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The Crystal and Molecular Structure of the Triethylammonium Salt of Cyclic Uridine-3', 5'-Phosphate

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(Received 27 November 1968)

The triethylammonium salt of cyclic uridine-3',5'-phosphate forms monoclinic crystals of space group $P2_1$, with $a = 15.51$, $b = 11.674$, $c = 11.044$ Å, $\beta = 98.6^\circ$; there are two molecules of the salt in the asymmetric unit. Three-dimensional diffractometer data were collected with Cu $K\alpha$ radiation. The structure was determined by Patterson and Fourier syntheses in conjunction with the tangent formula, and refined by block-diagonal least squares. The final R value was 5.8% for the 2712 reflections used in the refinement and 6.5% for all 2866 data.

The nucleotides pack with the pyrimidine rings nearly normal to c , arranged in hydrogen-bonded ribbons with the base planes near $z = 0$ and $z = \frac{1}{2}$. Adjacent anions are linked with a hydrogen bond between the C(2') hydroxyl group of the ribose and a phosphate oxygen atom.

The geometry of the molecule is in accord with previous studies of nucleotides. The C(4)–O(4) carbonyl bond on the uracil ring is bent towards N(3), as was found in most earlier studies. The torsion angles of the bases with respect to the sugars are -77° and -58° , both in the *anti* range. The ribose conformation is C(3')-*endo* in both anions. The conformation about the C(4')–C(5') bond is *trans-gauche* rather than *gauche-gauche* because of the geometrical requirements for forming the cyclic phosphate.

Introduction

Cyclic uridine-3',5'-phosphate (3',5'-UMP) is an interesting member of the class of mononucleotides. Uridine is a component of ribonucleic acid, and cyclic 2',3'-phosphate esters are intermediates in the breakdown of RNA. Cyclic 3',5'-ribonucleotides were used by Smith & Khorana (1959) in the synthesis of the specific 3',5'-phosphodiester linkage of a dinucleotide; some of these nucleotides (especially cyclic adenosine-3',5'-phosphate) are physiologically active. No detailed structure determinations have been reported for cyclic nucleotides, and this study was undertaken to elucidate the crystal structure of 3'-5'-UMP. Preliminary notes have appeared for this structure (Coulter, 1968) and for the adenosine analog, 3',5'-AMP (Watenpaugh, Dow, Jensen & Furberg, 1968). The chemical structure of 3',5'-UMP is given in Fig. 1 with the conventional numbering of the atoms.

Experimental

The triethylammonium salt of 3',5'-UMP was prepared and crystallized from an ethanol–water solution by Dr D. H. Rammner. The crystals were tabular plates, flattened on (001). They tended to fracture easily along (001). A roughly cube-shaped crystal 0.2 mm on a side was aligned about a^* and used for the determination of cell constants and the initial data collection. To this end, the specimen was placed on a General Electric goniostat with a single-crystal orienter and scintillation counter, and irradiated with nickel-filtered copper radiation. Cell dimensions were derived by a least-squares fit of 31 careful 2θ measurements, and were found to be:

$$a = 15.51 \pm 0.01 \quad b = 11.674 \pm 0.007, \quad c = 11.044 \pm 0.006 \text{ Å} \\ \beta = 98.6^\circ \pm 0.1; \quad \text{Cu } K\alpha: \lambda = 1.5418 \text{ Å}.$$

Systematic extinctions ($0k0$, $k = 2n + 1$) suggested space

group $P2_1$ for this optically active molecule, and the measured density of 1.38 g.cm^{-3} indicated four molecules of 3',5'-UMP and four triethylammonium ions in the cell ($d_{\text{calc}} = 1.38 \text{ g.cm}^{-3}$).

The data were collected for this crystal by the stationary-crystals stationary-counter technique, with 10-second counts for both peaks and backgrounds. Nickel attenuators were used when necessary. Integrated intensities were also measured for 100 peaks over the complete range of 2θ using a 2θ scan of ± 1 to 2° . The data were converted approximately to integrated intensities by the use of the mean ratios of integrated to peak intensity as a function of 2θ (Alexander & Smith, 1962). There are 3250 unique reflections with $2\theta \leq 125^\circ$; 2405 significant intensities (greater than twice background) were recorded from this crystal. Data collection was curtailed at this point because the intensities of all the reference reflections were falling. The variations in intensity of the six reference reflections were normal ($\pm 4\%$) for the first two thirds of the data collection, but then the total counts for these reflections began to decrease significantly. Data collection ceased when the monitored intensities had fallen by 10%. The losses were uniform for all measured peaks, and the effect was presumed to be a result of drift in the counting circuits (Arndt & Willis, 1966). Approximately 20% of the intensities were rescaled on the basis of remeasurement of

previously collected data at the time the new data were being measured. The maximum rescaled increase was 10%, and the average 6%. No absorption corrections were made. The linear absorption coefficient for Cu $K\alpha$ radiation is about 19 cm^{-1} ; this might give intensity differences of as much as 10% as a result of absorption. The intensity variation for $h00$ reflections measured at $\chi = 90^\circ$ was about 7% over the complete ϕ range, in agreement with the maximum variation estimate.

This initial set of data was used to solve the structure and partially refine it. After publication of a preliminary report on the structure (Coulter, 1968) a second

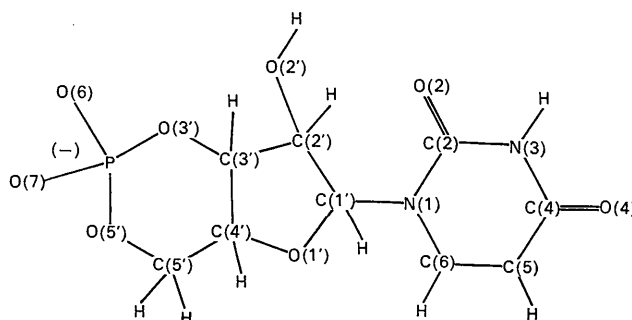


Fig. 1. Cyclic uridine-3',5'-phosphate.

Table 1. Positional parameters and their estimated standard deviations

	Molecule A			Molecule B		
	x	y	z	x	y	z
P	0.33302 (9)	0.05905 (16)	0.09453 (16)	0.18511 (10)	0.03386 (15)	0.57737 (16)
O(7)	0.4262 (3)	0.0556 (5)	0.0775 (5)	0.0907 (3)	0.0402 (5)	0.5347 (5)
O(6)	0.2943 (3)	0.1690 (4)	0.1246 (5)	0.2262 (3)	0.0786 (4)	0.6111 (5)
C(5')	0.2306 (4)	0.0654 (8)	0.2137 (6)	0.2937 (4)	0.1559 (7)	0.7357 (6)
O(5')	0.3199 (3)	0.0341 (4)	0.1979 (5)	0.2040 (3)	0.1197 (4)	0.6926 (4)
C(4')	0.1922 (4)	0.1140 (6)	0.0917 (6)	0.3250 (4)	0.2087 (5)	0.6274 (5)
C(3')	0.1902 (3)	0.0267 (5)	0.0096 (5)	0.3215 (3)	0.1243 (5)	0.5240 (6)
O(3')	0.2773 (2)	0.0056 (4)	0.0270 (4)	0.2346 (2)	0.0941 (4)	0.4748 (4)
C(2')	0.1358 (4)	0.0871 (5)	0.1183 (5)	0.3753 (4)	0.1832 (5)	0.4368 (6)
O(2')	0.1827 (3)	0.1680 (4)	0.1751 (4)	0.3263 (3)	0.2630 (4)	0.3589 (4)
C(1')	0.0709 (4)	0.1538 (6)	0.0485 (6)	0.4409 (4)	0.2512 (5)	0.5312 (6)
O(1')	0.1019 (3)	0.1448 (5)	0.0812 (4)	0.4161 (3)	0.2368 (4)	0.6500 (4)
N(1)	0.0201 (3)	0.1174 (4)	0.0684 (5)	0.5317 (3)	0.2193 (4)	0.5383 (5)
C(2)	0.0837 (4)	0.2019 (6)	0.0752 (6)	0.5902 (4)	0.3010 (5)	0.5069 (6)
O(2)	0.0668 (3)	0.3028 (4)	0.0730 (5)	0.5682 (3)	0.3954 (4)	0.4754 (6)
N(3)	0.1677 (3)	0.1624 (5)	0.0826 (5)	0.6746 (3)	0.2651 (5)	0.5117 (5)
C(4)	0.1951 (4)	0.0479 (7)	0.0867 (8)	0.7080 (4)	0.1568 (7)	0.5479 (8)
O(4)	0.2710 (3)	0.0232 (6)	0.0969 (8)	0.7843 (4)	0.1346 (6)	0.5439 (8)
C(5)	0.1233 (5)	0.0337 (7)	0.0791 (8)	0.6437 (4)	0.0781 (7)	0.5841 (7)
C(6)	0.0415 (4)	0.0020 (6)	0.0673 (7)	0.5603 (4)	0.1120 (6)	0.5755 (6)
N	0.5650 (4)	0.1926 (6)	0.1091 (6)	0.0276 (4)	0.4000 (6)	0.5851 (6)
Me(1)	0.6190 (11)	0.1348 (16)	0.2196 (16)	0.0787 (10)	0.3345 (15)	0.5048 (13)
Me(2)	0.6281 (9)	0.0187 (12)	0.2228 (13)	0.0848 (9)	0.3672 (14)	0.3853 (11)
Me(3)	0.6083 (10)	0.2080 (21)	0.0102 (12)	0.0401 (8)	0.3218 (13)	0.6373 (13)
Me(4)	0.5707 (11)	0.2083 (15)	0.1141 (11)	0.0002 (11)	0.2258 (14)	0.7127 (14)
Me(5)	0.5466 (12)	0.2942 (16)	0.1745 (21)	0.0934 (13)	0.4649 (19)	0.6597 (21)
Me(6)	0.4796 (9)	0.3531 (17)	0.1494 (18)	0.0749 (13)	0.5656 (19)	0.7212 (15)

crystal was located and aligned optically along the b axis. This crystal was a block $0.25 \times 0.2 \times 0.2$ mm in dimension. Ten levels of diffraction data ($h0l$ to $h9l$) were taken from this crystal with a Philips automatic linear diffractometer (PAILRED). The data were taken with copper $K\alpha$ radiation from a silicon monochromator with a scintillation counter. The peaks were scanned (in ω) and stationary backgrounds were sampled on either side of the peak. The data were collected to 100° in 2θ . After averaging the symmetry-related observa-

tions (average deviation from mean, 3%), 1893 unique intensities were available. No unusual behavior was noted for the reference reflections. The PAILRED data were eventually merged with the General Electric data using the two refined least-squares scale factors. The combined data consisted of 2712 non-zero intensities; these were used in the final refinement.

All calculations were carried out with the use of the IBM 7094/7040 system at the University of Chicago. The tangent program was written by Dewar, Coulter

Table 2. *Thermal parameters* ($\times 10^5$) *and their estimated standard deviations**

	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
P—A	207 (5)	686 (14)	1061 (16)	$\overline{18}$ (8)	62 (8)	$\overline{111}$ (13)
P—B	257 (6)	512 (12)	1087 (16)	$\overline{49}$ (7)	184 (8)	$\overline{98}$ (12)
O(7)—A	281 (18)	983 (49)	1796 (65)	$\overline{7}$ (27)	80 (28)	$\overline{260}$ (52)
O(7)—B	309 (18)	906 (46)	1657 (61)	$\overline{95}$ (27)	166 (27)	$\overline{191}$ (49)
O(6)—A	345 (20)	758 (41)	1278 (53)	$\overline{13}$ (24)	$\overline{40}$ (26)	$\overline{168}$ (39)
O(6)—B	530 (24)	659 (39)	1229 (51)	$\overline{74}$ (26)	433 (29)	$\overline{19}$ (38)
C(5)—A	377 (30)	1283 (83)	835 (64)	$\overline{87}$ (43)	10 (36)	200 (63)
C(5)—B	397 (30)	914 (64)	882 (63)	$\overline{104}$ (37)	191 (36)	$\overline{189}$ (54)
O(5)—A	340 (19)	915 (46)	1130 (50)	$\overline{33}$ (26)	$\overline{73}$ (25)	144 (40)
O(5)—B	291 (17)	734 (38)	1027 (44)	$\overline{91}$ (22)	230 (23)	$\overline{161}$ (35)
C(4)—A	263 (25)	769 (57)	924 (62)	$\overline{34}$ (32)	48 (32)	137 (49)
C(4)—B	294 (24)	576 (48)	778 (54)	$\overline{68}$ (29)	130 (30)	$\overline{98}$ (44)
C(3)—A	244 (23)	664 (50)	631 (50)	$\overline{36}$ (29)	84 (27)	30 (41)
C(3)—B	254 (23)	475 (45)	873 (58)	$\overline{24}$ (27)	60 (30)	22 (42)
O(3)—A	245 (17)	715 (38)	954 (43)	$\overline{94}$ (20)	89 (22)	$\overline{100}$ (33)
O(3)—B	279 (17)	687 (37)	880 (40)	$\overline{92}$ (21)	72 (21)	$\overline{29}$ (32)
C(2)—A	299 (24)	508 (46)	773 (54)	$\overline{61}$ (29)	102 (30)	26 (42)
C(2)—B	315 (26)	525 (50)	914 (60)	$\overline{12}$ (29)	115 (32)	97 (45)
O(2)—A	467 (22)	686 (39)	1008 (46)	$\overline{23}$ (25)	160 (26)	$\overline{86}$ (36)
O(2)—B	476 (22)	765 (41)	936 (44)	$\overline{60}$ (26)	9 (26)	222 (37)
C(1)—A	323 (27)	690 (55)	814 (59)	$\overline{35}$ (32)	60 (32)	144 (48)
C(1)—B	311 (26)	509 (47)	990 (62)	$\overline{33}$ (30)	107 (32)	$\overline{40}$ (47)
O(1)—A	359 (20)	1050 (48)	784 (41)	$\overline{108}$ (26)	127 (23)	190 (37)
O(1)—B	351 (19)	901 (42)	733 (37)	$\overline{144}$ (24)	179 (22)	$\overline{95}$ (35)
N(1)—A	259 (21)	526 (41)	979 (52)	$\overline{21}$ (24)	25 (26)	49 (37)
N(1)—B	282 (21)	515 (41)	1017 (53)	$\overline{81}$ (24)	111 (26)	55 (38)
C(2)—A	312 (26)	691 (55)	1032 (68)	$\overline{130}$ (33)	$\overline{38}$ (33)	122 (51)
C(2)—B	354 (26)	420 (44)	1027 (65)	$\overline{105}$ (30)	109 (34)	1 (44)
O(2)—A	384 (21)	465 (35)	1918 (71)	$\overline{50}$ (23)	48 (31)	231 (42)
O(2)—B	472 (25)	554 (40)	2214 (82)	$\overline{57}$ (26)	312 (36)	280 (48)
N(3)—A	230 (20)	791 (50)	1236 (63)	$\overline{42}$ (27)	11 (28)	46 (47)
N(3)—B	304 (21)	667 (45)	1010 (54)	$\overline{98}$ (26)	136 (28)	42 (42)
C(4)—A	333 (29)	961 (75)	1620 (97)	$\overline{20}$ (40)	6 (43)	$\overline{201}$ (71)
C(4)—B	373 (30)	802 (63)	1536 (91)	$\overline{37}$ (38)	80 (43)	99 (65)
O(4)—A	370 (24)	1210 (63)	3006 (117)	129 (34)	108 (42)	$\overline{338}$ (73)
O(4)—B	399 (25)	1318 (71)	3040 (115)	145 (35)	410 (43)	523 (79)
C(5)—A	429 (32)	620 (57)	1803 (102)	$\overline{2}$ (39)	24 (46)	$\overline{221}$ (67)
C(5)—B	415 (32)	767 (63)	1402 (84)	$\overline{72}$ (38)	79 (41)	264 (61)
C(6)—A	357 (28)	617 (56)	1303 (81)	$\overline{94}$ (33)	21 (38)	$\overline{81}$ (54)
C(6)—B	368 (29)	608 (51)	1154 (71)	$\overline{40}$ (33)	170 (36)	123 (51)
N—A	528 (30)	889 (57)	1072 (60)	$\overline{16}$ (36)	138 (34)	$\overline{28}$ (51)
N—B	497 (30)	919 (60)	1207 (66)	116 (36)	254 (36)	15 (53)
Me(1)—A	1396 (114)	2272 (224)	3348 (240)	$\overline{831}$ (138)	958 (134)	1041 (202)
Me(1)—B	1546 (107)	2405 (197)	2200 (171)	952 (129)	1139 (118)	1212 (164)
Me(2)—A	1219 (85)	1498 (136)	2589 (185)	$\overline{38}$ (92)	$\overline{364}$ (102)	643 (138)
Me(2)—B	1304 (90)	2447 (191)	1259 (118)	560 (112)	343 (83)	16 (122)
Me(3)—A	1209 (91)	5133 (388)	1603 (139)	$\overline{1550}$ (164)	535 (91)	$\overline{970}$ (200)
Me(3)—B	958 (69)	1924 (150)	2800 (192)	584 (89)	750 (94)	1015 (150)
Me(4)—A	1626 (117)	2327 (193)	1327 (122)	$\overline{373}$ (127)	$\overline{354}$ (98)	86 (130)
Me(4)—B	1574 (108)	1891 (177)	2548 (183)	479 (119)	1084 (120)	719 (159)
Me(5)—A	1521 (133)	1996 (210)	4407 (363)	397 (143)	256 (167)	$\overline{1347}$ (241)
Me(5)—B	1724 (137)	2486 (267)	4153 (349)	389 (172)	$\overline{1356}$ (180)	$\overline{363}$ (255)
Me(6)—A	951 (81)	2403 (230)	3686 (300)	377 (116)	655 (124)	247 (220)
Me(6)—B	1919 (154)	2990 (284)	2158 (180)	56 (180)	431 (136)	$\overline{1075}$ (206)

* Temperature factor = $\exp [-(B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + 2B_{12}hk + 2B_{13}hl + 2B_{23}kl)]$.

Table 3. Observed and calculated structure factors

The values listed are 10|F_o| and 10|F_c|. Unobserved reflections (less than twice the background) are marked U and assigned probable values where possible, or zero when this could not be done. Reflections marked * were omitted from the refinement because of possible measurement errors.

Table with 12 columns: H, K, Fobs, Fcalc, H, K, Fobs, Fcalc, H, K, Fobs, Fcalc, H, K, Fobs, Fcalc. It contains a large grid of numerical data representing structure factors for various reflections.

and Hawkinson; the least-squares program was a modified version of *ORFLS* (Busing, Martin & Levy, 1962). The Fourier program was written by Coulter (1964). Johnson's (1965) *ORTEP* program was used for Figs. 8 and 9, and Busing, Martin & Levy's (1964) *ORFFE* program was used for distance and angle calculations and corrections. Form factors for phosphorus, oxygen, nitrogen and valence carbon were taken from *International Tables for X-ray Crystallography* (1962), and the values of Stewart, Davidson & Simpson (1965) were used for hydrogen.

Determination of the structure

The structure was solved using the 2405 unique intensities measured with the General Electric XRD-5. These intensities were corrected and converted to $|E|$ values with a Wilson plot. A sharpened three-dimensional Patterson synthesis was calculated with $(|E|^2 - 1)$ coefficients. Harker peaks for the two unique phosphorus atoms were easily located and predicted the intermolecular P-P vector. Three phosphate oxygen atoms were placed on the basis of P-O vectors tetrahedrally surrounding the P-P vector. Patterson and Fourier methods were initially used to derive 13 atomic positions which appeared chemically and crystallographically feasible. The absence of resolved Patterson peaks which would permit positioning of the ribose atoms hampered the analysis. At this point, a general program for applying Karle and Hauptman's tangent formula (Karle & Hauptman, 1956; Karle & Karle, 1966) to crystallographic data became available. The phase angles based upon 13 atoms were assigned to the reflections with $|E| \geq 1.0$ and refined by the tangent formula. The phases converged to a self-consistent set within three cycles, and the resulting *E* map revealed the complete molecular structure. Two of the 13 atoms used initially were incorrect, thus explaining the earlier difficulties. The *E* map contained a small peak at one of the incorrect sites and nothing significant at the other site. The positions of the 40 atoms in the two 3',5'-UMP molecules in the asymmetric unit were derived from this *E* map. In a subsequent experiment, the tangent formula was used with phases based upon the five atoms initially located from the Patterson synthesis (a PO_3

group and the other phosphorus atom). After phase refinement the *E* map again revealed the structure clearly. Individual values for the refined phase angles were slightly different in this second refinement ($\pm 5^\circ$), and the reasons for the differences are still under investigation.

Refinement

The structure was partially refined by the use of a block-diagonal least-squares refinement program and a dif-

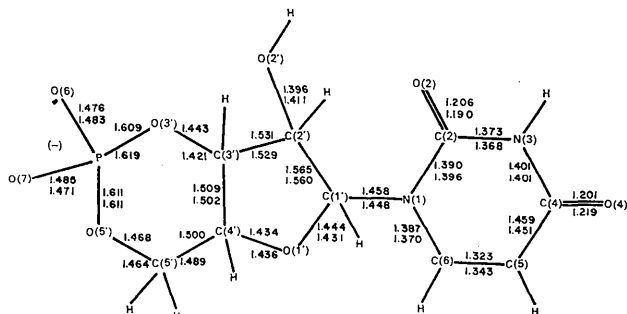


Fig. 2. Bond distances within the 3',5'-UMP anions. The upper figures refer to molecule *A*, the lower to *B* (Table 1). The estimated standard deviation for bond distances is 0.008 Å.

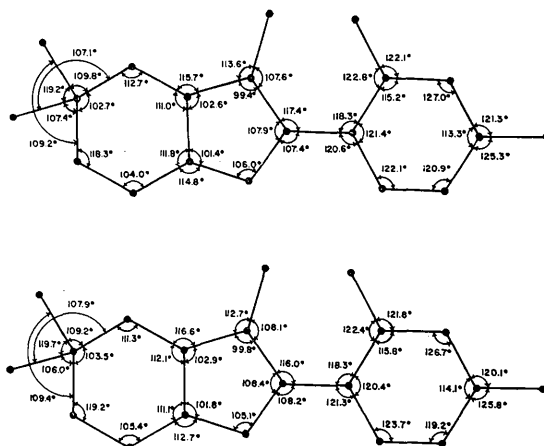


Fig. 3. Bond angles within the 3',5'-UMP anions. The estimated standard deviation of an angle is 0.7° .

Table 4. Hydrogen atom position parameters*

	Molecule <i>A</i>			Molecule <i>B</i>		
	<i>x</i>	<i>y</i>	<i>z</i>	<i>x</i>	<i>y</i>	<i>z</i>
H(C1')	0.072	-0.245	-0.073	0.435	0.342	0.509
H(C2')	0.102	-0.027	-0.185	0.408	0.121	0.383
H(C3')	0.156	0.049	0.015	0.355	0.047	0.561
H(C4')	0.231	-0.188	0.071	0.286	0.284	0.597
H1(C5')	0.230	-0.130	0.287	0.296	0.217	0.811
H2(C5')	0.193	0.007	0.238	0.334	0.083	0.769
H(N3)	-0.218	-0.227	-0.086	0.720	0.328	0.485
H(C5)	-0.139	0.125	-0.088	0.663	-0.007	0.620
H(C6)	0.011	0.061	-0.056	0.513	0.053	0.599

* The hydrogen atoms are 1.1 Å from the parent atoms and at tetrahedral or trigonal angles. These positions were calculated.

ference synthesis was calculated to locate the counter ions. The fourteen carbon and nitrogen atoms in the two unique triethylammonium ions were located after

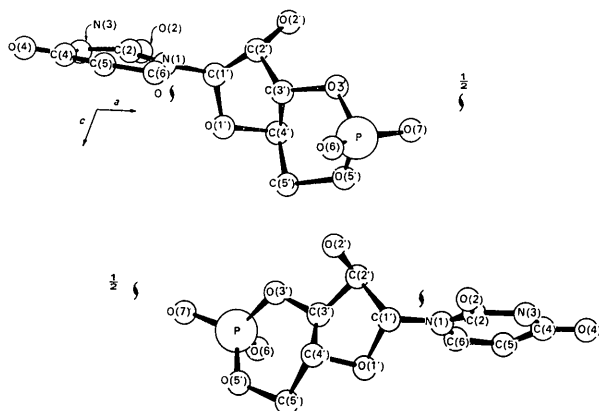


Fig. 4. The anions viewed down *b*. C(2) of the upper nucleotide was moved slightly to avoid overlap.

two least-squares refinement and Fourier synthesis cycles. The methylene groups in the triethylammonium ions did not give sharp peaks, presumably because of thermal vibration. The 54 atoms were refined with isotropic *B* values to an *R* of 16%. The thermal motions were clearly anisotropic, and vibration was especially marked in the direction normal to the pyrimidine rings. Several cycles of anisotropic refinement reduced the *R* value to 8%. At this point, the PAILRED data from the *b* axis crystal became available. These data were independently refined *via* block-diagonal least squares to an *R* of 7.6%. Half shifts were used in all least-squares cycles. The two refined structures were equivalent within the estimated errors of the analyses, and the two data sets were merged by the ratios of the two least-squares scale factors. The positional and thermal parameters were refined two further cycles of least-squares, and a difference map was calculated. Peaks of 0.2 to 0.5 e.Å⁻³ occurred at positions expected for most of the hydrogen atoms in the molecules, but other peaks as high as 0.4 e.Å⁻³ also appeared, especially in the

Table 5. Distances and angles in the triethylammonium ions

	Uncorrected		Thermally corrected*	
	<i>A</i>	<i>B</i>	<i>A</i>	<i>B</i>
N—Me(1)	1.53 Å	1.49 Å	1.64 Å	1.58 Å
N—Me(3)	1.38	1.57	1.51	1.62
N—Me(5)	1.44	1.43	1.57	1.58
Me(1)—Me(2)	1.36	1.39	1.62	1.70
Me(3)—Me(4)	1.41	1.48	1.71	1.66
Me(5)—Me(6)	1.24	1.41	1.58	1.73
Me(1)—N—Me(3)	115°	112°		
Me(1)—N—Me(5)	95	103		
Me(3)—N—Me(5)	117	124		
N—Me(1)—Me(2)	120	123		
N—Me(3)—Me(4)	126	114		
N—Me(5)—Me(6)	124	123		

* The nitrogen distances were corrected by the riding model of Busing & Levy (1964) with the methylene carbon atoms assumed to be riding on the nitrogen atom. The carbon-carbon distances were corrected assuming independent motion. *A* and *B* are the two molecules in the asymmetric unit.

Table 6. Root mean square displacements (Å) along crystal axes

	Molecule <i>A</i>			Molecule <i>B</i>		
	(μ^2) _{<i>a</i>} ^{1/2}	(μ^2) _{<i>b</i>} ^{1/2}	(μ^2) _{<i>c</i>} ^{1/2}	(μ^2) _{<i>a</i>} ^{1/2}	(μ^2) _{<i>b</i>} ^{1/2}	(μ^2) _{<i>c</i>} ^{1/2}
P	0.16	0.21	0.26	0.16	0.19	0.26
O(7)	0.18	0.25	0.34	0.18	0.25	0.32
O(6)	0.20	0.22	0.29	0.19	0.22	0.30
C(5')	0.20	0.22	0.31	0.20	0.21	0.27
O(5')	0.19	0.25	0.28	0.17	0.21	0.27
C(4')	0.18	0.21	0.25	0.17	0.19	0.23
C(3')	0.17	0.20	0.22	0.17	0.18	0.23
O(3')	0.16	0.22	0.25	0.17	0.23	0.23
C(2')	0.17	0.20	0.22	0.19	0.19	0.24
O(2')	0.21	0.23	0.25	0.19	0.25	0.27
C(1')	0.19	0.20	0.24	0.18	0.20	0.25
O(1')	0.18	0.22	0.28	0.18	0.21	0.27
N(1)	0.17	0.19	0.25	0.16	0.21	0.25
C(2)	0.17	0.22	0.27	0.15	0.22	0.25
O(2)	0.17	0.22	0.35	0.18	0.24	0.37
N(3)	0.16	0.23	0.28	0.17	0.23	0.25
C(4)	0.20	0.25	0.33	0.21	0.24	0.31
O(4)	0.20	0.29	0.44	0.20	0.28	0.44
C(5)	0.20	0.23	0.34	0.20	0.23	0.30
C(6)	0.18	0.23	0.29	0.19	0.22	0.27

neighbourhood of the triethylammonium ions. None of the sharp peaks could be assigned to the hydrogen atoms bound to the O(2') atoms of the sugars. The hydrogen atoms were placed at calculated positions 1.08 Å from their respective carbon or nitrogen atoms and assigned the thermal parameters of the atom to which they were bound. No hydrogens were placed bound to the triethylammonium ions or to the O(2') atoms of the sugars. Four additional block-diagonal least-squares refinement cycles were calculated before the weighted sum of the squares of the deviations leveled off. Hughes's (1941) weighting scheme was used, with $4F_{\min} = 15$. The shifts in position parameters on the final cycle averaged about 0.15 of the estimated standard deviations, with only one parameter shifting by as much as 0.25 σ [the x coordinate of C(4')]. The final position parameters are given in Table 1, the thermal parameters in Table 2, and the observed and calculated structure factors are listed in Table 3. The final R value is 5.8% for the observed reflections and 6.5% for all 2866 reflections. The weighted R value, $(\sum_h w_h (|F_o| - |F_c|)^2 / \sum_h w_h |F_o|^2)^{1/2}$, is 7.5%.

The position parameters for the hydrogen atoms included in the final refinement cycles are in Table 4.

Discussion

The distances and angles for the two molecules of 3',5'-UMP in the asymmetric unit are shown in Figs. 2 and 3. The distances and angles for the triethylammonium counter ions are given in Table 5. The large anisotropies reflected in the root mean square vibrations given in Table 6 give rise to concern about the need for thermal corrections to bond distances. For the 3',5'-UMP molecules, such corrections do not seem to be necessary since the distances and angles found here are directly comparable with those found in other studies.

The molecules are apparently vibrating rather rigidly normal to the pyrimidine rings (Fig. 4).

As a test, 'riding atom' corrections were calculated for the eight P-O distances: the maximum correction was 0.015 Å and this is within the errors estimated for the bond distances (± 0.008 Å). The triethylammonium ions are less rigid, and Table 5 lists thermally corrected bond distances as well as the uncorrected values.

The layers of molecules shown in Fig. 4 are linked within layers by a hydrogen bond between N(3) and O(6) of the adjacent screw-axis-related molecules. The only interlayer bond is a hydrogen bond between the O(2') hydroxyl group and O(6) of the other molecule. The two triethylammonium ions are hydrogen bonded to the two O(7) oxygen atoms rather symmetrically, as shown in Fig. 5. The hydrogen-bond distances are

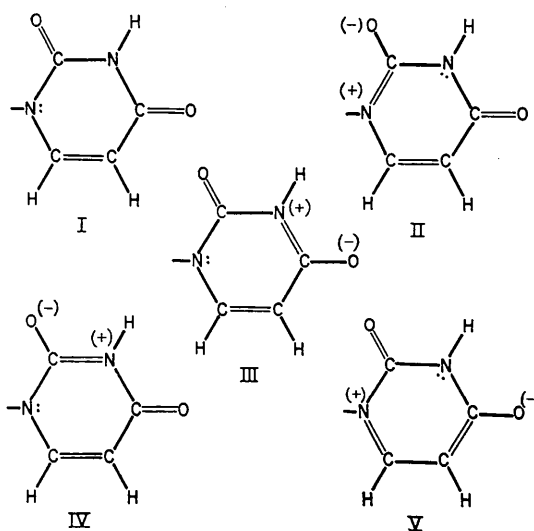


Fig. 6. Some valence bond structures for uracil.

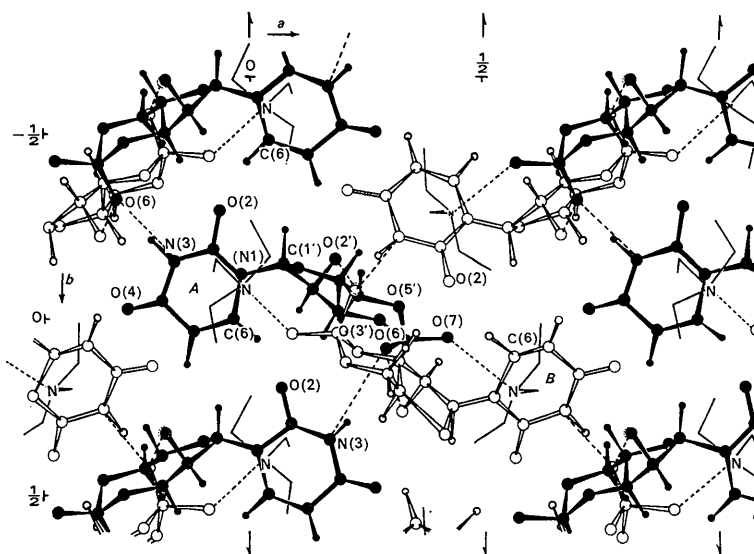


Fig. 5. The structure projected down c . Dark molecules are near $z=0$ and light molecules near $z=\frac{1}{2}$. Hydrogen bonds are dashed.

given in Table 7. The two molecules in the asymmetric unit can be approximately superimposed by the operation of a screw axis lying between the phosphorus atoms and parallel to *c*. It is not clear why this substance crystallizes as a dimer; cyclic adenosine 3',5'-phosphate also crystallized as a dimer (Watenpaugh *et al.*, 1968), but the two molecules have different conformations in that case. Some intermolecular contact distances are listed in Table 8. The most interesting of these are the close contacts between the carbonyl oxygen, O(2), and C(6) in a symmetry related molecule. Similar short C-H...O contacts were reported by Hoogsteen (1963) for 1-methylthymine and Parry (1954) for uracil. Sutor (1963) and Donohue (1958) have discussed these interactions.

Table 7. *Hydrogen bond distances**

O(6)(<i>A</i>)–O(2')(<i>B</i>)	2.785 Å
O(2')(<i>A</i>)–O(6)(<i>B</i> , 00 $\bar{1}$)	2.755
O(6)(<i>A</i>)–N(3)(<i>A'</i>)	2.768
O(6)(<i>B</i>)–N(3)(<i>B'</i> , 1 $\bar{1}$ 1)	2.855
O(7)(<i>A</i>)–N ⁺ (<i>A</i>)	2.66
O(7)(<i>B</i>)–N ⁺ (<i>B'</i> , 0 $\bar{1}$ 1)	2.66

* *A* and *B* are the reference molecules (Table 1); *A'* and *B'* are transformed to $-x$, $\frac{1}{2}+y$, $-z$; integers in parentheses correspond to translations along *a*, *b* and *c*. N⁺ is the triethylammonium nitrogen ion.

Table 8. *Intermolecular contact distances**

O(3')(<i>A</i>)–C(5')(<i>B</i> , 00 $\bar{1}$)	3.195 Å
O(3')(<i>B</i>)–C(5')(<i>A</i>)	3.424
C(3')(<i>A</i>)–O(2)(<i>A'</i>)	3.442
C(3')(<i>B</i>)–O(2)(<i>B'</i> , 1 $\bar{1}$ 1)	3.171
O(2)(<i>A</i>)–C(6)(<i>A'</i> , 0 $\bar{1}$ 0)	3.138
O(2)(<i>B</i>)–C(6)(<i>B'</i> , 101)	3.216
O(1')(<i>A</i>)–Me(4)(<i>B'</i> , 0 $\bar{1}$ 1)	3.32
O(1')(<i>B</i>)–Me(4)(<i>A</i> , 001)	3.28

* The labeling is as in Table 7.

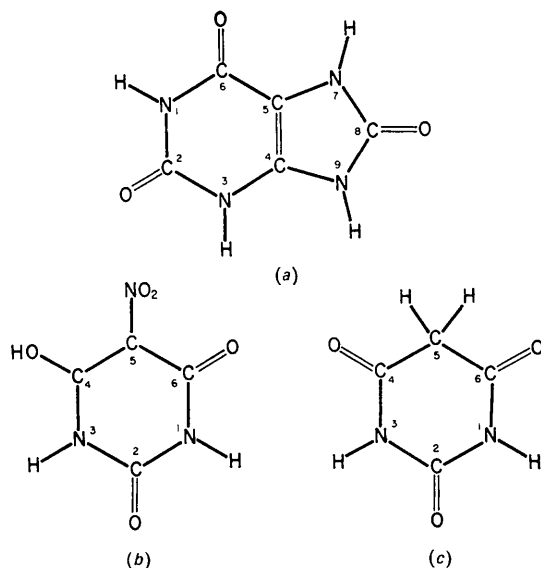


Fig. 7. (a) Uric acid; (b) dilituric acid; (c) barbituric acid.

The base

The dimensions of the base are comparable with those found in other studies of pyrimidines. The distances and angles are given in Figs. 2 and 3. The four carbonyl bond lengths average to 1.204 Å. None of these oxygens is involved in a hydrogen bond and the distance agrees well with that of 1.201 Å found in cytidylic acid *b* (Sundaralingam & Jensen, 1965). The C(4)–N(3) and the C(4)–C(5) bond distances are significantly longer than was found in uracil (1.371 and 1.430 Å; Stewart & Jensen, 1967) or in 1-methylthymine (1.375 and 1.432 Å; Hoogsteen, 1963), and the N(3)–C(4)–C(5) angle is smaller here (113.7°) than in uracil (115.5°) or 1-methylthymine (116.1°). This presumably is because O(4) is not hydrogen bonded in this structure but was in the other two. No other distances or angles differ by more than 2σ from those found in earlier studies. The non-equivalence of the external angles about the C(4) carbonyl group is interesting, and has been noted by others (Shefter & Trueblood, 1965). Table 9 lists values for the external angles about the C(2)–O(2) bond and the C(4)–O(4) bond for several pyrimidine and related structures. For thymine and uracil rings, the C(2)–O(2) angles are generally symmetric, whereas the C(4)–O(4) bond is bent towards N(3). When fluorine replaces the thymine methyl group on C(5), the angular difference is no longer significant.* This suggests an inductive effect, as the electronegativity of fluorine (4.0) is considerably greater than that of carbon (2.5) or hydrogen (2.1) (Pauling, 1960). Sobell (1966) pointed out that the *pK* of the hydrogen on N(3) followed the electronegativity of the C(5) substituent, so an effect on the C(4) carbonyl is not unexpected. The two carbonyl groups are in different electronic environments. Examination of the most likely resonance contributors for uracil (Fig. 6) reinforces this difference, since structure V, with a wide charge separation, is less likely to be a major contributor than structures I–IV. Valence bond structures tell us little about asymmetry in bond angles; however, a displacement of the electron-rich O(4) atom towards the more acidic N(3) seems reasonable. From the experimental data summarized in Table 9 we can conclude that the normal structure for thymine and uracil involves an N(3)–C(4)–O(4) angle of 120° and a C(5)–C(4)–O(4) angle of 125°. This situation persists in uric acid and dilituric acid (Fig. 7). In the two barbituric acid structures, with one symmetric carbonyl group analogous to C(2)–O(2) and two carbonyl groups similar to C(4)–O(4), the situation is less clear. From the Table values, one carbonyl appears to be significantly non-symmetric and the other symmetric in both structures. Barbituric acid differs from pyrimidines, uric acid, and dilituric acid in having a saturated carbon in the ring (Fig. 7).

* Sobell (unpublished) has observed a significant 5° difference in bond angles about the C(4)–O(4) carbonyl bond of 1-methyl-5-fluorouracil in the complex with 9-ethyl-2-aminopurine. The question of a possible inductive effect thus remains unresolved.

Table 9. Carbonyl angles with estimated standard deviations

Compound	N(1)-C(2)-O(2)	N(3)-C(2)-O(2)	N(3)-C(4)-O(4)	C(5)-C(4)-O(4)	e.s.d.	Reference
3', 5'- UMP	122.8°	122.1°	121.3°	125.3°	0.7°	This work
Thymine monohydrate	122.4	121.8	120.1	125.8		
Calcium thymidine 5'-phosphate	122.7	122.1	118.3	126.1	0.2	Gerdil (1961).
1-Methylthymine	123	121	118	129	1.4	Trueblood, Horn & Luzzati (1961).
Uracil	123.3	121.3	120.0	123.9	0.2	Hoogsteen (1963).
Barium uridine 5'-phosphate	123.7	122.3	119.2	125.3	0.2	Stewart & Jensen (1967).
5-Fluoro-2'-deoxyuridine	124	124	118	125	2	Shefter & Trueblood (1965).
Uric acid	122.8	123.4	123.3	124.1	0.7	Harris & Macintyre (1964).
Diluturic acid	121.4*	122.6*	122.3	126.4	0.3	Ringertz (1966).
Barbituric acid dihydrate‡	122.7	121.5	119.8†	126.3†	0.3	Bolton (1963b).
Barbituric acid‡	119.3	122.6	118.7	123.8	0.7	Jeffrey, Ghose & Warwicker (1961).
			119.6	121.9		
			118.1	121.4	0.5	Bolton (1963a).
			122.3	121.5		

* The C(2)-O(2) carbonyl group, adjacent to N(1).

† The C(6)-O(6) carbonyl group, adjacent to N(1).

‡ Barbituric acid has one carbonyl group symmetrically attached to two ring nitrogen atoms and two carbonyl groups bound to a nitrogen and a carbon atom (Fig. 7).

Table 10 summarizes the data on the planarity of the bases in 3',5'-UMP. The C(1') atom of the sugar is significantly out of the base plane in both molecules, as is O(4). Displacement of substituents, especially C(1'), from purine or pyrimidine ring planes seems to be a rule rather than an exception, as Sundaralingam & Jensen (1965) and Davies (1967) have pointed out.

C(3')-endo conformation. The fact that C(3') is 0.1 Å further from the best plane than C(5') in both molecules seems to reflect a necessary distortion of the C(5')-C(4') bond to form the cyclic ester.

Table 10. Least squares planes of the uracil rings*

	Distance out of plane in Å.	
	Molecule A	Molecule B
C(1')	0.099	-0.034
N(1)	-0.012	-0.010
C(2)	-0.005	0.016
O(2)	0.003	0.028
N(3)	0.013	-0.005
C(4)	-0.005	-0.012
O(4)	-0.026	-0.077
C(5)	-0.012	0.018
C(6)	0.020	-0.007

* Boldface atoms were used to define the planes.

Equations of the planes (x, y, z in fractional cell coordinates):

$$A: -1.216x - 0.0528y + 11.012z = -0.7104 \text{ \AA},$$

$$B: 0.7568x + 3.722y + 10.252z = 6.748 \text{ \AA}.$$

The sugar and the phosphate

Fig. 8 illustrates the ribose-phosphate area of 3',5'-UMP. The torsion angles, ϕ_{CN} , for the two molecules are -77° and -58° . These are both in the *anti* range, as expected. Table 11 summarizes the conformational data for the two β -ribose moieties. The best description of the sugar rings is in terms of the least-squares planes through C(1'), C(2'), O(1') and C(4'), with C(3') out of this plane on the same side as C(5'), or in the

Table 11. Least-squares planes for the sugars

Plane I*	Distance out of plane in Å.	
	Molecule A	Molecule B
C(1')	-0.105	0.127
C(2')	0.062	-0.075
O(2')	-1.206	1.182
C(3')	0.638	-0.600
O(3')	0.431	-0.378
C(4')	-0.068	0.080
C(5')	0.0507	-0.535
O(5')	0.280	-0.376
O(1')	0.110	-0.132
N(1)	0.770	-0.694

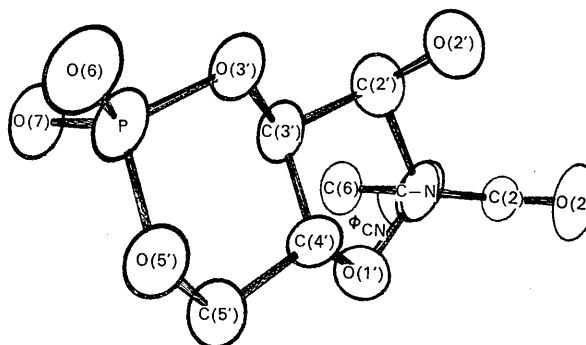


Fig. 8. A view down the glycosidic bond.

Table 11 (cont.)

Plane II		
C(1')	-0.140	0.145
C(2')	-0.414	0.343
O(2')	-1.793	1.715
C(3')	0.128	-0.133
O(3')	-0.417	0.398
C(4')	-0.226	0.235
C(5')	0.408	-0.423
O(5')	-0.158	0.049
O(1')	0.238	-0.247
N(1)	0.889	-0.828

* Boldface atoms were used to define the planes.

Equations of planes (x, y, z in fractional cell coordinates):

$$\begin{aligned} \text{I } A & -5.867x + 10.650y + 2.319z = -2.061 \text{ \AA} \\ \text{I } B & -5.141x + 10.676y - 1.971z = -0.7593 \\ \text{II } A & -8.691x + 8.936y + 4.372z = -2.062 \\ \text{II } B & -7.201x + 9.171y - 3.693z = -2.978 \end{aligned}$$

Fig. 9 is a view down the C(4')-C(5') bond for one of the 3',5'-UMP molecules. The values of the dihedral angles φ_{OO} and φ_{OC} are 184, 61 and 187, 59° for the two unique molecules. The conformation is thus *trans-gauche* in both cases; in the *gauche-gauche* conformation, which is usually found (Shefter & Trueblood, 1965), the cyclic link of O(3') and O(5') through the phosphate could not be made (Coulter, 1968). Similarly the P-O(3')-C(3') and O(3')-C(3')-C(4') dihedral angle must be about 60° (Sundaralingam, unpublished) in order to form the cyclic ester without greatly distorting the bond angles about C(3') (Fig. 8). These dihedral angles are 59 and 61° for the two 3',5'-UMP molecules. *Note added in proof:* - The best description of the sugar is with C(4') out of the plane.

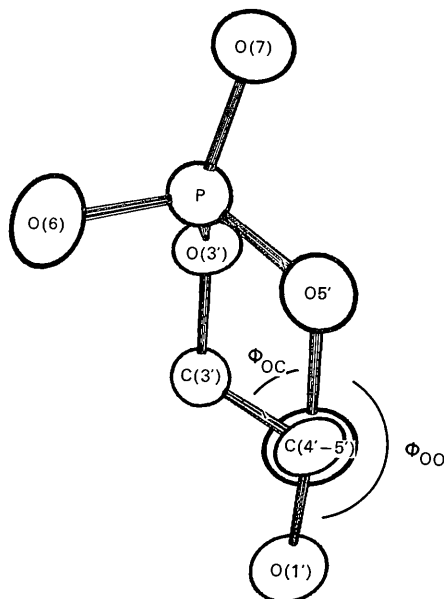


Fig. 9. A view down the C(5')-C(4') bond.

This research was supported by grants from the National Science Foundation (GB-8103) and the National Institutes of Health (FR-5367). I thank Dr Rammner for the crystals, Dr Everly Fleischer for the use of his diffractometers, and Dr Sundaralingam for making available his unpublished results. Dr Paul Moore, Stuart Hawkinson and Marsha Greaves provided helpful technical assistance.

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