

## Structure of the Potassium Salt of the Modified Nucleotide Dihydrouridine 3'-Monophosphate Hemihydrate: Correlation Between the Base Pucker and Sugar Pucker and Models for Metal Interactions with Ribonucleic Acid Loops

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### Abstract

The crystal structure of the potassium salt of dihydrouridine 3'-monophosphate hemihydrate,  $K^+ \cdot C_9H_{14}N_2O_9P^- \cdot \frac{1}{2}H_2O$ , a modified nucleotide, has been determined from three-dimensional X-ray crystallographic data. The nucleotide crystallizes in the space group  $C2$  with unit-cell dimensions  $a = 22.638 \pm 0.007$ ,  $b = 5.765 \pm 0.001$ ,  $c = 13.245 \pm 0.003$  Å, and  $\beta = 123.31 \pm 0.01^\circ$ ;  $Z = 4$ . Structural solution was obtained by use of the Patterson heavy-atom method followed by successive Fourier syntheses. Positional and thermal parameters were refined by block-diagonal least-squares techniques to a final  $R$  of 0.035. The dihydrouracil base is puckered, mainly at C(6), due to saturation of the C(5)–C(6) bond. The furanose ring has a C(2')-endo envelope conformation ( ${}^2E$ ) with  $P = 159.0^\circ$  and  $\tau_m = 39.7^\circ$ . A correlation has been found between the nature of the base pucker and the pucker of the sugar: a major pucker at C(6) of the base accommodates a C(2')-endo, O(4')-endo and the C(3')-endo sugar puckerings, but a major pucker at C(5) of the base preferentially co-exists with a C(3')-endo pucker. The nucleotide exhibits the *anti* conformation, with the glycosyl torsion angle  $\chi$  equal to  $71.2(4)^\circ$ . The conformation  $\psi$  about the exocyclic C(4')–C(5') bond is *gauche*<sup>+</sup>. The torsion angle  $\phi'$  [C(4')–C(3')–O(3')–O] is  $215.5(4)^\circ$  and falls within the continuous range of values ( $190$ – $270^\circ$ ) observed for the known 3'-nucleotides. One of the phosphate O atoms is in a nearly eclipsed conformation with respect to the C(3') atom. There is no base–base interaction. Eight ligands, at less than 3.2 Å, are coordinated to the K ion: two O(4) atoms of dihydrouracil bases, an O(2') atom of the ribose, four phosphate O atoms from two different nucleotide units, and one water O atom. The binding scheme observed here provides insight into possible modes of interaction of K and related metals with RNA's.

### Introduction

The presence of various modified nucleosides – in addition to the four that are generally found in ribo-

nucleic acids – has come to be characteristic of not only transfer RNA, but also 5S and other ribosomal RNA's, and eukaryotic messenger RNA as well as DNA. The proportion of modified nucleosides in tRNA is considerably greater than that found in other nucleic acids and there are more than fifty modified nucleosides that have been isolated and characterized thus far (McCloskey & Nishimura, 1977). The modified nucleosides show considerable structural variety; many of them result from a simple methylation of either the base or the O(2') hydroxyl of the ribose. A number of others, however, exhibit much more complex types of modification. Dihydrouridine is one of them, and is the only nucleic acid constituent with a puckered base; it is found exclusively in the dihydrouridine loop of tRNA's, in numbers varying from one to five. X-ray crystallographic studies of the rare base itself (Rohrer & Sundaralingam, 1970) and the corresponding nucleoside (Sundaralingam, Rao & Abola, 1971; Suck, Saenger & Zechmeister, 1972) have already revealed that the dihydrouracil base is puckered as a result of the saturation of the C(5)–C(6) bond. The nucleoside conformation indicated that dihydrouridine could be crucial to promoting loop formation in the sugar–phosphate chain of tRNA's. Here we report the structure of the potassium salt of dihydrouridine 3'-monophosphate. The study was undertaken to delineate further the role of the modified nucleotides in the structure of tRNA's (see also Sundaralingam, 1972). Knowledge gleaned about the conformation and metal-binding sites of the rare nucleotide, its hydrogen-bonding and base-stacking properties, should lead to a better understanding of the secondary and tertiary structures of tRNA's.

### Experimental

The hydrated potassium salt of dihydrouridine 3'-monophosphate was purchased from Calbiochem, LaJolla, California. Crystals were grown by slow evaporation of an aqueous ethanol solution containing a few drops of 2-methyl-2,4-pentanediol. A needle-like

Table 1. *Crystal data*

Chemical formula	$K^+ \cdot C_9H_{14}N_2O_9P^- \cdot \frac{1}{2}H_2O$
Molecular weight	373
Crystal system	Monoclinic
Systematic absences	$hkl, h + k = 2n + 1$
Space group	$C2$
Unit-cell dimensions	$a = 22.638 (7) \text{ \AA}$ $b = 5.765 (1)$ $c = 13.245 (3)$ $\beta = 123.31 (1)^\circ$
$Z$	4
Volume	$1444.6 \text{ \AA}^3$
$\rho_{obs}$	$1.719 \text{ Mg m}^{-3}$ (floatation in 2-propanol/ $CHBr_3$ )
$\rho_{calc}$	$1.714 \text{ Mg m}^{-3}$
Linear absorption coefficients	$\mu = 4.75 \text{ mm}^{-1}$
Crystal dimensions	$0.60 \times 0.09 \times 0.02 \text{ mm}$

crystal of dimensions  $0.60 \times 0.09 \times 0.02 \text{ mm}$  was chosen for study. The density of a typical crystal was measured by the floatation method, using a mixture of 2-propanol and bromoform. Oscillation and Weissenberg photographs indicated that the crystal was monoclinic with systematic absences ( $h + k = 2n + 1$ ) characteristic of a  $C$ -centered lattice and also provided the preliminary unit-cell constants. The space group was determined to be  $C2$ , in view of the optically active nature of the compound.

The crystal was mounted on a Picker FACS-1 diffractometer with the  $b$  axis coincident with the  $\phi$  axis of the goniostat. The angular settings of 25 strong nonaxial reflections with  $2\theta$  values ranging from  $30$  to  $60^\circ$  were carefully determined to obtain the refined cell constants. These and other pertinent crystal data appear in Table 1. Data were collected in two shells – high angle ( $2\theta = 40$ – $127^\circ$ ) and low angle ( $2\theta = 2$ – $42^\circ$ ) – using Ni-filtered  $Cu K\alpha$  radiation ( $\lambda = 1.5418 \text{ \AA}$ ) and a  $\theta$ – $2\theta$  scan technique with a scan speed of  $1^\circ \text{ min}^{-1}$  and a scan width of  $1.6^\circ$  in  $2\theta$ . The 133, 225, and 511 reflections were chosen as standards and their intensities were monitored periodically throughout the data collection. The overall drop in the intensities of the standards was 0.9% by the end of the data collection.

In addition to the correction for decay, standard Lorentz and polarization corrections were applied to the data, as well as an absorption correction based on an experimentally determined  $\phi$  curve. 1314 independent reflections were collected, of which 1264 (96%) were stronger than 1.5 times the standard deviations of their intensities and were subsequently used in the refinement. The structure factors for the 128 pairs of equivalent reflections agreed within 3.64%.

### Structure solution

An overall Wilson temperature factor ( $B = 1.67 \text{ \AA}^2$ ) and scale factor ( $k = 0.636$ ) were evaluated and used to compute normalized structure factors. An ( $E^2 - 1$ )

Patterson map was calculated and the strongest peaks in the map were observed to fall on the Harker section at  $v = 0$ , which suggested that the two heavy atoms – K and P – have the same  $y$  coordinate. Subsequent study of the four strongest peaks in the Patterson map showed the top two peaks to be the double-weighted potassium–phosphorus cross vectors, the third peak to be the potassium–potassium self vector, and the fourth peak to be the phosphorus–phosphorus self vector. The ( $x, z$ ) coordinates of K<sup>+</sup> and P were obtained from the interpretation of the Harker section, assignment of the  $y$  coordinates being arbitrarily chosen as zero. Starting with K<sup>+</sup> and P ( $R$  index of 0.549) the structure was developed by successive Fourier syntheses. As expected, the interpretation of the initial Fourier map presented some complications because of a pseudo mirror on  $y$  resulting from the identical  $y$  coordinates of the two heavy atoms.

In addition to the nucleotide atoms and the K<sup>+</sup> ion, a water O atom was found to be located on a twofold axis parallel to  $b$ , which was given an occupancy value of one half in subsequent refinement studies. The structure was refined by block-diagonal-matrix least-squares refinement with isotropic thermal parameters for the atoms and unit weights ( $R = 0.073$ ). Further refinement switching from unit weights to a weighting scheme based on counting statistics and including  $f''$  anomalous-dispersion scattering-factor correction terms and anisotropic temperature factors for the atoms reduced  $R$  to 0.052. At this point, difference Fourier maps were used to pin the positions of the H atoms. H atoms were assigned isotropic temperature factors one unit above the corresponding values of the

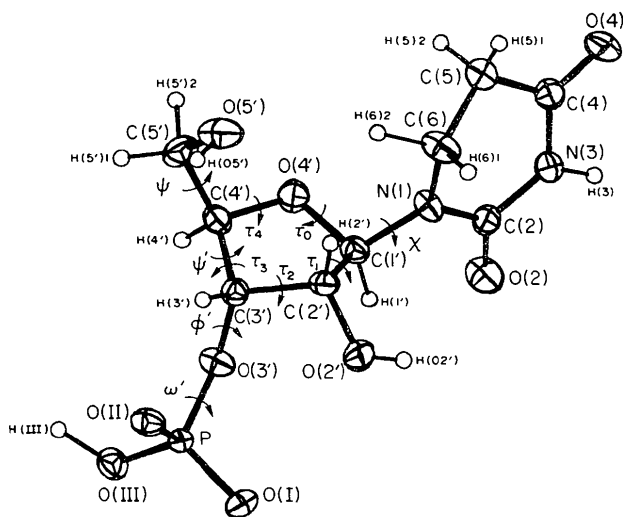


Fig. 1. Thermal-ellipsoid plot showing 50% probability ellipsoids of the nonhydrogen atoms of dihydrouridine 3'-monophosphate; H atoms are depicted by spheres of arbitrary diameter. Nomenclature and torsion-angle definitions are after Sundaralingam (1969).

heavy atoms to which they were attached. Continuation of the refinement of the heavy atoms with anisotropic thermal parameters and the H atoms with isotropic thermal parameters led to a final *R* index of 0.035. The parameter shifts were all less than 0.10 times their estimated errors.

The following weighting scheme was used during the refinement:

$$\text{weight} = 1/[\sigma_F^2 + (0.025 \times |F_{\text{obs}}|)^2],$$

where an adjustment factor of 0.025 was chosen to produce a flat curve when plotting average [weight  $\times (|F_o| - |F_c|)^2$ ] versus the corresponding range of  $|F_o|$  values.

Table 2. Positional parameters ( $\times 10^4$  for nonhydrogen atoms;  $\times 10^3$  for hydrogen atoms) and the isotropic temperature factors for all atoms in potassium dihydrouridine 3'-monophosphate hemihydrate

The equivalent isotropic temperature factors for the non-hydrogen atoms were computed using the expression  $B_{\text{iso}} = \frac{1}{3} \sum_{i,j} \beta_{ij} (\mathbf{a}_i \cdot \mathbf{a}_j)$  where  $\beta_{ij}$  are the anisotropic thermal parameters and  $\mathbf{a}_i$  and  $\mathbf{a}_j$  are direct-cell vectors.

	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i> (Å <sup>2</sup> )
K	9082.0 (4)	-107 (2)	9831 (1)	2.28 (4)
P	6921.0 (4)	120 (2)	676 (1)	1.51 (4)
N(1)	8485 (2)	5092 (7)	5213 (2)	2.02 (15)
C(2)	8433 (2)	4412 (8)	6140 (3)	2.07 (21)
O(2)	8146 (2)	2605 (7)	6137 (2)	3.29 (17)
N(3)	8741 (2)	5872 (7)	7129 (3)	2.47 (19)
C(4)	9223 (2)	7571 (9)	7389 (3)	2.38 (22)
O(4)	9548 (1)	8516 (7)	8382 (2)	3.63 (18)
C(5)	9340 (2)	8169 (9)	6414 (3)	2.46 (21)
C(6)	8730 (2)	7429 (9)	5193 (3)	2.60 (22)
C(1')	8290 (2)	3504 (7)	4234 (3)	1.84 (20)
C(2')	7825 (2)	4502 (7)	2959 (3)	1.79 (20)
O(2')	7099 (1)	4546 (6)	2516 (2)	2.39 (14)
C(3')	7989 (2)	2851 (7)	2248 (3)	1.74 (20)
O(3')	7607 (1)	708 (5)	1974 (2)	2.23 (16)
C(4')	8781 (2)	2324 (8)	3139 (3)	2.05 (20)
O(4')	8927 (1)	2811 (6)	4328 (2)	2.54 (15)
C(5')	9260 (2)	3734 (9)	2920 (4)	2.82 (30)
O(5')	9088 (1)	6142 (6)	2744 (2)	3.37 (19)
O(I)	6294 (1)	30 (7)	762 (2)	2.20 (14)
O(II)	6871 (1)	1812 (5)	-236 (2)	2.31 (24)
O(III)	7064 (1)	-2411 (6)	428 (2)	2.78 (21)
O(W)	10000 (0)	3293 (10)	10000 (0)	6.70 (38)
H(3)	869 (2)	563 (7)	778 (3)	0.7 (0.7)
H(5)1	941 (2)	984 (8)	639 (3)	1.0 (0.7)
H(5)2	978 (2)	733 (13)	658 (4)	5.4 (1.3)
H(6)1	833 (2)	852 (9)	482 (4)	4.2 (1.2)
H(6)2	892 (2)	720 (9)	457 (3)	2.6 (0.9)
H(1')	800 (2)	213 (10)	437 (4)	3.9 (1.1)
H(2')	800 (2)	605 (8)	294 (3)	1.9 (0.9)
H(O2')	697 (2)	514 (13)	287 (4)	5.7 (1.3)
H(3')	783 (2)	350 (9)	143 (3)	3.4 (1.1)
H(4')	881 (2)	78 (7)	301 (3)	1.1 (0.8)
H(5')1	928 (2)	320 (7)	221 (3)	0.6 (0.7)
H(5')2	980 (2)	354 (9)	360 (3)	2.9 (1.1)
H(O5')	878 (3)	654 (11)	191 (5)	4.2 (1.8)
H(III)	743 (5)	-250 (26)	14 (8)	3.2 (3.1)
H(W)	966 (3)	449 (17)	1000 (7)	6.7 (2.9)

The scattering factors used were from *International Tables for X-ray Crystallography* (1962). Final positional parameters for all atoms are listed in Table 2.\* Fig. 1 is an *ORTEP* (Johnson, 1965) drawing of the molecule showing the atom-numbering and the torsion-angle notations.

## Discussion of results

### Bond distances and bond angles

In Fig. 2 the bond distances and angles involving the nonhydrogen atoms are shown. The distances and angles for H atoms are in the usual range and are not tabulated. The average standard deviations in bond distances and bond angles are 0.004 Å and 0.2° respectively. Bond distances and angles found for the base are in agreement with those found for dihydrouracil (Rohrer & Sundaralingam, 1970) and dihydrouridine (Sundaralingam *et al.*, 1971; Suck *et al.*, 1972). Similarly, the geometry of the ribose agrees with previously determined values for the nucleoside.

### The base pucker

Table 3 shows the deviations of atoms from the least-squares planes through the base. Saturation of the C(5)–C(6) bond has caused the base to pucker to an

\* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 34826 (8 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

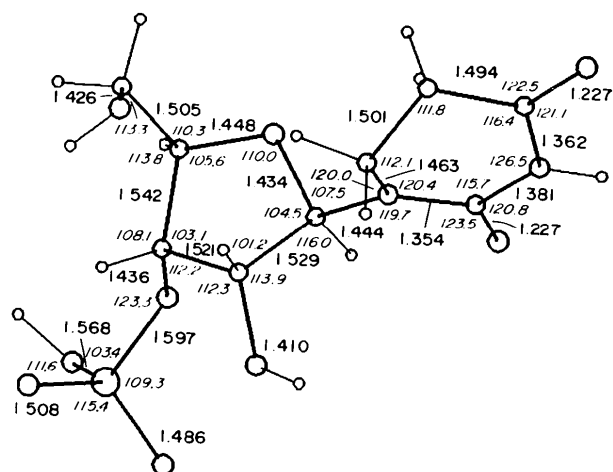


Fig. 2. The bond distances (Å) and bond angles (°) in potassium dihydrouridine 3'-monophosphate hemihydrate. The average e.s.d.'s in the bond distances and bond angles are 0.004 Å and 0.2° respectively.

Table 3. *Least-squares planes through the base in potassium dihydrouridine 3'-monophosphate hemihydrate*

The estimated standard deviations for distances to the planes are in the range 0.005–0.007 Å.

	Deviations from the plane (Å)		
	Plane I	Plane II	Plane III
N(1)	0.137*	−0.162	−0.031*
C(2)	0.079*	0.023*	0.065*
O(2)	0.237	0.262	0.281
N(3)	−0.148*	−0.046*	−0.069*
C(4)	−0.001*	0.042*	0.035*
O(4)	−0.018	0.185	0.114
C(5)	0.207*	−0.019*	0.072
C(6)	−0.274*	−0.655	−0.502
C(1')	0.596	0.118	0.322
r.m.s. deviation of fitted atoms	0.203	0.035	0.053

$$\text{Plane I } 0.739X - 0.613Y + 0.279Z = 11.073$$

$$\text{Plane II } 0.643X - 0.631Y + 0.435Z = 10.734$$

$$\text{Plane III } 0.679X - 0.631Y + 0.376Z = 10.819$$

\* Atoms used in fitting the least-squares planes.

Table 4. *Pseudorotation parameters (Altona & Sundaralingam, 1972) of the furanoid ring and Cremer & Pople (1975) puckering parameters of the dihydrouracil ring of dihydrouridine 3'-monophosphate and related nucleosides*

The atomic displacements of atoms C(5) and C(6) from the least-squares plane through N(1), C(2), N(3), and C(4) are also given. Dihydrouracil itself (Rohrer & Sundaralingam, 1970) and in its complex with mercury(II) chloride (Carrabine & Sundaralingam, 1971) give similar puckeringings.

	$P$ (°)	$\tau_m$ (°)	C(5) (Å)	C(6) (Å)	$Q$ (Å)	$\theta$ (°)	$\varphi$ (°)
Dihydrouridine 3'-monophosphate (this work)	159	39.7	0.07	−0.50	0.40	67	288.5
Dihydrouridine <sup>a</sup> (1)	153.5	43	0.17	−0.49	0.44	60	283
(2)	151	33	−0.02	0.65	0.49	114	113
Dihydrothymidine <sup>b</sup>	84	32	−0.27	0.41	0.45	119	95
5-Hydroxy-5,6- dihydrothymidine <sup>c</sup>	95	37	0.29	−0.36	0.44	65.5	272
Dihydroisocytidine <sup>d</sup>	37.5	37	0.46	−0.16	0.43	65.5	260
5,6-Dihydro-2- thiouridine <sup>e</sup>	10	36.5	0.32	−0.30	0.41	63	271
5,6-Dihydro-2,4- dithiouridine <sup>f</sup>	11	38	0.16	−0.50	0.45	66	283

References: (a) Sundaralingam *et al.* (1971); Suck *et al.* (1972). (b) Konnerth, Karle & Karle (1970). (c) Grand & Cadet (1978). The published coordinates correspond to the L-enantiomer. (d) Kojić-Prodić, Ružić-Toroš & Coffou (1976). The published coordinates of the base atoms C(2), N(2), N(3), C(4) and O(4) should be translated along *a* to be connected to the rest of the molecule. (e) Kojić-Prodić, Liminga, Šljukić & Ružić-Toroš (1974). The published coordinates correspond to the L-enantiomer. (f) Kojić-Prodić, Kvič & Ružić-Toroš (1976).

approximate half-chair conformation. The least-squares plane through the six ring atoms shows considerable deviations from planarity. The substituent atoms, O(2), O(4) and C(1'), are quite displaced from the base

planes. The C(5) and C(6) atoms show displacements of 0.072 and 0.502 Å respectively on opposite sides of the four-atom plane defined by the remaining ring atoms (plane III). In Table 4 the puckering parameters of Cremer & Pople (1975) of related compounds are given. It can be seen that the major pucker occurs preferentially at C(6) (see below).

#### Molecular conformation

The disposition of the base relative to the ribose is *anti* with a glycosyl torsion angle of 71.2°. The furanoid ring is in the C(2')-*endo* envelope conformation (<sup>2</sup>*E*) with pseudorotation parameters  $P$  and  $\tau_m$  (Altona & Sundaralingam, 1972) equal to 159 and 39.7°, respectively. Mid-range *anti* values of  $\chi$  are typically associated with C(2')-*endo* sugar pucker (Sundaralingam, 1969, 1973). The conformation about the exocyclic C(4')–C(5') bond described by the torsion angle  $\psi$  is *gauche*<sup>+</sup> (46.7°). The crystal structure of the nucleoside dihydrouridine (Sundaralingam *et al.*, 1971; Suck *et al.*, 1972) contains two independent molecules in the asymmetric unit which are both in the *anti* conformation ( $\chi = 65.6$  and 57.1°) with the furanoid ring in a C(2')-*endo*–C(1')-*exo* pucker ( $P$  and  $\tau_m$  equal to 153.5, 43.0 and 151.3, 33.1° respectively). However, in the nucleoside, one molecule has the *trans* conformation about C(4')–C(5') and the other shows disorder with two alternate staggered arrangements *gauche*<sup>−</sup> (88%) and *gauche*<sup>+</sup> (12%). A comparison with the common ribonucleosides and ribonucleotides, which favor strongly the conformational family C(3')-*endo*-low *anti-gauche*<sup>+</sup>, shows that the saturated base favors a C(2')-*endo*-mid *anti* conformation, which by itself reduces the population of the preferred *gauche*<sup>+</sup> conformation about C(4')–C(5') (Sundaralingam, 1973). NMR studies of nucleosides containing a saturated base have shown similar trends, *i.e.* preference for the C(2')-*endo* type pucker and a reduction in the population of the *gauche*<sup>+</sup> conformation about C(4')–C(5') (Ducolomb, Cadet, Taieb & Teoule, 1976).

The data presented in Table 4 indicate that the puckering of the saturated base could influence the ribose pucker because of the interactions of the C(6) methylene protons with the ribose. It appears that, while the base with the major pucker at C(6) can accommodate the C(2')-*endo*, O(4')-*endo*, and C(3')-*endo* puckers, the base with the major pucker at C(5) would co-exist preferentially with the C(3')-*endo* pucker. Table 5 lists the conformation angles describing the nucleotide and information concerning the sugar conformation. A comparison of the conformations of the known structures of 3'-nucleotides appears in Table 6. It can be seen that there are striking similarities; all but one display  $\chi$  values in the *anti* range. The sugar pucker is either 2'-*endo* or 3'-*endo* with the 2'-*endo*

pucker correlating with  $\chi$  values in the *syn* or middle to high *anti* range and the 3'-*endo* pucker correlating with the low *anti*  $\chi$  values (Sundaralingam, 1973). The conformation ( $\psi$ ) about the C(4')-C(5') bond is *gauche*<sup>+</sup> for all cases but one, which is *trans*. The torsion angle  $\phi'$  [C(4')-C(3')-O(3')-P] sweeps over the range of values 190-270° (or -90 to -170°) and the ideal *trans* conformation (180°) has not yet been observed. Alongside the  $\phi'$  values the companion torsion angles C(2')-C(3')-O(3')-P are also given in parentheses (Table 6). The eight structures known provide some idea of the range of conformational fluctuations about the C(3')-O(3') bond and further emphasize the point that the torsion angle H(3')-C(3')-O(3')-P, which is obtainable from NMR coupling-constant analysis, occupies a continuous range of values and that there are no discrete conformational states for  $\phi'$ , *viz* a three/two state equilibrium as has been commonly assumed (Davies, 1978).

Table 5. Conformation angles (°) for potassium dihydrouridine 3'-monophosphate hemihydrate

E.s.d.'s on torsion angles range from 0.2-0.4°.

Glycosyl torsion angle, $\chi$	71.2 <i>anti</i>
Backbone torsion angles	
$\psi$	46.7
$\psi'$	142.3
$\phi'$	-144.5
$\omega'$	133.7
Pseudorotation parameters	
Phase angle of pseudorotation, $P$	159.0 ( <sup>2</sup> <i>E</i> )
Maximum amplitude of puckering, $\tau_m$	39.7
Torsion angles involving the phosphate group	
C(3')-O(3')-P-O(I)	-112.2
C(3')-O(3')-P-O(II)	14.8
C(3')-O(3')-P-O(III)	133.7

### The phosphate group

Bond lengths and angles (Fig. 2) for the mono-anionic phosphate group are in the typical ranges. In particular, bond lengths agree well with the tabulated average values (Sundaralingam & Prusiner, 1978) for P-O(H) bonds ( $1.569 \pm 0.015$  Å) and P-O<sup>-</sup> or P=O bonds ( $1.493 \pm 0.010$  Å). The bond angle between the two O atoms sharing the negative charge is 115.4°.

Torsion angles describing the phosphate group appear in Table 5. One of the phosphate O atoms is close to being eclipsed with C(3').

### Metal coordination

Table 7 lists the eight ligands which are coordinated to the K<sup>+</sup> cation. All the ligands are O atoms. The coordination sphere is made up of two O(4) carbonyl atoms of the dihydrouracil base, one O(2') atom of the ribose, four phosphate O atoms from two different

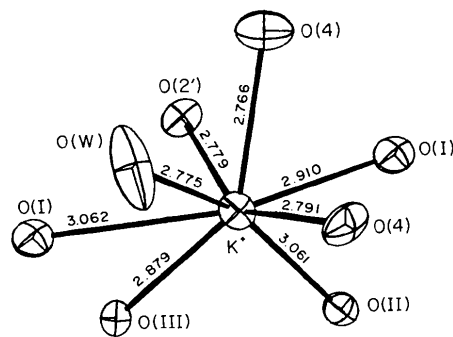


Fig. 3. K<sup>+</sup> ion coordination depicted by an ORTEP drawing (Johnson, 1965) showing 50% probability ellipsoids of the atoms. The average e.s.d.'s in the K...O distances (Å) are 0.003 Å.

Table 6. Conformational comparison of 3'-nucleotides

E.s.d.'s in torsion angles (given in degrees) for these structures are in the range 0.4-0.8°.

	$\chi$	Sugar pucker		$\psi$	$\phi'$	
		$P$	$\tau_m$			
3'-AMP.2H <sub>2</sub> O <sup>a</sup>	3.8 ( <i>anti</i> )	<sup>3</sup> <i>T</i> <sub>4</sub>	23.3	38.4	171.2 ( <i>trans</i> )	-123 (121)*
3'-CMP(O) <sup>b</sup>	41.8 ( <i>anti</i> )	<sup>2</sup> <i>T</i> <sub>3</sub>	169.4	40.2	43.9 ( <i>gauche</i> <sup>+</sup> )	-91.5 (157.7)
3'-CMP(M) <sup>c</sup>	39.3 ( <i>anti</i> )	<sup>2</sup> <i>T</i> <sub>3</sub>	171.7	38.7	45.5 ( <i>gauche</i> <sup>+</sup> )	-111.0 (137.7)
Na <sub>2</sub> .3'-UMP.4H <sub>2</sub> O <sup>d</sup>	45.1 ( <i>anti</i> )	<sup>2</sup> <i>T</i> <sub>1</sub>	152.7	38.1	42.9 ( <i>gauche</i> <sup>+</sup> )	-105.9 (143.9)
DPMA <sup>e</sup>	28.1 ( <i>anti</i> )	<sup>3</sup> <i>T</i> <sub>2</sub>	4.4	38.2	49.8 ( <i>gauche</i> <sup>+</sup> )	-164.9 (79.1)
8Me.3'-AMP.2H <sub>2</sub> O <sup>f</sup>	216.8 ( <i>syn</i> )	<sup>2</sup> <i>E</i>	161.3	35.6	54.4 ( <i>gauche</i> <sup>+</sup> )	-112.4 (136.3)
3'-UMP.H <sub>2</sub> O <sup>g</sup>	57.2 ( <i>anti</i> )	<sup>2</sup> <i>E</i>	165.5	39.6	55.6 ( <i>gauche</i> <sup>+</sup> )	-133.3 (116.1)
K.DHUMP.0.5H <sub>2</sub> O <sup>h</sup>	71.2 ( <i>anti</i> )	<sup>2</sup> <i>E</i>	159.0	39.7	46.7 ( <i>gauche</i> <sup>+</sup> )	-144.5 (102.6)

(a) 3'-AMP.2H<sub>2</sub>O, adenosine 3'-phosphate dihydrate (Sundaralingam, 1966); (b) 3'-CMP(O), cytidine 3'-phosphate (orthorhombic) (Sundaralingam & Jensen, 1965); (c) 3'-CMP(M), cytidine 3'-phosphate (monoclinic) (Bugg & Marsh, 1967); (d) Na<sub>2</sub>.3'-UMP.4H<sub>2</sub>O, disodium uridine 3'-phosphate tetrahydrate (Viswamitra, Reddy, James & Williams, 1972); (e) DPMA, 3'-deoxy-3'-(dihydroxyphosphinylmethyl)adenosine (Hecht & Sundaralingam, 1972); (f) 8Me.3'-AMP.2H<sub>2</sub>O, 8-methyladenosine 3'-phosphate dihydrate (Yasuniwa *et al.*, 1979); (g) 3'-UMP.H<sub>2</sub>O, uridine 3'-phosphate monohydrate (Srikrishnan, Andrusz & Parthasarathy, 1978); (h) K.DHUMP.0.5H<sub>2</sub>O, potassium dihydrouridine 3'-monophosphate hemihydrate, present work.

\* The values in parentheses refer to the torsion angle C(2')-C(3')-O(3')-P.

nucleotide units, and one water O. Four of the ligands are less than 2.8 Å from the  $K^+$  ion, two O atoms are at a distance of less than 3.0 Å, and the remaining two O atoms are slightly less than 3.1 Å from the cation. An ORTEP drawing (Fig. 3) depicts the full coordination about K. Besides the water molecule, four nucleotide units comprise the coordination sphere and this coordination environment of K is portrayed in Fig. 4. Similar schemes of interactions of polynucleotide chain segments with metals may generate or stabilize loop regions in nucleic acids (see also Swaminathan & Sundaralingam, 1979).

### Hydrogen bonding and molecular packing

Hydrogen-bond distances and angles are listed in Table 8. No self-pairing of the dihydrouracil bases is observed. Rather, the bases tend to hydrogen bond to either the ribose or the phosphate group.

The molecular packing is vividly illustrated in the stereoscopic packing diagram (Fig. 5). Potassium coordination is illustrated with thin lines and hydrogen bonds are represented as dotted lines. No stacking of the bases is evident. Base-ribose interaction *via* hydrogen bonds between O(2) of the base and O(2') of the neighboring ribose is shown in Fig. 6.

Table 7. Potassium coordination sphere in potassium dihydrouridine 3'-monophosphate hemihydrate

Contact <i>A</i> ... <i>B</i>	Symmetry code for <i>B</i> *	Translation for <i>B</i>			Distance (Å) <i>A</i> ... <i>B</i>
		<i>x</i>	<i>y</i>	<i>z</i>	
K...O(4)	1	0	$\bar{1}$	0	2.766 (3)
K...O(2')	4	1	$\bar{1}$	1	2.779 (3)
K...O(4)	2	2	$\bar{1}$	2	2.791 (3)
K...O(I)	4	1	$\bar{1}$	1	2.910 (3)
K...O(I)	4	1	0	1	3.062 (3)
K...O( <i>W</i> )	1	0	0	0	2.775 (3)
K...O(II)	4	1	$\bar{1}$	1	3.061 (3)
K...O(III)	4	1	0	1	2.879 (3)

\* Symmetry codes are: (1)  $x, y, z$ ; (2)  $\bar{x}, y, \bar{z}$ ; (3)  $\frac{1}{2} + x, \frac{1}{2} + y, z$ ; (4)  $\frac{1}{2} - x, \frac{1}{2} + y, \bar{z}$ .

Table 8. Hydrogen-bond distances and angles in potassium dihydrouridine 3'-monophosphate hemihydrate

E.s.d.'s in the distances are:  $A-H$  0.04,  $A...B$  0.004,  $H...B$  0.04 Å, and in angles 1°.

<i>A-H...B</i>	Symmetry code for <i>B</i> *	Translation for <i>B</i>			<i>A-H</i>	Distance (Å)		Angle (°) <i>A-H...B</i>
		<i>x</i>	<i>y</i>	<i>z</i>		<i>A...B</i>	<i>H...B</i>	
N(3)-H(3)...O(I)	4	1	0	1	0.94	2.879	1.94	173
O(2')-H(O2')...O(2)	4	1	0	1	0.76	2.772	2.05	160
O(5')-H(O5')...O(II)	4	1	0	0	0.96	2.824	1.88	171
O(III)-H(III)...O(II)	4	1	$\bar{1}$	0	1.09	2.598	1.57	156
O( <i>W</i> )-H( <i>W</i> )...O(I)	4	1	0	1	1.03	2.712	1.84	140

\* As in Table 7.

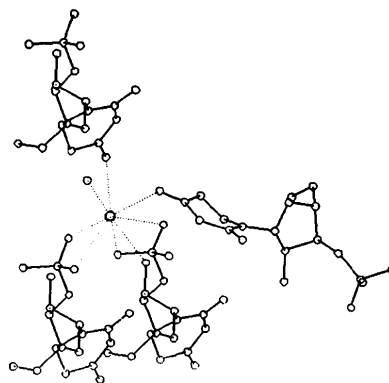


Fig. 4. View of the four nucleotide units and the water molecule making up the K coordination sphere.

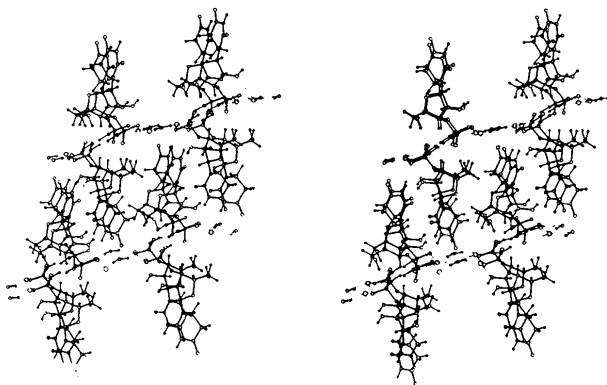


Fig. 5. Stereoscopic packing diagram showing K coordination and hydrogen bonding as viewed down the *y* axis.

### Conclusions

Both dihydrouridine and potassium dihydrouridine 3'-monophosphate fail to show any base-pairing, but rather tend to form hydrogen bonds between the base and neighboring ribose. In dihydrouracil, however, base-pairing is observed. In the nucleoside and nucleotide, when other more favorable interactions with ribose

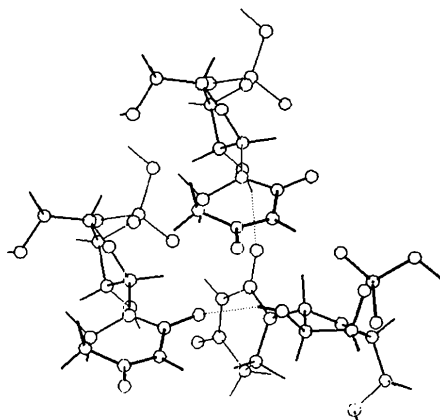


Fig. 6. Hydrogen bonding of base to neighboring ribose in potassium dihydrouridine 3'-monophosphate hemihydrate.

or phosphate O atoms are available, base-base interactions are perhaps disrupted. The presence of dihydrouridine in the dihydrouridine loop of tRNA and its evident reluctance to base-pair may be crucial for maintaining single-strand character in this region.

Also, the significance of the  $K^+$  ion coordination by four nucleotide units and water molecule could serve as a model for alkali metal or  $Mg^{2+}$  ion coordination in RNA's. The presence of chelated metal ions in RNA's may serve to promote or stabilize loop formation.

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