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The Conformations of the Inosine Molecule

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An independent determination of the structure of inosine dihydrate is described and the results compared with those of Bugg, Thewalt & Marsh for the same structure and with the results of Munns & Tollin for the structure of inosine.

Introduction

We wish to confirm the crystal and molecular structure of inosine dihydrate determined by Bugg, Thewalt & Marsh (1968, 1970) and to compare the conformations of the inosine molecules with that of inosine in another crystal form studied by two of us (Tollin & Munns, 1969; Munns & Tollin, 1970).

In the studies of nucleic acid components it is of interest to compare the conformation of the same nucleoside when it is in different environments in the crystalline state. Inosine is of particular interest in this respect because it crystallizes in at least three different crystal forms (Munns & Tollin, 1970) and in the dihydrate form there are two molecules in the asymmetric unit. Inosine is also of interest because of its occurrence in the third position in the anticodon triplet in a number of transfer ribonucleic acids, and because of the part it plays in Crick's 'wobble' hypothesis (Crick, 1966).

After we had determined the structure of inosine dihydrate we learned that the structure had previously been determined by Bugg, Thewalt & Marsh (1968) and after confirming that the structures were in agreement, we stopped the refinement process at an R index value of 0·09, where the R index is defined as $\sum_h ||F_{obs}|-|F_{calc}|| / \sum_h |F_{obs}|$. However, our method of structure determination was different from that of Bugg *et al.* and an outline of the procedure used will be described.

Experimental

Crystals were obtained by slow evaporation from partially sealed test tubes at 19 °C (Munns & Tollin, 1970). The crystals were of monoclinic symmetry with unit-cell dimensions $a=6{\cdot}65\pm0{\cdot}01$, $b=11{\cdot}26\pm0{\cdot}02$, $c=17{\cdot}58\pm0{\cdot}02$ Å and $\beta=98°\ 18'\pm5'$ and were found to belong to the space group $P2_1$. There are four molecules in the unit cell. Intensity data were collected on a Hilger & Watts linear diffractometer using molybdenum K radiation and balanced filters.

Structure determination

The inosine dihydrate structure was solved in an analogous manner to the procedure used in the case of inosine (Munns & Tollin, 1970). The method incorporates the rotation function (Rossmann & Blow, 1962) into the general Patterson function interpretation procedure and is fully discussed elsewhere (Tollin, Young & Munns, to be published).

The atoms of the purine group, together with atom C(1′) of the ribose sugar group, were assumed to be in a plane and its orientation in the unit cell was found using the $I(\theta, \varphi)$ function of Tollin & Cochran (1964). A disc of radius 4·5 Å and 2347 sharpened Patterson coefficients were used to compute this function. The results, plotted on a Sanson–Flamsteed projection, are shown in Fig. 1. This map contains only one large peak at $\theta=90°$, $\varphi=0°$. This indicates that all four purine planes in the unit cell lie parallel to the crystallographic bc plane. The rotation function was used to determine the azimuthal angle defining the orientation of the purine group in this plane. Sharpened Patterson coefficients were calculated for a model of the purine group. The line in the rotation function which corresponds to rotating the purine group in the plane defined by the $I(\theta, \varphi)$ function is given by $\theta_1=\varphi+\pi/2$ and $\theta_2=\theta$. This line through the rotation function, calculated using the model coefficients and the observed sharpened Patterson coefficients, is shown in Fig. 2(*b*). Symmetry considerations restrict the unique part to $0\leq\theta_3\leq\pi$. Fig. 2(*a*) shows the rotation of the model against itself, the rotation axis being the normal to the purine plane. The best interpretation of Fig. 2(*b*) corresponds to the superposition of two copies of Fig. 2(*a*) shifted by $\pm12\frac{1}{2}°$ and this is shown in Fig. 2(*c*).

The relative coordinates of the atoms of the purine groups were calculated from the values of the Eulerian angles θ_1, θ_2 and θ_3 so determined. The positions of the purine groups in the unit cell, relative to the 2_1 axis were found using the $Q(X_0, Z_0)$ function of Tollin (1966). The results from the rotation function cannot distinguish between two possible symmetry related orientations for the purine group. This ambiguity was

resolved by attaching a ribose sugar group to the purine group in approximately the same orientation as that found in inosine (Munns & Tollin, 1970). Two Q functions were calculated using the two possible positions for the sugar group and the correct choice was clearly indicated. The purine groups were found to be close to the planes $x=0$ and $x=\frac{1}{2}$. The position with respect to the y axis of the purine residue in the $x=0$ plane could be assigned arbitrarily. The position of the other purine group in the asymmetric unit was found by examining the section through the electron density map at $x=\frac{1}{2}$, computed using phases calculated on the basis of one inosine molecule.

Successive structure factor calculations and Fourier syntheses allowed all the ribose sugar heavy atoms and the water molecule positions to be found directly. Isotropic least-squares refinement reduced the R index to 0·14 and two anisotropic cycles reduced the R value to 0·11.

At this stage we learned that the structure had re-

Table 1. *Non-hydrogen atom positional parameters*

For direct comparison with Table 3 of Bugg *et al.* (1968) interchange the x and z columns of coordinates.

Molecule II*A*	x	y	z	Molecule II*B*	x	y	z
N(1)	0·5156	0·4459	0·4438	N(1)	1·0044	0·6982	0·4681
C(2)	0·5340	0·4296	0·3674	C(2)	1·0103	0·7367	0·3952
N(3)	0·5378	0·5115	0·3181	N(3)	1·0167	0·6662	0·3359
C(4)	0·5169	0·6237	0·3517	C(4)	1·0218	0·5508	0·3562
C(5)	0·5087	0·6497	0·4251	C(5)	1·0150	0·5018	0·4270
C(6)	0·5026	0·5553	0·4803	C(6)	1·0055	0·5789	0·4909
O(6)	0·4860	0·5621	0·5489	O(6)	0·9991	0·5530	0·5584
N(7)	0·4940	0·7702	0·4365	N(7)	1·0161	0·3814	0·4252
C(8)	0·4980	0·8149	0·3683	C(8)	1·0210	0·3561	0·3538
N(9)	0·5093	0·7306	0·3141	N(9)	1·0274	0·4544	0·3067
C(1′)	0·5095	0·7460	0·2313	C(1′)	1·0308	0·4565	0·2248
O(1′)	0·3412	0·6840	0·1909	O(1′)	1·1804	0·3754	0·2104
C(2′)	0·4797	0·8769	0·2057	C(2′)	0·8336	0·4069	0·1788
O(2′)	0·6574	0·9465	0·2184	O(2′)	0·6718	0·4918	0·1650
C(3′)	0·3873	0·8593	0·1204	C(3′)	0·9131	0·3759	0·1029
O(3′)	0·5401	0·8325	0·0739	O(3′)	0·9238	0·4753	0·0559
C(4′)	0·2576	0·7486	0·1232	C(4′)	1·1287	0·3330	0·1309
C(5′)	0·0293	0·7685	0·1281	C(5′)	1·1598	0·1983	0·1336
O(5′)	0·0012	0·8246	0·1973	O(5′)	1·0073	0·1381	0·1660
Water							
O(10)	0·3689	0·6509	0·6892	O(12)	0·6021	0·6152	1·0031
O(11)	0·7989	0·5807	0·6913	O(13)	0·2110	0·5230	0·9549

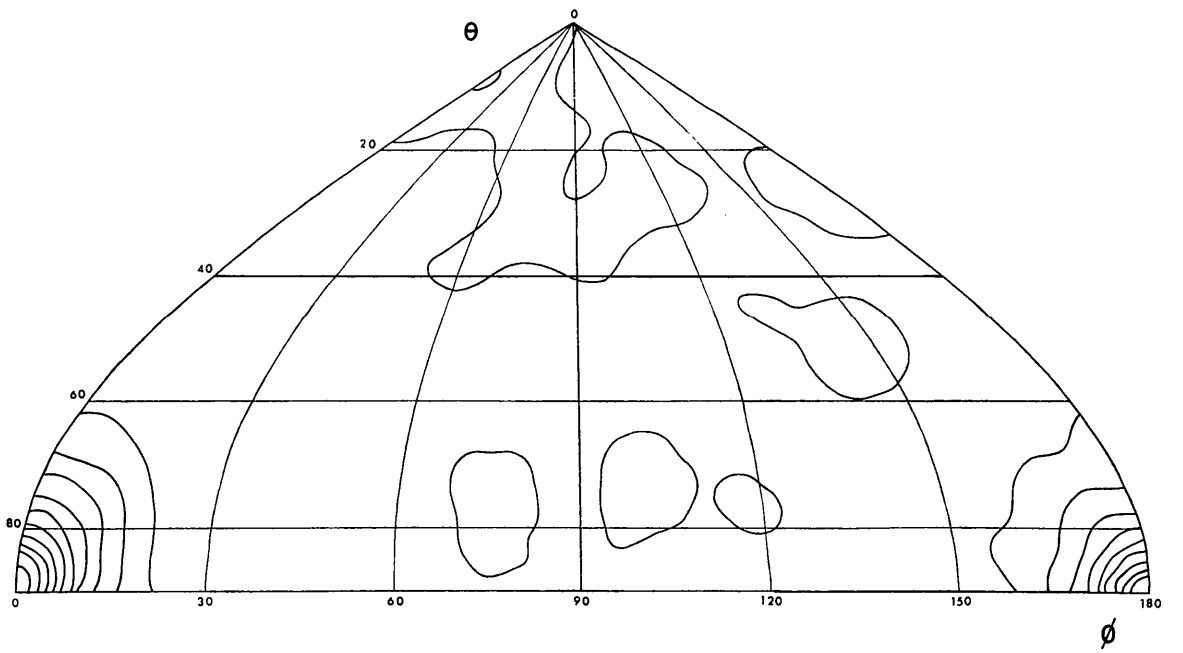

Fig. 1. Sanson–Flamsteed projection of the $I(\theta,\varphi)$ function, contoured at equal arbitrary intervals. $R=4{\cdot}5$Å.

cently been determined by Bugg *et al.* (1968). We carried out two further cycles of least-squares refinement with anisotropic temperature factors for the heavy atoms with isotropic thermal parameters on fixed hydrogen atoms, which reduced the *R* index to 0·09. The coordinates of the non-hydrogen atoms at this stage of refinement are listed in Table 1.

Discussion

We shall refer to the inosine molecule in the inosine crystal (Tollin & Munns, 1969) as molecule I and those in inosine dihydrate (Bugg, Thewalt & Marsh, 1968, 1970) as molecules II*A* and II*B*.

There are no significant differences between the bond lengths and angles in all three molecules. However, the deviations of the atoms from the mean plane of the nine atoms of the purine base differ slightly for the three molecules. The largest differences in deviation involve atoms O(6) and C(1′) which are displaced 0·15 and 0·16 Å respectively in molecule I, but only 0·04 and 0·09 Å in molecule II*A* and 0·004 and 0·03 Å in molecule II*B*. Similar variations in the deviations of atom C(1′) are observed in other nucleosides.

The relative orientation of the sugar and purine base can be described by the torsion angle φ_{CN} defined by Donohue & Trueblood (1960), or by Haschemeyer & Rich (1967). In all three inosine molecules the conformation is *anti*, with $\varphi_{CN} = -10{\cdot}6°$ in molecule I, $\varphi_{CN} = -121°$ in molecule II*A* and $\varphi_{CN} = -45°$ in molecule II*B*. Haschemeyer & Rich (1967) have calculated the allowed values of φ_{CN} for purine nucleosides puckered with C(3′) *endo* and with C(2′) *endo* by considering the steric barriers to rotation about the glycosidic bond. The allowed ranges of φ_{CN} are much larger than for pyrimidine nucleosides and the values for the three inosine molecules fall into the allowed ranges calculated by Haschemeyer & Rich.

The differences between the bond lengths of the sugar parts of the three inosine molecules are not significant but there is a difference between the type of pucker in molecule I and molecules II*A* and II*B*. In molecule I the pucker is C(3′) *endo* whereas it is C(2′) *endo* in molecules II*A* and II*B*. Similar differences in the type of pucker with change in environment is observed in the case of bromouridine (Iball, Morgan & Wilson, 1968). The difference in pucker results in similar differences between the angles around the C(2′) and C(3′) atoms to those that have already been discussed by Sundaralingam (1965) and Sundaralingam & Jensen (1966) based on a survey of nucleoside and nucleotide structures. The exocyclic C–C–O angles involving the out-of-plane carbon atom in Sundaralingam & Jensen's analysis were about 6° greater than those involving the in-plane carbon atom. In inosine I, the mean exocyclic C–C–O angle at atom C(3′) is 115·1° and the mean angle at C(2′) is 108·6°, whereas in molecules II*A* and II*B* the mean angle at C(3′) is 110·0° and at C(2′) it is 114·0°. The angle C(2′)–C(3′)–C(4′), however, is greater than angle C(1′)–C(2′)–C(3′) in all three inosine molecules so that only the results for molecules II*A* and II*B* agree with Sundaralingam's (1966) conclusion that the C–C–C angle involving the out-of-plane carbon atom is about 1·6° less than the angle involving the in-plane atom. In the case of molecule I, the angle at the in-plane C(2′) atom is 0·9° less than the angle at the out-of-plane C(3′) atom and it is probable that packing forces can therefore affect these angles (Munns & Tollin, 1970).

Sundaralingam & Jensen's (1965) analysis suggests that the exocyclic C–O bond involving the out-of-plane atom should be about 0·2 Å less than the bond involving the in-plane atom. However, there are no significant differences between the C(2′)–O(2′) and C(3′)–O(3′) bonds in any of the inosine molecules. The other bond lengths and angles, however, agree with

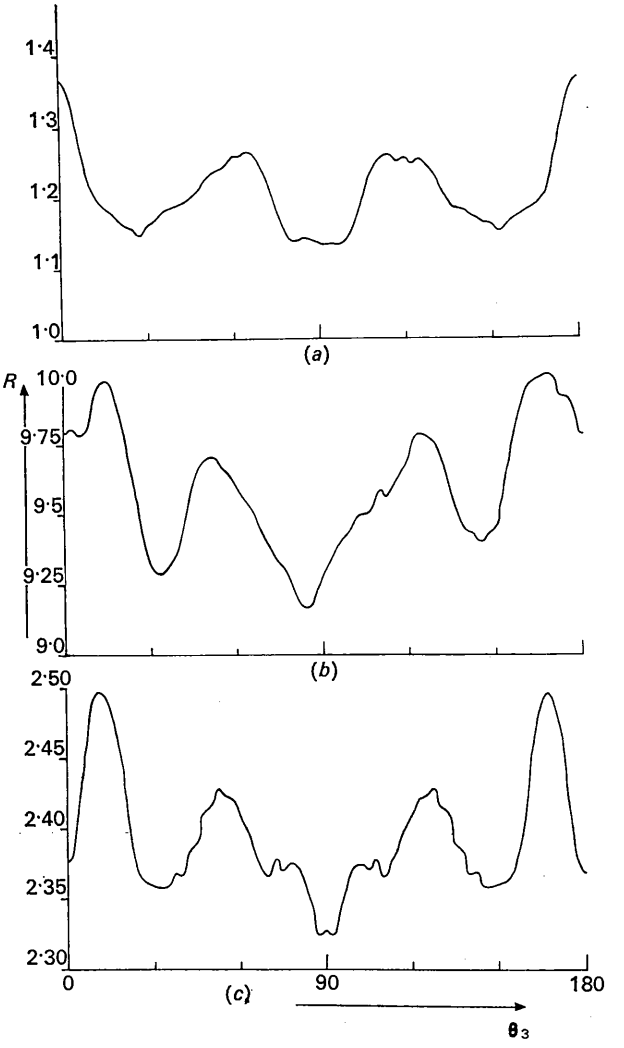

Fig. 2. (*a*) Rotation function of model *v.* model, (*b*) rotation function of model *v.* inosine, (*c*) addition of two superimposed copies of Fig. 2(*a*) shifted by $\pm 12\frac{1}{2}°$.

Sundaralingam & Jensen's conclusions. The C(1′)–O(1′) bond is significantly smaller than the C(4′)–O(1′) bond in all three molecules, the mean C(1′)–O(1′) bond being 1·414 Å and the mean C(4′)–O(1′) being 1·454 Å. Angles N(9)–C(1′)–O(1′) are consistently smaller than angles N(9)–C(1′)–C(2′), and angles C(3′)–C(4′)–C(5′) are smaller than angles O(1′)–C(4′)–C(5′).

Apart from the difference in pucker in the sugars, the other big difference in conformation between molecule I and molecules II*A* and II*B* is in the orientation of the C(5′)–O(5′) bond about the C(4′)–C(5′) bond. In both molecules II*A* and II*B* the conformation is such that C(5′)–O(5′) is *gauche* to C(4′)–O(1′) and to C(4′)–C(3′), but in inosine I, C(5′)–O(5′) is *gauche* to C(4′)–O(1′) and *trans* to C(4′)–C(3′).

In nucleoside structures, the pyrimidine and purine bases have a tendency to pack in parallel planes about 3·5 Å apart and to prefer hydrogen bonding to each other. Very often nucleoside structures possess a unit-cell dimension of about 4·8 Å, and in many cases, the bases which are parallel and 3·5 Å apart are those which are separated by this lattice translation. Therefore, it is possible to predict that the planes of the bases will make an angle of about 45° with this cell edge. Some examples of this include deoxyadenosine (Watson, Sutor & Tollin, 1965), thymidine (Young, Tollin & Wilson, 1969), and the 5-bromouridine–adenosine complex (Haschemeyer & Sobell, 1965).

The structure of inosine again shows this kind of packing, when the short axis is the *c* axis. The hydrogen bond between the bases is from N(1) to N(7). In the inosine dihydrate structure the bases are again connected by this hydrogen bond but have been rotated about it until they are coplanar and as a result produce sheets of hydrogen bonded bases. These sheets are stacked 3·3 Å apart as mentioned by Bugg *et al.* (1970).

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A Refinement of the Crystal Structure of $NH_4NO_3.2HNO_3$

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The structure of $NH_4NO_3.2HNO_3$ has been refined, using the original experimental results of Duke & Llewellyn. The structure consists of NH_4^+ cations and hydrogen-bonded $[O_2NO–H–(NO_3)–H–ONO_2]^-$ anions. The bond lengths in this anion are discussed in detail, and compared with relevant values for other molecules.

Introduction

There has been an increasing interest recently in the group of anions with the general formulae HX_2^- and HXY^-, in which two like or unlike uninegative anions (X^-, Y^-) are linked through a hydrogen bond. The study of such species offers many advantages in the investigation of simple isolated hydrogen-bonded systems. A number of spectroscopic studies have been reported for salts of a number of these anions, but bond length data are lacking in most cases (Tuck, 1968). In addition to the HX_2^- species, a small number of anions of the type $[H_MX_{M+1}]^-$ are also known. For X = F, the values of *n* range from 2 to 7, and the structure of $H_2F_3^-$ has been established by X-ray methods (Forester, Senko, Zalkin & Templeton, 1963).