

The Crystal and Molecular Structure of Inosine-5'-phosphate

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The crystal and molecular structure of inosine-5'-phosphate has been determined by the symbolic addition method. The crystal obtained from an aqueous methanol solution contains one water and one methanol molecule per nucleotide and belongs to space group $P2_12_12_1$ with $a = 23.3_3$, $b = 4.78_8$, $c = 15.4_4$ Å, $Z = 4$. The phosphate oxygen atoms are distributed at the two rotational positions around the P-O ester bond. The torsion angle of the base with respect to the sugar, ϕ_{CN} is -20° , in the *anti* conformation. The ribose puckering is C(3')-*endo*, which is different from the molecule of inosine-5'-phosphate in the salt crystals. The conformation about the C(4')-C(5') bond is *gauche-gauche*. Large tunnels run through the structure parallel to the b axis. Water and methanol molecules fill the tunnels, but their positions are considerably disordered.

Introduction

To date there have been reported structure determinations of inosine-5'-phosphate in the sodium and barium salts (Nagashima & Iitaka, 1968; Rao & Sundaralingam, 1969). It was reported by Arnott & Hukins (1969) and Sundaralingam (1969) that the conformational angles in the monomers of the nucleic acid are confined to narrow ranges, no matter what their environment in crystals may be. However, the conformations of nucleotides in the solid state determined by X-ray analysis and in solution, which were investigated by means of n.m.r. or o.r.d., are not always in good agreement with each other (Schrimmer, Davis, Noggle & Hart, 1972; Klee & Mudd, 1967). These differences in conformation indicate that there is considerable flexibility in the sugar-base conformation and ribose puckering.

No nucleotide structure has been solved in both the ionic and free-acid forms. This paper describes the structure of free inosine-5'-phosphate and compares it with that in the ionic forms.

Experimental

Crystallization of inosine-5'-phosphate (5'-IMP) from an aqueous solution was not successful, but good prismatic crystals elongated along the b axis were grown from the boundary of the layers of a methanol and a methanol-water solution. This freshly formed crystal contains one water and one methanol molecule per nucleotide, but the methanol of crystallization was partly lost on storage at room temperature.

The unit-cell parameters were obtained from precession photographs taken with Cu $K\alpha$ radiation. The density was measured by flotation in a chloroform-

tetrabromoethane mixture. Crystal data are listed in Table 1.

Table 1. *Crystal data*

Chemical formula	$C_{10}H_{13}N_4O_8P \cdot H_2O \cdot CH_3OH$
Space group	$P2_12_12_1$
Unit-cell	$a = 23.3_3$, $b = 4.78_8$, $c = 15.4_4$ Å
Density	$D_m = 1.53_9$, $D_x = 1.535$ g cm $^{-3}$
Absorption coefficient	$\mu = 21.2$ cm $^{-1}$ (for Cu $K\alpha$ radiation)

Using Cu $K\alpha$ radiation, equi-inclination Weissenberg photographs were taken for layers 0 to 3 about the b axis, and 0 to 6 about the c axis. The intensities were measured on the multiple-film Weissenberg photographs with a Narumi microphotometer. They were then corrected for Lorentz and polarization factors and put on a common scale by correlating the layers. The dimensions of the crystal were approximately $0.3 \times 0.2 \times 1.0$ mm. In view of the low linear absorption coefficient and the small size of the crystal, no absorption correction was applied. 1873 independent reflexions were obtained from 2267 reflexions within the range $2\theta < 165^\circ$.

Determination of the structure

Various attempts to solve the structure by the Patterson method were unsuccessful. The structure was finally solved by the direct method using the tangent formula. The starting set of phases are listed in Table 2, where E is the normalized structure factor.

Using the list of addition formulae involving the reflexions given in Table 2, it was immediately found that the phase difference between the symbols a and b must be π and the symbol c must be near 0 or π . Therefore,

Table 2. *Starting set of phases*

$ E $	h	k	l	φ	
2·806	14	0	11	$\pi/2$	} origin assignment
2·745	15	0	16	0	
2·277	0	3	12	$\pi/2$	
2·715	20	0	13	a	
2·625	8	0	19	b	$(\pm \pi/2)$
2·488	14	3	3	c	
2·258	0	1	3	$-\pi/2$	enantiomorph assignment

the following four combinations of the phases of a , b and c were assigned.

	a	b	c
(i)	$\pi/2$	$-\pi/2$	0
(ii)	$\pi/2$	$-\pi/2$	π
(iii)	$-\pi/2$	$\pi/2$	0
(iv)	$-\pi/2$	$\pi/2$	π

Each of the four sets of phases was used as a starting set for the phase refinement of 68 reflexions with $|E| > 1.7$ by the tangent formula. Two sets were soon rejected because of high values of the R index (defined by $R = \sum_h ||E_{h\text{obs}} - |E_{h\text{calc}}|| / \sum_h |E_{h\text{obs}}|$). The phase assignment and refinement were extended to involve the 243 reflexions with $|E|$ greater than 1.4.

The correct combination, (iv), was indicated by the lowest R index and the E map which showed the phos-

phorus and the remaining eight atoms. The Fourier map calculated with the phases based on these atoms revealed all the atoms of the nucleotide except the three terminal oxygen atoms of the phosphate group. These oxygen atoms along with that of the water molecule were found in a subsequent difference electron density map which showed that each phosphate oxygen atom is distributed in two positions corresponding to two different rotational positions of the phosphate group around the P–O (ester) bond. In this difference map, disorder of the methanol molecule was also noticed: the electron densities at the position expected for the methanol molecule were low and diffused along the b direction. The most dense position in these diffused regions was chosen, therefore, for the position of the methanol oxygen atom M , and the low occupancy factor, $m = 0.5$, was assigned.

The structure was refined by several cycles of block-matrix least-squares calculations and the R index was reduced to 0.108. The weighting scheme used was as follows:

$$\begin{aligned} \sqrt{w} &= 0.5 & |F_o| &\leq 5.0 \\ \sqrt{w} &= 1.0 & 5.0 < |F_o| &\leq 20.0 \\ \sqrt{w} &= 20/|F_o| & |F_o| &> 20.0. \end{aligned}$$

A difference electron density map showed a small positive peak at the $W(2)$ position. We put another water oxygen atom at this site to form a spiral arrangement of water molecules around the twofold screw axis.

Table 3. *Positional parameters in fractional coordinates and their estimated standard deviations in Å*

	x	y	z	m^*	$S(X)$	$S(Y)$	$S(Z)$
P	0.5199	-0.1661	0.5781	1.0	0.002 Å	0.002 Å	0.002 Å
O(I)	0.4961	-0.1647	0.4826	0.5	0.019	0.029	0.015
O(II)	0.5563	0.0909	0.5903	0.5	0.014	0.013	0.017
O(III)	0.5488	-0.4191	0.6062	0.5	0.012	0.013	0.016
O(I')	0.5278	0.0894	0.5187	0.5	0.015	0.014	0.014
O(II')	0.5663	-0.1617	0.6503	0.5	0.012	0.020	0.015
O(III')	0.5154	-0.4328	0.5263	0.5	0.016	0.014	0.012
O(6)	0.3018	0.7440	0.3197	1.0	0.007	0.008	0.007
O(1')	0.3490	-0.3360	0.6056	1.0	0.006	0.006	0.006
O(2')	0.2546	-0.1532	0.7260	1.0	0.006	0.007	0.006
O(3')	0.3514	0.0582	0.8035	1.0	0.006	0.007	0.006
O(5')	0.4609	-0.1076	0.6264	1.0	0.006	0.007	0.007
N(1)	0.2215	0.5729	0.3878	1.0	0.008	0.009	0.008
N(3)	0.2216	0.2150	0.4928	1.0	0.008	0.009	0.008
N(7)	0.3660	0.2879	0.4266	1.0	0.007	0.009	0.008
N(9)	0.3176	0.0377	0.5182	1.0	0.007	0.008	0.007
C(2)	0.1945	0.4017	0.4441	1.0	0.009	0.011	0.010
C(4)	0.2783	0.2088	0.4776	1.0	0.008	0.009	0.008
C(5)	0.3094	0.3664	0.4210	1.0	0.008	0.009	0.009
C(6)	0.2808	0.5786	0.3706	1.0	0.010	0.011	0.009
C(8)	0.3697	0.0985	0.4880	1.0	0.008	0.010	0.009
C(1')	0.3015	-0.1532	0.5920	1.0	0.008	0.009	0.008
C(2')	0.2923	0.0091	0.6747	1.0	0.008	0.009	0.008
C(3')	0.3530	0.0130	0.7132	1.0	0.008	0.008	0.008
C(4')	0.3740	-0.2791	0.6896	1.0	0.008	0.008	0.008
C(5')	0.4381	-0.3229	0.6841	1.0	0.008	0.009	0.009
W(1)	0.4564	0.5258	0.3626	0.5	0.015	0.020	0.017
W(2)	0.4477	0.7357	0.1920	0.5	0.050	0.067	0.048
M†	0.3762	0.2534	0.1388	0.5	0.028	0.092	0.038

* Occupancy factor

† Methanol oxygen atom

No conclusive result about the positions of the hydrogen and methanol carbon atoms could be obtained from the difference map. The final atomic parameters and their estimated standard deviations are listed in Tables 3 and 4, the observed and calculated structure factors in Table 5.

Results and discussions

The crystal packing and hydrogen bonding

The packing of the molecules and the probable hydrogen bonding are shown in Fig. 1.

A notable feature of the structure is that the two positions were found for the oxygen atoms of the phosphate group. One, consisting of O(I), O(II) and O(III), takes a eclipsed conformation with respect to the C(5')-O(5') bond and the other, consisting of O(I'), O(II') and O(III'), takes a staggered conformation. However, it was not possible to determine the manner of distribution of the phosphate-oxygen atoms on these positions. Assuming that the phosphate groups randomly adopt either of the two conformations, close contacts between the oxygen atoms of the neighbouring phosphate group in the **b** direction would occur as shown in Fig. 2. Thus, if they took the same conformation, the distance between O(II) and neighbouring O(III) would be 2.37 Å and that between O(I') and neighbouring O(III') would be 2.31 Å; both are much smaller than the normal van der Waals contact. It may

therefore be improbable to assume the same conformation for these neighbouring phosphate groups.

On the other hand, if we assume a succession of the alternative arrangement of eclipsed and staggered conformations of the phosphate groups along the **b** direction, this will give two kinds of reasonably strong hydrogen bond between the phosphate groups O(III) and the neighbouring O(I') atoms and that between O(III') and the neighbouring O(II) atoms. However, this was not confirmed by the X-ray diffraction pattern since the diffraction photographs, even though they were taken for fresh crystals and were heavily exposed, showed neither evidence for the double repeat distance along the **b** direction nor the diffuse scattering showing the partial order of the arrangement. It is therefore reasonable to assume that the packing of the molecules is governed by the base stacking. The bases are stacked with a separation of 3.275 Å along the *b* axis, but are tilted about 30° with respect to the (010) plane. The phosphate oxygen atom O(II') or O(III) is hydrogen bonded to the adjacent ribose hydroxy group O(3'), while O(I) or O(III') is hydrogen bonded to the water molecule.

There are two kinds of hydrogen bond between the purine rings and the ribose groups of the adjacent molecule: N(1)···O(3') and O(6)···O(2'). N(7) of the purine ring is hydrogen bonded to the water molecule. The hydrogen bond lengths in the crystal are given in Table 6.

Table 4. *Thermal parameters and their estimated standard deviations*

Temperature factors are of the form $T = \exp \{-10^{-4}(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)\}$.

	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
P	10 (0)	183 (8)	30 (1)	7 (2)	1 (1)	-4 (3)
O(I)	33 (5)	1344 (215)	34 (7)	113 (30)	-15 (5)	-90 (37)
O(II)	19 (3)	137 (51)	63 (8)	4 (11)	2 (4)	-6 (21)
O(III)	11 (2)	201 (56)	64 (8)	20 (10)	1 (4)	21 (21)
O(I')	20 (3)	277 (64)	38 (6)	-3 (12)	13 (4)	50 (19)
O(II')	9 (2)	728 (109)	46 (7)	28 (15)	-9 (3)	-48 (29)
O(III')	25 (3)	217 (56)	25 (5)	-2 (13)	4 (4)	-24 (17)
O(6)	20 (2)	325 (35)	41 (3)	-5 (6)	-8 (2)	45 (11)
O(1')	14 (1)	173 (25)	28 (2)	17 (5)	0 (1)	-3 (8)
O(2')	12 (1)	306 (32)	36 (3)	-8 (6)	5 (1)	11 (10)
O(3')	15 (1)	261 (29)	24 (2)	12 (5)	1 (1)	-42 (8)
O(5')	12 (1)	229 (30)	54 (4)	16 (5)	5 (2)	44 (10)
N(1)	17 (2)	303 (39)	34 (3)	20 (7)	-8 (2)	-11 (12)
N(3)	13 (1)	334 (42)	38 (4)	5 (7)	0 (2)	11 (12)
N(7)	13 (1)	340 (41)	29 (3)	9 (6)	0 (2)	10 (12)
N(9)	11 (1)	236 (33)	21 (3)	7 (6)	0 (2)	12 (10)
C(2)	15 (2)	357 (51)	37 (4)	14 (8)	-5 (2)	-6 (15)
C(4)	12 (1)	217 (39)	24 (3)	19 (7)	-2 (2)	-11 (11)
C(5)	12 (1)	232 (39)	25 (3)	14 (7)	-5 (2)	-10 (13)
C(6)	18 (2)	318 (48)	25 (3)	2 (8)	-7 (2)	-20 (13)
C(8)	9 (1)	348 (48)	28 (3)	-2 (7)	0 (2)	12 (13)
C(1')	12 (1)	177 (34)	30 (4)	11 (7)	0 (2)	13 (12)
C(2')	10 (1)	221 (38)	22 (3)	3 (7)	3 (2)	6 (11)
C(3')	10 (1)	115 (32)	28 (3)	8 (6)	0 (2)	0 (11)
C(4')	10 (1)	124 (33)	25 (3)	3 (6)	-2 (2)	2 (10)
C(5')	8 (1)	244 (39)	38 (4)	4 (7)	3 (2)	36 (13)
W(1)	20 (3)	621 (105)	59 (8)	-28 (17)	14 (4)	33 (30)
W(2)	80 (14)	3595 (375)	217 (33)	-272 (70)	-4 (16)	337 (117)
M	31 (7)	6004 (969)	157 (25)	-167 (77)	9 (10)	444 (164)

Table 5. Observed and calculated structure factors
For the reflexions marked with an asterisk were attached the weight other than unity.

Table with multiple columns of numerical data representing structure factors. The columns are labeled with Miller indices (h, k, l) and 'F' for observed and 'FC' for calculated values. The data is organized in a grid-like format with some rows containing asterisks to indicate specific reflexions.

Table 6. *Hydrogen-bond lengths*

The number in parentheses after the second atom in each case indicates the atomic position as follows:

- (1) x, y, z ; (2) $x, 1+y, z$; (3) $1-x, -\frac{1}{2}+y, \frac{1}{2}-z$;
 (4) $1-x, -\frac{1}{2}+y, 1+\frac{1}{2}-z$; (5) $\frac{1}{2}-x, 1-y, -\frac{1}{2}+z$.

O(II)····O(III)	(2)	2.37* Å
O(II)····O(III')	(2)	2.66
O(III)····O(3')	(4)	2.72
O(I')····O(III)	(2)	2.76
O(I')····O(III')	(2)	2.31*
O(II')····O(3')	(4)	2.45
O(6)····O(2')	(5)	2.77
N(1)····O(3')	(5)	2.78
W(1)····O(1)	(2)	2.55
W(1)····O(III')	(2)	2.88
W(1)····N(7)	(1)	2.59
W(1)····W(2)	(1)	2.83
W(1)····W(2)	(3)	2.77
W(2)····M	(1)	2.96
W(2)····M	(2)	3.10

* These hydrogen bonds do not seem to exist.

Large tunnels are seen in the structure along the b axis. In these tunnels, are situated the water and methanol molecules and hydrogen bonds between the water molecules form a continuous spiral along the b axis. The electron densities at the region M where the methanol molecules seem to exist are very diffuse and low but the distances between $W(2)$ and M are 2.96 and 3.10 Å. We therefore assume the methanol molecules are

bound to the water molecules through weak hydrogen bonds and are loosely trapped in the tunnels so that the methanol molecules are volatile and can escape easily from the crystal. It was confirmed by means of n.m.r. that the methanol of crystallization was partly lost on storage at room temperature. In fact, about half seem to have been lost during the data collection.

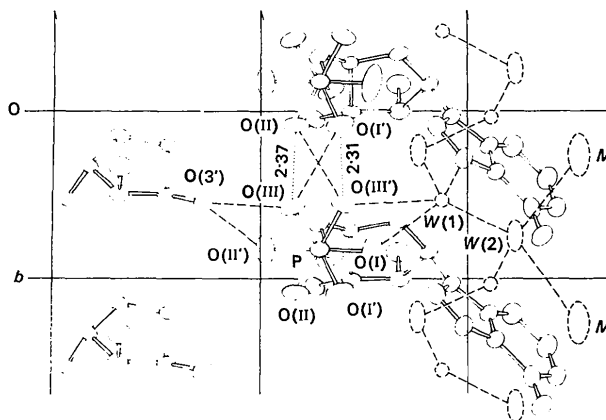


Fig. 2. The molecular packing in the crystal viewed along the direction of the P-O(5') bond. Hydrogen bonds are shown by dashed lines and the other close contacts listed in Table 6 are shown by dotted lines.

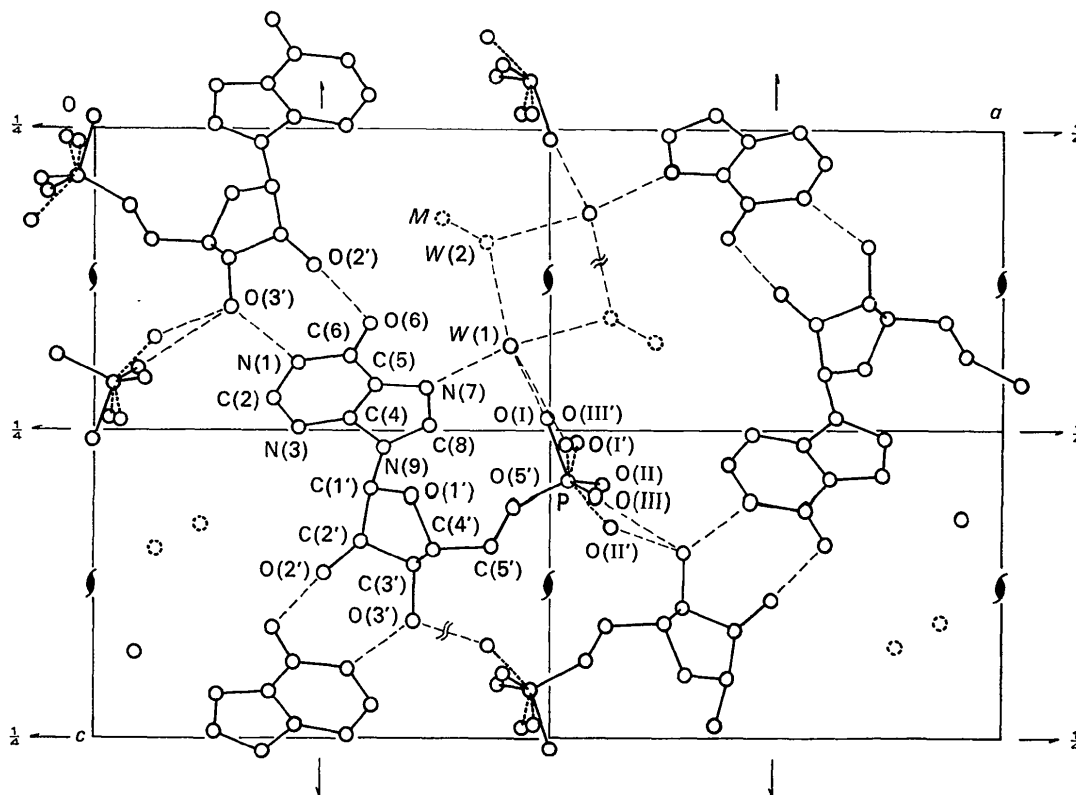


Fig. 1. The crystal structure viewed down the b axis. Possible hydrogen bonds are shown by dashed lines.

The water content may also be variable. These results indicate that the water and methanol of crystallization play no important role in the construction of the crystal lattice, although it is interesting that even such loosely bound and labile methanol molecules are important in the crystallization, because the crystals have never been obtained from an aqueous solution.

It was also found that 5'-IMP crystallized from another mixed solution of organic solvent and water, *i.e.* an aqueous ethanol or an aqueous acetone solution *etc.* These crystals are found to have a similar structure by the X-ray powder diffraction pattern.

The present crystal has a lower density than the crystals of other nucleotides, as shown in Table 7, and the low density reflects the particular feature of the structure such as the existence of large tunnels filled with the labile methanol and water molecules.

Bond lengths and angles

The bond lengths and angles of inosine-5'-phosphate are shown in Fig. 3. The estimated standard deviations are about 0.02 Å for the P-O bond lengths, 0.014 Å for other bond lengths and about 1° for bond angles. Most of the bond lengths and angles have reasonable values, and there are no significant deviations from the value found in the sodium salt crystal.

Table 7. Densities of nucleotides (g cm^{-3})

Nucleotide	D_m	D_x	Reference
5'-IMP. $\text{H}_2\text{O} \cdot \text{CH}_3\text{OH}$	1.539	1.535	1
5'-IMP. $2\text{Na} \cdot n\text{H}_2\text{O}$ ($n=7\sim 8$)	1.62	1.616	2
5'-AMP. H_2O	1.64	1.647	3
5'-GMP. $3\text{H}_2\text{O}$	1.644	1.650	4
5'-GMP. $2\text{Na} \cdot 6\text{H}_2\text{O}$	1.632		5
3'-CMP (orthorhombic)	1.64		6
3'-CMP (monoclinic)	1.72	1.73	7
3'-AMP		1.698	8

1. Inosine-5'-phosphate (present work).
2. Disodium inosine-5'-phosphate hydrate (Nagashima & Iitaka, 1968).
3. Adenosine-5'-phosphate monohydrate (Kraut & Jensen, 1963).
4. Guanosine-5'-phosphate trihydrate (Murayama, Nagashima & Shimizu, 1969).
5. Disodium guanosine-5'-phosphate hexahydrate (unpublished data).
6. Cytidine-3'-phosphate (orthorhombic form) (Sundaralingam & Jensen, 1965).
7. Cytidine-3'-phosphate (monoclinic form) (Bugg & Marsh, 1967).
8. Adenosine-3'-phosphate (Sundaralingam, 1966).

The C(6)-O(6) bond length and C(6)-N(1)-C(2) angle indicate that the nucleotide is in a lactam form, as was found in the crystal of the disodium salt. The deviations of atoms from the least-squares plane

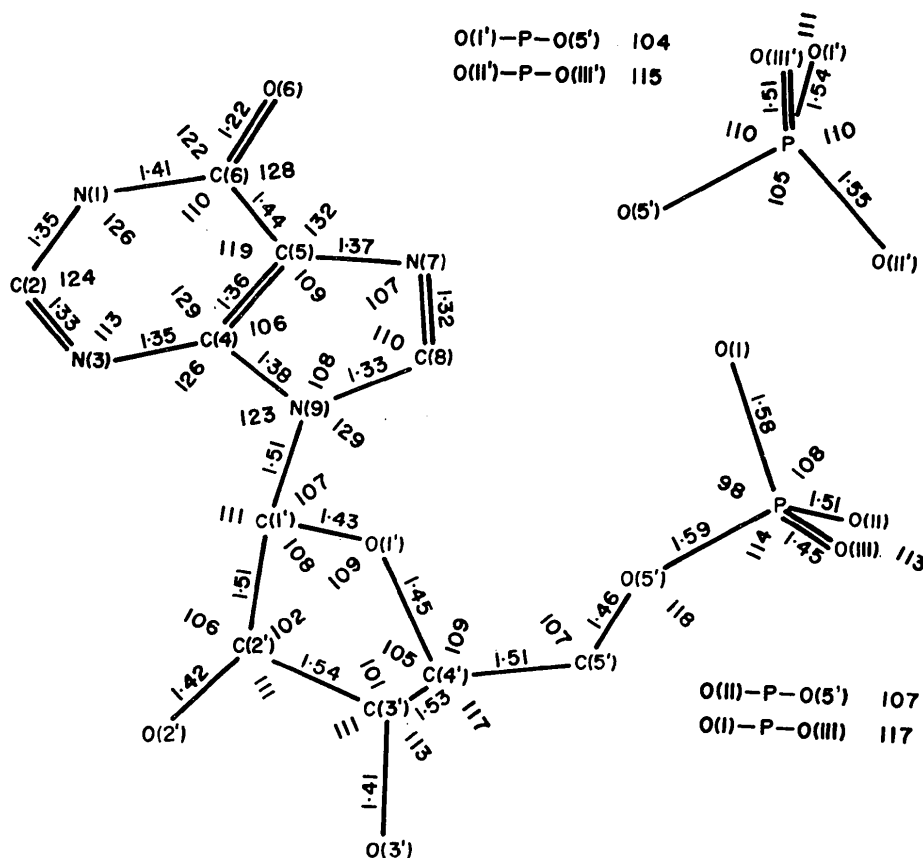


Fig. 3. The bond lengths and angles in the inosine molecule.

through the purine ring are listed in Table 8, and show that the ring is planar within experimental error.

Table 8. *Deviations from the purine ring plane*

N(1)	0.011 Å
C(2)	-0.012
N(3)	0.000
C(4)	-0.008
C(5)	-0.020
C(6)	0.026
N(7)	-0.037
C(8)	0.035
N(9)	0.005
O(6)*	0.072
C(1')*	0.146

Equation of the plane is:

$$0.1356X + 0.6840Y + 0.7167Z = 6.857,$$

where $X||\mathbf{a}$, $Y||\mathbf{b}$ and $Z||\mathbf{c}$, measured in Å.

* Atoms excluded from the calculation of the least-squares plane.

The conformation of the nucleotide

The torsion angles in the nucleotide are listed in Table 9. Table 10 lists the deviations of the individual atoms from the least-squares plane through the ribose

Table 9. *Torsion angles*

C(8)-N(9)-C(1')-O(1')	(φ_{CN})	-20.2°
C(8)-N(9)-C(1')-C(2')		96.8
C(4')-O(1')-C(1')-C(2')		-5.5
O(1')-C(1')-C(2')-C(3')		27.7
C(1')-C(2')-C(3')-C(4')		-37.9
C(2')-C(3')-C(4')-O(1')		35.7
C(3')-C(4')-O(1')-C(1')		-19.5
N(9)-C(1')-C(2')-C(3')		-88.8
N(9)-C(1')-C(2')-O(2')		154.6
O(2')-C(2')-C(3')-O(3')		-45.0
O(2')-C(2')-C(3')-C(4')		74.8
C(2')-C(3')-C(4')-C(5')		156.5
O(3')-C(3')-C(4')-C(5')		-84.9
C(5')-C(4')-O(1')-C(1')		-145.7
O(1')-C(4')-C(5')-O(5')	(φ_{OO})	66.4
C(3')-C(4')-C(5')-O(5')	(φ_{OC})	-52.3
C(4')-C(5')-O(5')-P	(φ_{OP})	-155.8
C(5')-O(5')-P—O(I)		116.0
C(5')-O(5')-P—O(II)		-132.5
C(5')-O(5')-P—O(III)		-7.6
C(5')-O(5')-P—O(I')		175.6
C(5')-O(5')-P—O(II')		-69.3
C(5')-O(5')-P—O(III')		56.4

ring. The conformation of the ribose ring is C(3')-endo, which is different from that found in the disodium salt but similar to that found in 5'-GMP (Murayama, Nagashima & Shimizu, 1969) and 5'-AMP (Kraut & Jensen, 1963).

Table 10. *Deviations from the ribose ring plane*

O(1')	-0.030 Å
C(1')	0.029
C(2')	-0.018
C(3')*	0.600
C(4')	0.018

* Atom excluded from the calculation of the least-squares plane.

The glycosidic torsional angle φ_{CN} is -19.9° , which lies in the *anti* range. But this value is considerably different from the value for 5'-IMP in the disodium salt crystal. This difference may be due to the ribose conformation, *i.e.* in the case of C(3')-endo, the repulsion between the hydrogen atoms at the C(8) and C(3') atoms would reduce the φ_{CN} angle. The conformation about the C(4')-C(5') bond is *gauche-gauche*. The torsion angle C(4')-C(5')-O(5')-P, $\varphi_{\text{OP}} = -156^\circ$, is somewhat different from the value generally found in 5'-IMP salt crystals ($160-170^\circ$).

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