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Crystal Structure of a Naturally Occurring Dinucleoside Phosphate: Uridylyl 3′,5′-adenosine phosphate Model for RNA Chain Folding

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The crystal structure of uridylyl 3′,5′-adenosine phosphate (UpA) has been determined and refined to an R value of 0·062. There are two UpA molecules per asymmetric unit in a monoclinic unit cell with dimensions $a = 16{\cdot}91$, $b = 12{\cdot}37$, $c = 11{\cdot}25$ Å and $\beta = 96{\cdot}0°$. The conformation of the phosphodiester linkage is different for the two molecules; one UpA suggests a model for a sharp turn in a single-stranded nucleic acid structure and the other for a helical structure. All the nucleoside units have similar conformations. There is no complementary base pairing between the adenines and uracils but rather layers of base-paired adenines and base-paired uracils separated by 3·4 Å. The predominant form of base stacking involves the ribosyl oxygen atom and the bases.

1. Introduction

Nucleic acids have a fundamental role in life as we know it. They are the storehouse of genetic information and are involved in virtually all aspects of its expression. To have a full understanding of how nucleic acids function their three-dimensional structure must be known. This is seen most vividly in the double helical structure of DNA (Watson & Crick, 1953) which immediately predicts a model of gene replication. However, nucleic acids can also fold into other three-dimensional structures; in these forms they participate in various stages of gene expression, e.g. as messenger RNA, ribosomal RNA and transfer RNA.

In order to understand the structure of nucleic acids, it is necessary to know the conformations of the sugar residues, of the sugar/base linkages and of the sugar/phosphate linkages. Until now our structural information has been based mostly on crystal structures of monomeric subunits such as nucleosides and mononucleotides, the interpretation of diffraction patterns of oriented nucleic acid fibres and molecular model building. Extensive reviews have been written on the subject of the

sugar and sugar base geometries (Sundaralingam, 1965; Haschemeyer & Rich, 1967), but there are few data about the sugar/phosphate conformations in polynucleotide strands. In the nucleic acids the nucleosides are connected through the phosphodiester bonds which are composed of the O3′ atom of the ribose, the phosphorus atom of the phosphate and the O5′ atom of the next ribose. The smallest molecule which contains this bonding sequence is a 3′,5′-dinucleoside phosphate. Uridylyl 3′,5′-adenosine phosphate is the first such molecule whose structure has been determined by X-ray crystallographic techniques (Seeman, Sussman, Berman & Kim, 1971). We present here the structure and its features in detail.

2. Experimental

(a) *Crystal data*

Powdered uridylyl 3′,5′-adenosine phosphate (UpA) was purchased from Gallard Schlesinger Chemical Mfg. Corp., New York. The crystals grew, upon slow evaporation, as long beautiful needles from acidic (initially 10^{-3} M-HCl) aqueous solution. There are two crystallographically independent UpA molecules and one water molecule in the asymmetric portion of the unit cell. The crystal data are given below.

Empirical formula: $2(C_{19}H_{24}N_7O_{12}P)$, H_2O: 79 non-hydrogen atoms per asymmetric unit.

Unit-cell dimensions: $a = 16{\cdot}91$ Å (2)
$b = 12{\cdot}37$ Å (1)
$c = 11{\cdot}25$ Å (1)
$\beta = 96{\cdot}0°$ (1)

Calculated density: 1·65 g/cm³, measured density: 1·63 g/cm³. Crystal system: monoclinic. Space group: $P2_1$ from systematic absences $0k0$, k = odd. Linear absorption coefficient: 18·1 cm^{-1}. 4 UpA's plus 2 H_2O's per unit cell. Mol. wt = 1164 daltons/asymmetric unit. $F(000) = 1212$.

2680 unique reflections were measured on a single crystal with the $\theta/2\theta$ scan technique on a Hilger–Watts automatic four-circle diffractometer using CuKα radiation to a resolution of 1 Å. Of these reflections, 397 were considered weak or unobserved using systematic absences and counting statistics as the criteria. The data were reduced using a program written by Shiono (1969). No absorption or extinction corrections were made because of the low value of the linear absorption coefficient.

(b) *Structure determination and refinement*

The positions of the phosphorus atoms were obtained by a comparison of a Patterson synthesis of all data with one calculated using only those 1612 reflections of resolution greater than 1·5 Å. The atomic scattering factor of the phosphorus atom falls off much less rapidly as the resolution increases than those of the other atoms in this structure. Thus the high resolution Patterson synthesis emphasizes the phosphorus/phosphorus vectors over the other large peaks in the Patterson synthesis which result from the overlap of lighter atom vectors. Patterson superpositions based on the phosphorus atom positions showed the orientations of the two independent phosphate groups. Successive cycles of Fourier refinement using normalized structure factors (Hauptman & Karle, 1953) as coefficients revealed the locations of 78 atoms. The water oxygen was located in a difference Fourier synthesis. The disagreement index† at this stage was 0·29.

Direct method and orientation and translation search for molecular fragments were only partially successful. We attribute these failures to the existence of pseudosymmetry (Sussman, 1972) (see section on packing).

Refinement proceeded using the full matrix least-squares program of Busing, Martin & Levy (1962). The quantity minimized was $w(F_o - F_c)^2$ where $w = 2/(50 + F_o^2)$. The unobserved reflections were given zero weights. Because there were so many parameters,

† The disagreement index, or R factor, is defined as: $R = \dfrac{\Sigma||F_o| - |F_c||}{\Sigma|F_o|}$.

the refinement had to be done in four overlapping blocks. The scattering factors used were from the *International Tables* (1962). Refinement using isotropic temperature factors reduced the R factor to 0·12. Anisotropic refinement of the temperature factors and the positional co-ordinates further reduced it to 0·097. At this stage a difference Fourier was calculated. Of the 34 hydrogen atoms whose positions can be predicted by known stereochemistry, 23 appeared on the difference synthesis among the high peaks. The rest were placed in calculated positions and another difference synthesis that included the 34 hydrogens was calculated in which eight more hydrogen atoms were located. A final three cycles of anisotropic refinement of the heavier atoms holding the parameters of the 42 hydrogen atoms fixed reduced the R factor to 0·081. The R factor for observed reflections only is 0·062.

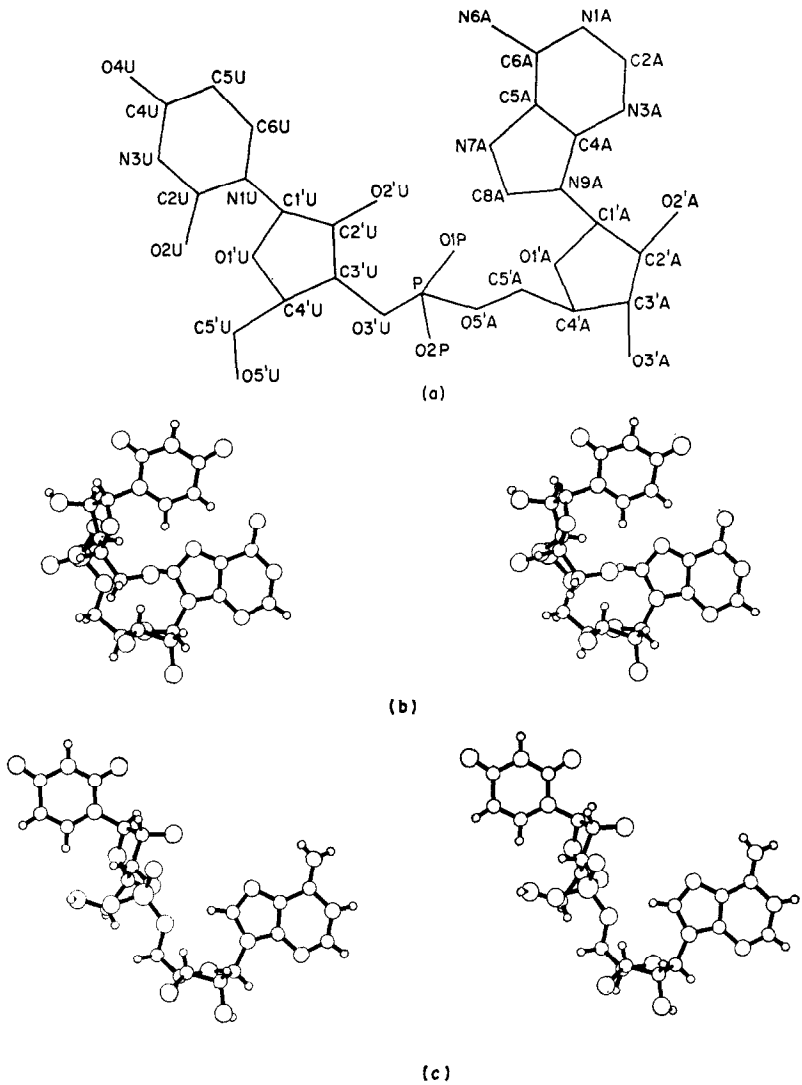


FIG. 1. (a) The atomic labeling system used for UpA. The labels are given by three symbols. The first is the atom type, the second is the atom number and the third designates whether the atom is in the adenosine (A) or uridine (U) portion of the molecule. The prime is on the atoms belonging to the sugar residues. Stereo drawings of UpA 1 (b) and UpA 2 (c) in the crystal structure with similar views of the ribose of the adenosine portions.

TABLE 1

Atomic parameters and their estimated standard deviations in the single-crystal structure of UpA

	UpA1																	
	X		Y		Z		b^{11}		b^{22}		b^{33}		b^{12}		b^{13}		b^{23}	
C6U	−0.0772	(5)	−0.2810	(7)	−0.7124	(7)	51	(4)	63	(7)	70	(7)	20	(4)	08	(4)	−05	(6)
C5U	−0.0961	(6)	−0.3803	(7)	−0.7503	(8)	58	(4)	63	(8)	98	(9)	34	(4)	06	(5)	−16	(7)
C4U	−0.1591	(5)	−0.3961	(8)	−0.8381	(7)	35	(3)	70	(8)	71	(7)	07	(4)	−01	(4)	20	(7)
O4U	−0.1806	(4)	−0.4826	(5)	−0.8868	(5)	60	(3)	63	(5)	89	(5)	03	(3)	−03	(3)	12	(4)
N3U	−0.1972	(3)	−0.3004	(5)	−0.8785	(5)	34	(2)	44	(5)	42	(4)	−05	(3)	03	(3)	15	(4)
C2U	−0.1791	(4)	−0.1971	(7)	−0.8424	(5)	22	(2)	68	(7)	33	(5)	12	(4)	14	(3)	32	(5)
O2U	−0.2138	(3)	−0.1207	(4)	−0.8825	(4)	24	(2)	52	(4)	75	(5)	12	(2)	−06	(2)	−13	(4)
N1U	−0.1142	(3)	−0.1889	(5)	−0.7538	(5)	28	(2)	55	(5)	52	(5)	11	(3)	−05	(3)	04	(4)
C1'U	−0.0848	(4)	−0.0773	(6)	−0.7222	(6)	35	(3)	65	(7)	50	(6)	−07	(4)	09	(3)	06	(5)
C2'U	−0.1374	(4)	−0.0206	(5)	−0.6374	(6)	22	(2)	39	(5)	51	(6)	−04	(3)	−05	(3)	02	(4)
O2'U	−0.1313	(3)	0.0909	(4)	−0.6538	(5)	31	(2)	51	(4)	79	(5)	−05	(2)	−00	(2)	02	(4)
C3'U	−0.0932	(4)	−0.0551	(6)	−0.5149	(5)	25	(2)	73	(6)	30	(5)	−04	(3)	−10	(3)	09	(5)
C4'U	−0.0046	(5)	−0.0565	(8)	−0.5407	(7)	36	(3)	84	(7)	85	(8)	−12	(4)	15	(4)	07	(6)
C5'U	0.0488	(4)	−0.1277	(9)	−0.4639	(7)	20	(3)	134	(10)	56	(7)	06	(4)	−01	(3)	06	(7)
O5'U	0.0191	(4)	−0.2343	(6)	−0.4637	(5)	55	(3)	104	(6)	81	(6)	37	(4)	−27	(3)	−10	(5)
O1'U	−0.0092	(2)	−0.0848	(4)	−0.6674	(4)	24	(2)	75	(4)	42	(4)	−03	(2)	−00	(2)	06	(3)
O3'U	−0.1035	(2)	0.0226	(4)	−0.4235	(4)	30	(3)	59	(4)	50	(4)	−10	(2)	04	(2)	−19	(3)
P	−0.1762	(1)	0.0048	(1)	−0.3426	(1)	23	(0)	20	(1)	39	(1)	−02	(1)	−01	(1)	−07	(1)
O1P	−0.2446	(2)	−0.0489	(4)	−0.4141	(4)	21	(2)	33	(3)	62	(4)	05	(2)	−06	(2)	−02	(3)
O2P	−0.1863	(3)	0.1088	(4)	−0.2833	(4)	33	(2)	40	(3)	44	(4)	03	(2)	−07	(2)	−09	(3)
O5'A	−0.1436	(3)	−0.0817	(3)	−0.2447	(4)	49	(2)	15	(3)	45	(4)	07	(4)	−18	(2)	−14	(2)
C5'A	−0.0948	(5)	−0.0505	(6)	−0.1412	(7)	58	(4)	23	(5)	70	(7)	04	(4)	−16	(4)	−22	(5)
C4'A	−0.0456	(4)	−0.1444	(5)	−0.0897	(6)	33	(3)	27	(5)	67	(6)	01	(3)	−02	(3)	−07	(5)
C3'A	0.0048	(4)	−0.2010	(7)	−0.1741	(6)	18	(2)	64	(6)	56	(6)	−13	(3)	−08	(3)	−03	(5)
O3'A	0.0789	(3)	−0.1559	(6)	−0.1849	(5)	39	(3)	104	(6)	116	(6)	−16	(3)	−03	(3)	42	(5)
C2'A	0.0101	(4)	−0.3159	(6)	−0.1311	(5)	32	(3)	50	(6)	31	(5)	04	(3)	−07	(3)	02	(4)
O2'A	0.0633	(2)	−0.3299	(5)	−0.0240	(4)	24	(2)	76	(4)	65	(4)	07	(2)	−12	(2)	05	(4)
C1'A	−0.0718	(4)	−0.3294	(5)	−0.0873	(5)	28	(2)	17	(4)	31	(5)	03	(3)	−05	(3)	02	(4)
O1'A	−0.1003	(3)	−0.2302	(4)	−0.0592	(4)	30	(2)	37	(4)	44	(4)	−04	(2)	−05	(2)	−16	(3)
N9A	−0.1290	(3)	−0.3798	(4)	−0.1828	(5)	24	(2)	33	(4)	58	(5)	−02	(2)	−07	(3)	−01	(4)
C4A	−0.1403	(4)	−0.4896	(5)	−0.2027	(6)	23	(2)	27	(5)	53	(6)	02	(3)	−10	(3)	−12	(5)
N3A	−0.1087	(3)	−0.5703	(4)	−0.1318	(5)	37	(2)	16	(4)	57	(5)	−01	(3)	−04	(3)	−12	(4)
C2A	−0.1332	(5)	−0.6651	(6)	−0.1778	(6)	49	(4)	32	(6)	54	(7)	09	(3)	15	(4)	08	(5)
N1A	−0.1796	(4)	−0.6807	(4)	−0.2821	(4)	43	(3)	26	(4)	26	(4)	03	(3)	00	(3)	02	(3)
C6A	−0.2118	(4)	−0.5984	(5)	−0.3535	(5)	24	(2)	28	(5)	33	(4)	04	(3)	−04	(3)	−00	(4)
N6A	−0.2530	(3)	−0.6179	(5)	−0.4543	(5)	23	(2)	52	(5)	46	(5)	−00	(2)	01	(3)	−06	(4)
C5A	−0.1920	(4)	−0.4927	(5)	−0.3075	(5)	30	(3)	21	(5)	39	(6)	−05	(3)	−07	(3)	−19	(4)
N7A	−0.2107	(3)	−0.3942	(4)	−0.3533	(5)	35	(2)	13	(4)	48	(4)	−01	(2)	−02	(3)	−15	(4)
C8A	−0.1716	(4)	−0.3276	(5)	−0.2768	(6)	30	(3)	19	(5)	67	(7)	01	(3)	−07	(4)	09	(5)
HC6U	−0.039		−0.280		−0.644		3.3											
HC5U*	−0.069		−0.440		−0.716		4.5											
HN3U*	−0.241		−0.309		−0.937		2.7											
HC1'U	−0.090		−0.037		−0.799		3.6											
HC2'U	−0.192		−0.040		−0.656		2.5											
HC3'U	−0.109		−0.123		−0.490		2.7											
HC4'U	0.015		0.016		−0.532		3.3											
HC5'U*	0.053		−0.095		−0.385		4.2											
HC5'U	0.100		−0.120		−0.493		4.2											
HC5'A	−0.126		−0.023		−0.083		3.2											
HC5'A	−0.059		0.004		−0.163		3.2											
HC4'A	−0.013		−0.120		−0.022		3.4											
HC3'A	−0.024		−0.207		−0.250		3.0											
HC2'A*	0.031		−0.359		−0.191		2.5											
HC1'A	−0.068		−0.373		−0.017		2.0											
HC2'A	−0.120		−0.730		−0.136		4.0											
HC8A	−0.171		−0.251		−0.285		2.7											
HO2'U	−0.180		0.125		−0.650		3.0											
HO3'A	0.080		−0.120		−0.070		3.0											

Positional parameters are expressed as fractions of unit cell edges. Anisotropic temperature factors are expressed as:

$$\exp(-b^{11}h^2 - b^{22}k^2 - b^{33}l^2 - 2b^{12}hk - 2b^{13}hl - 2b^{23}kl).$$

In this Table the b^{ij}'s are multiplied by 10^4. The isotropic temperature factors are of the form

TABLE 1—*continued*

UpA2

	X		Y		Z		b^{11}		b^{22}		b^{33}		b^{12}		b^{13}		b^{23}	
C6U	-0.5865	(4)	-0.6426	(6)	-0.7041	(3)	26	(3)	52	(7)	58	(6)	01	(3)	-11	(3)	-08	(5)
C5U	-0.6193	(5)	-0.7368	(6)	-0.7384	(7)	52	(4)	41	(6)	62	(7)	04	(4)	11	(4)	-06	(5)
C4U	-0.6665	(4)	-0.7379	(6)	-0.8535	(7)	24	(2)	25	(6)	86	(8)	-07	(3)	06	(3)	-26	(5)
O4U	-0.6962	(3)	-0.8218	(4)	-0.9016	(5)	48	(2)	41	(4)	72	(5)	-03	(3)	-03	(3)	-02	(4)
N3U	-0.6799	(5)	-0.6399	(5)	-0.9100	(5)	35	(2)	24	(5)	63	(5)	07	(3)	-13	(3)	-06	(4)
C2U	-0.6468	(4)	-0.5443	(5)	-0.8692	(5)	30	(3)	18	(5)	41	(6)	-02	(3)	02	(3)	-06	(5)
O2U	-0.6604	(3)	-0.4609	(5)	-0.9240	(5)	46	(2)	50	(5)	71	(5)	-01	(2)	-17	(3)	-16	(4)
N1U	-0.6006	(3)	-0.5464	(4)	-0.7613	(4)	37	(2)	19	(4)	21	(4)	-02	(2)	-03	(2)	02	(3)
C1'U	-0.5603	(4)	-0.4419	(6)	-0.7210	(6)	26	(2)	39	(5)	45	(6)	-07	(3)	-03	(3)	-08	(4)
C2'U	-0.6124	(4)	-0.3696	(5)	-0.6520	(6)	21	(2)	19	(5)	45	(6)	07	(3)	-05	(3)	-08	(4)
O2'U	-0.5916	(3)	-0.2600	(4)	-0.6708	(4)	30	(2)	31	(4)	69	(4)	-03	(2)	-00	(2)	-07	(3)
C3'U	-0.5874	(3)	-0.4036	(5)	-0.5261	(5)	22	(2)	31	(5)	26	(5)	-06	(3)	-09	(3)	09	(4)
C4'U	-0.4987	(4)	-0.4266	(6)	-0.5293	(5)	20	(2)	49	(5)	30	(6)	-14	(3)	-08	(3)	-11	(4)
C5'U	-0.4615	(4)	-0.5010	(6)	-0.4324	(6)	30	(3)	42	(6)	55	(6)	01	(3)	-05	(3)	14	(5)
O5'U	-0.5039	(3)	-0.6041	(4)	-0.4408	(4)	36	(2)	48	(4)	70	(4)	-02	(2)	-09	(2)	11	(4)
O1'U	-0.4911	(2)	-0.4693	(4)	-0.6460	(4)	18	(2)	50	(4)	49	(4)	12	(2)	01	(2)	-16	(3)
O3'U	-0.5963	(3)	-0.3292	(4)	-0.4358	(4)	34	(2)	29	(3)	53	(4)	02	(2)	11	(2)	-13	(3)
P	-0.6641	(1)	-0.3492	(1)	-0.3471	(1)	24	(1)	23	(1)	32	(1)	01	(1)	05	(1)	02	(1)
O1P	-0.7398	(2)	-0.3060	(3)	-0.4035	(4)	19	(1)	20	(3)	89	(4)	03	(2)	-05	(2)	08	(3)
O2P	-0.6612	(3)	-0.4623	(4)	-0.3050	(3)	42	(2)	31	(3)	28	(3)	01	(2)	06	(2)	05	(3)
O5'A	-0.6350	(3)	-0.2688	(3)	-0.2407	(3)	37	(2)	27	(3)	22	(3)	07	(2)	-06	(2)	-06	(3)
C5'A	-0.5806	(4)	-0.2988	(6)	-0.1393	(6)	23	(2)	45	(5)	45	(5)	01	(3)	03	(3)	09	(5)
C4'A	-0.5789	(4)	-0.2126	(5)	-0.0446	(6)	25	(2)	33	(5)	58	(6)	-06	(3)	-12	(3)	-21	(5)
C3'A	-0.6591	(4)	-0.1785	(5)	-0.0031	(5)	33	(3)	34	(5)	28	(5)	13	(3)	-03	(3)	01	(4)
O3'A	-0.6773	(3)	-0.2363	(4)	0.0979	(4)	41	(2)	59	(4)	45	(4)	-03	(2)	07	(2)	09	(3)
C2'A	-0.6487	(5)	-0.0581	(6)	0.0279	(6)	49	(3)	35	(5)	52	(6)	01	(3)	18	(4)	14	(5)
O2'A	-0.6157	(4)	-0.0449	(4)	0.1436	(4)	71	(3)	54	(4)	40	(4)	-19	(3)	-09	(3)	-08	(3)
C1'A	-0.5897	(4)	-0.0190	(5)	-0.0603	(6)	41	(3)	21	(5)	47	(6)	-02	(3)	-07	(3)	-01	(4)
O1'A	-0.5465	(2)	-0.1143	(4)	-0.0926	(4)	22	(2)	42	(4)	76	(4)	-03	(2)	-08	(2)	17	(3)
N9A	-0.6298	(3)	0.0283	(4)	-0.1709	(4)	28	(2)	20	(4)	37	(4)	-03	(2)	-10	(2)	02	(3)
C4A	-0.6278	(4)	0.1352	(5)	-0.2040	(6)	33	(3)	22	(5)	46	(6)	13	(3)	10	(3)	18	(4)
N3A	-0.5923	(3)	0.2187	(4)	-0.1369	(5)	28	(2)	33	(4)	41	(4)	-14	(2)	-07	(2)	-10	(4)
C2A	-0.6061	(4)	0.3103	(5)	-0.1881	(6)	37	(3)	20	(5)	55	(6)	-16	(3)	05	(3)	-01	(5)
N1A	-0.6450	(3)	0.3295	(4)	-0.2960	(4)	28	(2)	24	(4)	37	(4)	-01	(2)	-06	(2)	02	(3)
C6A	-0.6801	(4)	0.2457	(6)	-0.3664	(6)	26	(3)	38	(5)	49	(6)	-01	(3)	09	(3)	-01	(5)
N6A	-0.7190	(3)	0.2630	(4)	-0.4706	(4)	42	(2)	27	(4)	37	(5)	-04	(2)	-13	(3)	09	(3)
C5A	-0.6687	(4)	0.1422	(5)	-0.3135	(5)	31	(3)	32	(5)	24	(5)	-02	(3)	03	(3)	-03	(4)
N7A	-0.6972	(3)	0.0420	(4)	-0.3537	(4)	28	(2)	32	(4)	43	(5)	-02	(2)	01	(3)	-02	(4)
C8A	-0.6719	(3)	-0.0241	(5)	-0.2662	(5)	22	(2)	45	(5)	15	(5)	-03	(3)	-11	(3)	03	(4)
H_2O	-0.7887	(3)	-0.3693	(5)	-0.0946	(5)	34	(2)	72	(5)	83	(5)	01	(2)	-04	(2)	-03	(4)
HC6U*	-0.551		-0.647		-0.635		2.4											
HC5U*	-0.610		-0.798		-0.692		3.7											
HN3U*	-0.712		-0.639		-0.984		2.9											
HC1'U	-0.549		-0.402		-0.791		3.2											
HC2'U	-0.667		-0.380		-0.680		1.9											
HC3'U	-0.618		-0.466		-0.511		1.9											
HC4'U	-0.469		-0.362		-0.518		1.7											
HC5'U*	-0.465		-0.469		-0.357		2.8											
HC5'U	-0.406		-0.511		-0.444		2.8											
HC5'A	-0.530		-0.311		-0.164		1.4											
HC5'A*	-0.600		-0.365		-0.106		1.4											
HC4'A*	-0.544		-0.240		0.023		2.1											
HC3'A	-0.698		-0.190		-0.067		2.8											
HC2'A	-0.704		-0.030		0.022		3.6											
HC1'A*	-0.555		0.032		-0.023		3.4											
HC2A	-0.586		0.372		-0.149		2.0											
HC8A'	-0.686		-0.099		-0.266		1.3											
HO2'A	-0.560		-0.020		0.120		3.0											
HO5'U	-0.540		-0.620		-0.400		3.0											
HN1A	-0.640		0.400		-0.320		3.0											
HN6A	-0.700		0.320		-0.520		3.0											
HN6A	-0.740		0.220		-0.520		3.0											
HH2O'	-0.780		-0.400		-0.200		3.0											

$\exp(-B\sin^2\theta/\lambda^2)$ with B values in Å^2. The temperature factors assigned to the hydrogens attached to the C and N atoms were the same as those of the atoms to which they are attached. The hydroxyl hydrogen temperature factors were arbitrarily assigned a value of 3 Å^2. An asterisk indicates that the atom did not appear in the difference map. The estimated standard deviations are given in parentheses and correspond to the last decimal place.

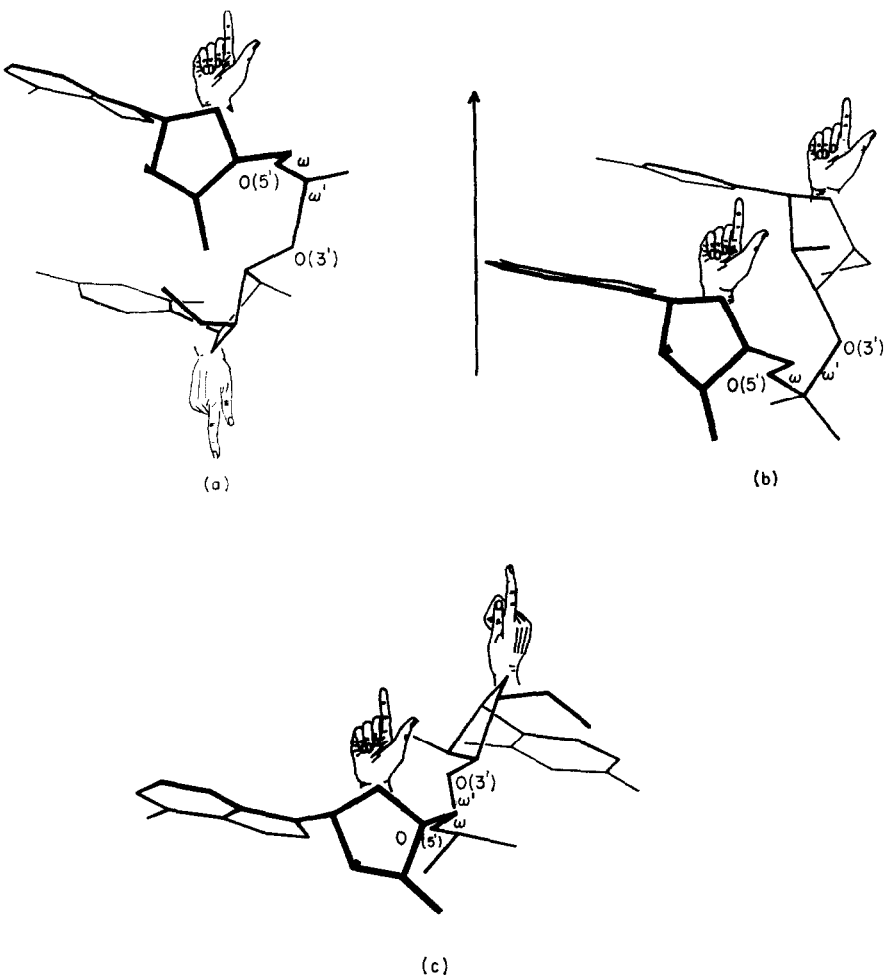


FIG. 2. Views of (a) UpA 1; (b) RNA with eleven base pairs per turn (RNA-11); and (c) UpA 2 oriented so that the bases of the adenosine portions are similarly aligned. In order to clarify the conformational differences among the molecules we have attached a hand to the ribose ring oxygen. Note that both fingers in (b) are pointing in the direction of the helix axis which is shown by an arrow. In UpA 1 (a) they point in opposite directions and in UpA 2 (c) both fingers are pointing in the same direction. Note carefully the orientation of the hands. A right-handed rotation of 109° of the uridine portion of (c) will align the molecule so that it looks like RNA-11.

The observed and calculated structure factors are listed in the thesis of Sussman (1972). The positional and thermal parameters of all the atoms are given in Table 1. Fig. 1(a) shows chemical structure and labeling system.

3. Results and Discussion

(a) *UpA 1 and UpA 2 are different*

We knew at the outset that there were two UpA molecules in the asymmetric unit and we did not expect large differences between the two; however, we found that the two molecules, UpA 1 and UpA 2, have strikingly different conformations as shown in Figure 1(b) and (c). The major differences are found in the conformation of the phosphodiester linkage. The conformation of UpA 1 (Fig. 1(b)) appears deceptively similar to models of helical nucleic acids: the two bases are approximately parallel and in the *anti*-conformation (Donohue & Trueblood, 1960) with respect to the ribose sugars. However, the conformation about the phosphodiester linkage O3'U—P—O5'A is very different from any proposed repetitive helical nucleic acid structure, as shown in Figure 2(a) and (b), which make a comparison of UpA 1 and an RNA helical structure. In a helical nucleic acid structure all the sugar residues must be aligned similarly with respect to the helix axis as in Figure 2(b). In UpA 1 the sugar residues are oppositely aligned. This conformation allows a sharp bend to occur in a single strand of RNA or DNA. It is therefore likely that the conformation of UpA 1 or its minor variations will be found in the loop structure of RNA or DNA such as the anticodon loop of tRNA.

The extended appearance of UpA 2 (Fig. 1(c)) does not resemble any of the proposed helical nucleic acid structures. But it can be approximately converted into a

TABLE 2

Sugar phosphate torsion angles in the crystal structure of UpA compared with other proposed nucleic acid structures

Nucleic acid structure	ω	ϕ	ψ	ψ'	ρ	ρ'	ϕ'	ω'
				(degrees)				
DNA (Watson & Crick, 1953)	182	179	155		100		186	295
DNA *B* (Langridge *et al.*, 1960)	281	212	58		130		147	282
DNA *C* (Marvin *et al.*, 1961)	315	143	48		168		211	212
DNA *A* (Fuller *et al.*, 1965)	283	167	67		76		221	279
RNA-11 (Arnott *et al.*, 1969)	282	165	74		95		216	273
RNA-10 (Arnott *et al.*, 1969)	257	188	88		80		203	285
Poly(A) (Rich *et al.*, 1961)	285	168	69		83		216	293
2',5'-ApU (Shefter *et al.*, 1969)	313	170	57	45	84	148	244	232
UpA 1	82	203	55	53	85	86	206	81
UpA 2	271	192	54	58	93	77	224	164

See Fig. 3 for the definition and symbols of these torsion angles.

TABLE 3

Torsional angles in sugars and of glycosidic bonds in the crystal structure of UpA

Angle	UpA 1		UpA 2		Reported range of values for 3′-endo sugars (Sundaralingam, 1969)
	Adenosine	Uridine	Adenosine	Uridine	
			(degrees)		
χ, χ'	37·0	12·1	44·1	19·5	3·8–50·0 (A) to 10·0–42·0 (U)
τ_0, τ_0'	4·5	10·7	6·8	4·9	−34·0 to 29·0
τ_1, τ_1'	−24·3	−29·0	−23·6	−26·2	−46·0 to −14·0
τ_2, τ_2'	34·1	35·0	31·1	35·3	21·0 to 46·0
τ_3, τ_3'	−32·4	−30·0	−27·7	−34·2	−45·0 to −22·0
τ_4, τ_4'	17·3	12·8	13·0	18·2	−14·0 to 26·0

See Fig. 3 for the definition and symbols of torsional angles.

structure resembling a right-handed RNA helix with eleven base pairs per turn by rotation around the P→O3′U bond of about 109° as can be seen by comparing Figure 2(b) and (c) (see Figure 3 for definition and rotational sense). As in UpA 1 the bases are in nearly parallel planes and are in the *anti*-conformation with respect to the sugars.

UpA 1 can be approximately converted to UpA 2 by the following operations. (1) Rotate about P→O5′A directed bond by −171°. This rotation allows the fingers in Figure 2(a) to point in the same direction. (2) Rotate about P→O3′U directed bond by 83°. This rotation makes the orientation of the hands in Figure 2(a) similar to those in 2(c); however, to make a more precise match the other conformation angles have to be slightly adjusted (see Fig. 3 and Tables 2 and 3 for the definition and values of all backbone and ribose torsion angles).

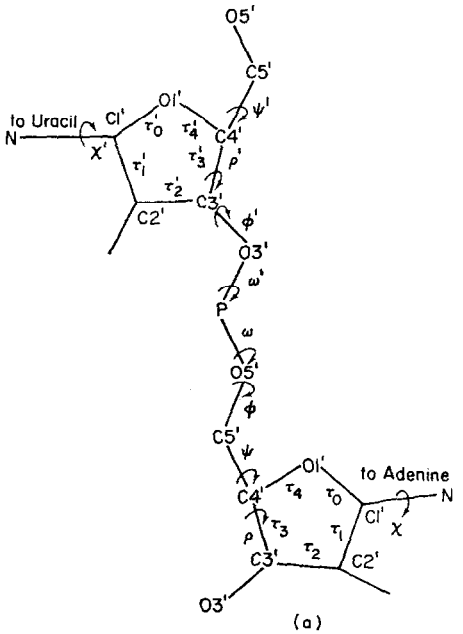
All four sugars display the 3′-endo 2′-exo conformation (Table 3) and exhibit the features associated with this conformer, i.e. C3′ is 0·55 Å out of the least-squares plane of C2′, C1′, O1′, C4′ on the same side as C5′ while C2′ is 0·55 Å out of the plane of C1′, O1′, C4′, C3′ on the opposite side from C5′ (see Table 4).

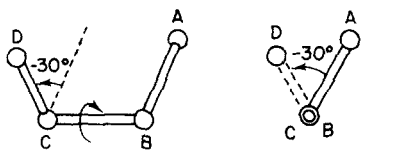
TABLE 4

Deviations, in Ångstrom units, of the atoms from the best four atom least-squares plane of the ribose rings in the crystal structure of UpA

Atom	Ribose of uridine		Ribose of adenosine	
	UpA 1	UpA 2	UpA 1	UpA 2
C1′	−0·05	−0·03	−0·02	−0·04
C2′	+0·03	+0·02	+0·01	+0·02
C4′	−0·03	−0·02	−0·01	−0·02
O1′	+0·06	+0·03	+0·02	+0·04
C3′	−0·55	−0·56	−0·53	−0·48
O3′	−0·04	−0·20	−0·22	+0·08
C5′	−0·82	−0·79	−0·89	−0·91

The best least-squares plane contains atoms C1′, C2′, C4′ and O1′. The atoms excluded from the least-squares plane are underlined. When C1′, C3′, C4′ and O1′ are used to define a plane, C2′ deviates +0·55 Å from the plane.





Angle	A	B	C	D	
X	O1'	C1'	N	C8	(A)
X'	O1'	C1'	N	C6	(U)
ρ	C5'	C4'	C3'	O3'	(A)
ρ'	C5'	C4'	C3'	O3'	(U)
ψ	O5'	C5'	C4'	C3'	(A)
ψ'	O5'	C5'	C4'	C3'	(U)
φ	P	O5'	C5'	C4'	
φ'	C4'	C3'	O3'	P	
ω	O3'	P	O5'	C5'	
ω'	C3'	O3'	P	O5'	
τ_0	C4'	O1'	C1'	C2'	
τ_1	O1'	C1'	C2'	C3'	
τ_2	C1'	C2'	C3'	C4'	
τ_3	C2'	C3'	C4'	O1'	
τ_4	C3'	C4'	O1'	C1'	

(c)

FIG. 3. Conformation angles. (a) The labeling system employed for the conformation angles of a dinucleoside phosphate are indicated. (b) They are each defined in terms of 4 consecutive atoms ABCD. The positive sense of the rotation is clockwise from A to D while looking down the BC bond. The value of the conformation angle indicated by ABCD is $+330°$ or $-30°$. (c) The definition of each angle is given in terms of the four consecutive atoms. The prime on the Greek letters indicates that the conformation angle is on the O3′ side of the phosphorus atom.

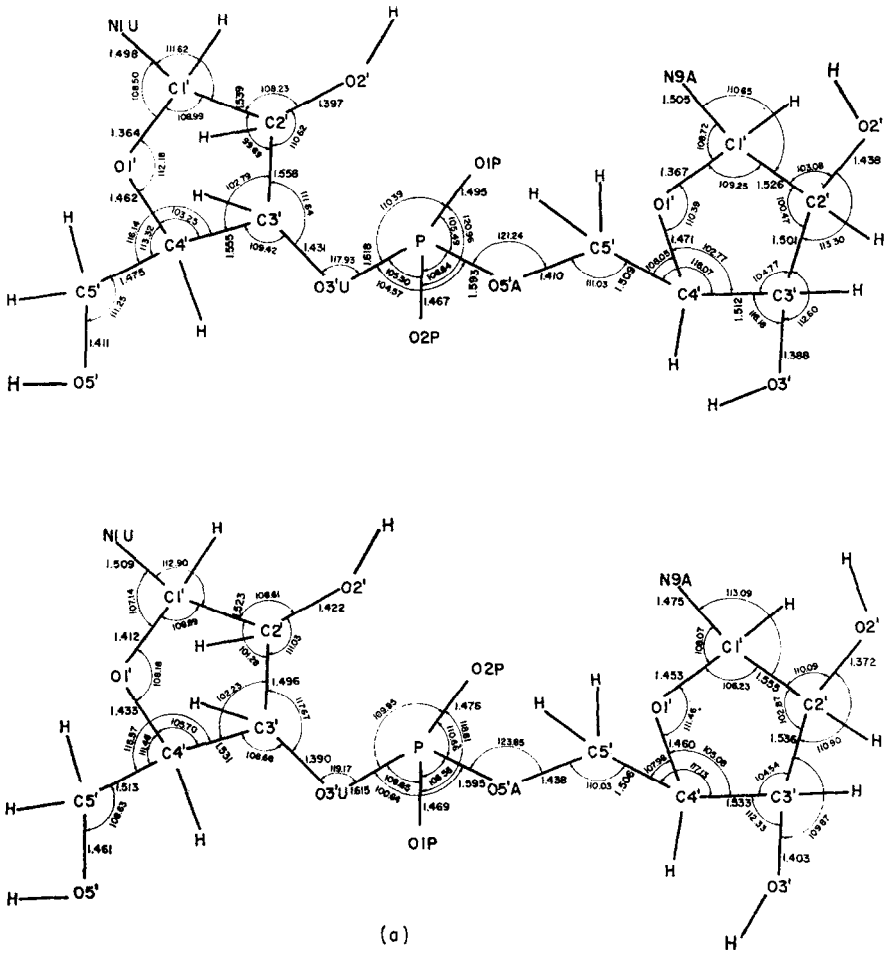
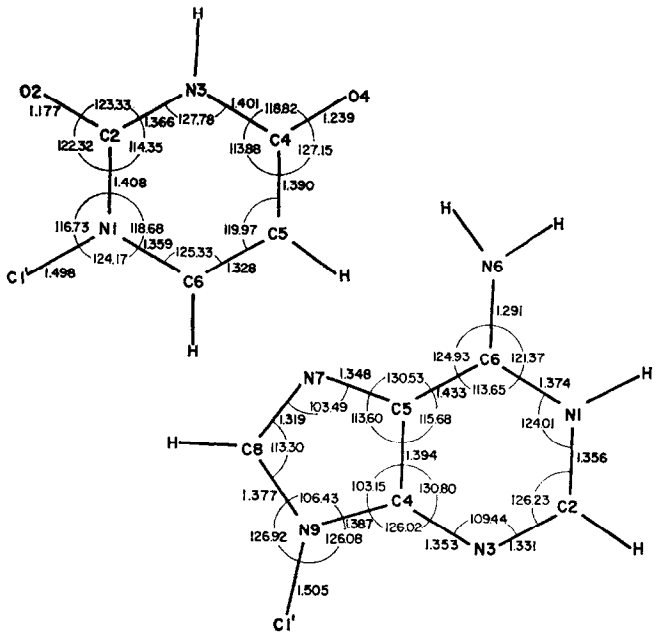
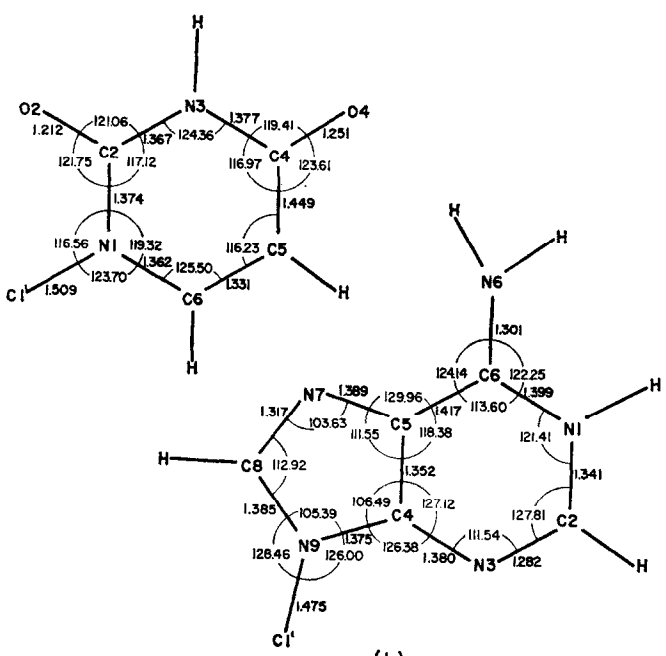


FIG. 4. Bond distances and angles in the single crystal structure of UpA. The average error in the phosphorus to oxygen bond lengths is 0·005 Å. All other bond lengths have an estimated standard deviation of 0·015 Å. The average error in the bond angles is 0·5° for the phosphate angles and 1° for the others. (a) The distances and angles in the ribose–phosphate–ribose part of UpA 1 (upper) and UpA 2 (lower). (b) The distances and angles in the adenines and uracils of UpA 1 (upper) and UpA 2 (lower).


H
O2
N3
O4
1.177
123.33
1.401
118.82
1.239
1.366
127.78
C2
C4
122.32
114.35
113.88
127.15
1.408
1.390
116.73
N1
118.68
119.97
C5
1.359
125.33
124.17
1.328
Cl'
1.498
C6
H
H
H
N6
1.291
124.93
C6
121.37
N7
1.348
130.53
1.374
1.433
113.65
H
103.49
C5
N1
1.319
113.60
115.68
124.01
H
C8
113.30
1.394
1.356
103.15
130.80
1.377
106.43
C4
126.23
C2
1.387
126.02
10944
126.92
N9
126.08
1.353
N3
1.331
H
1.505
Cl'


H
O2
N3
O4
1.212
121.06
1.377
119.41
1.251
1.367
124.36
C2
C4
121.75
117.12
116.97
123.61
1.374
1.449
116.56
N1
119.32
116.23
C5
1.362
125.50
123.70
1.331
Cl'
1.509
C6
H
H
H
N6
1.301
124.14
C6
122.25
N7
1.389
129.96
1.399
1.317
113.60
H
103.63
C5
N1
1.417
111.55
118.38
121.41
H
C8
112.92
1.352
1.341
106.49
127.12
1.385
105.39
C4
127.81
C2
1.375
111.54
128.46
N9
126.00
126.38
1.380
N3
1.282
H
1.475
Cl'

(b)

In both molecules the glycosidic torsion angles for the uracils (χ') are smaller than those for the adenines (χ). This may be in part due to the fact that the O5'U is free, i.e. not participating in a phosphoester linkage (see further discussion of this in the intramolecular hydrogen bonding section).

(b) *The bond distances and angles are not unusual*

The bond distances and angles of the two independent phosphate groups are internally consistent within experimental error (Fig. 4(a)). They show the general features characteristic of other organic phosphate compounds (Li & Caughlan, 1965): the phosphoester bonds are longer (average 1·61 Å) than the other phosphorus/oxygen bonds (average 1·48 Å). The O3'U—P—O5'A angle averages 103°, while the O1P—P—O2P angle averages 120°. This is again in agreement with other known structures, in particular adenosyl 2',5'-uridine phosphate (2',5'-ApU) (Shefter, Barlow, Sparks & Trueblood, 1969). It is clear from these results that the phosphate group is not protonated, since the O1P and O2P bond lengths of both molecules are much shorter than those expected (1·55 Å) for a P—OH bond.

TABLE 5

Average distances and angles in four ribose moieties from the crystal structure of UpA

Bond	Average distance (Ångstrom units)	Reported average (Sundaralingam, 1965) (Ångstrom units)
C4' O1'	1·46±0·01	1·450
O1' C1'	1·40±0·03	1·427
C1' C2'	1·53±0·01	
C2' C3'	1·52±0·02	
C3' C4'	1·53±0·02	
C2' O2'	1·41±0·02	1·429
C3' O3'	1·40±0·01	1·404
C4' C5'	1·50±0·02	1·516
C5' O5'	1·43±0·02	
C1' N	1·50±0·01	

Angle	Average angle (degrees)	Reported average (Sundaralingam, 1965) (degrees)
C1' C2' C3'	101±1	
C2' C3' C4'	103±1	
C3' C4' O1'	104±1	
C4' O1' C1'	111±1	109·3
O1' C1' C2'	108±1	
C1' C2' O2'	108±2	
O2' C2' C3'	112±1	
O3' C3' C4'	112±3	
C2' C3' O3'	113±3	
C3' C4' C5'	116±0·5	116
C5' C4' O1'	110±2	
O1' C1' N	108±0	107·6
C2' C1' N	113±1	112·9

± shows the mean deviation from the average.

The average distances and angles in the ribose part of the molecule are summarized in Table 5 and, where appropriate, compared with those given by Sundaralingam (1965). When the average parameters are compared with those of other known structures, there are no remarkable discrepancies; however, there are a few individual bond lengths (see Fig. 4(a)) which seem out of line, particularly O1'U—C1'U and O1'A—C1'A of UpA 1 which are shorter by about 0·06 Å than average O1'—C1' bond length of 1·43 Å.

The geometry of the adenine and uracil bases is not unusual with the following exceptions (see Fig. 4(b)): the C4U—C5U distance in UpA 1 appears to be shorter than that found in other structures (Voet & Rich, 1970), while the C6A—N1A and C4A—N3A bond lengths of UpA 2 are longer. The bond angles are consistent and normal.

(c) *UpA is a zwitterion*

Since the crystals were obtained at pH 3 to 4†, one would expect both phosphate groups to be negatively charged. Because the pK of N1A is 4·3, protonation of this atom, thereby conferring a positive charge on the adenine, would be a possible way to maintain electrical neutrality. It is likely that N1A is protonated for the following reasons.

(1) One hydrogen was located near N1A in UpA 2 in a difference Fourier synthesis.

(2) There is a close contact or hydrogen bond between N1A and O2P in both molecules (see Fig. 5(a)) as in 2',5'-ApU.

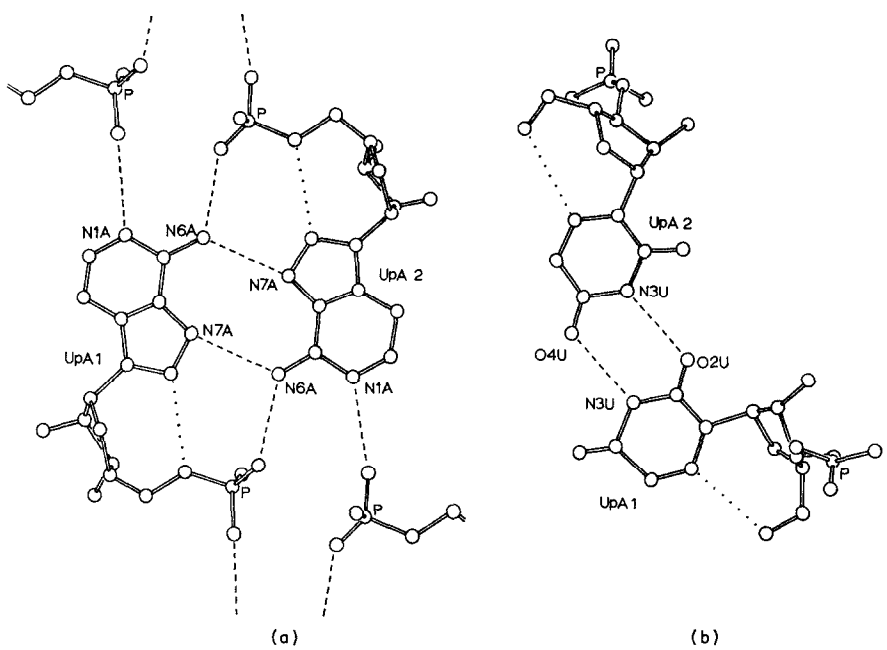


FIG. 5. Base pairing of the (a) adenines and (b) uracils in the crystal structure of UpA. The dashed lines indicate the possible intermolecular hydrogen bonds, while the dotted lines indicate intramolecular close contacts (or possible hydrogen bonds) between the bases and the O5' of the riboses.

† The pH probably increased from its initial value of 3 because the crystallizing medium was exposed to air.

(3) The lengths of P—O2P and P—O1P are shorter than would be expected for a P—OH bond (see discussion of the bond distances and angles).

(4) The angle C2A—N1A—C6A should be about 125° for a protonated N1A and 117° when unprotonated (Voet & Rich, 1970). In UpA 1, this angle is 124° and in UpA 2 it is 121°.

(d) *The bases are not exactly parallel*

The deviations of the bases from planarity are significant (Table 6), but not greater than expected for purines and pyrimidines in nucleotide and nucleoside structures (Voet & Rich, 1970). The angle between the two least-squares planes in UpA 1, one through adenine and the other through uracil, is 18° and in UpA 2 it is 11°. The angle between the best planes of the adenines in the adenine base pair is 14° and between the uracils in the uracil base pair 16°. Thus, the bases are not perfectly parallel but are tilted with respect to one other.

(e) *Intramolecular hydrogen bonding*

In both UpA 1 and UpA 2 there are close contacts, possibly hydrogen bonds, between C8A and O5'A and between C6U and O5'U. The positions of the hydrogen

TABLE 6

Least-squares planes of adenine and uracil bases in the crystal structure of UpA

Atom	Deviation from adenine plane (Å)	
	UpA 1	UpA 2
N9A	+0·03	−0·03
C4A	+0·01	+0·02
N3A	+0·02	+0·03
C2A	−0·02	−0·03
N1A	0·00	−0·02
C6A	−0·02	0·00
N6A	+0·05	0·00
C5A	−0·05	+0·03
N7A	−0·04	0·00
C8A	+0·02	−0·02

Equation for the least-squares plane of adenine in UpA 1: $0{\cdot}86x - 0{\cdot}01y - 0{\cdot}50z = -0{\cdot}63$; in UpA 2: $0{\cdot}89x - 0{\cdot}13y - 0{\cdot}42z = -8{\cdot}5$.

Atom	Deviation from uracil plane (Å)	
	UpA 1	UpA 2
C6U	0·00	+0·03
C5U	−0·01	−0·05
C4U	−0·03	0·00
O4U	+0·04	+0·03
N3U	−0·03	−0·03
C2U	−0·01	+0·01
O2U	+0·01	0·00
N1U	+0·02	0·00

Equation for the least-squares plane of uracil in UpA 1: $0{\cdot}71x + 0{\cdot}09y - 0{\cdot}70z = 4{\cdot}90$; in UpA 2: $0{\cdot}86x - 0{\cdot}15y - 0{\cdot}47z = -2{\cdot}88$.

atoms on C8A and C6U bring them to about 2·2 Å from the O5′ oxygens. From deuterium experiments (Schweizer, Chan, Helmkamp & T'so, 1964) and proton magnetic resonance measurements (T'so, Kondo, Schweitzer & Hollis, 1969) the hydrogen on C8 of purines and C6 of pyrimidines are known to be partly acidic and to interact with the phosphoester oxygen in solution.

The possible hydrogen bond distances in the uracils (3·14 Å, 3·18 Å) are longer than those found in the adenines (3·09 Å, 3·10 Å). This is also reflected in the differences in the glycosidic torsion angles as noted earlier in the paper. This difference may be due to the fact that the O5′U is not involved in a phosphoester linkage while the O5′A is. However, one cannot discount the possibility that the environment of C6U may be less electronegative than that of C8A. This difference may manifest itself in a smaller induced positive charge resident on the hydrogen of C6U.

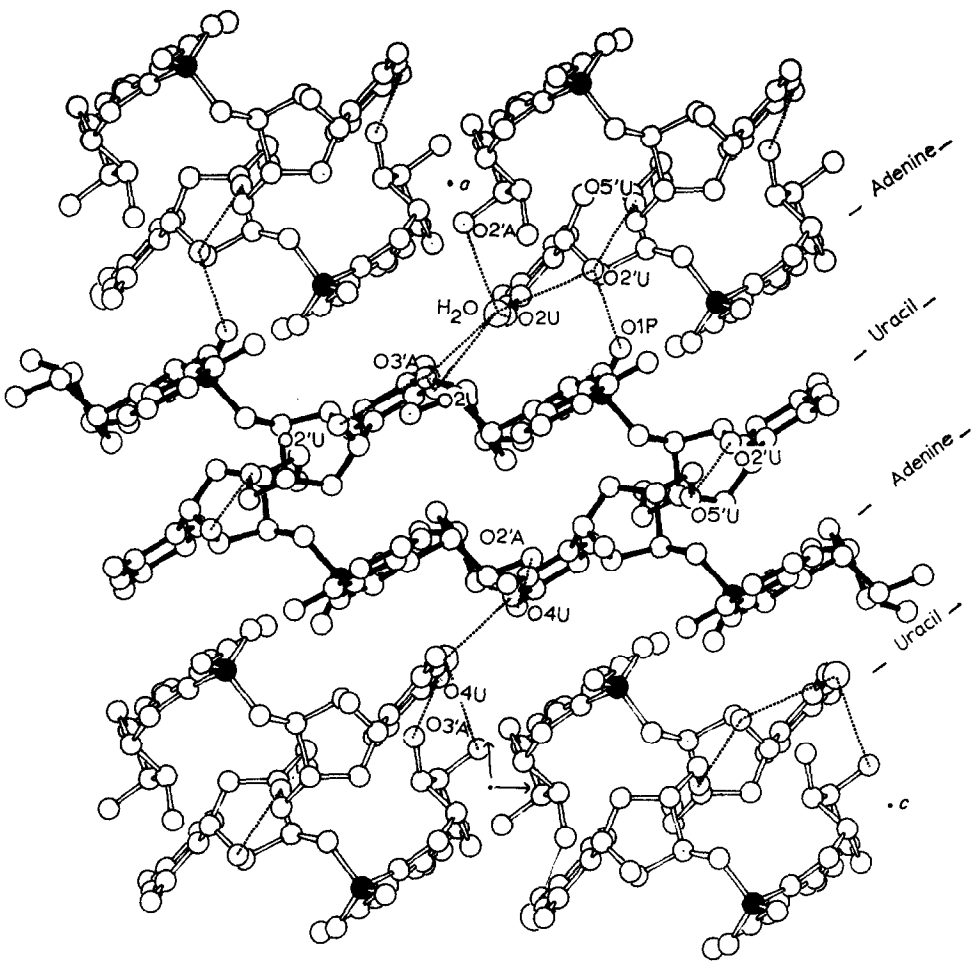


FIG. 6. A view of the crystal structure of UpA down the *b* axis showing the hydrogen bonding between the adenine layers and uracil layers. The UpA 2 molecules are shown with dark bonds. The phosphorus atoms are also shaded. The atoms most likely involved in the interplane interactions are labeled and the hydrogen bonds or close contacts are indicated by dotted lines. The base planes are shown at the edge of the picture.

(f) *There is no complementary base pairing*

There is no complementary base pairing between the adenines and the uracils of the type proposed by Watson & Crick (1953) or that found by Hoogsteen (1963). Indeed, the former type of base pairing is precluded if our hypothesis of the protonation of N1A is true. All the adenines lie approximately in one plane, the uracils in another and the planes are approximately parallel with a separation of 3·4 Å (see Fig. 6). The adenine of UpA 1 is hydrogen bonded to the adenine of UpA 2 in the same manner as in polyadenylic acid (Rich, Davies, Crick & Watson, 1961) (Fig. 5(a)). The uracils form hydrogen-bonded dimers: N3U of UpA 2 donates a hydrogen to O2U of UpA 1 and N3U of UpA 1 donates a hydrogen to O4U of UpA 2 (Fig. 5(b)). This type of uracil·uracil base pairing, in which there is no local 2-fold axis perpendicular to the plane of the hydrogen bonds, has not been previously observed. Moreover, the spatial relation of the two nucleotides involved in the U·U pair is such to allow the formation of an anti-parallel double helical structure of two poly(U) strands.

(g) *Interplanar hydrogen bonds*

A view of the crystal structure down the *b* axis (Fig. 6) shows the essential features of the packing of UpA molecules. There are two types of layering in the structure. First there are the separate sheets of adenine bases and uracil bases which are hydrogen bonded as described above. Furthermore, the two conformers of UpA are separated, as can be seen in this projection, so that there are symmetry-related UpA 1 molecules around the screw axes at $x = 0$ and UpA 2 molecules around the axes at $x = \frac{1}{2}$. In the *b* axis projection UpA 1 and UpA 2 are related by a pseudo 2-fold axis at $x = \frac{1}{4}$, $z = 0{\cdot}0$.

Each conformer of UpA is connected to a symmetry-related molecule by a hydrogen bond between O2′U and O5′U. It is remarkable that both UpA 1 and UpA 2 have this type of bonding.

The water molecule serves as a link between the UpA molecules. It is in the uracil layer and is in close contact with five atoms which are in both the adenine and uracil layers.

There are a few interactions which are difficult to categorize involving O5′U—N1A, O2′A—O4U, and O3′A—O2U in UpA 2, and O2′A—C2A in UpA 1. The last one, however, is another example of CH···O type hydrogen bond.

(h) *Little base stacking*

Although the bases lie in approximately parallel planes it is surprising to find little base/base stacking (see Table 7). The dominant interaction between the planes is found between the sugars and the bases. The O1′ of all four riboses points directly at a base in an adjacent plane (see Fig. 6 and Table 7). Other occurrences of this type of interaction have been summarized by Bugg, Thomas, Sundaralingam & Rao (1971).

4. General Conclusions

Although the two UpA molecules look very different, the most striking feature of the structure† is the fact that all four independent nucleosides have very similar conformations:

† We are pleased to note that our structure is confirmed by another laboratory at the level of their present refinement (Rubin, Brennan & Sundaralingham, 1971).

TABLE 7

Hydrogen bonds and close contacts in the crystal structure of UpA

i	*j*	Symmetry operation of *j*			Distance in Å
A. Hydrogen bonds or close contacts involved in base pairing of the adenines and the uracils (Fig. 5(a) and (b))					
N3U(1)	O4U(2)	−1−x	½+y	−2−z	2·919
N3U(2)	O2U(1)	−1−x	−½+y	−2−z	2·806
N6A(1)	N7A(2)	−1−x	−½+y	−1−z	2·985
N6A(2)	N7A(1)	−1−x	½+y	−1−z	2·938
N6A(1)	O1P(2)	−1−x	−½+y	−1−z	2·819
N6A(2)	O1P(1)	−1−x	½+y	−1−z	2·704
N1A(1)	O2P(1)	x	−1+y	z	2·606
N1A(2)	O2P(2)	x	1+y	z	2·591
B. Hydrogen bonds or close contacts among the ribose sugars, the bases, and the phosphate atoms (Fig. 6)					
O3′A(1)	O4U(1)	−x	½+y	−1−z	2·814
O2′U(1)	O5′U(1)	−x	½+y	−1−z	3·083
O2′U(1)	O1P(2)	−1−x	½+y	−1−z	2·661
O2′A(2)	O4U(2)	x	1+y	1+z	3·096
O3′A(2)	O2U(2)	x	y	1+z	2·806
O2′U(2)	O5′U(2)	−1−x	½+y	−1−z	2·736
O2′U(2)	O3′A(2)	x	y	−1+z	2·860
O5′U(2)	N1A(2)	x	−1+y	z	3·134
O2′A(1)	C2A(1)	−x	½+y	−z	3·187
C. Intramolecular hydrogen bonds (Fig. 5(a))					
C6U(1)	O5′U(1)	x	y	z	3·142
C6U(2)	O5′U(2)	x	y	z	3·178
C8A(1)	O5′A(1)	x	y	z	3·094
C8A(2)	O5′A(2)	x	y	z	3·098
D. Close contacts or hydrogen bonds involving the water molecule (Fig. 6)					
O of H_2O	O2U(1)	−1−x	−½+y	−1−z	3·121
	O2′U(1)	−1−x	−½+y	−1−z	3·049
	O2′A(1)	−1+x	y	z	2·746
	O3′A(2)	x	y	z	3·179
	O2U(2)	x	y	1+z	2·969
E. Stacking interactions					
Ribose/base interactions less than 3·3 Å					
O1′A(1)	N3U(1)	x	y	1+z	2·874
	C2U(1)	x	y	1+z	2·926
	O2U(1)	x	y	1+z	3·203
O1′A(2)	N1U(2)	−1−x	−½+y	−1−z	3·231
O1′U(1)	C4A(1)	−x	½+y	−1−z	3·267
	C2A(1)	−x	½+y	−1−z	3·268
	N3A(1)	−x	½+y	−1−z	3·168
O1′U(2)	N9A(2)	−1−x	−½+y	−1−z	3·048
	C4A(2)	−1−x	−½+y	−1−z	3·043
	C5A(2)	−1−x	−½+y	−1−z	3·109
	N7A(2)	−1−x	−½+y	−1−z	3·187
	C8A(2)	−1−x	−½+y	−1−z	3·097
Base/base stacking less than 3·3 Å					
N3U(1)	C4A(2)	−1−x	−½+y	−1−z	3·289
O4U(1)	C8A(2)	−1−x	−½+y	−1−z	3·214
O4U(2)	N9A(1)	−1−x	−½+y	−1−z	3·276
O4U(2)	C8A(1)	−1−x	−½+y	−1−z	3·155

The numbers in parenthesis, (1) or (2), indicate whether a particular atom is found in UpA 1 or UpA 2.

(1) all four bases are in the *anti*-conformation;
(2) all four sugars are in the 3'-endo 2'-exo conformations;
(3) all four nucleosides have possible intramolecular hydrogen bonds between O5' and the bases (noted earlier by Sutor (1963)).

If these constraints are general, nucleosides can be considered to a first approximation as roughly rigid structures and the conformational degrees of freedom of nucleotides in nucleic acids may be much more limited than has previously been assumed. The phosphodiester bond contains the two major conformational parameters in nucleic acid structures.

There are likely to be only a few sets of energetically favorable conformations about the phosphodiester bonds. This crystal structure reveals examples from two such sets.

(1) UpA 1 set gives a model for a sharp turn in single-stranded nucleic acid structure.
(2) UpA 2 set gives a model for a very open nucleic acid structure.

The identification and characterization of conformation sets will be published later.

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Note added in proof: In Fig. 1(b) and (c), only those hydrogens which were included in structure factor calculations are shown.