

The Crystal and Molecular Structure of 2,5'-Anhydro-2',3'-isopropylidene Cyclouridine*

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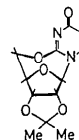
The compound 2,5'-anhydro-2',3'-isopropylidene cyclouridine crystallizes in the space group $P2_1$ with $Z=2$; the unit-cell dimensions are $a=10.344$ (1), $b=6.407$ (1), $c=9.077$ (1) Å and $\beta=93.69$ (1)°. The intensity data were collected on a four-circle diffractometer with Mo $K\alpha$ radiation and a graphite monochromator; 1645 reflexions with $2.5^\circ \leq 2\theta \leq 60^\circ$ had intensities $> 3\sigma$. In the initial attempt to solve the structure by direct methods, an E map was computed which contained a fragment of the molecule in an incorrect position in the unit cell. The portion of the molecule was translated to its correct position by use of a difference function [Delbaere & James (1973), *Acta Cryst.* B29, 404–407]. The complete structure was refined to an R index of 0.034 (weighted R 0.044). The ribose ring conformation is C(2')-*exo*, C(3')-*exo* with displacements of 0.852 (2) and 0.979 (2) Å, respectively, from the plane through C(1'), O(1') and C(4'). The best four-atom mean plane (planar within 0.06 Å) of the ribose ring is through the four carbon atoms with O(1') displaced 0.522 (2) Å from this plane on the opposite side of C(5'). The glycosidic torsion angle O(1')–C(1')–N(1)–C(6) is 246.4° and therefore falls in the *syn* region. The final difference electron density map contains an unusual bonding-electron-density distribution which correlates with anomalous [to that expected for a simple Karplus (*J. Chem. Phys.* (1959) 30, 11–15) relationship] ^{13}C -vicinal ^1H coupling constants obtained from the solution ^{13}C magnetic resonance spectrum of the compound with ^{13}C -enriched at the uracil ring 2-position.

Introduction

The use of ^{13}C n.m.r. spectroscopy as a probe into molecular conformations in solution studies has been a subject of much interest in recent years. Measurement of the ^{13}C -vicinal ^1H coupling constants of a number of uracil and uracil nucleoside derivatives (^{13}C -enriched at the 2-position of the uracil ring) has shown that an approximate Karplus (1959) relationship exists between these coupling constants and the relevant torsional angles estimated from molecular models (Lemieux, Nagabhushan & Paul, 1972). Crystal structure determinations of two *rigid* cyclonucleosides that were used in this solution n.m.r. study were undertaken to obtain accurate torsional angles for these compounds in order to provide experimental verification of this Karplus relationship. Furthermore, the maximum observed coupling constant, 8.7 Hz, in the spectrum of the title compound did not seem to correspond to the expected torsional angle of $\sim 160^\circ$ and appeared anomalous. A short communication describing the essential results correlating the n.m.r. work and the X-ray results has been published (Delbaere, James & Lemieux, 1973).

As a consequence of the polycyclic fused-ring nature of these two nucleoside derivatives one would expect that the torsional angles observed in the crystalline state would remain essentially unchanged in solution. In fact, this lack of conformational flexibility was demonstrated in the crystal structure determination of the first of these compounds, 2,2'-anhydro-1- β -D-*ara*-

bino-furanosyl uracil (Delbaere & James, 1973*a*) in which the two crystallographically independent molecules, although having differing crystalline environments, had essentially the same conformation. This paper describes the results of the X-ray structure analysis of the second compound, 2,5'-anhydro-2',3'-isopropylidene cyclouridine:



In addition to providing accurate torsional angles, this work would also reveal the effects of 2',3'-isopropylidene formation and cyclization on the ribose moiety of the molecule. Hydrogen bonding, which might be expected to affect the conformational properties in the crystalline state, is not possible in this cyclonucleoside.

Experimental

The sample of 2,5'-anhydro-2',3'-isopropylidene cyclouridine was given to us by Dr R. U. Lemieux of the Chemistry Department, University of Alberta. Recrystallization was accomplished by slow vapour diffusion of ether into a dry methanolic solution of the compound. Preliminary oscillation and Weissenberg photographs showed $2/m$ symmetry with the only systematic absence $0k0$; $k=2n+1$. The crystal density was measured by flotation in a mixture of bromobenzene and chlorobenzene. The unit-cell dimensions were determined from the accurately centred 2θ values of 12 reflexions in the range $35^\circ < 2\theta < 45^\circ$ ($\lambda=0.70926$ Å,

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Mo $K\alpha_1$) by a least-squares procedure. The unit-cell dimensions and other pertinent crystal data are included in Table 1.

Table 1. *Crystal data for 2,5'-anhydro-2',3'-isopropylidene cyclouridine*

Molecular formula: $C_{12}H_{14}N_2O_5$	Cell dimensions:
M.W. 266.3	$a = 10.344$ (1) Å
$d_{obs} = 1.46$ g cm $^{-3}$	$b = 6.407$ (1)
$d_{calc} = 1.47$ g cm $^{-3}$	$c = 9.077$ (1)
Crystal system: monoclinic	$\beta = 93.69$ (1)°
Space group: $P2_1$	$V = 600.3$ Å 3
Linear absorption coefficient (Mo $K\alpha$):	$Z = 2$
$F(000) = 270e$	1.2 cm $^{-1}$
2θ range explored: $2.5^\circ \leq 2\theta \leq 60^\circ$	
Temperature during data collection: $20 \pm 2^\circ C$	

The intensity data were collected from a crystal measuring $0.2 \times 0.4 \times 0.2$ mm with a Picker FACS-1 diffractometer and a θ - 2θ scan. The scan speed was 2° min^{-1} over a scan range of $(1.5 + 0.692 \tan \theta)^\circ$ and 10 s fixed-position background counts were measured. The data were collected in the range $2.5 \leq 2\theta \leq 60^\circ$ with Mo $K\alpha$ radiation ($\lambda = 0.71069$ Å) monochromatized from a highly-oriented graphite crystal. The total number of reflexions measured in this range was 1899 and of these 1645 (87%) had net intensities greater than $3\sigma(I)$. The estimated standard deviation in intensity was calculated from $\sigma(I) = \sqrt{[T + t^2B + (0.04I)^2]}$,

where T is the total peak count, B is the total background count and t is the ratio of the peak scan time to the total background time. Lorentz and polarization corrections were applied but no absorption corrections were made since the calculated transmission factors, for all of the reflexions, differed by less than 0.2%.

Structure determination and refinement

The observed structure amplitudes were converted to E 's by means of a Wilson (1942) plot. The overall isotropic temperature factor B is 2.93 Å 2 . The starting set of reflexions was

h	k	l	E	Phase
5	0	7	2.5	0
2	0	5	2.4	0
$\overline{10}$	1	1	2.6	0
$\overline{5}$	4	5	2.4	a
$\overline{7}$	2	5	2.2	b
$\overline{2}$	5	0	2.7	c
$\overline{3}$	4	7	2.2	d

Symbolic addition by hand (Karle & Karle, 1966) on a Σ_2 listing was employed using the relationship

$$\varphi_h \simeq \langle \varphi_k + \varphi_{h-k} \rangle_{kr}$$

The most commonly occurring relationships between symbols were $2b = a = c = \pi + d$ and $2b = \pi + a = \pi + c = d$. Setting $b = \pi/4$, $3\pi/4$ radians fixes the enan-

Table 2. *Atomic parameters and their estimated standard deviations (e.s.d.)*

(a) Non-hydrogen atoms (parameters $\times 10^4$)

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

	x/a	y/b	z/c	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
O(2)	6678 (1)	4158 (4)	1424 (2)	71 (1)	188 (4)	127 (2)	17 (2)	33 (1)	-24 (2)
O(4)	3330 (2)	1874 (4)	3950 (2)	92 (1)	204 (5)	151 (2)	-21 (2)	28 (1)	12 (3)
O(1')	6390 (1)	8749 (4)	806 (2)	65 (1)	241 (4)	88 (2)	22 (2)	5 (1)	26 (2)
O(2')	8466 (1)	9502 (4)	3420 (2)	61 (1)	244 (5)	100 (2)	-35 (2)	22 (1)	-31 (2)
O(3')	9233 (1)	9172 (4)	1164 (1)	69 (1)	219 (4)	91 (2)	-13 (2)	22 (1)	15 (2)
N(1)	5481 (1)	6609 (0)	2620 (2)	49 (1)	143 (4)	91 (2)	3 (2)	11 (1)	-11 (2)
N(3)	4986 (2)	3013 (4)	2646 (2)	66 (1)	150 (4)	98 (2)	2 (2)	6 (1)	-8 (2)
C(2)	5662 (2)	4571 (4)	2241 (2)	55 (1)	156 (4)	88 (2)	13 (2)	7 (1)	-18 (3)
C(4)	3947 (2)	3360 (4)	3518 (2)	59 (2)	173 (5)	82 (2)	-3 (2)	0 (1)	0 (3)
C(5)	3647 (2)	5514 (4)	3843 (2)	51 (1)	190 (5)	96 (2)	11 (2)	12 (1)	-2 (3)
C(6)	4412 (2)	7048 (4)	3409 (2)	50 (1)	162 (5)	95 (2)	15 (2)	9 (1)	-14 (3)
C(1')	6411 (2)	8297 (4)	2322 (2)	51 (1)	145 (4)	88 (2)	4 (2)	9 (1)	4 (3)
C(2')	7831 (2)	7718 (4)	2801 (2)	51 (1)	175 (5)	72 (2)	-5 (2)	7 (1)	7 (2)
C(3')	8456 (2)	7356 (4)	1321 (2)	53 (1)	193 (5)	87 (2)	3 (2)	17 (1)	1 (3)
C(4')	7304 (2)	7368 (5)	192 (2)	68 (2)	270 (6)	70 (2)	2 (3)	12 (1)	0 (3)
C(5')	6735 (2)	5211 (5)	5 (2)	85 (2)	301 (8)	98 (2)	-23 (3)	26 (2)	-58 (4)
C(6')	9592 (2)	9862 (5)	2617 (2)	56 (2)	196 (5)	107 (2)	-12 (2)	15 (1)	11 (3)
C(7')	9842 (3)	12187 (5)	2581 (4)	101 (2)	210 (7)	169 (4)	-24 (4)	35 (3)	6 (3)
C(8')	10732 (2)	8630 (6)	3285 (3)	62 (2)	303 (8)	160 (3)	-5 (3)	-7 (2)	46 (5)

(b) Hydrogen atoms (coordinates $\times 10^3$)

	x/a	y/b	z/c	B_{iso}		x/a	y/b	z/c	B_{iso}
H(5)	292 (2)	577 (5)	437 (3)	3.4 (5)	H(5'')	584 (2)	531 (5)	-47 (3)	3.4 (5)
H(6)	424 (2)	844 (5)	359 (3)	2.6 (4)	H(7'')	1012 (4)	1289 (9)	349 (5)	8.7 (12)
H(1')	603 (2)	951 (5)	281 (2)	2.7 (4)	H(7''')	1039 (3)	1262 (7)	183 (4)	6.1 (8)
H(2')	789 (2)	656 (5)	351 (2)	2.6 (4)	H(7''')	903 (4)	1303 (10)	237 (5)	8.9 (12)
H(3')	895 (2)	614 (4)	128 (3)	2.4 (4)	H(8'')	1098 (3)	925 (8)	432 (4)	6.8 (9)
H(4')	750 (2)	783 (5)	-71 (3)	2.9 (5)	H(8''')	1149 (3)	903 (7)	271 (3)	5.4 (7)
H(5')	730 (3)	434 (6)	-58 (3)	4.7 (6)	H(8''')	1056 (3)	714 (7)	326 (4)	5.6 (8)

tiomorph and gives four possible solutions. A chemically reasonable unit of 17 atoms was chosen from the E map of one of these solutions but attempted least-squares refinement of the structure was unsuccessful. The fragment of the molecule was incorrectly located in the unit cell; the vector shift required to bring this portion to its correct position was determined by use of a difference function proposed by Karle (1972). The

details involved in this method have been published (Delbaere & James, 1973*b*).

The phase values of the reflexions having $E_{\min} \geq 1.7$ and $|F_c| \geq 0.5|F_{\text{obs}}|$ for this correctly shifted molecular fragment were used as a starting point in the tangent formula; all of the non-hydrogen atoms of the molecule were located in the resulting E map. The initial structure factor calculation had a residual R of 0.187;

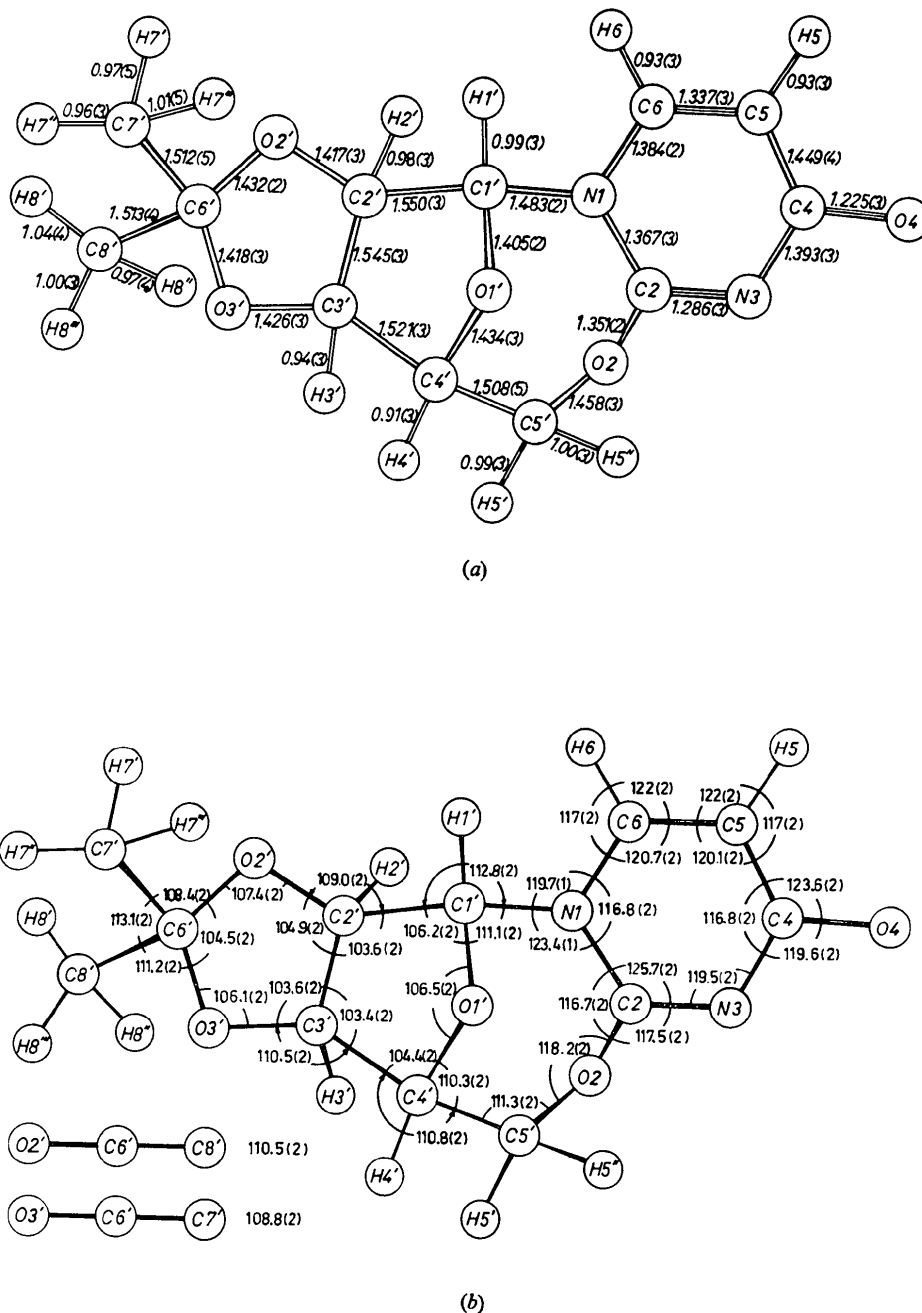


Fig. 1. (a) Bond distances found in the crystal structure of 2,5'-anhydro-2',3'-isopropylidene cyclouridine with the estimated standard deviations in parentheses. (b) Bond angles for 2,5'-anhydro-2',3'-isopropylidene cyclouridine as determined in the crystal structure.

$R = \sum ||F_o| - |F_c|| / \sum |F_o|$. The data with $I > 3\sigma(I)$ were used in this and subsequent least-squares cycles. Three cycles of full-matrix isotropic refinement gave $R = 0.099$ and a subsequent difference map revealed the hydrogen-atom positions. The function $\sum w(|F_o| - k|F_c|)^2$ was minimized. All of the atoms included in the structure factor calculation gave an R index of 0.083 and two further cycles of full-matrix isotropic refinement lowered R to 0.078. With anisotropic temperature factors for the non-hydrogen atoms and isotropic B values for the hydrogen atoms, two more cycles of full-matrix least-squares refinement converged to $R = 0.034$ (weighted $R_w = 0.044$). The ratios of the largest and the average shift/e.s.d. for the parameters in this last cycle were 0.16 and 0.02 respectively, for the non-hydrogen atoms and 0.48 and 0.08, respectively, for the hydrogen atoms. Initially, unit weights were employed in the refinement and after two least-squares cycles, observational weights [$w = 2F_o/\sigma(I)$] were used. The intense low-angle reflexions 002, 101, 111, $\bar{1}02$ and 202 were removed from the data set and the final cycles of refinement, since they appeared to suffer from secondary extinction. A secondary extinction correction was then applied as: $F_{\text{calc}}(\text{corrected}) = F_{\text{calc}} / (1 + C\beta I_{\text{obs}})$, according to Zachariasen (1963). The extinction parameter C was varied and converged to a value of $1.3 (1) \times 10^{-7}$ in two further cycles of full-matrix least-squares refinement; all of the data with $I > 3\sigma(I)$ were included in this refinement and the resulting R index was 0.033. The secondary extinction correction improved the agreement between the observed and calculated structure amplitudes for the very intense reflexions; the largest change, due to the extinction correction, was a 14% decrease in the F_{calc} of the 202 reflexion. Since the refined atomic parameters were within one e.s.d. of their values before this correction was applied, the structure factor table and the results quoted in this paper are based on the parameters which were obtained prior to the secondary extinction correction.* The scattering factors of Cromer & Mann (1968) were used for the non-hydrogen atoms and that of Stewart, Davidson & Simpson (1965) was used for the hydrogen atoms. The carbon, nitrogen and oxygen curves were corrected for anomalous scattering (Cromer & Liberman, 1970). The NRC Crystallographic Programs (Ahmed, Hall, Pippy & Huber, 1966) and ORFLS program (Busing, Martin & Levy, 1962) were used in the computations.

Results and discussion

The final atomic parameters and their estimated standard deviations are given in Table 2. The bond lengths and angles are in Fig. 1(a) and (b), respectively, and the torsion angles around the ribose ring are given as

* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 30358 (19 pp.). Copies may be obtained through the Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Newman projections in Fig. 2. A stereo drawing of the molecule is in Fig. 3. The absolute configuration of the molecule was not determined in this study but the tabulated atomic parameters correspond to the conventional β -D-ribose configuration.

Ribose-uracil linkages and the uracil ring

According to the definition of Sundaralingam (1969), the glycosidic torsion angle, χ_{CN} , O(1')-C(1')-N(1)-C(6) is 246.4° . The seven-membered ring, C(1')-O(1')-C(4')-C(5')-O(2)-C(2)-N(1), adopts a chair conformation at this value of χ_{CN} . The second possible value, $\chi_{\text{CN}} \sim 180^\circ$ would make the seven-membered ring boat-shaped with an eclipsed conformation about the C(4')-C(5') bond, i.e. the C(4')-O(1') bond would eclipse the C(5')-O(2) bond. In addition to steric repulsions, such a conformation would also result in a large electrostatic repulsion between a lone pair of electrons on atom O(1') and a lone pair of electrons on atom O(2). Thus, this latter conformation would be energetically less favoured than that found in the present crystal structure.

Although χ_{CN} falls close to that occurring in other *syn* (Donohue & Trueblood, 1960) nucleosides and nucleotides (Sundaralingam, 1969; Arnott & Hukins, 1972), the 2,5'-anhydro-cyclization must considerably restrict the range of this torsion angle. The 2,2'-anhydro cyclization limits χ_{CN} in 2,2'-anhydro-1- β -D-arabino-furanosyl uracil (Delbaere & James, 1973a) and in 2,2'-anhydro-1- β -D-arabino-furanosyl cytosine hydrochloride (Brennan & Sundaralingam, 1973); $\chi_{\text{CN}} = 294.5^\circ$ and 290.0° for the two molecules in the asymmetric unit of the former compound and $\chi_{\text{CN}} = 299^\circ$ in

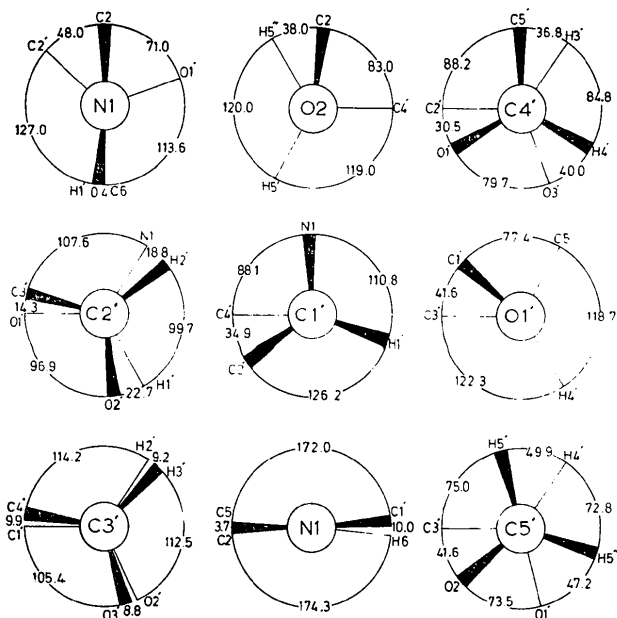


Fig. 2. Conformational angles for the ribose ring shown in Newman projections.

the latter. The glycosidic bond length, C(1')–N(1), of 1.483 (2) Å is similar to the average value of 1.476 Å quoted for other ribofuranose derivatives (Voet & Rich, 1970). The formation of the 2,5'-anhydro derivative of uridine causes a keto form of the uracil ring to shift to the enol tautomer. This tautomeric shift is similar to that in 2,2'-anhydro-1- β -D-arabino-furanosyl uracil and the corresponding bond distances are reasonably similar. However, the bond angles involving the sugar–uracil linkages, in these two cyclonucleosides, differ greatly owing to the fact that there is a seven-membered ring in the former compound, but a five-membered ring in the latter, which is fused to the uracil ring.

Although the internal angles at N(1), C(2) and N(3) in the uracil ring [see Fig. 1(b)] differ greatly from that found in most uracil residues whose crystal structures have been determined (Voet & Rich, 1970), the uracil ring is within 0.03 Å of being planar (Table 3). The other bond angles subtended at these atoms also change to maintain this near-planar configuration. The greatest change in bond angle, compared to the average values found in other uracil residues, is +10.3° in the N(1)–C(2)–N(3) bond angle; this value is similar to the 11.2° and 11.9° increase in the same bond angle in the two independent molecules of 2,2'-anhydro-1- β -D-arabino-furanosyl uracil.

Ribose ring conformation

The bond distances and angles for the ribose moiety are similar to those found in related structures (Arnott & Hukins, 1972; James & Matsushima, 1973) except for three bond angles. The C(1')–O(1')–C(4') angle of 106.5 (2)° is 3–4° smaller than usual. The O(2')–C(2')–C(3') and O(3')–C(3')–C(2') angles of 104.9 (2)° and 103.6 (2)° are also smaller than the average owing to the isopropylidene formation. The isopropylidene

formation also causes the very close O(2')...O(3') contact of 2.253 (2) Å with an almost eclipsed conformation about the C(2')–C(3') bond (Fig. 2). This conformation also occurs in the X-ray structure of uridine 2',3'-O,O-cyclophosphorothioate (Saenger & Eckstein, 1969). In the latter the C(2')C(3')O(3') and O(2') atoms are planar with the phosphorus atom displaced 0.23 Å from the plane. In 2,5'-anhydro-2',3'-isopropylidene cyclouridine the best four-atom plane (within 0.05 Å of being planar, Table 3) is through these same atoms and C(6') is displaced 0.495 (2) Å from this mean plane.

The conformation of the ribose ring referred to the C(1')–O(1')–C(4') plane is C(2')-*exo*, C(3')-*exo* with displacements of 0.852 (2) and 0.979 (2) Å from this plane on the opposite side of C(5'). The large values of both of these latter distances are also due to the isopropylidene formation. A deoxyribose ring system which has the same ring puckering occurs in deoxycytidine 5'-phosphate monohydrate (Viswamitra, Reddy, Lin & Sundaralingam, 1971); the corresponding displacements are 0.222 Å for C(2') and 0.647 Å for C(3') in this nucleotide. The arabinose ring in both of the molecules of 2,2'-anhydro-1- β -D-arabino-furanosyl uracil (Delbaere & James, 1973a) also possess the same ring puckering; the displacements of C(2') and C(3') are 0.366 (2) and 0.675 (2) Å, respectively for one molecule and 0.237 (2) and 0.688 (2) Å, respectively, for the other. The best four-atom plane of the ribose ring in the 2,5'-anhydro cyclouridine is through the carbon atoms (within 0.06 Å of being planar, Table 3) with O(1') 0.522 (2) Å [on the opposite side of C(5')] from this mean plane.

The conformation about the C(4')–C(5') bond is the usual *gauche-gauche* (Shefter & Trueblood, 1965) type with $\varphi_{OO} = +73.5^\circ$ and $\varphi_{OC} = -41.6^\circ$. With regard to the methyl groups, there is a staggered conformation about the C(6')–C(7') and C(6')–C(8') bonds (see Fig. 3).

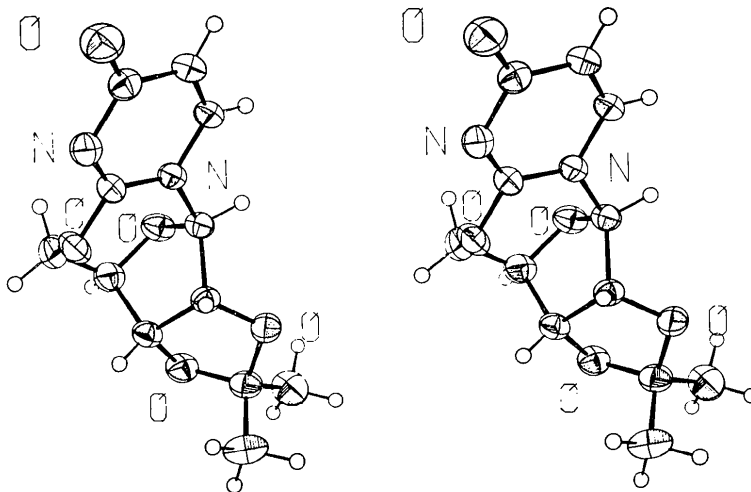


Fig. 3. Stereoscopic drawing of the molecule of 2,5'-anhydro-2',3'-isopropylidene cyclouridine as determined in the crystal structure. The thermal ellipsoids include 50% probability and the hydrogen atoms were given temperature factors of 0.7 Å². The program ORTEP (Johnson, 1965) was used for this drawing and for Fig. 4.

Table 3. *Least-squares planes*

(a) Ribose

	Distance from plane		χ^2
	Plane I	Plane II	
C(1')	0.0* Å	0.040 (3)* Å	
C(2')	0.852 (2)	-0.060 (3)*	
C(3')	0.979 (2)	0.060 (3)*	
C(4')	0.0*	-0.041 (3)*	
O(1')	0.0*	0.522 (2)	
C(5')	-1.380 (3)	-1.480 (3)	
r.m.s. Δ	-	0.051	
I	0.7126X + 0.6787Y + 0.1779Z - 8.6105 = 0		-
II	0.2202X + 0.9659Y + 0.1361Z - 6.2385 = 0		1390.8

(b) Isopropylidene ring

	Distance from plane
C(2')	-0.049 (2)* Å
C(3')	0.049 (2)*
O(2')	0.034 (2)*
O(3')	-0.034 (2)*
C(6')	0.495 (2)
r.m.s. Δ	0.042

Equation of the plane:

$$0.7345X - 0.5326Y + 0.4205Z - 4.3119 = 0 \quad \chi^2 \quad 1938.8$$

(c) Uracil ring

	Distance from plane
N(1)	0.032 (2)* Å
C(2)	-0.024 (2)*
N(3)	-0.010 (2)*
C(4)	0.033 (2)*
C(5)	-0.024 (2)*
C(6)	-0.008 (2)*
O(2)	-0.036 (2)
O(4)	0.095 (2)
C(1')	0.228 (2)
H(5)	-0.052 (26)
H(6)	-0.053 (24)
r.m.s. Δ	0.024

Equation of the plane:

$$0.5339X - 0.0923Y + 0.8405Z - 4.5170 = 0 \quad \chi^2 \quad 1159.3$$

* Indicates atoms which are included in calculating the least-squares plane.
X, Y and Z refer to the orthogonal coordinate system (Å units) with X along a, Y along b and Z along c*.

Molecular packing

Fig. 4 is a stereo view along the *b* axis of the unit cell. The intermolecular contacts less than 3.5 Å between non-hydrogen atoms are given in Table 4. Several close contacts occur between molecules involving both base-base and ribose-base interactions. These interactions are shown in Fig. 5 which is a projection onto the mean plane of the uracil ring of the molecule in the centre. The molecule on the left is below this plane whereas that on the right is above. As is common with uracil derivatives (Bugg, Thomas, Sundaralingam & Rao, 1971), the overlap of the bases involves a single carbonyl oxygen atom. The electronegative oxygen atom forms close contacts with the aromatic ring system of the base of another molecule (Table 4). The angle between the two base planes is 10.6°. There are also interactions between the edges of base planes along the *y*

axis; the uracil ring of each molecule forms close contacts with the uracil ring of the molecule above and below.

Table 4. *Intermolecular non-bonded contacts*

O(4)···C(6')	3.239 (3) Å
O(4)···N(1')	3.277 (2)
C(4)···C(6')	3.280 (3)
O(4)···C(2'')	3.300 (3)
O(4)···C(6''')	3.335 (4)
N(3)···C(1''')	3.383 (3)
O(1')···N(3''')	3.390 (2)
O(1')···C(5''')	3.400 (3)
C(4')···N(3''')	3.410 (3)
C(5')···N(3''')	3.411 (3)
O(1')···C(2''')	3.418 (2)
O(4)···C(8''')	3.420 (4)
C(5)···O(2')	3.474 (2)
O(4)···C(1')	3.498 (3)
O(4)···H(6''')	2.43 (3)
N(3)···H(1''')	2.49 (3)
H(5')···H(8''')	2.38 (4)

Symmetry code

I	1 - x	-½ + y	1 - z
II	x	-1 + y	z
III	1 - x	½ + y	-z
IV	-1 + x	-1 + y	z
V	2 - x	-½ + y	-z

Final difference electron density

Two difference electron density maps were computed. The first map included the data with $I > 3\sigma(I)$ except those five reflexions which were deemed suffering most from secondary extinction effects (see above). The second computation was carried out on all data with $I > 3\sigma(I)$ subsequent to the refinement including the secondary extinction correction. Both maps have very similar features; in particular the positions and heights of the peaks corresponding to bonding electron densities between atomic sites were identical on both maps. The major difference between the two maps is associated

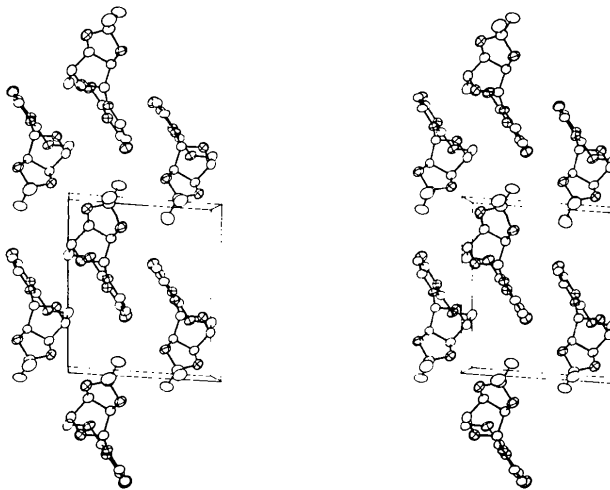


Fig. 4. Stereoscopic packing diagram projected along the *b* axis of the unit cell.

with the size of the negative region in the centre of the uracil ring. In the map which included the correction for secondary extinction in F_{calc} this negative region has a value of $-0.13 \text{ e } \text{Å}^{-3}$ whereas the corresponding region on the map which excluded the contributions of the five reflexions had a minimum of $-0.17 \text{ e } \text{Å}^{-3}$.

The section of the second difference electron density map through the uracil ring and exocyclic atoms is in Fig. 6. A skeletal outline of these atoms is superimposed on the map. The O(2)–C(5') bond is indicated by a dashed line since C(5') is displaced below the plane of the drawing. The e.s.d. of the electron density in this map is $0.025 \text{ e } \text{Å}^{-3}$ calculated by the Cruickshank (1967) formula. Positive contours start at $0.09 \text{ e } \text{Å}^{-3}$ (negative contours at $-0.09 \text{ e } \text{Å}^{-3}$) and the contour interval is $0.02 \text{ e } \text{Å}^{-3}$. Negative contours are denoted as broken curves.

A comparison of the residual electron density associated with the uracil ring in 2,5'-anhydro-2',3'-isopropylidene cyclouridine can be made with that residual density observed in the uracil ring of 2,2'-anhydro-1- β -D-arabino-furanosyl uracil (Delbaere & James, 1973a). This comparison must be made with caution; for the two molecules in the asymmetric unit of the latter compound exhibit differences in their environments

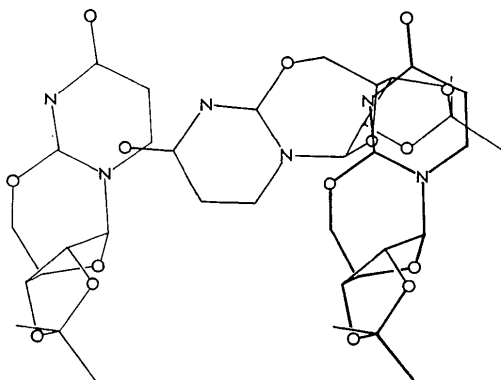


Fig. 5. Projection onto the mean plane of the uracil ring of the molecule in the centre. The molecule on the left is below this plane whereas that on the right is above.

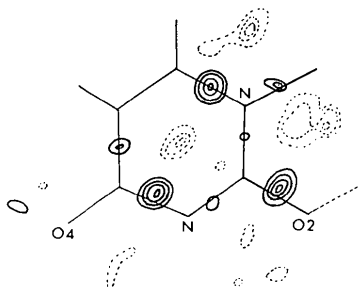


Fig. 6. A section of the final difference map of 2,5'-anhydro-2',3'-isopropylidene cyclouridine. The features shown commence at $0.09 \text{ e } \text{Å}^{-3}$ (also at $-0.09 \text{ e } \text{Å}^{-3}$) and the contour interval is $0.02 \text{ e } \text{Å}^{-3}$. Negative contours are denoted as broken curves.

and in particular there are significant differences between the residual bonding electron densities associated with the two uracil rings. In this paper we choose to compare the residual electron density in the uracil ring of molecule *B* of 2,2'-anhydro-1- β -D-arabino-furanosyl uracil with the residual density in the uracil ring of the present compound, because there is no hydrogen bond to N(3) of the uracil ring in molecule *B*.

The standard deviations of residual electron density as calculated by Cruickshank's (1967) formula are comparable in magnitude for both structures. The differences that we shall discuss are greater than 3σ (the value of σ used in this comparison is $0.025/2$ or $0.035 \text{ e } \text{Å}^{-3}$) for both compounds except for one difference. Firstly, comparison of the residual electron density in the bonds N(1)–C(2) and C(2)–N(3) in both compounds shows that in 2,5'-anhydro-2',3'-isopropylidene cyclouridine there is less electron density in these bonds (differences of $0.12 \text{ e } \text{Å}^{-3}$ and $0.10 \text{ e } \text{Å}^{-3}$, respectively). Secondly there is an increase of $0.06 \text{ e } \text{Å}^{-3}$ in the electron density associated with the C(2)–O(2) bond in 2,5'-anhydro-2',3'-isopropylidene cyclouridine with respect to the corresponding bond of 2,2'-anhydro-1- β -D-arabino-furanosyl uracil. Whereas in the former two bonds [N(1)–C(2) and C(2)–N(3)] these differences are significant, the increase in peak height of $0.06 \text{ e } \text{Å}^{-3}$ for the C(2)–O(2) bond is only 2σ and is marginally significant. However, Fig. 6 does not portray the whole situation as the peak associated with this bond has a much larger volume than the other peaks associated with the other bonding electron densities in the uracil moiety. On the basis of these arguments and the comparison of these two structural studies, we propose that electron density is transferred mainly from the N(1)–C(2) and C(2)–N(3) bonds to the C(2)–O(2) bond of 2,5'-anhydro-2',3'-isopropylidene cyclouridine.

Distortions in electron density arising from the formation of a hydrogen bond have been shown by the very careful study of sucrose by Hanson, Sieker & Jensen (1972, 1973). Additional evidence for a shift in concentration of bonding electron density, from bonding orbitals to a lone-pair orbital on a nitrogen atom, on the formation of a hydrogen bond is provided by the structure of 2,2'-anhydro-1- β -D-arabino-furanosyl uracil (Delbaere & James, 1973a). The present work gives reasonable evidence of a shift in concentration of bonding electron density from bonding orbitals in the N(1)–C(2) and C(2)–N(3) bonds to the C(2)–O(2) bond. A possible explanation for this phenomenon may reside in the geometrical differences between the two compounds particularly in the region of the uracil-furanose linkages.

The ^{13}C n.m.r. spectra of these two cyclonucleosides (with ^{13}C -enriched at the uracil ring 2-position) indicate that the $^{13}\text{C}2$ -vicinal ^1H coupling constants and the relevant torsion angles of the 2,2'-anhydro compound follow a simple Karplus (1959) relationship whereas two $^{13}\text{C}2$ -vicinal ^1H coupling constants of the cyclouridine are anomalous to this curve (Delbaere, James

& Lemieux, 1973). The increased electron density for the C(2)–O(2) bond in the latter compound may account for the anomalously large ^{13}C -vicinal ^1H coupling constant, $^3J_{^{13}\text{C}2, \text{H}5'}$, which occurs across a three-bond system containing this bond, because coupling constants are considered to be transmitted by the bonding electrons (Ramsey & Purcell, 1952). Correspondingly, an anomalously small coupling constant for this compound, $^3J_{^{13}\text{C}2, \text{H}1'}$, occurs across a three-bond system from which much of this aforementioned electron density appears to be withdrawn, *i.e.* the N(1)–C(2) bond.

The following data from the X-ray analysis of 2,5'-anhydro-2',3'-isopropylidene cyclouridine suggest that there is considerable strain in the molecule: the C(2)–O(2)–C(5') bond angle of $118.2(2)^\circ$ is much larger than the tetrahedral value, C(2') and C(3') both have unusually large displacements [$0.852(2)$ and $0.979(2)$ Å, respectively] from the C(1')–O(1')–C(4') plane and there is a very close contact between O(2') and O(3') of $2.253(2)$ Å. In addition, the geometry of the molecule is such that there is possible overlap of electron density from atom C(2) and the antibonding orbital on atom C(5') which lies in the opposite direction to the C(5')–H(5') bond. The residual electron density associated with the C(2)–O(2) bond is extended in this direction (reaching a peak height of $0.17 \text{ e } \text{Å}^{-3}$). The coupling of $^{13}\text{C}(2)$ to H(5') could be transmitted through this system and the coupling of $^{13}\text{C}(2)$ to H(5') would not be affected. The strain in the molecule, and this possible overlap of orbitals, may lead to the abnormal residual electron densities which, in turn, may cause the coupling constants that are anomalous to the Karplus (1959) relationship.

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