THE CRYSTAL AND MOLECULAR STRUCTURE OF 5-FLUORO-2'-DEOXY-β-URIDINE

DAVID R. HARRIS *and* WALTER M. MACINTYRE
From the Chemistry Department, University of Colorado, Boulder. Dr. Harris' present address is the Biophysics Department, Roswell Park Memorial Institute, Buffalo

ABSTRACT The crystal structure of 5-fluoro-2'-deoxy-β-uridine (FUDR) has been solved and the details of the molecular structure determined by x-ray analysis. The bond lengths and angles have been measured and are compared with the dimensions found for calcium thymidylate and adenylic acid. In all three compounds the bonds formed by the nitrogen atom involved in the glycosidic bond are not coplanar. Furthermore, it is found that in FUDR the C2' carbon atom is out of the plane of the ribose ring. The possible effect of these observations on nucleic acid structures is discussed.

A number of synthetic analogs of nucleic acid purines and pyrimidines have been shown to be incorporated into nucleic acids *in vivo* with a variety of biological effects on the host cell. Among these compounds are 5-fluorouracil (1) (FU), 5-fluoroorotic acid (1), 5-iodo-2'-deoxy-β-uridine (2), 8-azaguanine (3), and 6-mercaptopurine (4). Even though the biological effects of these chemicals on the host cell may not be directly attributable to this incorporation, it is still of interest to speculate on the effects of such incorporation on the secondary structure of the nucleic acids. Furthermore, an exact knowledge of the hydrogen-bonding capabilities of the molecules permits a much more detailed study of enzyme-substrate complex formation at various points in the metabolic cycle of the drug.

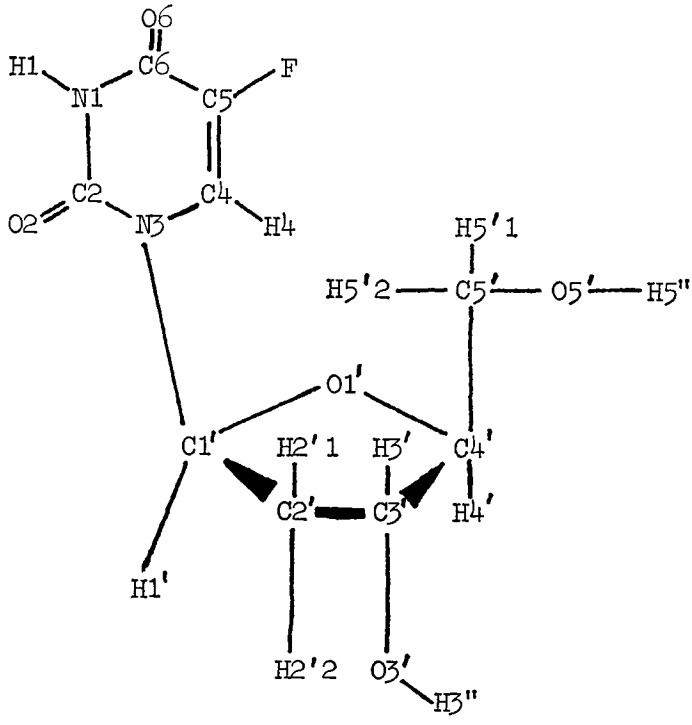
The current theories of nucleic acid structure (5, 6) impose rather exacting geometrical requirements on the secondary bonding to the nucleic acid bases. Accordingly, one might ask the following questions about each of these purine or pyrimidine analogs.

(*a*) Is it sufficiently similar to a natural base to be able to adopt the same hydrogen-bonding scheme on incorporation into a nucleic acid and to fit into the same stereochemical environment?
(*b*) If so, will the hydrogen bond lengths be the same?
(*c*) Is no other hydrogen-bonding scheme available to the synthetic base which might be preferred?

(*d*) If the synthetic base cannot be fitted into the hydrogen-bonding scheme of a natural base, what hydrogen-bonding scheme is it likely to use?

Answers to these questions are essential to an understanding of the effect of the synthetic bases on the molecular structure of the nucleic acid into which they are incorporated.

The nature of the hydrogen-bonding system preferred by small molecules can frequently be elucidated by x-ray analysis of their crystal structure. This method has the additional advantage of giving exact bond lengths and angles for the hydrogen bonds in addition to providing exact molecular dimensions. In this laboratory we have begun the x-ray crystal structure analysis of the five compounds listed above or their corresponding nucleoside. This paper describes the crystal structure of 5-fluoro-2′-deoxy-β-uridine, hereafter referred to as FUDR. The structural formula of this molecule and the atom-numbering scheme adopted in this paper is:



It was decided to examine the crystal structure of FUDR rather than that of the simpler FU for a variety of reasons. In FUDR hydrogen bonds cannot be formed to the N3 which is blocked by the sugar ring, and the possible hydrogen-bonding schemes are restricted to those available in the nucleic acid. Any electronic effects arising from substitution of the deoxyribose ring on N3 will make the electron dis-

tribution on the pyrimidine ring of FUDR closer to that existing in the nucleic acid than would be the case in FU. There was the further compelling reason that FUDR formed crystals suitable for x-ray analysis while FU did not.

FU was found to be incorporated into the RNA of a human anaplastic lung tumor, mouse liver, spleen, Sarcoma 180, and Ehrlich ascites carcinoma (1). In a later paper it was shown that FU and FUDR interfered with the action of bacterial thymidylate synthetase (7). In general, FUDR behaves in a manner analogous to 5-fluorouracil in biological systems.

EXPERIMENTAL

The following crystal data were found for FUDR:

System: Orthorhombic		Space group: $P2_12_12_1$
$a = 19.38 \pm 0.03$ A		$Z = 4$
$b = 11.76 \pm 0.02$ A		$\rho_o = 1.556$ gm/cc
$c = 4.61 \pm 0.01$ A		$\rho_o = 1.556$ gm/cc

The axial lengths were calculated from General Electric single crystal orienter measurements of 2θ for a number of reflections and agreed to within 0.5 per cent with the cell constants previously determined from film data.

The only systematic absences observed were ($h00$) with h odd, ($0k0$) with k odd and, $00l$ with l odd. Space group $P2_12_12_1$ was assigned to the crystal on the basis of these absences.

The assumption of four molecules of FUDR in the crystal cell gave exact agreement between observed and calculated densities. Since the space group has a multiplicity of 4, there is one molecule of FUDR in the asymmetric unit.

Two different sets of intensity data were used in the analysis. The first set was a mixture of scintillation counter data, collected using the Evans Weissenberg attachment (8) ($hk0$ zone only), together with equi-inclination integrating Weissenberg film data recorded from a c axis setting of the crystal using CuKα radiation. This was the set used for the solution of the structure and for the initial refinement. The final refinement of the structure was carried out using data collected with a modified General Electric single crystal orienter (9). Use of this equipment enables one to collect all reflections accessible to the radiation used from a single setting of the crystal, and therefore all are on the same scale.

Data were taken with CuKα radiation out to the limit of the modified instrument ($2\theta = 150°$). This corresponds to roughly 90 per cent of the CuKα range and a total of 1303 reflections. All reflections, except those systematically absent, were observed although the standard deviation of the smaller F's is much larger in comparison to their magnitude than are those of the larger ones.

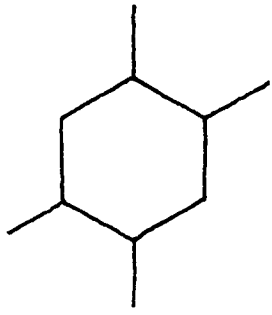
The crystal used for data collection was approximately $0.1 \times 0.2 \times 0.4$ mm in size and was mounted with the (110) direction vertical. An empirical absorption correction was applied to the data, the maximum correction to any intensity being about 14 per cent. The data were also corrrected for α_1, α_2 splitting since the stationary crystal- stationary counter technique was used.

STRUCTURE DETERMINATION

In view of the 4.61 A *c* axis, it was decided to attempt to solve the structure first in projection (the short axis indicates that there will be little overlap of electron density when viewing the molecule along the *c* axis).

Furthermore, the projection looking down a twofold axis is centrosymmetrical (phase angles are zero or π) provided the origin is chosen at the twofold axis. Previous structure analyses of similar compounds, cytidine (10) and calcium thymidylate (11), have shown that the planes of sugar and pyrimidine rings are inclined to each other at approximately 75°. Making this assumption for FUDR, it was possible to obtain a model in which all atoms were well resolved and which fitted within the 4.61 A *c* axis repeat distance. Trial and error methods using this model gave unsatisfactory progress and it was decided to extend the analysis to three dimensions.

Since some information as to the relative orientation of the ring systems was available from the two-dimensional Patterson, it was hoped that interpretation of special sections of the three-dimensional Patterson function might be sufficient to determine the structure. The Harker sections $P(uv\frac{1}{2})$, $P(u\frac{1}{2}w)$, $P(\frac{1}{2}vw)$ were calculated using data measured photographically from equi-inclination integrating Weissenberg films. Data for five levels were taken about the *c* axis. A search was made on these sections for sets of vectors of the figure;



i.e., the pyrimidine ring and the four non-hydrogen atoms substituted on it.

From the Harker sections it was possible to deduce four independent sets of coordinates for the pyrimidine ring.

There remained the problem of determining the point of attachment of the deoxyribose to the pyrimidine ring, and their relative orientation. The Patterson projection $P(uv)$ had revealed two peaks which might represent vectors from $O6$ to $C1'$ one of them along the *b* axis and the other at 60° to the first. Neither of these possibilities was inconsistent with any of the four sets of coordinates for the pyrimidine ring. The vector at 60° to the *b* axis was eventually deduced to be the correct one, on the basis of packing considerations, as follows.

The projection $P(uv)$ had also revealed that the pyrimidine ring had essentially

zero inclination to the a axis, confirmed by inspection of the section $P(u0w)$, but was inclined at approximately 30° to the b axis. Now there is no room along the very short c axis (4.61 A) to accommodate a pyrimidine ring (inclined at 30° to b) and a deoxyribose ring (at 75° to the pyrimidine ring). Thus the structures involving a nucleosidic linkage parallel to b could be eliminated.

On the basis of the three-dimensional Patterson function there remained four possible structures. These were tested first on the ($hk0$) reflections. Structure factors were calculated for all ($hk0$) reflections for which $\sin\theta < 0.5$, and $|F_0| > 5$. The four structures gave reliability indices (R) of 0.60, 0.77, 0.58, and 0.69. Two-dimensional Fourier refinement eventually showed the structure with $R = 0.58$ to be correct.

The projection of the electron density on to the (001) plane, computed using the phases calculated from the structure with $R = 0.58$, showed the molecule clearly, and x and y coordinates for all the atoms could be measured. When these coordinates were used to calculate a new set of structure factors R was reduced to 0.44 for these ($hk0$) planes with $\sin\theta < 0.5$. At this point R was calculated for all the ($hk0$) planes observed (192 in all) and was found to be 0.49. Eight more cycles of Fourier refinement were carried out using the ($hk0$) planes. After the last cycle R became 0.25. On excluding (200) and (020) from consideration, since they appeared to suffer from secondary extinction, R was 0.19.

Since approximate z coordinates had been obtained from the Harker sections it was clear, at this point, that the phase problem had in fact been solved.

REFINEMENT OF STRUCTURE

Refinement began with a series of three-dimensional Fourier syntheses designed to improve the z coordinates of the atoms. Four cycles of refinement gave an R factor over the entire set of data of 0.16.

These Fourier syntheses were followed by a number of cycles of least squares refinement. The least squares refinement was carried out in two ways, one involving the use of isotropic temperature factors, the other involving anisotropic temperature factors.

The photographic data were used only to refine individual isotropic temperature factors and atomic coordinates. After four cycles of refinement R was 0.13 for the 891 observed planes.

The counter data, collected on the General Electric single crystal orienter, were then used to refine individual anisotropic temperature factors for the non-hydrogen atoms and atomic coordinates.

Initially only planes with $\sin\theta$ (CuKα) > 0.4 were included in the refinement. This was done partly to accelerate convergence and partly to minimize the effect of secondary extinction. Secondary extinction appeared to affect the planes 200, 020, and 110 quite severely.

Of the 1303 reflections measured 1187 had sin $\theta > 0.4$. Of these 1187 reflections 55 were considered unobserved in the sense that their intensities were not significantly different from the background. They were considered to have intensities less than the smallest measurement considered significant. Thus, numbers could be obtained which represented upper limits for the values of the structure factors. These unobserved planes were included in the least squares refinement with "observed" structure factors equal to one-half of these upper limits.

After eight cycles of refinement using these data the changes in atomic coordinates and anisotropic thermal parameters were all well within the corresponding estimated standard deviations. The R factor for the 1187 reflections included in the refinement was 0.100. Including the contributions of the low order reflections gave $R = 0.097$. Since the 55 unobserved planes are included these R values are probably slightly too high.

The atomic coordinates and thermal parameters of the heavier atoms in the molecule were now determined as precisely as the data would allow. Refinement was therefore terminated at this point. There remained the question of the location of the eleven hydrogen atoms in the molecule. Using the structure factors calculated with the final set of heavy atom parameters, a three-dimensional difference Fourier synthesis was computed. Reasonable locations for these atoms could be calculated from the positions of the heavy atoms to which they were attached. Peaks of appropriate height were found in the difference map at all of these positions (Fig. 1). These peaks were therefore identified as hydrogen atoms.

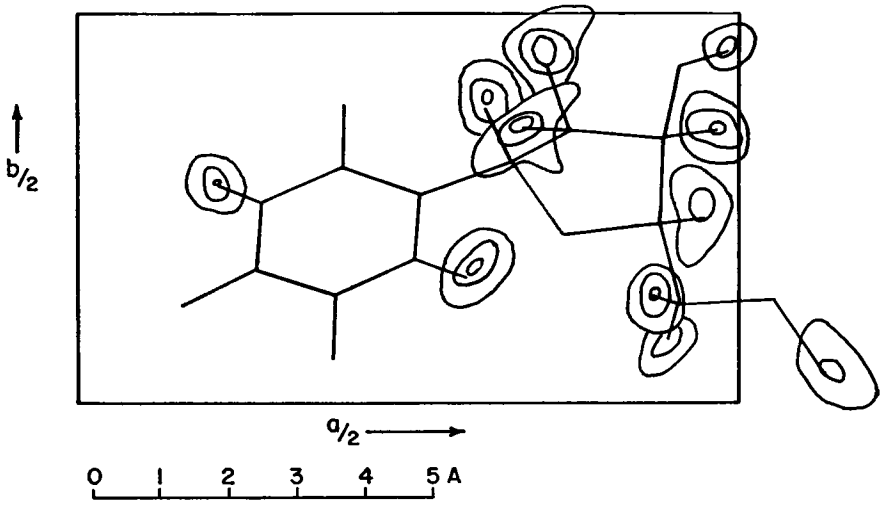


FIGURE 1 A superposition of sections of electron density through the hydrogen atoms from the final three-dimensional difference Fourier synthesis. The contours are at intervals of 0.12 $e.$ A^{-3}. The zero level contour is omitted.

Only the position of H5″ seems a little uncertain. The length of the O5′—H5″ bond is found to be 1.33 A (Table VI) which is too long.

A final structure factor calculation in which these hydrogen atoms were included was then carried out. This gave a final over-all $R = 0.092$. A list of calculated and observed structure factors may be obtained from one of us (W. M. M.) on request. A list of observed structure factors ($\times$ 10) is given in the Appendix.

COMPUTING METHODS

The computing facilities available to us changed very rapidly in the course of this analysis and a variety of machines and programs was used. The first two-dimensional Patterson functions and the three Harker sections were evaluated on X-RAC. The first two-dimensional structure factor calculations and Fourier syntheses were carried out on a desk calculator. After our IBM 1620 computer arrived the structure factor calculations were carried out by a program written by us (12), and the Fourier syntheses were evaluated using a program written by D. van der Helm (13). When refinement was started our IBM 709 computer was not completely operational and the initial refinement was carried out on an IBM 7090 computer at Los Alamos Scientific Laboratory. The isotropic least squares refinement was carried out there using an unpublished full-matrix program devised by D. T. Cromer, F. H. Kruse, and A. C. Larson. The final refinement of the anisotropic thermal parameters and atomic coordinates was performed on the University of Colorado IBM 709 using a block diagonal least squares program originally written by P. K. Gantzel and K. N. Trueblood at the University of California at Los Angeles, but later modified by R. Deverill of this laboratory. This program contains a provision for weighting the observations by a function of the form $1/(1 + a|F_o| + b|F_o|^3)$. This weighting function was used in the refinement with the values $a = -0.55 \times 10^{-3}$ and $b = 0.213 \times 10^{-4}$. The standard deviations of the final atomic parameters were calculated in the usual way by this program. The final difference Fourier synthesis was performed on the IBM 709 using the van den Hende modification of the Shoemaker-Sly program (14).

RESULTS

The final atomic positions of the non-hydrogen atoms and their estimated standard deviations (ESD) are given in Table I. Their anisotropic thermal parameters with the ESD are given in Table II. The atomic coordinates of the hydrogen atoms are given in Table III. The intramolecular bond lengths and their ESD are in Table IV. The intramolecular bond angles and their ESD are in Table V. The lengths of bonds involving hydrogen atoms are given separately in Table VI.

DISCUSSION

Molecular Dimensions. Similarly detailed analyses of other pyrimidine nucleosides have not been reported in the literature. However, structure analyses based on two two-dimensional projections have been reported for cytidine (10), cytidylic acid (15), and 5′-bromo-5′-deoxythymidine (16). Furthermore, a rather precise analysis of calcium thymidylate has been published recently (11).

TABLE I

FINAL ATOMIC POSITIONS IN FUDR AND THEIR STANDARD DEVIATIONS

The atomic positions were given by the last cycle of least squares refinement. All quantities are expressed as fractions of the unit cell edges.

Atom	x	$\sigma(x) \times 10^4$	y	$\sigma(y) \times 10^4$	z	$\sigma(z) \times 10^4$
C2	0.1989	3.42	0.30820	5.85	0.4823	20.0
C4	0.2544	3.76	0.1855	6.57	0.8324	19.4
C5	0.1945	3.67	0.1362	6.41	0.8968	19.9
C6	0.1310	3.55	0.1670	6.51	0.7587	19.6
C1′	0.3246	3.27	0.3170	5.76	0.5364	18.4
C2′	0.3689	3.58	0.3623	5.83	0.7869	18.2
C3′	0.4409	3.20	0.3486	5.49	0.6571	17.1
C4′	0.4353	3.05	0.2359	5.74	0.5061	17.4
C5′	0.4541	3.41	0.1344	6.11	0.6921	20.1
N1	0.1387	2.81	0.2548	5.52	0.5651	16.5
N3	0.2572	2.68	0.2703	5.14	0.6307	15.3
O2	0.2005	2.70	0.3809	4.87	0.3005	16.1
O6	0.0783	2.66	0.1221	5.32	0.8126	16.3
O1′	0.3638	2.21	0.2245	4.00	0.4177	11.8
O3′	0.4528	2.49	0.4403	3.94	0.4584	13.7
O5′	0.5273	2.49	0.1421	4.97	0.7455	16.9
F	0.1912	2.65	0.0525	4.49	1.0981	15.0

TABLE II

FINAL ANISOTROPIC THERMAL PARAMETERS OF NON-HYDROGEN ATOMS IN FUDR AND THEIR STANDARD DEVIATIONS

These quantities are defined by the expression:

$$T = \exp - (h^2b_{11} + k^2b_{22} + l^2b_{33} + hkb_{12} + hlb_{13} + klb_{23})$$

where T is the anisotropic temperature factor applied to the structure factors. The b_{ij} and their standard deviations are multiplied by 10^5 in the table.

Atom	b_{11}	$\sigma(b_{11})$	b_{22}	$\sigma(b_{22})$	b_{33}	$\sigma(b_{33})$	b_{12}	$\sigma(b_{12})$	b_{13}	$\sigma(b_{13})$	b_{23}	$\sigma(b_{23})$
C2	109	16	379	45	4470	424	94	44	−112	159	457	266
C4	149	18	482	50	3460	397	−6	51	−206	163	347	257
C5	136	17	454	49	4660	429	−63	51	−225	155	1070	289
C6	104	16	513	50	4830	432	−171	49	65	155	583	292
C1′	88	15	368	43	3930	388	2	43	331	142	85	255
C2′	122	16	359	43	3840	362	−2	44	126	145	−558	264
C3′	88	14	294	42	3550	351	−22	41	68	137	−66	218
C4′	71	13	390	43	3010	297	21	42	168	125	144	233
C5′	100	16	355	45	4860	425	−17	45	−70	154	368	278
N1	75	13	537	44	4940	390	−1	42	−257	124	841	269
N3	59	12	471	42	4060	327	14	39	120	117	571	234
O2	131	14	624	43	6220	388	91	40	−85	139	1990	250
O6	110	13	792	49	5930	378	−151	42	−133	133	1570	278
O1′	67	11	417	32	3370	251	57	30	−97	89	−559	175
O3′	125	12	303	32	4400	297	−80	32	156	112	−57	180
O5′	86	12	613	42	6780	412	5	37	−145	125	1480	272
F	185	13	744	43	7030	377	−163	39	−180	127	3010	230

TABLE III

ATOMIC COORDINATES, ISOTROPIC THERMAL PARAMETERS, AND PEAK HEIGHTS OF THE HYDROGEN ATOMS

The atomic coordinates and peak heights were obtained from the final difference Fourier synthesis. The thermal parameters are the values for the atom to which the hydrogen atom is bonded. The atomic coordinates are given as fractions of the unit cell edges.

	x	y	z	$B(A^2)$	(e/A^3)
H4	0.297	0.174	0.948	2.2	0.37
H1′	0.311	0.369	0.393	1.7	0.37
H2′1	0.353	0.435	0.813	1.9	0.38
H2′2	0.340	0.345	0.995	1.9	0.33
H3′	0.483	0.336	0.743	2.1	0.35
H4′	0.471	0.244	0.313	2.0	0.29
H5′1	0.434	0.125	0.924	2.0	0.36
H5′2	0.446	0.079	0.555	2.0	0.33
H1	0.102	0.280	0.491	2.2	0.46
H3″	0.503	0.438	0.416	2.0	0.20
H5″	0.566	0.051	0.696	2.7	0.21

TABLE IV

INTRAMOLECULAR BOND LENGTHS, NOT INVOLVING HYDROGEN ATOMS, IN FUDR AND IN CALCIUM THYMIDYLATE AND THEIR STANDARD DEVIATIONS; BOND LENGTHS GIVEN FOR URACIL

The standard deviation of bond lengths in uracil is quoted to be of the order of 0.01 A (18). These data are given in Angstroms.

	FUDR		Calcium thymidylate (11)		Uracil (18)
Bond	Bond length	Standard deviation	Bond length	Standard deviation	Bond length
C2—O2	1.198	0.010	1.247	0.018	1.230
C2—N1	1.377	0.010	1.377	0.018	1.384
C2—N3	1.394	0.010	1.365	0.018	1.344
N3—C4	1.365	0.010	1.367	0.018	1.341
C4—C5	1.331	0.011	1.310	0.022	1.408
C5—C6	1.433	0.011	1.452	0.021	1.411
C5—F	1.355	0.010			
C6—O6	1.235	0.010	1.218	0.019	1.241
C6—N1	1.373	0.010	1.414	0.018	1.374
N3—C1′	1.481	0.009	1.466	0.018	
C1′—O1′	1.432	0.008	1.438	0.018	
C1′—C2′	1.536	0.010	1.527	0.028	
C2′—C3′	1.527	0.010	1.550	0.019	
C3′—O3′	1.433	0.008	1.409	0.018	
C3′—C4′	1.502	0.010	1.491	0.022	
C4′—C5′	1.514	0.010	1.526	0.022	
C5′—O5′	1.442	0.010	1.472	0.019	
C4′—O1′	1.451	0.008	1.441	0.017	

TABLE V

INTRAMOLECULAR BOND ANGLES, NOT INVOLVING HYDROGEN ATOMS, FOR FUDR, CALCIUM THYMIDYLATE, AND URACIL AND THE STANDARD DEVIATIONS FOR FUDR

These data are given in degrees. The standard deviation of the calcium thymidylate angles was quoted as *ca.* 1.4° (11). The standard deviation of the uracil angles was quoted as 0.6-0.8°.

			FUDR			
Angle			Magnitude	Standard deviation	Calcium thymidylate (11)	Uracil (18)
Pyrimidine ring—internal angles						
C6	N1	C2	128.0	0.7	125.7	124.7
N1	C2	N3	113.9	0.6	115.8	115.6
C2	N3	C4	122.4	0.6	121.4	123.6
N3	C4	C5	120.3	0.7	123.3	121.5
C4	C5	C6	122.7	0.7	120.3	116.4
C5	C6	N1	112.6	0.7	113.4	118.1
Pyrimidine ring—external angles						
N1	C2	O2	122.8	0.7	120.8	119.9
O2	C2	N3	123.4	0.7	123.2	124.5
C2	N3	C1′	117.0	0.6	117.6	
C1′	N3	C4	120.2	0.6	121.0	
C4	C5	F	120.7	0.7		
F	C5	C6	116.5	0.7		
C5	C6	O6	124.1	0.7	128.8	123.6
O6	C6	N1	123.3	0.7	117.7	118.3
Sugar ring—internal angles						
O1′	C1′	C2′	104.8	0.6	107.2	
C1′	C2′	C3′	100.3	0.6	103.3	
C2′	C3′	C4′	102.0	0.6	101.8	
C3′	C4′	O1′	106.3	0.5	106.7	
C4′	O1′	C1′	109.2	0.5	108.9	
Sugar ring—external angles						
N3	C1′	O1′	107.5	0.6	107.4	
C2′	C3′	O3′	108.6	0.6	114.3	
O3′	C3′	C4′	112.3	0.6	113.7	
C3′	C4′	C5′	114.6	0.6	117.1	
C4′	C5′	O5′	106.5	0.6	109.4	
C5′	C4′	O1′	108.5	0.6	107.6	

Estimated standard deviations of the bond lengths were given for calcium thymidylate and so it is possible to make an exact comparison of the corresponding molecular dimensions in FUDR. The values of appropriate bond lengths and bond angles quoted for calcium thymidylate are also listed in Tables IV and V. It is clear that the corresponding bond lengths are identical in the two molecules to within experimental error except in the case of C2—02. In C2—02 the difference

TABLE VI

LENGTHS OF COVALENT BONDS FORMED BY HYDROGEN ATOMS

The bond lengths are in Angstroms. The estimated standard deviation of these bond lengths is about 0.15 A.

Bond	Bond length
	A
C4—H4	1.0
C1′—H1′	0.9
C2′—H2′1	0.9
C2′—H2′2	1.1
C3′—H3′	0.9
C4′—H4′	1.1
C5′—H5′1	1.1
C5′—H5′2	0.9
N1—H1	0.8
O3′—H3″	1.0
O5′—H5″	1.3

is only slightly greater than the sum of the two ESD and is probably not significant.[1]

The agreement between corresponding angles is not so marked. The large differences in the values of C5 $\widehat{C6}$ O6, O6 $\widehat{C6}$ N1, and C2′ $\widehat{C3}$′ O3′ are probably significant. C5 $\widehat{C6}$ O6 is 4.7° larger in calcium thymidylate than in FUDR. This is a reasonable result in view of the fact that the methyl group in thymidine has a larger van der Waals radius (2.0 A) than F (1.35 A) (17) and will tend to push the 06 atom away from itself, thus increasing the C5 $\widehat{C6}$ O6 angle slightly. This same effect will cause 06 $\widehat{C6}$ N1 to be smaller in calcium thymidylate than in FUDR, as is indeed found, the difference being 5.6°.

The discrepancy between the two values for C2′ $\widehat{C3}$′ O3′ (5.7°) is probably associated with the difference in the conformation of the ribose ring in the two compounds as discussed below.

The other corresponding angles agree rather well. This is a remarkable demonstration of the invariance of the molecular parameters of these pyrimidine nucleosides under differences in molecular environment. The calcium thymidylate crystals contain six water molecules per formula weight and so the thymidylate ion is in an aqueous medium. When incorporated into nucleic acids in a cell the nucleosides presumably are in a similarly aqueous medium. It is thus comforting to find that bond lengths and angles determined from such non-aqueous crystals as cytidine and FUDR are very probably the same as those obtaining when the nucleoside is incorporated into a nucleic acid, where the secondary bonding pattern can be somewhat different from that found in the crystals.

[1] In this paper the conventional atom-numbering scheme for thymidine is used. This is identical to that for FUDR except that the fluorine atom is replaced by a methyl group whose carbon atom is referred to as C7.

When FU is incorporated into RNA it is probably incorporated in place of uracil, since fluorine and hydrogen atoms are quite similar sterically. Therefore, it is appropriate to compare the dimensions of the fluorouracil ring in FUDR with those given by Parry for uracil (18). The data published by Parry are reproduced in Tables IV and V.

It is apparent that there is a large measure of disagreement between the results of the two analyses. In FUDR the two angles made by C2—O2 with the ring are equal to within experimental error. Likewise, the angles made by C6—06 with the ring are equal. In uracil these angles were found to be significantly different. There is an important difference in the lengths of the C4—C5 bond.

The precision of this uracil analysis has been questioned by Trueblood *et al.* (11). They are of the opinion that the standard deviations given by Parry were seriously underestimated. Some of the results of the uracil analysis themselves suggest that a substantial increase in ESD would be appropriate. There is no physical reason why the angles C5 $\widehat{C6}$ O6 and N1 $\widehat{C6}$ O6 should be different in uracil, nor the angles N1 $\widehat{C2}$ O2 and N3 $\widehat{C2}$ O2. Furthermore, the C4—C5 distance found in FUDR is equal, to within experimental error, to the distance found in calcium thymidylate and thymine (19), while the distance quoted for uracil is well outside this range.

However, one important conclusion from the uracil analysis, the equality of the two C—O bonds and their double bond character, is substantiated by the FUDR analysis.

Conformation of Ribose. The glycosidic linkage along the C1′—N3 bond was found to be β as expected.

It is anticipated that of the five atoms in the ribose ring four will be coplanar and one will be about 0.5 A out of the plane. Spencer (20) predicts that the out-of-plane carbon atom will be C2′ or C3′. In cytidine (10), 5′-bromo-5′-deoxythymidine (16), calcium thymidylate (11), and adenosine-5′-phosphate (21)[2], O1′, C1′, C2′, and C4′ are coplanar and C3′ is out of the plane by approximately 0.5 A. However, in cytidine-3′-phosphate (15) it was found that the C2′ carbon atom was out of the plane.

In FUDR the atoms O1′, C1′, C3′, and C4′ are approximately coplanar. The best plane through these four atoms was found to have the equation:

$$-0.1522\,X - 0.4830\,Y + 0.8620\,Z = -0.6180$$

(XYZ) are the coordinates of a point on the plane with respect to the crystallographic axes and are expressed in A. The distances of the atoms from the plane are as follows:

C1′, 0.023 A; C3′, −0.022 A; C4′, 0.036 A; O1′ −0.038 A.

The standard deviation of these distances is 0.031 A. Since each of the atoms has

[2] In this paper when referring to atoms in adenosine-5′-phosphate the conventional atom-numbering scheme is used, not that adopted by Kraut and Jensen. This is:

a rms (root mean square) standard deviation of position of less than 0.01 A it is clear that C1′, C3′, C4′, and O1 are only approximately coplanar.

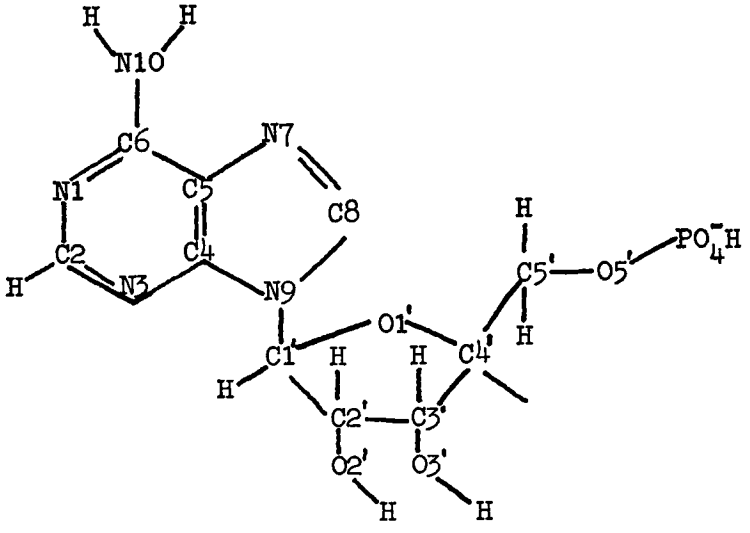
The distance of C2′ from this plane is 0.592 A.

It was noted earlier that the angle C2′ $\widehat{C3'}$ O3′ in FUDR was significantly different from that in calcium thymidylate. The angle in adenosine-5′-phosphate is equal to the value found in calcium thymidylate (115.4° and 114.3°, respectively) to within the experimental error. It is conjectured that the difference between the angle in FUDR and these two nucleotides is associated with the different conformation of the ribose ring. This conclusion is supported by the fact that in cytidine-3′-phosphate (15), which also has C2′ out of the plane, C2′ $\widehat{C3'}$ O3′ has a value of 110° which is equal to the value in FUDR (108.6°) to within the experimental error.

The plane of the deoxyribose ring and the plane of the pyrimidine ring have a dihedral angle of 71.9°. This is close to the values in calcium thymidylate (11) (75°), cytidine (10) (75°), and adenosine-5′-phosphate (21) (76°). It is, however, quite different from that in cytidine-3′-phosphate (computed by Kraut and Jensen (21)) where the angle is 62°.

The torsion angle, as defined by Donohue and Trueblood (22), is −60°. This confirms that FUDR is in the *anti* conformation, like other nucleosides and nucleotides studied so far.

Hydrogen Bonding. FUDR forms only three hydrogen bonds. The molecule has only three atoms which can act as proton donors in a hydrogen bond; these are N1, O3′, and O5′. All three of these atoms do form hydrogen bonds in this way. In addition O3′ and O5′ form second hydrogen bonds in which they act as proton acceptors. The only other atom involved in hydrogen bonding is O6.



The hydrogen bonds formed, their bond lengths and ESD, the bond angles formed and their ESD are listed in Table VII.

The only hydrogen bond FUDR has in common with calcium thymidylate is the hydrogen bond between 03′ and 06. This bond has the same bond length in both crystals (2.75 A in calcium thymidylate (11)). The other two hydrogen bond

TABLE VII

LENGTHS AND ANGLES OF HYDROGEN BONDS AND THEIR STANDARD DEVIATIONS

Bond	Length	ESD	Angle	Magnitude of Angle	ESD
	A	*A*		*degrees*	*degrees*
O3′—H · · · O6	2.773	0.008	C3′ O3′ O6	91.3	0.5
N1—H · · · O5′	2.861	0.008	N1 O5′ C5′	132.5	0.6
O5′—H · · · O3′	2.765	0.008	C5′ O5′ O3′	85.3	0.5

lengths have the expected values. The hydrogen-bonding scheme is shown in the (001) projection in Fig. 2. It will be observed that the hydrogen atoms do not lie on the line joining the two atoms held together by the hydrogen bonds. This is becoming a common observation in such systems (11, 21).

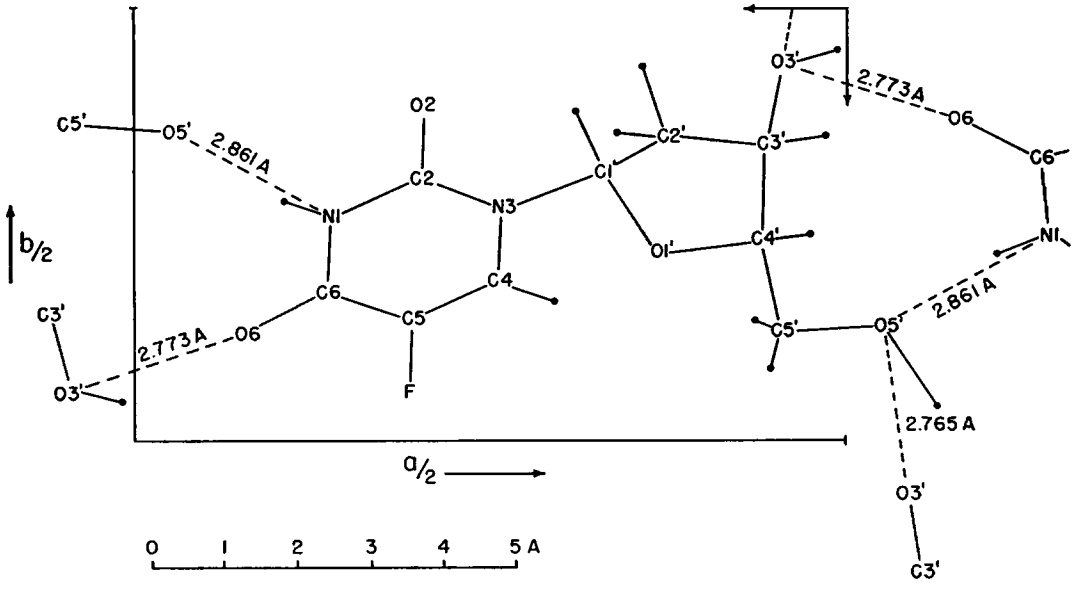


FIGURE 2 Projection of one molecule of FUDR down the *c* crystallographic axis showing the hydrogen bonding. Hydrogen bonds are indicated by dashed lines. The small solid circles represent hydrogen atoms. The large open circles represent the heavier atoms.

It is of interest to note that fluorine is not involved at all in the hydrogen bonding.

In uracil Parry (18) observed that O2 forms no hydrogen bonds, the only hydrogen bonds being formed by O6, N1, and N3. In FUDR we observe that O2 likewise forms no hydrogen bond. N3 cannot form hydrogen bonds in the nucleic acid. Thus if uracil or fluorouracil enters into a hydrogen-bonded base pair with, say, adenine, the only atoms on the pyrimidines which are likely to take part in the hydrogen bonding are N1 and O6.

It seems very likely then that fluorouracil and uracil will adopt identical hydrogen-bonding schemes in nucleic acids.

Planarity of Bases in Nucleosides. In building models of nucleic acids it has been the custom in the past to assume that the purine and pyrimidine bases are planar and that the glycosidic C—N bond is in the plane of the base. This assumption was justified by earlier structure analyses of these bases and a few nucleosides. These analyses have been reviewed by Spencer (20).

Now, in the last few years development of modern computers has revolutionized x-ray crystallography. This effect has been twofold. It has now become possible to refine structures to whatever extent the data warrant, including individual anisotropic temperature factors, etc., and so to increase the precision with which molecular parameters are determined. Secondly, it is possible to include all the observed intensities in the refinement since the arithmetic involved in handling several thousand reflections is performed automatically.

Only three structure analyses of nucleosides are known to us in which the full power of modern computing machines has been brought to bear. These are the analyses of calcium thymidylate (11), adenosine-5′-phosphate (21), and FUDR.

In each case there is evidence that the base and its substituents are not coplanar.

In calcium thymidylate C7 (methyl group) is out of the mean plane of the pyrimidine ring by 0.061 A, and O6 is out of the plane, in a direction opposite to C7, by 0.064 A. C1′ and O2 are also out of the plane, in opposite directions, by 0.050 A and 0.101 A, respectively.

In adenosine-5′-phosphate it is reported that N1 is out of the purine plane by 0.051 A and C5 by 0.043 A in the opposite direction. C1′ is out of the plane by 0.211 A. However, for the least squares plane quoted, the r.m.s. deviation of all atoms from the plane is 0.026 A. Since the r.m.s. standard deviation of position for each of the atoms in the plane is of the order of 0.01 A, it is clear that the adenine part of the molecule is only approximately planar in any case.

For FUDR we calculated the equation of the plane which best fitted the positions of all the atoms in the pyrimidine ring and of the fluorine atom and the two oxygen atoms substituted on it. The distances of all the atoms from this plane, with the exception of N1 and O2, were less than their root mean square standard deviations of position. N1 was 0.035 A above the plane while its rms standard deviation of position was 0.006 A. O2 was 0.020 A below the plane and its rms standard

deviation of position was also 0.006 A. These differences appeared significant enough to cast doubt on whether N1 was in fact in the plane of the other atoms.

Accordingly a second plane was fitted to the positions of the ring atoms, O2, O6, and F, but excluding N1. This plane has the equation,

$$-.01657\,X + 0.6814\,Y + 0.7129\,Z = 3.421.$$

(XYZ) are coordinates of points on the plane with respect to the crystallographic axes and expressed in Angstroms. The distances of the ring atoms from this plane are given in Table VIII. The standard deviation of the distances from the mean

TABLE VIII

DISTANCES OF PYRIMIDINE RING ATOMS, AND THE ATOMS SUBSTITUTED ON THE RING, FROM BEST PLANE THROUGH ATOMS

Atoms N1 and C1′ were not included in the calculation which fitted the plane.

Atom	Distance	Atom	Distance
	A		*A*
C2	0.009	C6	0.002
N3	0.004	O2	−0.010
C4	−0.003	O6	0.000
C5	0.004	F	−0.005
N1	+0.047	C1′	−0.150

plane is 0.006 A. The individual distances from the mean plane are all less than the r.m.s. standard deviation of position of the atoms, including O2. However, N1 is 0.047 A from the plane. This distance is about eight times the r.m.s. standard deviation of position of the atom. Thus atom N1 is slightly, but significantly, out of the plane of the pyrimidine ring.

Atom C1′ is 0.150 A from the mean plane, and on the opposite side from N1. The r.m.s. standard deviation of position of C1′ is 0.01 A, and so it too is significantly out of the plane of the pyrimidine ring.

In these three compounds intramolecular steric hindrance is at least partly, if not wholly, responsible for the deviations of the base from planarity.

Since all three bases are in the *anti* configuration with respect to their sugars, the hydrogen atom on C1′ is pointing in approximately the direction of O2 in calcium thymidylate and FUDR and approximately in the direction of N3 in adenosine-5′-phosphate. Thus the CH group at C1′ will have the van der Waals radius of a methyl group with respect to these atoms. A comparison of some relevant intramolecular distances and the sums of the corresponding van der Waals radii is given in Table IX.

The observed distances between the pairs of atoms listed in Table IX are con-

TABLE IX

SOME INTRAMOLECULAR DISTANCES IN CALCIUM THYMIDYLATE (11), ADENOSINE-5′-PHOSPHATE (21), AND FUDR AND THE CORRESPONDING VAN DER WAALS APPROACHES.

The sums of the van der Waals radii were obtained from Pauling (17).

		Distance observed	Sum of van der Waals radii
		A	*A*
Calcium thymidylate	(*a*) C7—O6	2.90	3.4
	(*b*) C1′—O2	2.74	3.4
Adenosine-5′-phosphate	C1′—N3	3.04	3.5
FUDR	C1′—O2	2.75	3.4

siderably smaller than the corresponding van der Waals approaches. Thus, there must be large repulsive forces between the atoms in each pair. In calcium thymidylate the repulsion between the C7 (methyl group) and O6 causes the atoms to move out of the plane of the ring, C7 to one side and O6 to the other. Similarly C1′ and O2 are forced out of the plane of the ring to opposite sides of the ring.

In adenosine-5′-phosphate the repulsive force between C1′ and N3 results in a movement of C1′ out of the plane of the ring.

In FUDR the repulsion between O2 and C1′ results in a displacement of C1′ from the plane of the pyrimidine ring. Interestingly O2 is not moved from the plane of the pyrimidine ring by any significant amount, contrary to what is found in calcium thymidylate.

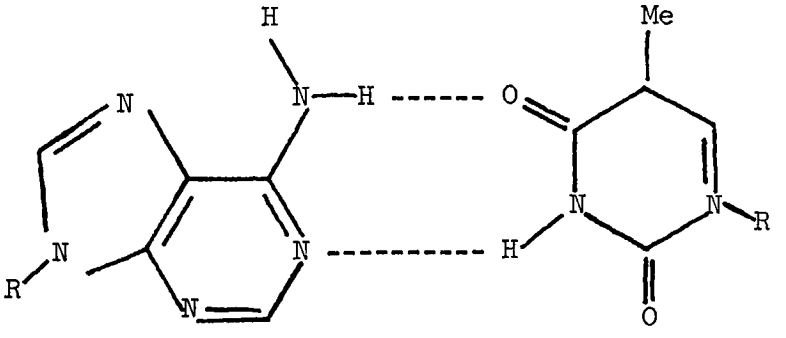
It is more difficult to explain the departure of the position of N1 from the plane of the pyrimidine ring in FUDR. The N1 atom does not appear to be pulled out of the ring by the hydrogen bond it forms to 05′, since N1 and 05′ are on opposite sides of the pyrimidine plane. No other simple explanation suggests itself to explain the deviation of N1 from the pyrimidine plane.

In nucleic acids it is believed (6) that the orientation of sugar and base in the nucleosides is similar to that found in crystals of the nucleosides. Therefore, the CH group at C1′ will interact with the base in nucleic acid nucleosides also. It seems reasonable to conclude that the displacement of C1′ from the base plane will persist when the nucleosides are incorporated into the nucleic acids. Furthermore, the displacement of C7 and O6 from the thymine plane in calcium thymidylate is unlikely to change in a different environment and so these displacements will also persist on incorporation of the thymine into nucleic acids.

The deviations from planarity of the purine and pyrimidine bases will have an effect on the structure of nucleic acids. Existing structures will be affected in two

ways. In the first place, base pairs are not necessarily coplanar. Secondly, the orientation of the base pairs to the sugar phosphate chains will be changed. We will consider the planarity of the base pairs first.

In an adenine-thymine pair the suggested hydrogen-bonding scheme (20) is,



and the planes of the bases are assumed to be coplanar. Adenine would presumably form similar base pairs with uracil and fluorouracil under the proper conditions.[3] Since O6 is out of the plane of the thymine ring in calcium thymidylate (11) and N1O is out of the plane of adenine in adenosine-5′-phosphate (21) by an almost equal amount and in the same sense, it is possible for the above pairing scheme to take place and still have the bases coplanar. However, the plane of the bases will not coincide with the plane of the hydrogen bonds.

On the other hand, if the NH_2 group on adenine should be out of the plane on the other side of the mean plane of the base, which seems perfectly feasible energetically, then the bases will no longer be coplanar when paired. We calculate that the two bases would have a dihedral angle of *ca.* 5° under these circumstances.

A third possibility is that the displacement of the nitrogen of the amino group from the mean adenine plane is due only to the nature of the hydrogen bonding in the adenosine-5′-phosphate crystal, and that in the nucleic acid the amino nitrogen atom is in the mean plane of the adenine molecule. In such a case the dihedral angle would be reduced to *ca.* 2°.

Similar conclusions can be drawn about base pairing between adenine and fluorouracil, and possibly uracil, since we find N1 and FUDR to be displaced from the pyrimidine plane by the same amount that O6 is displaced in calcium thymidylate.

There is a similar small, but significant, effect on the orientation of the base pairs and the sugar phosphate chains. Kraut and Jensen (21) calculate that the C1′—N9 bond in adenosine-5′-phosphate makes an angle of 8° with its projection on the

[3] Although fluorouracil is not incorporated into DNA it is incorporated into RNA. The recently proposed structure of transfer RNA (6) does imply some base pairing in that molecule as well as in DNA. Therefore it is permissible to discuss base pairs involving uracil and fluorouracil.

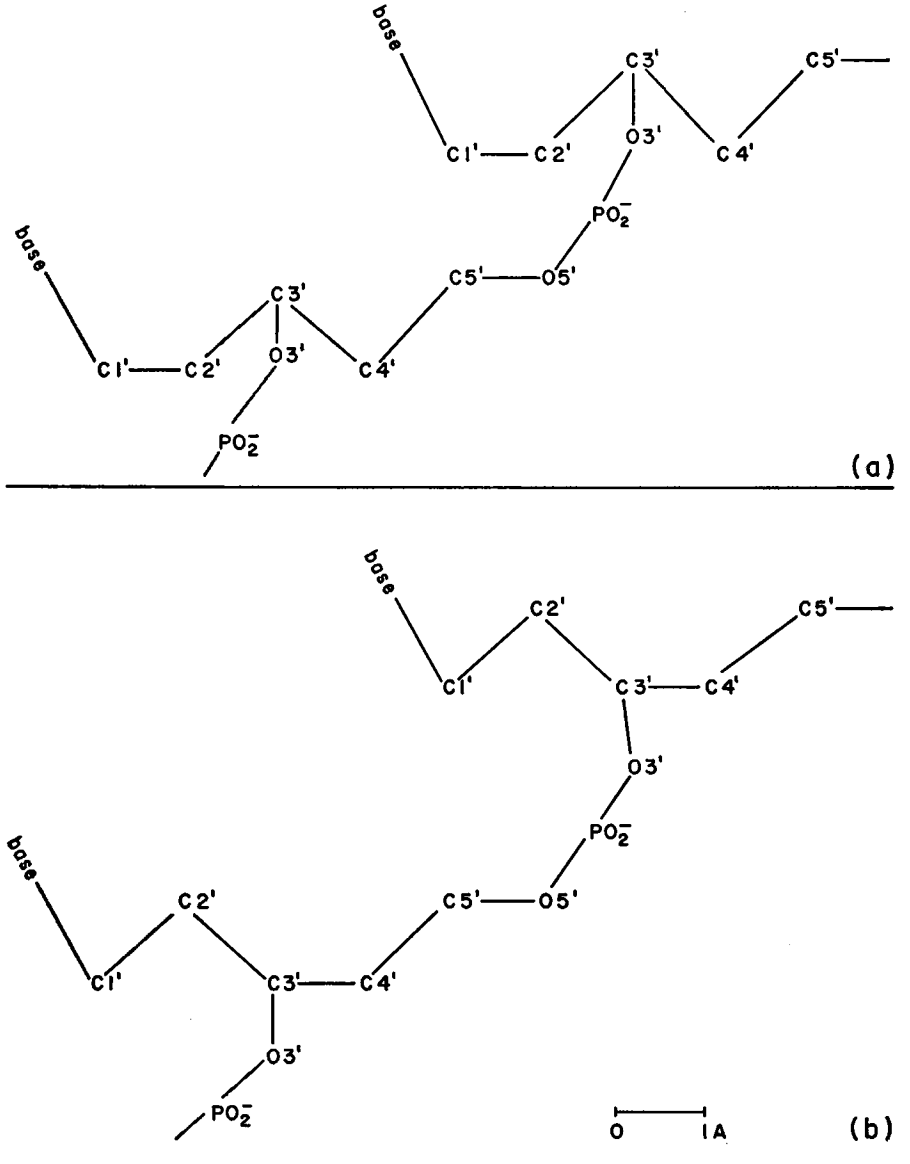


FIGURE 3 A dideoxyribotide is shown with the mean plane of the sugar normal to the plane of the paper. O1′ is omitted from the diagram but should be considered in the plane of the sugar and behind the paper. Hydrogen atoms are omitted. (*a*) C3′ out-of-the-mean plane of the sugar, (*b*) C2′ out-of-the-mean plane of the sugar.

The distance between the pairs of sugar rings is greater in (*b*) than in (*a*) since in (*b*) O3′ projects below the plane of the sugar by about 1 A.

mean plane of the adenine base. The corresponding angle in FUDR (*i.e.* involving the bond C1′—N3) we calculate to be *ca.* 6°, and in calcium thymidylate it is *ca.* 2°.

If deviations of atoms from the planes of the bases, such as those in FUDR and adenosine-5′-phosphate, exist in nucleotides when they are incorporated into nucleic acids, the net effect will be to translate the mean plane of the base pairs along the sugar-phosphate chains by an amount 0.1 to 0.2 A relative to their position in the model of Langridge *et al.* (5). This model requires a certain amount of distortion of the base pairs from strict planarity in order to fit them into the double helix with a minimum of steric hindrance. However, it is clear that such distortions are consistent with, and indeed follow from, the nucleoside structures discussed above and may well represent the rule in nucleic acid structure rather than an exception.

It seems appropriate in this discussion of nucleic acid structure to consider the conformation of the ribose ring.

The ribose ring may have either C2′ or C3′ out-of-the-mean plane. Since both conformations are found in nucleosides and nucleotides, it is reasonable to assume they will be found also in nucleic acids.

However, in nucleic acids these are not equivalent conformations as demonstrated in Fig. 3.

Fig. 3*a* represents a dideoxyribotide viewed end on to the plane of the sugar. The sugars have C3′ out of the ribose plane with the result that O3′ is approximately in the plane.

Fig. 3*b* is a similar representation of a dideoxyribotide but with C2′ out of the ribose plane. In this case O3′ is below the plane of the sugar by 1 to 1.2 A.

Since the position of O5′, relative to the plane of the sugar, is the same in the two conformations, the distance between the planes of the two rings, which are joined by a phosphate group through O3′ and O5′, will depend upon whether O3′ is or is not in the plane of the sugar. The actual difference in the distances will depend on the inclination of the planes of two neighboring sugar rings to each other. In the extreme case when the two rings are parallel the difference in the distance between them could be as much as 1.2 A. Similarly, the distance between the bases will depend on which sugar conformation is present.

It is unlikely that the double helix of a nucleic acid molecule could have one sugar conformation on one strand and the other sugar conformation on the other strand. If the two strands were to have different conformations, the base pairs would not meet at regular intervals and the formation of hydrogen-bonded base pairs would involve considerable strain. For similar reasons, one would not expect to find the two different sugar conformations in the same strand of the double helix.

Thus it is possible that the two different sugar conformations, with C2′ or C3′ out of plane, will lead to two different nucleic acid structures with different distances between the hydrogen-bonded base pairs.

APPENDIX

TABLE OF OBSERVED STRUCTURE FACTORS (× 10) FOR FUDR

The entry "A" denotes a reflection absent by virtue of the crystal symmetry. The entry "U" denotes other unobserved reflections.

H	K	L=0	1	2	3	4	5
0	0	5120	A	58	A	59	A
0	1	A	898	126	215	36	29
0	2	716	707	128	201	22	39
0	3	A	639	450	43	78	63
0	4	534	114	746	151	50	U
0	5	A	446	318	80	36	10
0	6	21	495	297	78	81	U
0	7	A	190	36	100	46	17
0	8	201	145	249	33	U	
0	9	A	26	122	116	113	
0	10	325	19	43	62	U	
0	11	A	62	16	46		
0	12	108	91	35	37		
0	13	A	22	80			
0	14	36	91				
1	0	A	347	94	198	123	63
1	1	84	477	51	163	154	41
1	2	335	629	75	149	31	27
1	3	215	312	181	107	84	46
1	4	346	373	337	U	50	41
1	5	29	287	379	162	31	23
1	6	26	262	127	76	47	37
1	7	13	58	101	101	42	25
1	8	222	128	124	114	U	
1	9	96	217	138	42	80	
1	10	196	126	96	106	36	
1	11	47	100	176	30		
1	12	23	66	64	53		
1	13	19	39	44			
1	14	42	108				
2	0	220	606	203	44	150	53
2	1	62	448	223	177	55	27
2	2	358	194	127	47	67	49
2	3	295	767	155	U	63	87
2	4	356	169	154	128	85	25
2	5	123	164	145	215	84	38
2	6	32	60	269	180	98	23
2	7	63	269	65	142	29	U
2	8	164	69	86	47	28	
2	9	42	115	91	104	14	
2	10	184	112	28	22	63	
2	11	43	122	31	29		
2	12	U	120	36	14		
2	13	74	77	29			
2	14	96	45				
3	0	A	134	118	29	89	19
3	1	162	485	233	179	22	27
3	2	443	392	193	163	49	43
3	3	308	495	147	73	109	57
3	4	58	324	212	81	101	44
3	5	62	331	255	57	127	23
3	6	195	254	188	107	25	27
3	7	96	182	45	63	51	18
3	8	306	176	18	196	60	
3	9	365	145	89	21	34	
3	10	22	136	19	42	31	
3	11	12	117	48	30		
3	12	42	50	90	U		
3	13	35	43	54			
3	14	U	48				
4	0	36	319	150	46	71	63
4	1	533	212	159	112	48	75
4	2	271	414	349	16	48	43
4	3	396	345	54	106	118	65
4	4	228	87	208	225	U	45
4	5	317	100	253	44	114	38
4	6	91	193	169	203	156	18
4	7	31	33	33	115	65	
4	8	95	59	95	111	26	
4	9	145	90	43	116	17	
4	10	29	82	U	38	57	
4	11	97	20	61	46		
4	12	36	51	26	34		
4	13	98	66	35			
4	14	13	61				
5	0	A	567	80	U	76	46
5	1	161	341	210	130	65	120
5	2	390	235	459	109	139	38
5	3	175	133	187	75	87	25
5	4	273	187	64	290	44	36
5	5	87	104	174	95	45	46
5	6	467	119	144	90	47	44
5	7	198	86	135	33	47	
5	8	86	42	158	94	29	
5	9	110	66	48	61	87	
5	10	29	32	112	52	73	
5	11	100	64	90	33		
5	12	71	98	68	U		
5	13	118	66	46			
5	14	31	34				
6	0	79	574	157	53	57	19
6	1	116	71	215	97	90	45
6	2	134	212	91	69	106	36
6	3	349	191	208	106	133	30
6	4	71	82	15	127	35	20
6	5	269	227	66	31	113	22
6	6	103	160	76	138	82	U
6	7	77	156	179	116	11	
6	8	91	37	94	27	29	
6	9	38	156	100	29	26	
6	10	55	246	90	39	U	
6	11	195	78	55	62		
6	12	31	79	37	29		
6	13	91	47	35			
6	14	12	48				
7	0	A	248	71	176	60	U
7	1	250	405	414	100	82	29
7	2	60	430	110	151	125	50
7	3	428	103	138	41	191	51
7	4	206	239	78	184	124	36
7	5	114	75	91	291	102	30
7	6	98	123	120	196	35	17
7	7	29	64	230	39	U	
7	8	27	102	140	69	26	
7	9	162	U	134	41	59	
7	10	99	75	26	47		
7	11	149	48	28	27		
7	12	115	52	38			
7	13	58	55	41			
7	14	22					

(Table continued on following page)

TABLE OF OBSERVED STRUCTURE FACTORS (× 10) FOR FUDR (*concluded*)

H	K	L=0	1	2	3	4	5
8	0	520	199	253	64	53	27
8	1	268	31	136	67	52	120
8	2	526	237	59	15	123	35
8	3	322	115	85	64	16	41
8	4	486	62	86	99	183	31
8	5	22	243	122	177	36	22
8	6	43	221	130	91	90	
8	7	31	168	333	47	31	
8	8	50	212	229	87	9	
8	9	52	172	86	69	36	
8	10	58	258	31	30		
8	11	68	35	39	26		
8	12	101	77	32			
8	13	86	31				
9	0	A	14	38	23	34	15
9	1	353	221	106	89	59	37
9	2	259	349	146	110	51	33
9	3	205	63	133	18	84	U
9	4	73	65	101	158	U	11
9	5	331	70	183	152	45	
9	6	69	95	85	179	48	
9	7	90	75	44	53	U	
9	8	64	57	221	45	29	
9	9	30	116	96	23	49	
9	10	123	76	74	74		
9	11	214	62	41	36		
9	12	U	49	U			
9	13	80	42				
10	0	466	274	28	30	25	39
10	1	41	339	109	33	106	47
10	2	427	85	41	96	95	43
10	3	188	96	108	129	66	42
10	4	258	104	120	226	28	30
10	5	138	306	138	80	41	
10	6	116	117	235	45	45	
10	7	104	97	161	130	U	
10	8	69	81	118	55	31	
10	9	12	195	55	80		
10	10	180	143	98	59		
10	11	113	132	41	30		
10	12	76	39	22			
10	13	114	80				
11	0	A	43	119	214	25	71
11	1	151	431	222	U	139	21
11	2	111	52	145	116	91	38
11	3	312	194	145	108	95	18
11	4	62	261	152	165	21	
11	5	98	161	140	124	42	
11	6	36	103	U	111	U	
11	7	96	52	150	49	26	
11	8	92	150	117	11	37	
11	9	112	125	11	44		
11	10	107	41	36	U		
11	11	26	39	39			
11	12	32	59	14			
11	13	60					
12	0	488	101	U	U	52	36
12	1	466	144	160	50	28	25
12	2	70	185	57	U	15	
12	3	143	73	113	43	102	
12	4	175	71	233	84	28	
12	5	21	75	169	115	38	
12	6	19	86	125	60	74	
12	7	29	73	19	34	51	
12	8	71	57	109	67		
12	9	141	38	19	30		
12	10	163	26	60	30		
12	11	32	44	30			
12	12	70	37				
13	0	A	245	99	69	82	
13	1	58	116	21	161	30	
13	2	133	77	197	U	67	
13	3	158	159	57	30	64	
13	4	175	268	94	130	29	
13	5	174	224	145	31	9	
13	6	246	110	101	38	51	
13	7	41	51	51	58	21	
13	8	42	158	53	96		
13	9	120	147	92	U		
13	10	99	86	103			
13	11	66	85	49			
13	12	54	60				
14	0	47	166	25	U	54	
14	1	15	100	54	81	39	
14	2	148	136	149	36	35	
14	3	66	211	U	65	49	
14	4	58	81	134	31	51	
14	5	37	133	134	23	63	
14	6	106	52	80	86	45	
14	7	87	114	35	130		
14	8	201	51	56	81		
14	9	103	42	63	103		
14	10	27	117	37			
14	11	58	41				
14	12	17					
15	0	A	166	97	71	50	
15	1	U	226	98	16	86	
15	2	189	217	211	112	31	
15	3	19	162	149	76	61	
15	4	74	38	188	78	52	
15	5	35	194	165	86	21	
15	6	277	148	36	86		
15	7	89	140	44	46		
15	8	294	29	53	81		
15	9	22	82	63			
15	10	27	47	28			
15	11	30	54				
16	0	498	162	66	46	27	
16	1	391	228	43	70	37	
16	2	206	53	36	U	42	
16	3	164	270	144	75	12	
16	4	55	16	122	15	25	
16	5	94	129	125	U		
16	6	21	28	80	99		
16	7	U	123	102	89		
16	8	U	61	46	78		
16	9	58	57	74			
16	10	81	52				
16	11	19					
17	0	A	41	104	130	82	
17	1	158	161	115	41	45	
17	2	247	124	66	161	29	
17	3	36	128	111	29		
17	4	141	100	162	17		
17	5	59	98	61	27		
17	6	17	76	152	13		
17	7	126	47	U	51		
17	8	41	52	71			
17	9	84	U	45			
17	10	10	16				
18	0	433	141	96	26		
18	1	130	85	99	57		
18	2	192	235	43	81		
18	3	197	229	95	34		
18	4	U	113	140	17		
18	5	12	82	65	93		
18	6	35	129	87	61		
18	7	43	139	55			
18	8	115	92	25			
18	9	28	29				
19	0	A	41	61	U		
19	1	17	90	63	32		
19	2	101	63	67	66		
19	3	39	67	19	52		
19	4	16	63	83	22		
19	5	36	55	39	22		
19	6	83	70	70			
19	7	90	20	57			
19	8	U	45				
19	9	65					
20	0	75	19	90	31		
20	1	54	36	71	51		
20	2	45	81	37	69		
20	3	43	49	46	21		
20	4	57	27	37			
20	5	36	44	48			
20	6	64	18	106			
20	7	32	18				
20	8	36					
21	0	A	55	33			
21	1	32	21	48			
21	2	U	73	37			
21	3	48	94	U			
21	4	122	52	53			
21	5	U	49	61			
21	6	48	35				
21	7	U	18				
22	0	U	46	58			
22	1	35	81	93			
22	2	U	96	51			
22	3	41	31	28			
22	4	65	U				
22	5	26	U				
22	6	43					
23	0	A	108				
23	1	U	70				
23	2	65	69				
23	3	18	17				
23	4	58	65				
24	0	14					
24	1	123					
24	2	21					
24	3	142					

The authors take great pleasure in acknowledging the support of this work by the National Cancer Institute of the National Institutes of Health, United States Public Health Service through their research grant number CA-04315. Los Alamos Scientific Laboratory of the University of California made us a grant of IBM 7090 time for which we express our thanks. The Graduate School Computing Center of the University of Colorado made us a large grant of computing time on their IBM 709 without which the final refinement could not have been carried out. It is a pleasure to acknowledge the helpful discussions of the structure with Dr. Leonard S. Lerman, Dr. Kenneth N. Trueblood, and Dr. Theodore T. Puck. We would like to thank Dr. R. Duschinsky of Hoffmann-LaRoche, Inc. for supplying us with the sample of FUDR.

Received for publication, July 2, 1963.

REFERENCES

1. CHAUDHURI, N. K., MONTAG, B. J., and HEIDELBERGER, C., *Cancer Research*, 1958, **18**, 318.
2. PRUSOFF, W. H., *Cancer Research*, 1960, **20**, 92.
3. BROCKMAN, R. W., BENNETT, L. L., JR., SIMPSON, M. S., WILSON, A. R., THOMSON, J. R., and SKIPPER, H. E., *Cancer Research*, 1959, **19**, 856.
4. WELCH, A. D., *Cancer Research*, 1961, **21**, 1475.
5. LANGRIDGE, R., MARVIN, D. A., SEEDS, W. E., WILSON, H. R., HOOPER, C. W., WILKINS, M. H. F., and HAMILTON, L. D., *J. Mol. Biol.*, 1960, **2**, 38.
6. SPENCER, C., FULLER, W., WILKINS, M. H. F., and BROWN, G. L., *Nature*, 1962, **194**, 1014.
7. EIDENOFF, M. L., and RICH, M. A., *Cancer Research*, 1959, **19**, 521.
8. EVANS, H. T., *Rev. Scient. Instr.*, 1953, **24**, 156.
9. FURNAS, T., Single Crystal Orienter Manual, Milwaukee, X-Ray Department, General Electric Company, 1957.
10. FURBERG, S., *Acta Cryst.*, 1950, **3**, 325.
11. TRUEBLOOD, K. N., HORN, P., and LUZZATI, V., *Acta Cryst.*, 1961, **14**, 965.
12. MACINTYRE, W. M., Paper No. B-12, American Crystallographic Association Meeting, Boulder, 1961.
13. VAN DER HELM, D., JOHNSON, C. K., and PATTERSON, A. L., Paper No. H-9, American Crystallographic Association Meeting, Boulder, 1961.
14. VAN DEN HENDE, J., Esso Research Laboratories, Linden, New Jersey, private communication.
15. ALVER, E., and FURBERG, S., *Acta Chem. Scand.*, 1959, **13**, 910.
16. HUBER, M., *Acta Cryst.*, 1957, **10**, 129.
17. PAULING, L., Nature of the Chemical Bond, Ithaca, Cornell University Press, 3rd edition, 1960, 260.
18. PARRY, G. S., *Acta Cryst.*, 1954, **7**, 313.
19. GERDIL, R., *Acta Cryst.*, 1961, **14**, 333.
20. SPENCER, M., *Acta Cryst.*, 1959, **12**, 59.
21. KRAUT, J., and JENSEN, L. H., *Acta Cryst.*, 1963, **16**, 79.
22. DONOHUE, J., and TRUEBLOOD, K. N., *J. Mol. Biol.*, 1960, **2**, 363.