpreted by the thermal anisotropy of the oxygen atom, O(4). The hydrogen atom, H(10) was centred in the former electron density which had a gentle slope along the line joining the oxygen atoms. In this situation the hydrogen atom is poorly determined by the least squares refinement and such large thermal motion makes the arguments of the hydrogen atom position less meaningful. It is another possibility that the apparent asymmetry of the hydrogen bond could come from the uninterpretable electron density beside the oxygen atom; O(4).

As a result the most plausible model is one in which the hydrogen atom lies in a single, broad and probably symmetric energy well, in spite of the fairly large difference between the calculated distances from the hydrogen position to the oxygen positions. This result would be supported by the symmetric geometry of the maleate molecule, (Fig. III 1-7). The geometry of the right side of the molecule agreed well with that of the left side. There were no significant differences between the bond lengths of C(6) - O(2) and C(9) - O(4), and between the corresponding angles around the carbon atoms, C(6) and C(9). These facts suggest that the electronic states at the oxygen atoms would be similar and that the hydrogen atom, H(10), would be equally affected by these two carboxyl groups.

C=O...H-N	distance O...N	distance O...H	angle O...H-N	angle C=O...H
C(6)=O(1)...H(3)-N(3)	2.789(2)Å	1.89(2)Å	171(1)°	109.3(3)°
C(9)=O(3)...H(1)'-N(1)'	2.797(2)Å	1.92(2)Å	170(1)°	108.8(4)°

H(1)' and N(1)' are operated on by a symmetry operation of x = -x, y = 2.0, z = -z.

Table III 1-10. Geometries of the intermolecular hydrogen bonds of imidazolium maleate.

(iii) molecular packing

The maleate anions are arrayed in a plane that is parallel to the bc-plane at x/a = 0 and the plane of the maleate moiety is almost perpendicular to this bc-plane. The imidazolium cations are arrayed in another bc-plane at x/a = ½ and the plane of the imidazolium moiety is also perpendicular to the plane. But this plane at x/a = ½ is charged positively. The negatively charged planes of the arrayed maleate moieties and the positively charged planes of the imidazolium moieties are stacked alternatively along the a-axis.

The similarity of the geometries of the right and left sides of the maleate molecule has already been discussed. This similarity of the geometries can be extended to the environments around both sides of the molecule. A group of two imidazolium ions and two maleate ions encircle a center of symmetry in the crystal (Fig. III 1-5). Two hydrogen bonds were accepted by the oxygens of O(1) and O(3). The geometries of the intermolecular hydrogen bonds are listed in Table III 1-10. The distances between the nitrogen and oxygen atoms were 2.789(2) Å and 2.797(2) Å. The angles of C = O....H were 109.3(5)° and 108.8(4)°. These geometries are very similar with each other sides of the molecule. The dimensions of other close contacts around the carboxyl groups are listed on Table III 1-11. The oxygen atoms of O(2) and O(4) were close to the carboxyl carbon atoms in a centrosymmetrically related maleate anion. The distances were 3.414(2) Å and 3.401(2) Å. The relatively close approaches of carbon and hydrogen atoms in the positively charged

A	B	C		distances B...C	angles A-B-C
	C(6)-O(2)...C(9)		i)	3.414Å	89.4°
	C(9)-O(4)...C(6)		i)	3.401	90.0
	C(6)-O(1)...C(4)		ii)	3.146	168.2
	C(9)-O(3)...C(5)		iii)	3.247	133.5
	C(6)-O(1)...N(1)		iv)	3.326	115.8
	C(9)-O(3)...N(3)		i)	3.305	113.4
	C(6)-O(1)...C(2)		iv)	3.220	106.8
	C(9)-O(3)...C(2)		i)	3.294	104.1
	H(3)...O(1)...N(1)		iv)		79.9
v)	H(1)...O(3)...N(3)		i)		80.2
	H(3)...O(1)...C(2)		iv)		102.9
v)	H(1)...O(3)...C(2)		i)		103.0
iv)	N(1)...O(1)...C(2)		iv)		23.1
i)	N(3)...O(3)...C(2)		i)		23.0

i) -x, 1.0-y, -z

ii) 1.0-x, y-0.5, 0.5-z

iii) -x, 1.0-y, z

iv) x, y-1.0, z

v) -x, 2.0-y, -z

Table III 1-11. The dimensions of close contacts around the carboxyl groups.

imidazolium cation to carboxyl groups were found for both sides of the maleate anion. These distances between the oxygen and the carbon atoms were 3.146(2) Å from O(1) and 3.237(2) Å from O(3). Other close contacts in both sides were found with the carbon and nitrogen atoms of the imidazolium cations which were shifted by one unit cell. The distances were 3.326(2) Å and 3.305(2) Å from the oxygen atoms to the nitrogen atoms and 3.220(2) Å and 3.294(2) Å to the carbon atoms. The differences between the corresponding angles around the oxygen atoms were less than 2.7° except those of C = O...C. These relations are shown in a stereoscopic diagram of Fig. III 1-9.

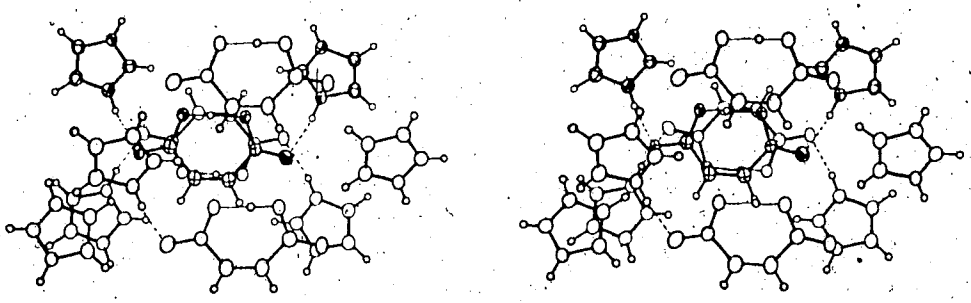


Fig. III 1-9 . A stereoscopic diagram of the packing structure of imidazolium maleate.

Since the same number and kinds of atoms surrounded both the carboxyl groups at similar distances and in similar directions, it may be concluded that the two carboxyl groups of the maleate anion are in almost identical environments. The crystal structure of IMIMAL suggests that the short intramolecular hydrogen bond is symmetric in the symmetric environment. But the symmetry of the hydrogen bond would be variable in different environments such as the three crystal structures, Bromopheniramine hydrogen maleate, chlorpheniramine hydrogen maleate and Methorypromazine, where one of the carboxyl groups contributed to an intermolecular hydrogen bond and the other remained free. The theoretical calculations (Murthy, Bhat & Rao, 1970) assumed that the distance between both oxygen atoms of the hydrogen bond would be the only factor which determines whether the bond should be symmetric or not. These authors did not take into account the environmental effects on the atoms comprising the two cis-carboxyl groups. When these effects were taken into account in the system of p-toluidinium bifluoride, the hydrogen bond of the bifluoride became asymmetric (Ostlund & Ballenger, 1975).

Since the carboxyl groups in the maleate anion have a conjugated electron system which may amplify the environmental effects on their geometries, the differences between them could be observed by a crystal structure analysis of the present accuracy. If a crystal could be grown that had different environments of the two carboxyl groups, these geometrical differences could differentiate between a symmetric or a non-symmetric hydrogen bond.

It is proposed that the crystal structure of IMIMAL should be refined with the data from neutron diffraction, because it is outstanding that the molecules are in general positions and have an almost symmetric environment. Another proposition is the structure determination of one side of the maleate monoanion with a hydrogen bond and the other free in small molecular combination. One such structure is pyridinium maleate and an attempt at the preparation of this compound is presented in part 2, chapter III.

Part 2

The Molecular and Crystal Structure of N-succinopyridine

2-1 Introduction

The crystal structure of imidazolium maleate showed that the two nitrogen atoms of the imidazolium ring made two hydrogen bonds in the crystals. In this structure the maleate molecules were present in a symmetric environment with the result that the geometry of the molecule was symmetric. In order to get an accurate structure of the maleate monoanion in an asymmetric environment, crystals of pyridinium maleate were grown in the hope that the pyridinium molecule would make only one hydrogen bond with the maleate monoanion. The hydrogen bond could break the symmetry of the environment and the maleate molecule would hopefully be placed in a general position of the crystal's space group. At the beginning of the experiment, two modifications of these crystals were discovered. One of them came from a methanolic solution of pyridine and maleic acid. There was no addition of water in this solution. The crystals were thought to belong to the triclinic system and had interplanar unit cell spacings of approximately 5 Å. But the crystals decomposed in air during the X-ray exposure.

The other modification of the crystals was grown from water solution. The crystals did not decompose during the experiments. The calculated and observed densities of the crystals agree well with the

assumption of a formula $C_5H_6N^+ : C_4H_3O_4^-$ for the asymmetric unit. A spectroscopic experiment, for example IR, could have confirmed the reaction product expected. The actual molecular structure was revealed only after the structure was solved. However the subsequent structure analysis of these crystals showed that the expected hydrogen bonded complex between the pyridinium ion and the maleate monoanion was not the isolated product.

2-2 Experimental

Solid anhydrous maleic acid (1.161g 0.01 mole) was mixed together with 0.80 ml (0.01 moles) of pyridine in a beaker. The coagulated mixture was subsequently dissolved in small amount of warm water. This solution was set in a styroform box and allowed to cool slowly to room temperature. Colorless crystals were deposited in 5 hours. These crystals were shown to be of high X-ray reflectivity and were examined by preliminary oscillation and Weissenberg photograph which showed that the crystals were orthorhombic with the systematical absences h00=2n+1, 0k0=2n+1 and 001=2n+1. The density of the crystals; Do=1.442 g/cm^3, was measured by the flotation method in a mixture of carbon tetrachloride (ρ=1.58 g/cm^3) and chlorobenzene (ρ=1.10 g/cm^3) at room temperature 21^{o}C. The cell dimensions were calculated from 12 accurately centered reflexions by a least-squares method at the beginning of the intensity data collection. The crystal data are in Table III 2-1. A crystal, 0.23 X 0.25 X 0.43 mm, was mounted with the a axis coincident with the ϕ axis of a Picker FACS-1 diffractometer. The incident radiation was graphite monochromatized Mo Kα (λ=0.71069 Å). The 2θ of the monochrometer was 12.09^o. Two octants, h k l and h k $\bar{l}$ reflexions within the angular range $3.0^o \leq 2\theta \leq 55.0^o$ were searched by the θ-2θ scan mode over a basic peak width of 1.5^o in 2θ. This value was varied by the function 0.692 tan θ to account for $\alpha_1\alpha_2$ splitting. A total of 23 reflexions were considered to be affected by counter coincidence loss and were

molecular formula	$C_9H_9NO_4$
molecular weight	195.17 Daltons
space group	$P2_12_12_1$
a	7.775(2) Å
b	14.974(3) Å
c	7.730(2) Å
V	899.9 $Å^3$
Z	4
D_o	1.442 g/cm^3
D_c	1.439 g/cm^3
$\bar{\mu}$	1.24 cm^{-1}
2θ range explored	3^o~55^o h k l and h k $\bar{l}$
no. unique reflexions	1221
no. reflexions for refinement	1037 (84.9%)
final unweighted R	0.032
final weighted R_w	0.048

Table III 2-1 Physical constants and other data for N-succinopyridine

corrected with an attenuator in position. Since the value of μR was very small, and the crystal was almost cylindrical, absorption corrections were not applied on the data. During the intensity data collection, three standard reflexions were measured after every 50 reflexions to detect moving or decomposition of the crystal and to check the stability of the diffractometer. No significant effects were detected. After averaging the two independent sets of reflexions, a total of 1037 reflexions had intensities more than $3\sigma(I)$. The same statistical criteria as described in the experimental section of imidazolium maleate were employed to examine whether the net counts of each reflexions were significantly above background.

The intensities were reduced to structure factors by the application of the appropriate Lorentz and polarization factors for normal-beam equatorial geometry.

2-3 Solution and Refinement

An overall isotropic temperature factor of 3.209 $e/\mathring{A}^2$ and a scale factor of 0.1969 were computed by Wilson's method (Wilson, 1942). The structure factors were reduced to the normalized structure factors (Karle and Karle, 1966). The statistics and distribution are listed in Table III 2-2.

	theoretical		experimental
	centric	acentric	
average value of $\|E\|$	0.798	0.886	0.852
average value of $\|E^2\|$	1.000	1.000	1.000
average value of $\|E^2-1\|$	0.968	0.736	0.820

	centric %	acentric %	%	no. of reflexions
$\|E\|>3.0$	0.27	0.01	0.10	2
2.5	1.24	0.19	0.46	8
2.0	4.55	1.83	3.13	46
1.8	7.19	3.92	5.55	75
1.6	10.96	7.73	9.18	116
1.4	16.15	14.09	14.71	187
1.2	23.01	23.69	23.29	291
1.0	31.73	36.79	35.47	435
0.0	100.00	100.00	100.00	1221

Table III 2-2 The theoretical and experimental statistics of the $|E|$ values of N-succinopyridine

A total of 117 reflexions with |E| values greater than 1.60 were selected for the calculation of a Σ_2 list of phase relations. Hand phase determinations were carried out on this Σ_2 list. The reflexions, 2,0,7(|E|= 3,28); 0,3,1(|E|= 2.56) and 3,4,0(|E|= 2.23) for the origin determining reflexions and 7,0,1(|E|= 2.09) for the enantiomorph reflexion were chosen. During the hand phasing the phase of reflexion 1,13,3 had a strong indication that it was close to 90° or 270°. The phase of reflexion 4,10,3 which had a large |E| value in general index reflexions could not be determined, because of no interactions with others in the hand phase determination. The tangent refinements (Karle & Hauptman, 1956) were computed with eight sets of starting phase combinations in which the phase of reflexion 1,13,3 was either 90° or 270°, and the phases of reflexion 4,10,3 were 45°, 135°, 225° and 315°. One of the conbinations, phase of 1,13,3 = 90° and phase of 4,10,3 = 45°, had the lowest R_k value of 0.17. The resulting phases for all 117 reflexions were well distributed over 360°. The R_k values of other phase combinations were all more than 0.24.

The E map corresponding to the phases from the best combination showed the molecular figure of 14 non-hydrogen atoms as the 14 largest peaks. The 10 carbon and 4 oxygen positions with the isotropic temperature factors of 3.21 e/A^{o2} and the scale factor of 0.1969 were applied in the structure factor calculation using only the data out to $2\theta=40^{\circ}$. The R factor was 0.20 for these 507 reflexions. The introduction of the nitrogen scattering factors and all of the reflexion data resulted in an R factor of 0.11 after three cycles

of full-matrix least-squares calculations. A difference map showed eight out of the nine hydrogen atoms expected. The one hydrogen position that did not show on this map belonged to a carboxyl group. After one cycle of least-squares calculations with anisotropic temperature factors for the non-hydrogen atoms, the carboxyl hydrogen atom appeared at the expected position in a difference map. The atomic scattering factors of the half negatively charged oxygens for O(1) and O(2) were used in the following structure factor calculations with the weighting scheme, $w = (2|Fo|^{\frac{1}{2}}/\sigma(I))$ The final R factor was 0.032 and R_w factor was 0.048. The maximum and average shift with respect to the corresponding error were 0.05 and 0.02 for the non-hydrogen atomic parameters in the final cycle of least-squares calculations, respectively. Those for the hydrogen atomic parameters were 0.32 and 0.05, respectively. The final F_o and F_c values are listed in Table III 2-3 and the final atomic parameters are given in Table III 2-4, (a), (b) and (c).

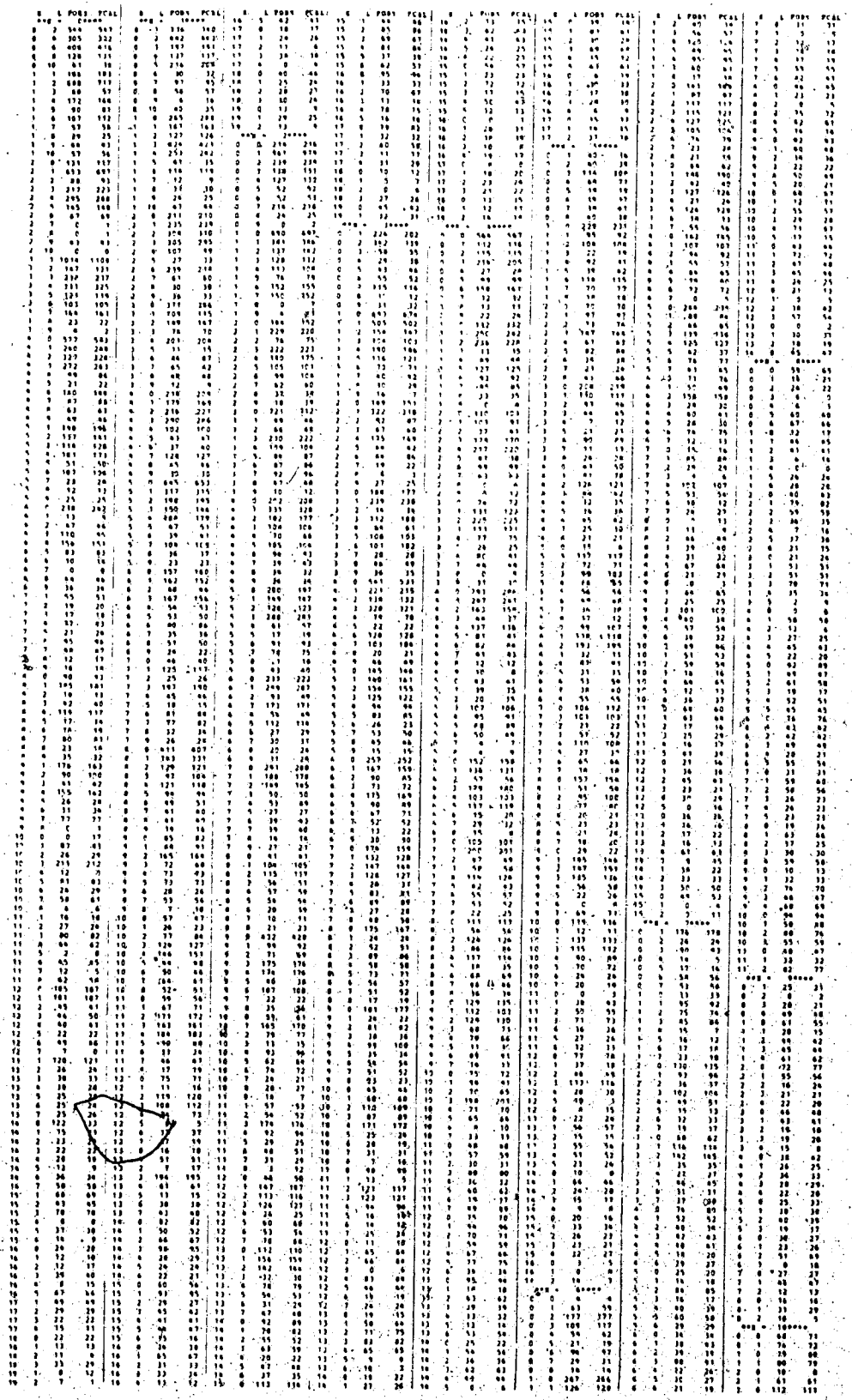


Table III 2-3 The observed and calculated structure factors (absolute scale X 10) which were included in the refinements in the crystal structure of N-succino-pyridine

atom	x/a	y/b	z/c
C1	8203(2)	1013(1)	4448(3)
C2	6363(2)	1354(1)	4080(2)
C3	5083(2)	596(1)	4342(3)
C4	3239(2)	920(1)	4194(3)
N5	6204(2)	1750(1)	2317(2)
C6	5995(3)	2634(1)	2155(3)
C7	5898(5)	3012(2)	531(4)
C8	6013(4)	2490(2)	-917(3)
C9	6221(5)	1581(2)	-717(3)
C10	6314(4)	1222(1)	912(3)
O1	8524(2)	888(1)	5994(2)
O2	9153(2)	856(1)	3165(2)
O3	2838(2)	1693(1)	4447(3)
O4	2161(2)	289(1)	3767(3)

Table III 2-4(a) Positional parameters ($X10^4$) of the non-hydrogen atoms of N-succinopyridine

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C1	219(8)	306(8)	419(9)	-15(6)	-58(7)	24(7)
C2	253(8)	305(8)	289(7)	7(6)	-13(6)	-5(7)
C3	240(8)	331(8)	396(9)	16(7)	=0(7)	53(7)
C4	266(8)	380(9)	362(8)	18(8)	28(7)	28(8)
N5	252(6)	272(7)	337(7)	17(6)	-2(5)	15(5)
C6	531(12)	275(9)	477(10)	52(9)	2(9)	15(7)
C7	814(21)	360(10)	590(14)	74(12)	0(14)	167(9)
C8	688(17)	659(15)	430(11)	112(15)	47(11)	212(11)
C9	785(18)	582(13)	340(10)	126(14)	47(11)	-4(9)
C10	596(14)	352(8)	356(9)	106(10)	11(10)	-12(7)
O1	422(8)	467(7)	410(8)	51(7)	-108(6)	44(6)
O2	246(7)	764(12)	516(9)	74(8)	5(6)	49(8)
O3	345(8)	414(8)	732(11)	83(7)	59(8)	-25(7)
O4	265(7)	479(9)	853(12)	5(7)	-52(7)	063(8)

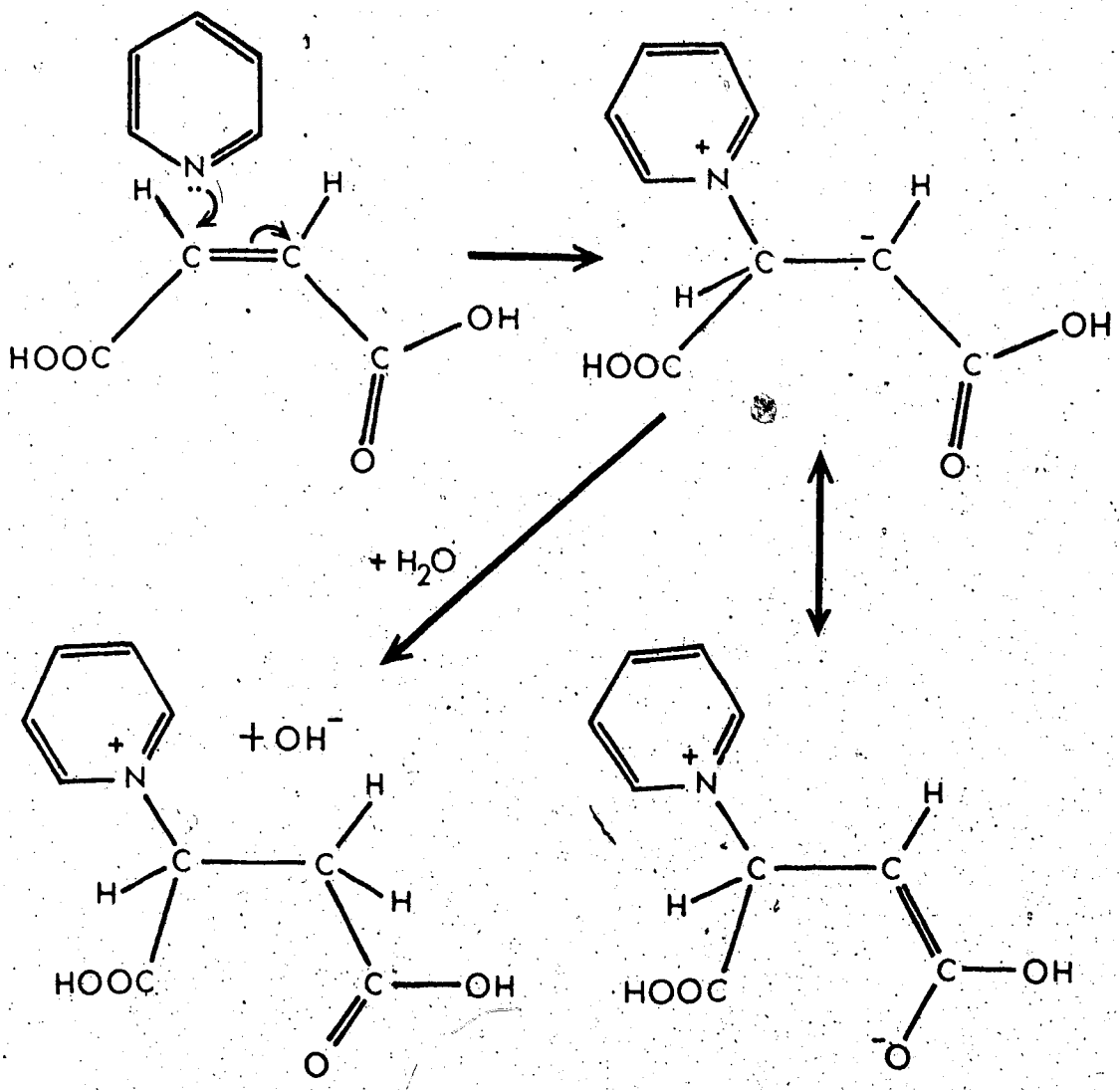
Table III 2-4(b) Thermal parameters ($X10^4$) of the non-hydrogen atoms of N-succinopyridine

Atom	x/a	y/b	z/c	U_{iso}
H2	615(5)	179(2)	481(4)	43(7)
H31	524(4)	34(2)	542(4)	39(6)
H32	529(4)	14(2)	346(3)	34(6)
H4	121(7)	52(3)	365(5)	62(10)
H6	584(6)	291(2)	319(5)	55(8)
H7	569(7)	359(3)	42(6)	76(12)
H8	596(6)	284(3)	-209(5)	67(10)
H9	642(5)	120(2)	-175(5)	57(9)
H10	645(4)	61(2)	111(3)	37(7)

Table III 2-4(c) Positional and thermal parameters ($X10^3$) of the hydrogen atoms of N-succinopyridine

2-4 Results and Discussion

The molecule that appeared in the E map proved to be a covalently bonded product. The reaction that had taken place was a nucleophilic addition reaction of pyridine to the double bond in maleic acid in water solution as shown below.



A simplified reaction pathway shows the nucleophilic attack of pyridine on maleic acid to produce the resonance stabilized structure. Removal of a proton from water produces the product

found in the crystals. The molecule exists as a zwitterion in the solid state with the pyridine ring positively charged and the α-carboxyl group negatively charged. The atomic numbering scheme used in this crystal structure analysis is given in Fig. III 2-1, and the molecular conformation is shown in Fig. III 2-2 which represents a stereoscopic drawing of the molecule of N-succinopyridine It can be seen in this diagram that the intramolecular hydrogen bond of the maleic acid moiety has been destroyed.

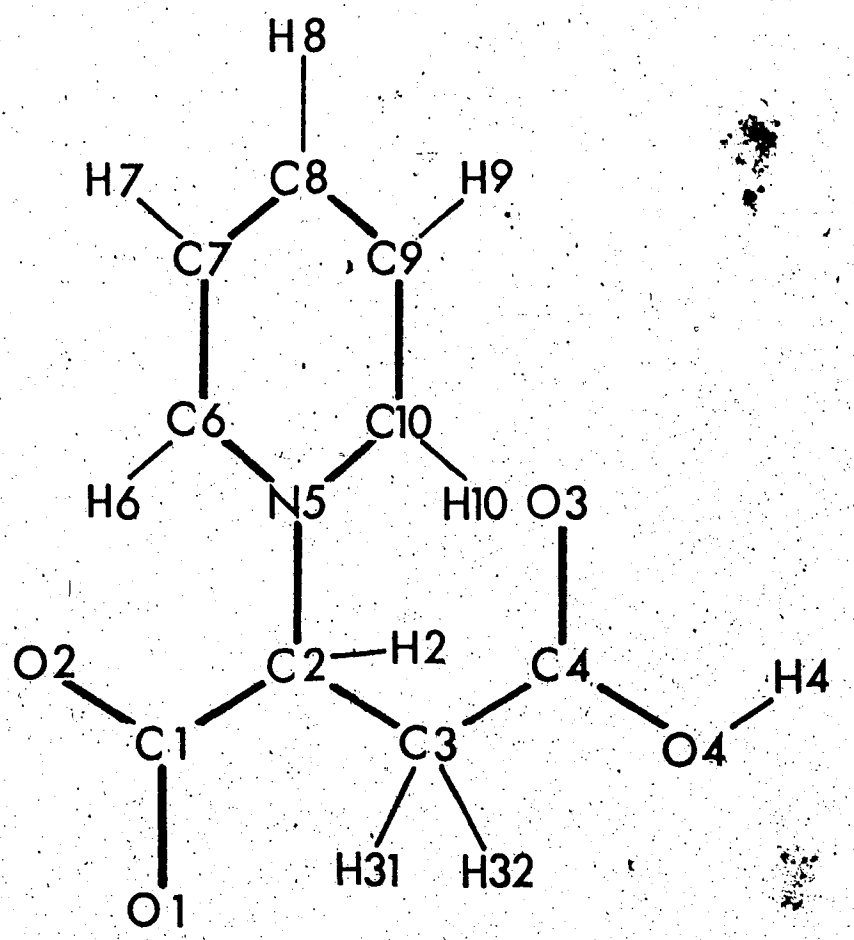


Fig. III 2-1 The molecular form and atomic numbering of N-succinopyridine

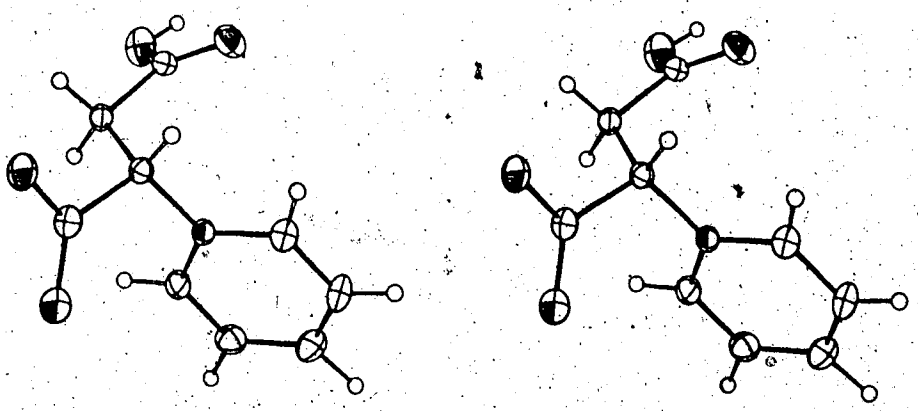


Fig. III 2-2 A stereoscopic view of N-succinopyridine molecule (Johnson, 1965)

As this reaction produced an asymmetric carbon atom C(2) in the succinyl moiety the question of stereospecificity of the reaction was raised. In order to test whether or not the reaction was stereospecific a batch of the crystals was dissolved in water and an ORD experiment showed no optical activity (K. Oikawa kindly ran this spectrum). Since the space group was non-centrosymmetric ($P2_12_12_1$), the isolated product was one of the enantiomorphs (D or L) of N-succinopyridine. The anomalous dispersion data for the

oxygen atoms were not used to determine which enantiomorph was present in the crystal used for intensity data collection.

The bond lengths and angles are illustrated in Fig. III 2-3(b) and Fig. III 2-4(b). The estimated standard deviations are 0.003Å for C-C bonds, except 0.004Å for C-C bonds in the pyridine moiety, 0.002Å for C-N bonds, 0.003Å for C-O bonds and 0.04Å for bonds involving hydrogen atoms. The estimated standard deviations for angles are 0.2° between non-hydrogen atoms and 2° for those angles involving hydrogen atoms.

i) succino moiety

The bond lengths of the succino moiety are compared with those of succinic acid (Broadly, Cruickshank, Morrison, Robertson & Shearer, 1959), potassium hydrogen succinate (McAdam, Currie & Speakman, 1971), dilithium succinate (Klapper & Kupper, 1973), DL-methyl succinic acid (Schouwstra, 1973), potassium hydrogen methyl succinate (Schouwstra, 1972) and two aspartic acid molecules (Rao, 1973; Derissen, Endeman & Peerdeman, 1968) in Table III 2-5. These several derivatives of succinic acid are different in their ionization states and the substituents on the succinic acid back bone. Aspartic acid has the most similar molecular form and ionization state because it has a positively charged nitrogen atom (α-NH_3^+) and a negatively charged carboxyl group (α-COO^-), and the β-carboxyl group retains its proton. In fact the most similar geometries of this compound were found in those of aspartic acid molecules. The positively charged nitrogen atom affects the bond

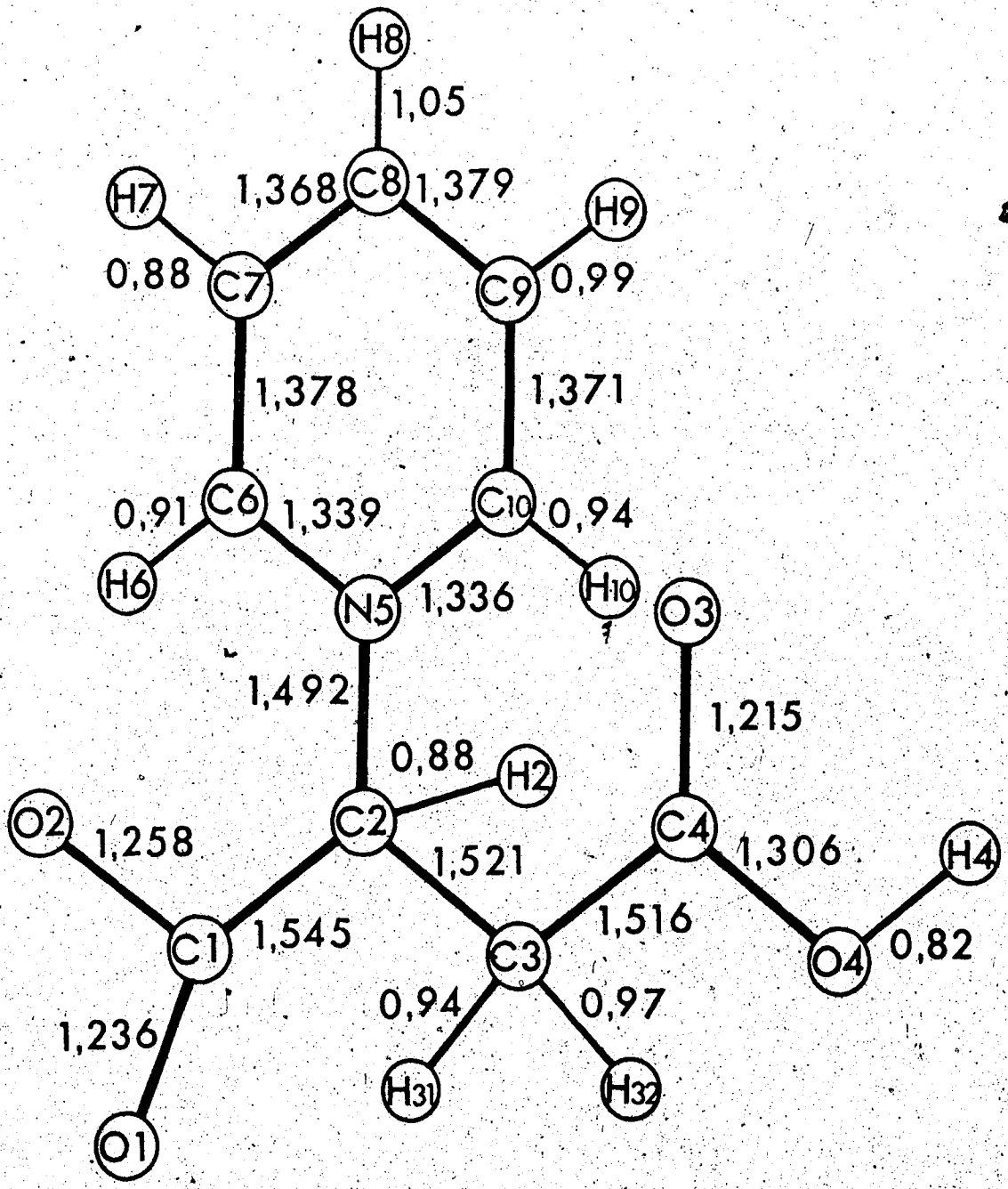


Fig. III 2-3(a) The bond lengths of N-succinopyridine

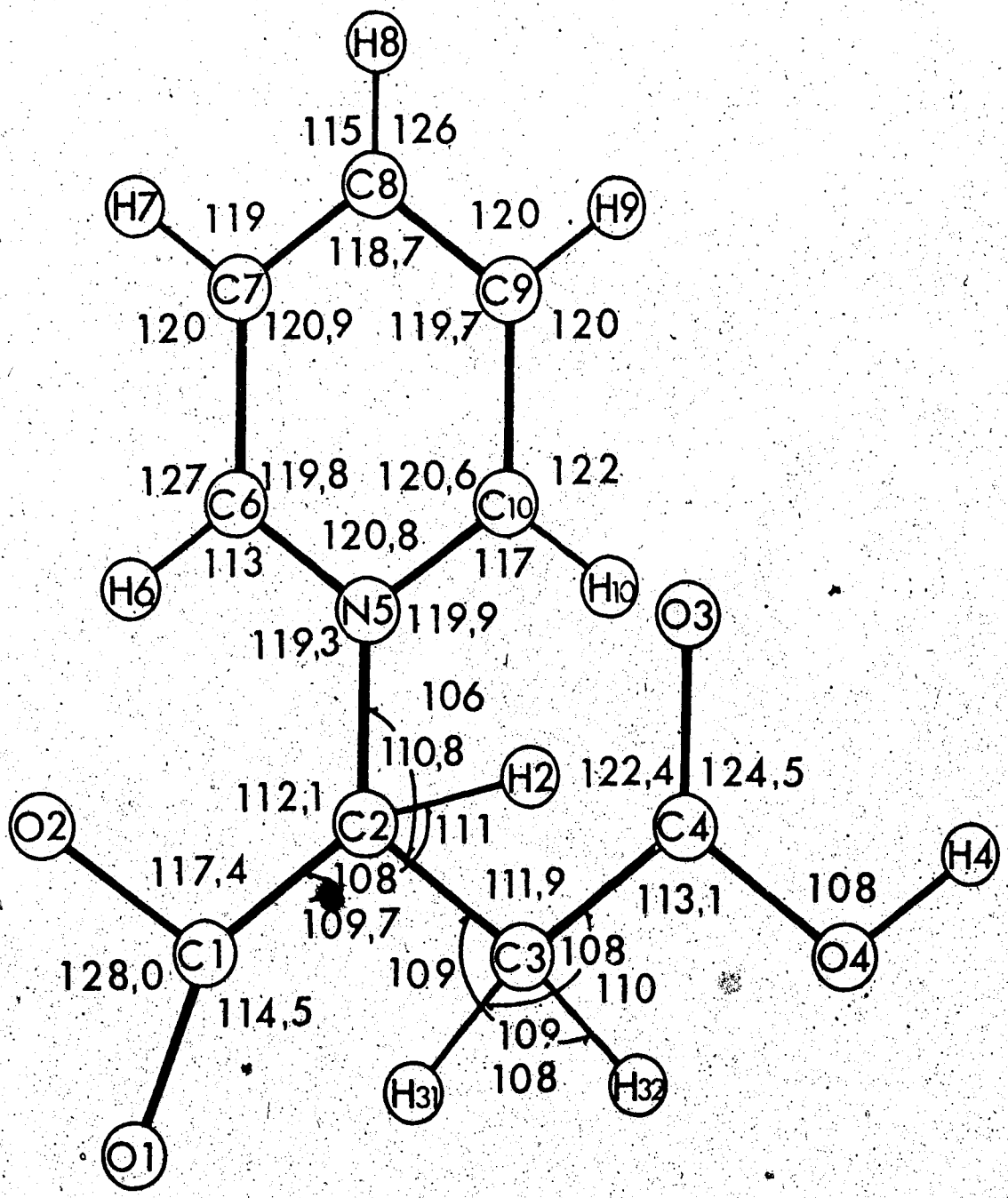


Fig. III 2-3(b) The bond angles of N-succinopyridine

	C1-O1	C1-O2	C1-C2	C2-C3	C3-C4	C4-O3	C4-O4	C2-X	
succinic acid	1.252(11)	1.322(12)	1.485(13)	1.533(19)	1.485(13)	1.252(11)	1.322(12)	-	$\bar{1}$
KH succinate	1.225(4)	1.310(2)	1.512(3)	1.510(5)	1.512(3)	1.225(4)	1.301(2)	-	$\bar{1}$
Li_2 succinate	1.251(1)	1.254(2)	1.518(2)	1.525(2)	1.518(2)	1.253(1)	1.254(2)	-	$\bar{1}$
DL-Methyl Succinic acid	1.245(5)	1.282(5)	1.517(6)	1.525(6)	1.493(6)	1.228(5)	1.301(5)	1.529(5)	$X=CH_3$
K hydrogen methyl succinate	1.242(3)	1.270(3)	1.524(4)	1.516(4)	1.504(4)	1.213(3)	1.313(3)	1.533(3)	$X=CH_3$
DL-aspartic acid	1.250(3)	1.251(2)	1.538(3)	1.519(3)	1.505(3)	1.219(3)	1.305(3)	1.491(3)	$X=NH^+$
L-aspartic acid	1.242(4)	1.252(4)	1.543(4)	1.518(4)	1.512(4)	1.202(4)	1.306(4)	1.495(4)	$X=NH_3^+$
average values of aspartic acid	1.247(2)	1.251(2)	1.540(2)	1.518(2)	1.508(2)	1.213(2)	1.305(2)	1.492(2)	

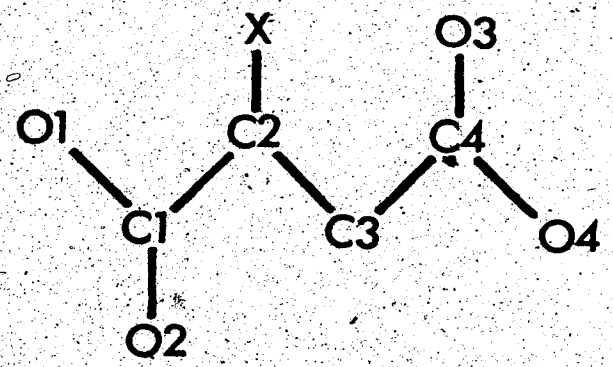


Table III 2-5 The bond lengths of succinic acid derivatives

length C(1) - C(2), 1.545(3) Å in N-succinopyridine and 1.540(2) Å in aspartic acid both of which are longer than those of the methyl-substituted molecules. The bond length C(3) - C(4), 1.516(3) Å, is close to that of C - C = O system, 1.516(5) Å (International Tables for X-ray Crystallography, vol III). The bond length C(2) - C(3), 1.521(3) Å, is not affected by the substituents. In the α-carboxyl group the bond lengths C - O are 1.236(3) Å and 1.258(3) Å, and the angles of C - C - O are 114.5(2)° and 117.4(2)°. The two bonds and the two angles are similar to each other and the values agree with those of negatively charged carboxyl groups in which the oxygen atoms share the charge (refer here to imidazolium maleate). Although it is difficult to explain clearly the reason why the bond length of C(1) - O(2) is significantly longer than that of C(1) - O(1), one reason may be that the oxygen atom O(2) accepts a hydrogen bond giving the C(1) - O(2) bond more single bond character. On the other hand the carbon oxygen bonds in β-carboxyl group have marked differences in bond lengths and angles. The bond lengths are 1.215(3)Å for C(4) - O(3) and 1.306(3) Å for C(4) - O(4) and the bond angles are 122.4(2) Å for C(3) - C(4) - O(3) and 113.1(2) Å for C(3) - C(4) - O(4). These values correspond to those expected for a non-dissociated carboxyl group and coincide with the attachment of the hydrogen atom H(4) on oxygen O(4).

The torsion angles are illustrated in Fig. III 2-4. The angle between the negatively charged oxygen atom O(2) and the positively charged nitrogen atom N(5) is 20.7°. The comparable torsion angles

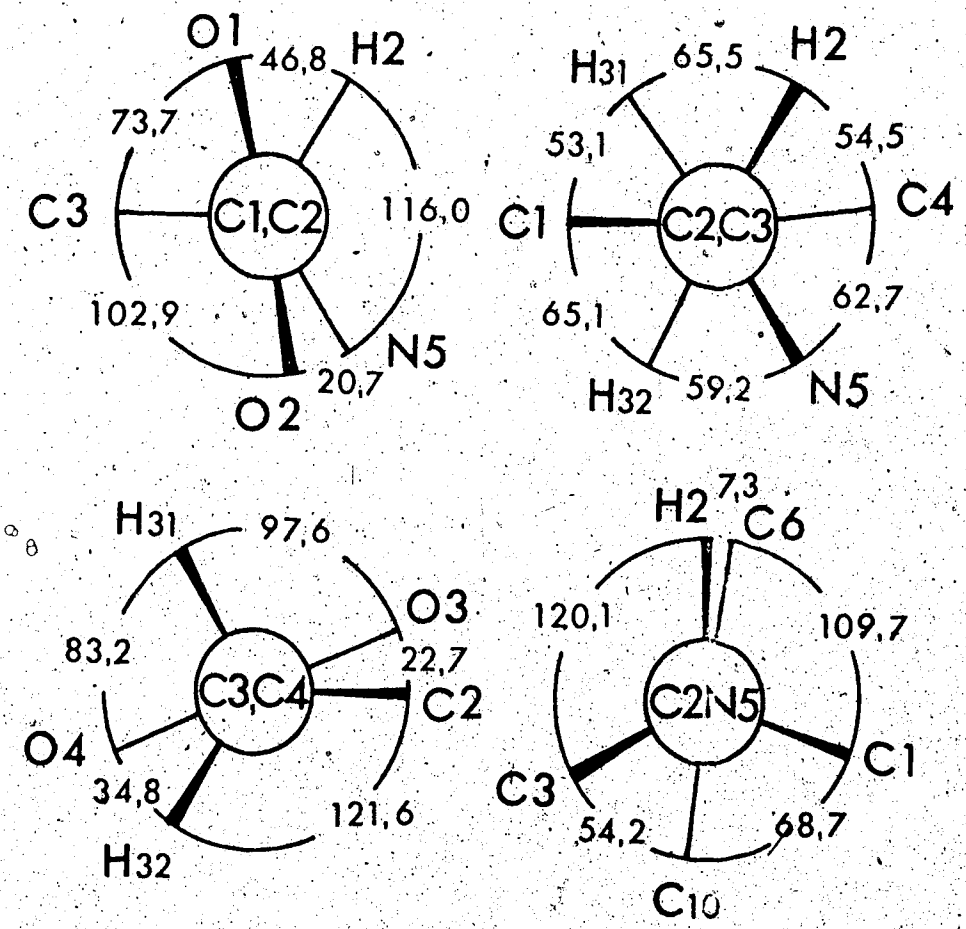


Fig. III 2-4 Newman projections of the torsion angles of N-succinopyridine

of DL-and L-aspartic acid were 7.3° and 37.8°, respectively, (Rao, 1973). These three values are very similar especially when one considers that the three aspartate moieties are in different environment in three separate crystal structures. In addition the torsion angles C(1) - C(2) - C(3) - C(4) and C(2) - C(3) - C(4) -O(3) are 173.1° and 22.7° in this structure. The corresponding angles were 174.2° and 3.1° in the structure of DL-aspartic acid, and 178.2° and 131.4° in the structure of L-aspartic acid. The torsion angles of the carbon back bone retain almost the same angle in these three structures, but the torsion angles around the bond C(3) - C(4) can be different in the different crystals, reflecting the effects of crystal packing and specific hydrogen bond formation.

(ii) Pyridinium moiety

The bond lengths and angles of neutral pyridine rings were summarized by Dr. G.J.B. Williams (1972). These averaged values and the values of pyridinium rings are listed in Table III 2-6. The bond lengths between the non-hydrogen atoms in this structure are not significantly different from those of other pyridine rings, but the bond angles show that the angles C - N - C and N - C - C in the pyridinium rings are different from those in neutral pyridine. The averaged angles of the neutral rings were 117.4° for C - N - C, 124.3° and 121.8° for N - C - C and 119.1° at the para position with respect to the nitrogen atom. The angles of pyridine by a spectroscopic determination agreed well with these above angles. Serewiez et al., observed in the structure of pyridine hydrogen nitrate that when the

	a	b	c	d	e	f	σ(1)	ref
average bond length of neutral pyridine	1.343	1.335	1.373	1.379	1.374	1.392	0.0014	(1)
pyridine	1.340	1.340	1.395	1.395	1.394	1.395	0.001	(2)
Glucetol:pyridine	1.329	1.330	1.364	1.359	1.364	1.382	0.006	(3)
Pyridinium moiety	1.346	1.337	1.362	1.398	1.376	1.361	0.007	(4)
Pyridine hydrogen nitrate	1.349	1.307	1.357	1.335	1.347	1.349	0.016	(5)
average bond lengths	1.346	1.332	1.361	1.388	1.371	1.359	0.006	
N-succinopyridine	1.339(2)	1.346(2)	1.378(4)	1.368(4)	1.379(4)	1.371(4)		

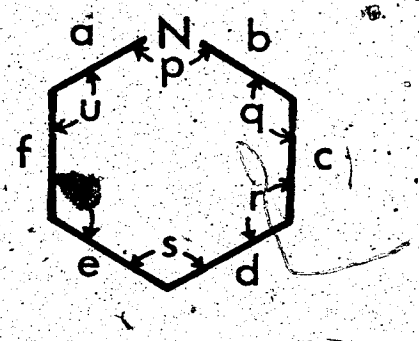


Table III 2-6 The bond lengths and angles of pyridine moieties.

continued...

	p	q	r	s	t	u	σ
average bond angles of neutral pyridine	117.4	124.3	118.0	119.1	119.3	121.8	0.12
pyridine	116.8	123.9	118.5	118.3	118.5	123.9	0.1
Glucitol:pyridine	116.5	123.2	119.7	118.8	118.1	123.7	0.4
Pyridinium moiety	123.1	119.5	119.1	119.1	120.2	118.8	0.5
Pyridine hydrogen nitrate	120.4	120.1	120.0	120.3	118.8	120.4	1.1
average bond angles	122.6	119.6	119.3	119.3	120.0	119.1	0.4
N-succinopyridine	120.8	119.8	120.4	118.7	119.7	120.6	0.2

References (1) Williams, 1972
(2) Bak, Hansen-Nygaard & Rastrup-Anderson, 1958
(3) Kim, Jeffery & Rosenstein, 1971
(4) Flynn & Boer, 1969.
(5) Serewiez, Robertson & Meyers, 1965.

lone pair electrons of the nitrogen in the ring were covalently bonded with another atom, the angles C - N - C and C - C_{para} - C increased and the angles N - C - C decreased (Serewiez, Robertson & Meyers, 1965). This structure did not show the increase of the angle at the para position, but the angle C - N - C in the ring increases to 120.8° and the angles N - C - C decreased to 120.2°. These changes of geometry agree with those observed for the averaged angles of N-substituted nicotinamide molecules (Table III 2-7). The bond length C(2) - N(5), 1.492(2)Å, agrees with those corresponding bonds in the aspartic acid structures, and also with the averaged value, 1.489(2) Å in the N-substituted nicotinamide derivatives.

Atom C(10) of the pyridine ring is in the guache-gauche conformation with respect to the carbon atoms C(1) and C(3) of the succinyl moiety. The torsion angle around the bond C(2) - N(5) is 68.7° for C(1) - C(2) - N(5) - C(10) and -54.2° for C(3) - C(2) - N(5) - C(10) (Fig. III 2-4). The least-squares plane of the pyridine ring is listed in Table III 2-8. Carbon atom C(2) lies 0.045 Å above this plane(0.036 Å above the plane through the atoms N(5), C(6) and C(10)). This atom may be significantly out of the planes, but the displacement is less than those of the atoms C(1') from the imidazole planes in IMR and AIMR.

(iii) Molecular Packing

A molecular packing diagram is shown in Fig. III 2-5. A hydrogen bond is formed between the oxygen atom O(4) and the oxygen atom O(2) which belongs to the molecule translated by one unit cell along the a axis. The dimension of the hydrogen bond is shown in Table III 2-9.

N(1)-C(7)	N(1)-C(2)	N(1)-C(6)	<C(2)-N(1)-C(6)	ref.
1.494(5)	1.348(5)	1.341(5)	120.0(4)	(1)
1.496(7)	1.348(6)	1.335(7)	121.8(5)	(2)
1.491(9)	1.348(8)	1.361(10)	120.5(5)	(3)
1.474(5)	1.361(5)	1.354(5)	119.8(2)	(4)
1.489(5)	1.354(5)	1.361(5)	120.3(2)	(5)
1.496(6)	1.339(6)	1.355(6)	120.8(4)	(5)
1.489(2)	1.351(2)	1.351(2)	120.2(1)	average values

references (1) Voet, 1973
(2) Johnson, Maier & Paul, 1973
(3) Johnson, Frank & Paul, 1973
(4) Frank, Thayer & Paul, 1973
(5) Herriott, Camerman, Deranleau, 1974

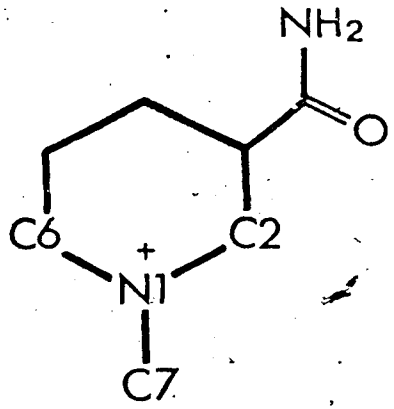


Table III 2-7 The bond lengths and angle of N-substituted nicotinamides

atoms	distance from the plane
N(5) *	0.002 Å
C(6) *	-0.001
C(7) *	-0.006
C(8) *	0.002
C(9) *	-0.001
C(10)*	-0.001
C(2)	0.045
standard deviation	0.001

* atoms defining the least-squares plane.

equation of the plane 0.99284i + 0.11925j - 0.00627k = 5.08858 in orthogonal angstrom space

Table III 2-8 The least-squares plane through pyridine moiety.

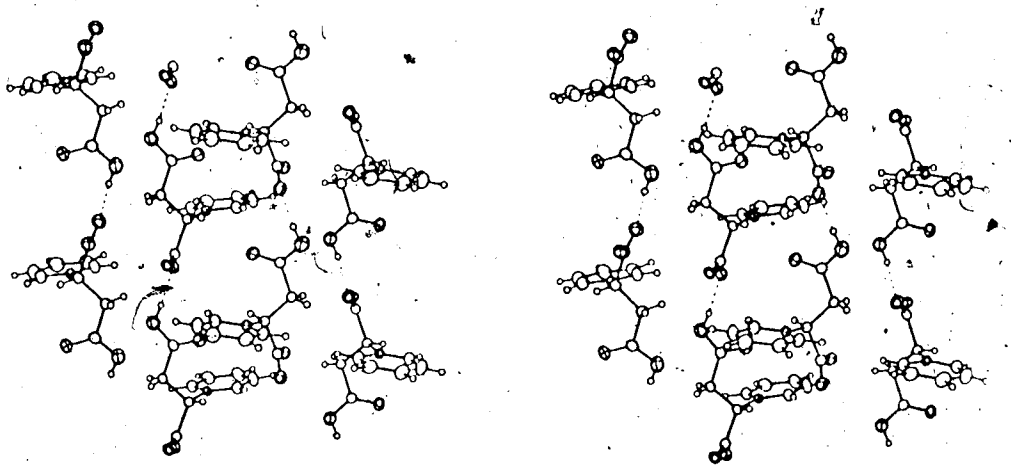


Fig. III 2-5 Molecular packing in the crystals of N-succinopyridine (Johnson, 1965). The vertical direction is along the a axis and the horizontal direction is approximately along the b axis.

	distance O...O	distance H...O	angle O-H...O
O(4)-H(4)...O(2)	2.531(2)Å	1.72(5)Å	171(4)°

Table III 2-9 The dimension of the hydrogen bond in the structure of N-succinopyridine

Since the hydrogen atom H(4) is the only available hydrogen atom to form a hydrogen bond in the molecule, these hydrogen bonds link the molecules and form infinite one dimensional chains of molecules parallel to the crystallographic a axis. The pyridine moieties partially overlap with each other and are related by a symmetry operation of a 2_1 screw axis along the a axis at x, ¼, 0 (Fig. III 2-6).

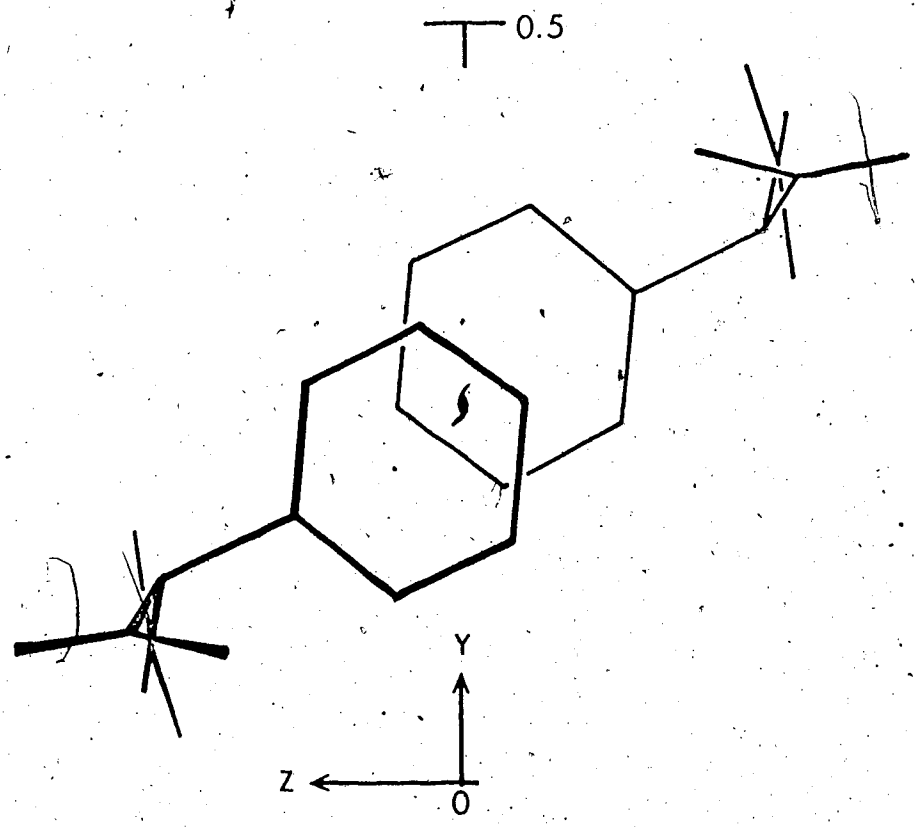


Fig. III 2-6 The projected diagram on bc plane of the structure of N-succinopyridine

There is another intermolecular interaction occuring between the positively charged pyridine ring and several oxygen atoms. A

stereoscopic view showing the environment of the pyridine ring is shown in Fig. III 2-7.

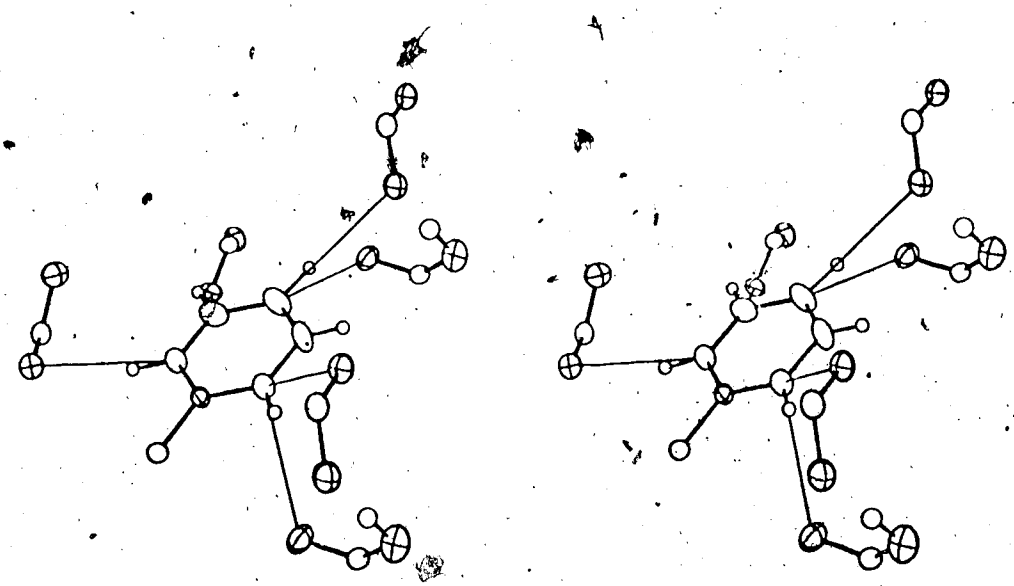


Fig. III 2-7 A stereoscopic view around the positively charged pyridine ring (Johnson, 1965).

A distance scan of all intermolecular contacts within a 3.5 Å radius from each of the carbon atoms of the pyridine ring was calculated. The only atoms found in this scan are two carbonyl oxygen atoms and four negatively charged oxygen atoms. None of the hydroxyl oxygen atoms are close to the ring. The distances between the carbon atoms and the oxygen atoms are listed in Fig. III 2-8.

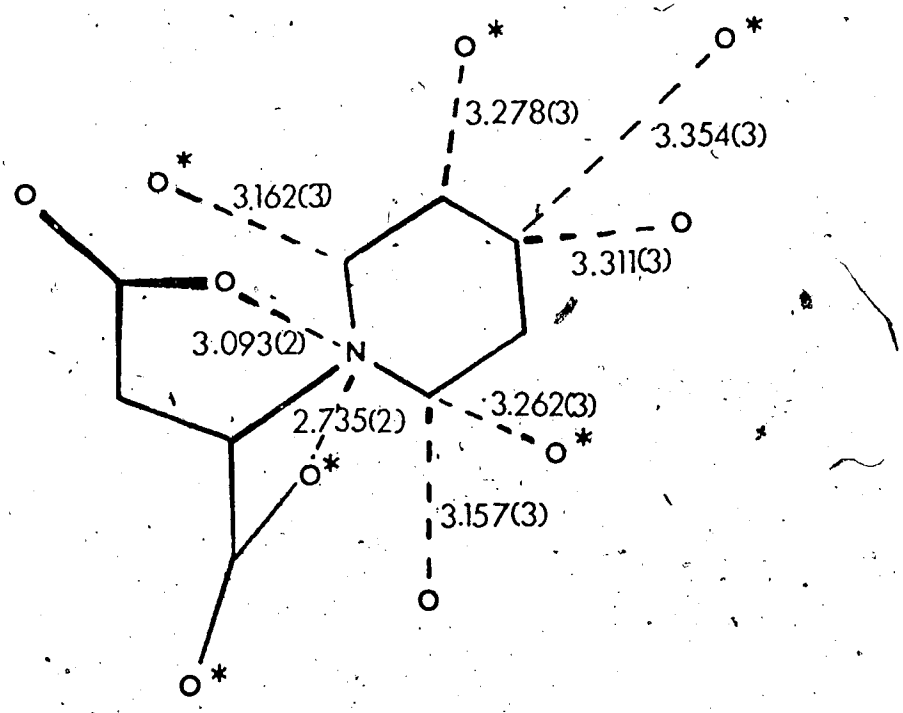


Fig. III 2-8 The distances between the carbon atoms and the oxygen atoms around the pyridine ring. The asterisks above O mean the oxygen atoms which have a half of negative charge.

The angles and the displacements from the least-squares plane of pyridine ring are listed in Table III 2-10. The signs of the displacements indicate the arbitrary directions along the perpendicular to the plane. Two oxygen atoms O(3) and O(1) are close to one of the ortho carbon atom C(6). The distances are 3.157(3) Å and 3.262(3) Å. The other ortho carbon atom, C(10), is close to an oxygen atom, O(1), which is almost on the plane of pyridine ring. The distance is 3.162(2) Å. The para carbon atom, C(8), is approached by two oxygen atoms, O(3) and O(2). The distances are 3.311(3) Å and 3.354(3) Å. Only one of the meta carbon atoms, C(9), is close

N5-C6...03(i)	100.5°	C7-C8...03(iii)	119.4
C7-C6...03(i)	130.6°	C9-C8...03(iii)	114.0
C9...C6...03(i)	146.3	N5...C8...03(iii)	151.5
N5-C6...01(ii)	134.5	C7-C8...02(iv)	88.5
C7-C6...01(ii)	95.1	C9-C8...02(iv)	146.9
C9...C6...01(ii)	141.9	N5...C8...02(iv)	142.7
C3(i)...C6...01(iii)	71.7	03(iii)...C8...02(iv)	59.0
C8-C9...01(v)	124.0	C9-C10...01(vi)	114.3
C10-C9...01(v)	106.8	N5-C10...01(vi)	125.0
C6-C9...01(v)	148.1	C7...C10...01(vi)	173.2

	displacement from the pyridine ring
03(i)	1.526 Å
01(ii)	-1.653
03(iii)	1.572
02(iv)	-1.127
01(v)	1.669
01(vi)	-0.253

Symmetry transformation for the following notation

(i)	1/2 + x, 1/2 - y, 1 - Z
(ii)	x - 1/2, 1/2 - Z, 1 - Z
(iii)	1/2 + x, 1/2 - y, -Z
(iv)	x - 1/2, 1/2 - y, -Z
(v)	x, y, Z - 1
(vi)	3/2 - x, -y, 1/2 + Z

Table III 2-10 The angles and displacements of the oxygen atoms around the pyridine ring.

to an oxygen atom O(1) to which the distance from the carbon atom is 3.278(3)Å. The fact that these oxygen atoms were oriented primarily towards both of the ortho carbon atoms and the para carbon atom in the pyridinium ring suggests that these atoms are involved with the charge delocalization. This packing also suggests that the positive charge on the nitrogen atom can reduce the electron densities on the carbon atoms at the ortho and para positions and that these carbon atoms have a slight positive charge.

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Glossary

absorption coefficient — a rate that X-ray beam is transmitted through 1 cm thick substance. This coefficient is dependent on the material and the wave length of the X-ray beam.

absorption of X-rays — X-ray beam is absorbed by material in its path and this reduces the intensity of the transmitted X-ray beam.

aglycon — a substituent on an anomeric carbon atom.

anisotropic temperature factors — elements of a matrix which describe atomic thermal motion as ellipsoidal harmonic vibration.

anomeric carbon — a carbon atom, C1, which is bonded to the ring oxygen atom and also to an electronegative atom in saccharides.

anomeric effect — a phenomenon which causes an electronegative and neutral aglycon to prefer an axial orientation with respect to a saccharide ring.

anomers — a pair of saccharide diastereoisomers which differ only in the configuration of their anomeric carbon atoms.

coenzyme — a molecule which is small and non-protein, and which is required in an enzymatic reaction and can reversibly be modified by other enzyme systems.

difference map — a map calculated by Fourier summations with coefficients (Fo-Fc) and calculated phases. This map shows differences between the actual and the calculated model of electron densities.

E (normarized structure factor) — This value is a ratio of a structure amplitude to the root square of the sum of the squared atomic scattering factors in a unit cell, assuming no atomic thermal vibrations

full-matrix and block-diagonal-matrix refinement — Full-matrix refinement takes all correlations between the parameters into account. Block-diagonal-matrix refinement neglaects correlations between parameters of different atoms.

isotropic temperature factor — thermal factor which describes atomic motion as isotropic harmonic vibration.

Lorentz factor — This factor takes account of the dependency of the diffraction time of a reflexion under the observational condition of constant angular velocity of a crystal. The diffraction time is dependent on Bragg angle.

least-squares refinement — a summation of weighted squared values of differences between observed and calculated values is minimized.

monochrometer — a single crystal (eg. quartz, graphite) placed in the path of an incident X-ray beam in order to produce a monochromatic beam. Such an X-ray beam can be selected by a diffraction from a crystal because Bragg angle from a set of parallel planes is dependent on wave length.

polarization factor — This factor corrects the intensity of a reflexion for polarization.

R (residual index) — one of the indicaters which show correctness of a model used.

reverse anomeric effect — a phenomenon where an equational orientation is preferred by an aglycon which is electronegative and has a positive charge.

secondary extinction — This arises for reflexions of such intensity that an appreciable amount of the incident radiation is reflected at a given instant by the first planes encountered by the beam. The deeper planes receive less incident intensity and therefore reflect less power than would otherwise have been the case.

structure factor — an amplitude and phase of a reflexion from a crystal. This factor includes the information about the atomic parameters in a unit cell of the crystal.

synplanar — if D eclipses A in a system A-B-C-D where B, C, D and E are on a plane, the conformation is termed as synplanar.

weighting — a method of taking the reliability of observations into account in least-squares refinement calculations.

Weissenberg camera — a camera which is designed to take X-ray photographs in which X-ray diffractions from a selected layer line are recorded in a two-dimensional array.