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## The Structure of Dihydrothymidine\*

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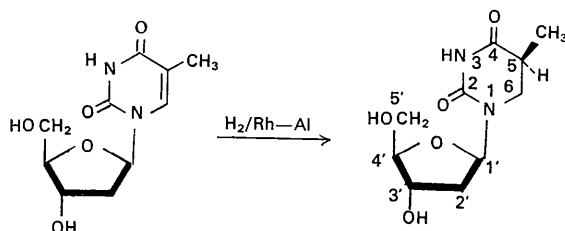
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Dihydrothymidine formed by the catalytic reduction of thymidine exists as only a single diastereoisomer. The six-membered ring in the dihydrothymine moiety has the half-chair conformation and the CH<sub>3</sub> group on C(5) is equatorial to the ring. In the furanose ring the four carbon atoms are coplanar while the O(1') atom is displaced by 0.42 Å from this plane. The O(5')H group in the sugar moiety is disordered with a 2:1 distribution. The material crystallizes in space group *P*2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> with *a* = 9.554 ± 0.001, *b* = 21.992 ± 0.002, *c* = 5.475 ± 0.001 Å and *Z* = 4. The structure was determined by application of the symbolic addition procedure for phase determination. The final *R* value was 5.1%.

### Introduction

The nucleoside thymidine is of particular interest since most radiation damage to deoxyribonucleic acid (DNA) involves the thymine moiety. Catalytic reduction of thymidine (Cohn & Doherty, 1956) yields dihydrothymidine:



From chemical and spectroscopic evidence, Kondo & Witkop (1968) have shown that in the above reaction only one diastereoisomer was produced and they have determined the stereoconfiguration about C(5). The purpose of this investigation was to confirm the configuration independently by means of a crystal struc-

ture analysis. The structure analysis has also shown that the OH group in the CH<sub>2</sub>OH chain of the sugar is disordered.

The irradiation by  $\gamma$ -rays of dihydropyrimidines in solution and in the solid state and the resulting conversion to unsaturated pyrimidines has been studied by Snipes & Bernhard (1968). A possibly similar effect of X-radiation on dihydrothymidine will be discussed.

### Experimental

Crystals of dihydrothymidine were supplied to us by Dr B. Witkop of the National Institutes of Health. From the first sample obtained, intensity data were collected along two axes with the multiple-film, equi-inclination Weissenberg technique. The intensities were estimated by visual comparison with a calibrated film strip. Corrections were made for Lorentz and polarization factors and spot size, the data from the two axes were cross-correlated, and normalized structure factors  $|E|$  were derived. These data were used for the phase determination from which the structure was derived. Least-squares refinement of the coordinates and thermal factors showed that the unit cell was a 1:1 mixture of dihydrothymidine and thymidine. Since a reaction caused by X-radiation was suspected, a crystal from a new sample of dihydrothymidine was used to collect the intensities of the *hkl* and  $\bar{h}kl$  reflec-

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tions in a four-circle automatic diffractometer using the  $\theta-2\theta$  technique with a  $2^\circ + 2\theta(\alpha_2) - 2\theta(\alpha_1)$  scan over  $2\theta$ . These data were corrected for Lorentz and polarization factors and absorption (program *ACACA*, Prewitt, 1966) and the  $hkl$  and  $\bar{h}kl$  data were averaged.

Space group, cell dimensions and other data for the crystals are listed in Table 1.

### Phase determination and refinement

Phases were determined by the symbolic addition procedure for noncentrosymmetric crystals (Karle & Karle, 1964, 1966). The phase determination was started with the relationship:

$$\varphi_h \approx \langle \varphi_k + \varphi_{h-k} \rangle_{kr} \quad (1)$$

To implement (1), three phases were assigned to specify the origin, and a fourth phase was assigned to specify the enantiomorph (Hauptman & Karle, 1956). During the determination it was found necessary to denote the phase of another reflection with a symbol. This symbol,  $p$ , may have any value between  $-\pi$  and  $\pi$ , Table 2. In this manner a total of 61 phases for reflections with  $E > 1.4$  were obtained. There were several indications that the value of  $p$  was near  $\pi$ , which proved to be correct.

Table 2. Phase assignments for specifying the origin and enantiomorph and implementing equation (1)

$h$	$\varphi_h$	$ E $
0 4 1	0	4.03
11 5 0	$+\pi/2$	4.47
8 1 0	0	4.71
0 9 2	$+\pi/2$	3.65
4 14 2	$p$	3.27

The initial set of 61 phases was refined and expanded with the tangent formula (Karle & Hauptman, 1956):

$$\tan \varphi_h \approx \frac{\sum |E_k E_{h-k}| \sin(\varphi_k + \varphi_{h-k})}{\sum |E_k E_{h-k}| \cos(\varphi_k + \varphi_{h-k})} \quad (2)$$

Phases for 333 reflections with  $|E| > 1.0$  were obtained from which a three-dimensional  $E$  map was computed. The maxima from the  $E$  map are shown in Fig. 1. The 16 largest peaks on the map correspond to 16 of the 17 non-hydrogen atoms. An oxygen atom of one of the

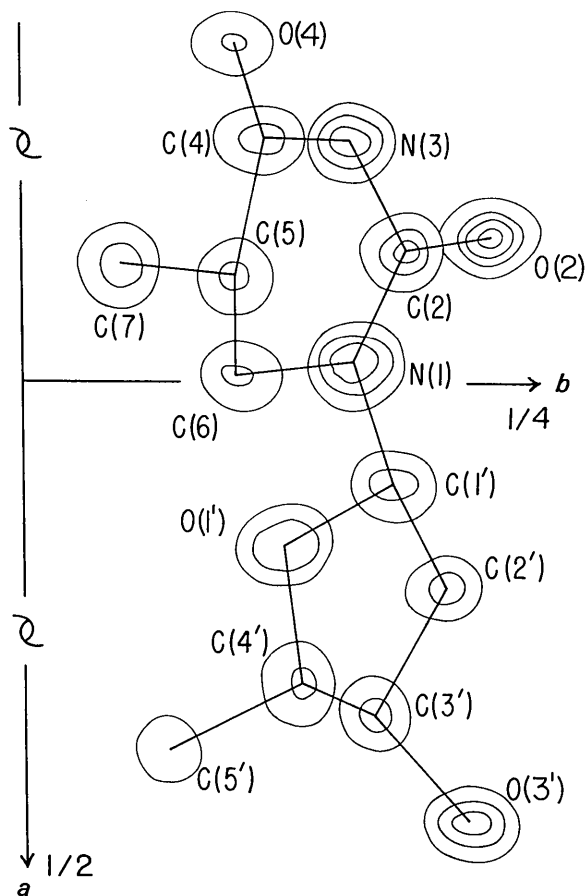


Fig. 1. Sections from the initial three-dimensional  $E$  map computed with 333 reflections with  $|E| > 1.0$  and phases determined by the symbolic addition procedure.

Table 1. Physical constants

	Thymidine-dihydrothymidine mixture	Dihydrothymidine
Molecular formula		$C_{10}O_5N_2H_{16}$
Molecular weight		244.4
Melting point		$155-156^\circ$
$[\alpha]_D^{23}$		$-20.5^\circ$
Habit		plates
Crystal size	plates { $0.4 \times 0.15 \times 1.0$ mm $0.6 \times 0.2 \times 0.2$ mm	$0.650 \times 0.104 \times 1.450$ mm
Space group	$P2_12_12_1$	$P2_12_12_1$
$a$	$9.55 \pm 0.02$ Å	$9.554 \pm 0.001$
$b$	$22.10 \pm 0.04$	$21.992 \pm 0.002$
$c$	$5.42 \pm 0.01$	$5.475 \pm 0.001$
$V$	$1143.9$ Å <sup>3</sup>	$1150.3$ Å <sup>3</sup>
$\rho_{calc}$		$1.419$ g.cm <sup>-3</sup>
Radiation	Cu $K\alpha$ , $1.5418$ Å	Cu $K\alpha$ , $1.5418$ Å
No. of independent reflections	1236	1188
Molecules per unit cell	4	4

hydroxyl groups was not found. The two highest peaks on a difference Fourier synthesis could be associated with a disordered hydroxyl oxygen atom, O(5').

Least-squares refinement on coordinates, anisotropic thermal parameters and occupancy for the two positions O(5') showed that the occupancy was 2:1 with the favored position in the *trans* conformation with respect to the C(3')-C(4') bond,  $\varphi_{CC} = 169^\circ$ , Fig. 2.

The refinement resulted in  $R = 10.5\%$ . An inspection of the bond lengths and angles showed a short C(5)-C(6) distance of 1.37 Å and bond angles about C(5) and C(6) intermediate between trigonal and tetrahedral, suggesting that the crystal was not composed of a single type of molecule. A closer inspection of the parameters indicated that the thermal ellipsoids of C(5), C(6), and O(2) were quite elongated normal to the plane determined by the rest of the dihydrothymine moiety, Fig. 2.

The two possibilities considered were that the crystal was composed of either a mixture of thymidine and dihydrothymidine or a mixture of two diastereoisomers formed by the hydrogenation of the C(5)-C(6) double bond. Molecules of a mixture of thymidine and dihydrothymidine or a mixture of diastereoisomers of dihydrothymidine may be nearly superimposed except for the atoms corresponding to C(5), C(6) and O(2). Similar crystallographic evidence for dihydrothymine (Furberg & Jensen, 1968) has been interpreted in terms of a mixture of D and L isomers.

The sample used for data collection was returned to The National Institutes of Health. Spectral analysis revealed the presence of double bonds in the irradiated crystal, although none had been present before irradiation. A least-squares refinement was carried out on a model comprised of thymidine and dihydrothymidine superimposed except for the atoms corresponding to C(5), C(6) and O(2). The parameters for the 15

superimposed atoms were taken from the previous refinement. The three atoms in question were split to give reasonable distances and angles for a saturated and unsaturated C(5)-C(6) bond and each split atom was given half weight. Alternate cycles of refinement were performed on the positional and anisotropic thermal parameters of the split atoms. To prevent the parameters from coalescing, it was necessary to refine the parameters for one type of molecule at a time. The multiplicity factors remained nearly  $\frac{1}{2}$  when included in the refinement, indicating a 1:1 mixture.

Data collection was repeated using a crystal from another sample of material. The second crystal proved to be entirely dihydrothymidine although the 2:1 disorder of O(5') in the sugar moiety remained. All hydrogen atoms except those on the disordered hydroxyl oxygen atom were located in a difference map. With the hydrogen atoms included as constant parameters, the resultant conventional  $R$  index was 5.1%. The full-matrix least-squares refinement was carried out on the function  $\sum w(|F_o| - |F_c|)^2$  where  $w = 0.5$  for  $F_o = 0$ ,  $w = 1.0$  for  $|F_o| < 10$ , and  $w = 15/|F_o|$  for  $|F_o| \geq 10$ . The atomic scattering factors used were those listed in *International Tables for X-ray Crystallography* (1963). Observed and calculated structure factors are given in Table 3. The fractional coordinates and thermal parameters for the heavy atoms are listed in Table 4 and the approximate coordinates for the hydrogen atoms are given in Table 5.

### Discussion

The mixture of thymidine and dihydrothymidine found in the first crystal suggested that the X-ray beam was causing the dihydrothymidine to decompose to thymidine. Such reactions with X-rays have been observed with dihydropyrimidines (Snipes & Bernhardt, 1968). The second crystal from a different sample, but pre-

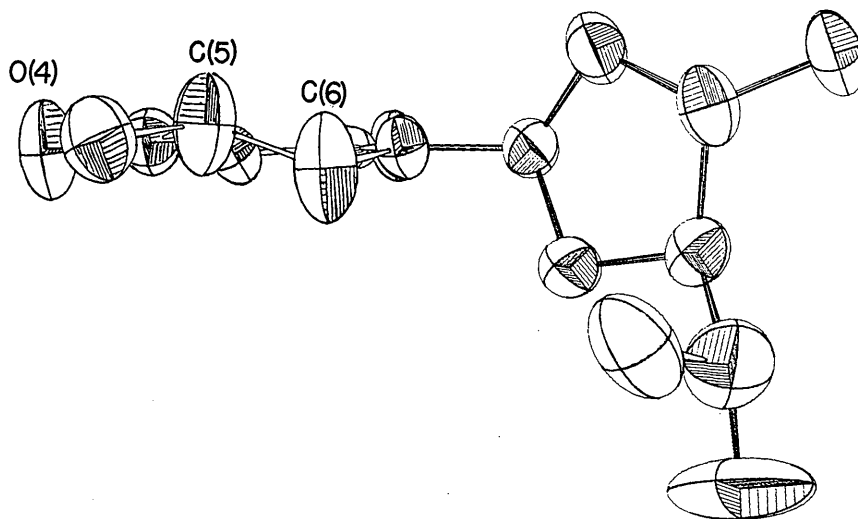


Fig. 2. An ORTEP drawing of the 'average' molecule in the thymidine-dihydrothymidine crystal.

Table 3. Observed and calculated structure factors

The columns are the index  $h, |F_o|, |F_c|$  and  $\phi$  (radians).

h	F <sub>o</sub>	F <sub>c</sub>	φ
0 0 L	4.9	4.0	-3.14
0 0 1	4.0	3.4	-0.00
0 1 1	1.3	0.6	-0.34
0 1 L	13.0	13.2	1.97
0 2 1	13.1	10.1	1.33
0 2 L	3.0	1.6	1.97
0 3 1	1.7	1.4	-1.97
0 3 L	19.3	10.8	1.00
0 4 1	3.9	3.9	-3.14
0 4 2	1.5	1.5	-0.00
0 4 3	4.0	4.0	-3.14
0 4 4	1.0	1.0	-3.14
0 4 5	4.2	4.1	0.00
0 4 6	3.0	3.0	-3.14
0 4 7	1.2	0.0	0.34
0 4 8	42.9	44.1	0.00
0 4 9	3.0	4.0	-3.14
0 4 10	1.2	0.0	0.34
0 4 11	34.2	14.8	-3.14
0 4 12	5.0	5.0	0.00
0 5 1	17.2	17.7	-1.97
0 5 2	10.7	10.3	-1.97
0 5 3	11.4	10.7	1.57
0 5 4	14.9	11.9	-1.97
0 5 5	4.0	4.0	0.00
0 5 6	2.0	1.0	-1.97
0 5 7	0.4	0.4	-1.97
0 6 1	13.3	12.9	-3.14
0 6 2	137.8	133.0	0.00
0 6 3	45.7	43.6	-3.14
0 6 4	18.7	18.0	-3.14
0 6 5	4.0	4.0	0.00
0 6 6	4.0	4.0	0.00
0 6 7	4.0	4.0	0.00
0 6 8	4.0	4.0	0.00
0 6 9	4.0	4.0	0.00
0 6 10	4.0	4.0	0.00
0 6 11	4.0	4.0	0.00
0 6 12	4.0	4.0	0.00
0 6 13	4.0	4.0	0.00
0 6 14	4.0	4.0	0.00
0 6 15	4.0	4.0	0.00
0 6 16	4.0	4.0	0.00
0 6 17	4.0	4.0	0.00
0 6 18	4.0	4.0	0.00
0 6 19	4.0	4.0	0.00
0 6 20	4.0	4.0	0.00
0 6 21	4.0	4.0	0.00
0 6 22	4.0	4.0	0.00
0 6 23	4.0	4.0	0.00
0 6 24	4.0	4.0	0.00
0 6 25	4.0	4.0	0.00
0 6 26	4.0	4.0	0.00
0 6 27	4.0	4.0	0.00
0 6 28	4.0	4.0	0.00
0 6 29	4.0	4.0	0.00
0 6 30	4.0	4.0	0.00
0 6 31	4.0	4.0	0.00
0 6 32	4.0	4.0	0.00
0 6 33	4.0	4.0	0.00
0 6 34	4.0	4.0	0.00
0 6 35	4.0	4.0	0.00
0 6 36	4.0	4.0	0.00
0 6 37	4.0	4.0	0.00
0 6 38	4.0	4.0	0.00
0 6 39	4.0	4.0	0.00
0 6 40	4.0	4.0	0.00
0 6 41	4.0	4.0	0.00
0 6 42	4.0	4.0	0.00
0 6 43	4.0	4.0	0.00
0 6 44	4.0	4.0	0.00
0 6 45	4.0	4.0	0.00
0 6 46	4.0	4.0	0.00
0 6 47	4.0	4.0	0.00
0 6 48	4.0	4.0	0.00
0 6 49	4.0	4.0	0.00
0 6 50	4.0	4.0	0.00
0 6 51	4.0	4.0	0.00
0 6 52	4.0	4.0	0.00
0 6 53	4.0	4.0	0.00
0 6 54	4.0	4.0	0.00
0 6 55	4.0	4.0	0.00
0 6 56	4.0	4.0	0.00
0 6 57	4.0	4.0	0.00
0 6 58	4.0	4.0	0.00
0 6 59	4.0	4.0	0.00
0 6 60	4.0	4.0	0.00
0 6 61	4.0	4.0	0.00
0 6 62	4.0	4.0	0.00
0 6 63	4.0	4.0	0.00
0 6 64	4.0	4.0	0.00
0 6 65	4.0	4.0	0.00
0 6 66	4.0	4.0	0.00
0 6 67	4.0	4.0	0.00
0 6 68	4.0	4.0	0.00
0 6 69	4.0	4.0	0.00
0 6 70	4.0	4.0	0.00
0 6 71	4.0	4.0	0.00
0 6 72	4.0	4.0	0.00
0 6 73	4.0	4.0	0.00
0 6 74	4.0	4.0	0.00
0 6 75	4.0	4.0	0.00
0 6 76	4.0	4.0	0.00
0 6 77	4.0	4.0	0.00
0 6 78	4.0	4.0	0.00
0 6 79	4.0	4.0	0.00
0 6 80	4.0	4.0	0.00
0 6 81	4.0	4.0	0.00
0 6 82	4.0	4.0	0.00
0 6 83	4.0	4.0	0.00
0 6 84	4.0	4.0	0.00
0 6 85	4.0	4.0	0.00
0 6 86	4.0	4.0	0.00
0 6 87	4.0	4.0	0.00
0 6 88	4.0	4.0	0.00
0 6 89	4.0	4.0	0.00
0 6 90	4.0	4.0	0.00
0 6 91	4.0	4.0	0.00
0 6 92	4.0	4.0	0.00
0 6 93	4.0	4.0	0.00
0 6 94	4.0	4.0	0.00
0 6 95	4.0	4.0	0.00
0 6 96	4.0	4.0	0.00
0 6 97	4.0	4.0	0.00
0 6 98	4.0	4.0	0.00
0 6 99	4.0	4.0	0.00
0 6 100	4.0	4.0	0.00



Table 4. *Fractional coordinates and thermal parameters\* with standard deviations*

	<i>x</i>	<i>y</i>	<i>z</i>	$\beta_{11}$	$\beta_{22}$	$\beta_{33}$	$\beta_{12}$	$\beta_{13}$	$\beta_{23}$
N(1)	-0.0269 (3)	0.1533 (1)	0.3945 (5)	34 (3)	16 (1)	230 (13)	2 (1)	8 (5)	15 (3)
C(2)	-0.1438 (3)	0.1779 (1)	0.2332 (7)	50 (3)	13 (1)	246 (12)	0 (1)	5 (6)	-2 (3)
O(2)	-0.1613 (3)	0.2185 (1)	0.0373 (5)	64 (3)	17 (1)	352 (11)	5 (1)	14 (5)	30 (2)
N(3)	-0.2577 (3)	0.1547 (1)	0.3533 (7)	34 (3)	18 (1)	310 (12)	2 (1)	1 (6)	8 (3)
C(4)	-0.2733 (4)	0.1177 (2)	0.5545 (8)	65 (4)	21 (1)	239 (14)	-1 (2)	28 (7)	9 (3)
O(4)	-0.3810 (3)	0.1010 (2)	0.6410 (7)	62 (3)	43 (1)	527 (17)	-5 (2)	63 (7)	64 (4)
C(5)	-0.1283 (4)	0.1922 (2)	0.6599 (8)	79 (4)	21 (1)	273 (14)	-2 (2)	2 (8)	7 (3)
C(6)	-0.0229 (4)	0.0930 (2)	0.4576 (8)	66 (4)	20 (1)	342 (15)	5 (2)	12 (8)	25 (3)
C(7)	-0.1337 (5)	0.0461 (2)	0.8151 (8)	121 (5)	24 (1)	291 (15)	-9 (2)	-7 (9)	23 (3)
C(1')	0.1027 (3)	0.1739 (2)	0.1936 (8)	42 (3)	17 (1)	291 (14)	-2 (1)	23 (6)	2 (3)
C(2')	0.2123 (4)	0.1949 (2)	0.3753 (9)	66 (4)	27 (1)	373 (19)	-12 (2)	10 (8)	-30 (4)
C(3')	0.3483 (3)	0.1648 (2)	0.2937 (8)	52 (3)	18 (1)	303 (16)	-5 (1)	-15 (7)	26 (3)
O(3')	0.4614 (2)	0.2060 (1)	0.2549 (6)	37 (2)	21 (1)	452 (13)	-7 (1)	-32 (5)	21 (3)
C(4')	0.3096 (3)	0.1309 (2)	0.0591 (8)	44 (3)	17 (1)	317 (15)	1 (1)	30 (7)	8 (3)
C(5')	0.3753 (5)	0.0680 (2)	0.0415 (11)	126 (6)	22 (1)	658 (26)	10 (2)	97 (11)	2 (4)
O(5')‡	0.3462 (14)	0.0326 (5)	0.2575 (32)	145 (17)	17 (3)	889 (89)	0 (6)	88 (39)	61 (14)
O(5')‡	0.3591 (9)	0.0436 (3)	-0.1953 (14)	288 (13)	28 (1)	688 (35)	15 (4)	70 (20)	-61 (6)
O(1')	0.1605 (2)	0.1233 (1)	0.0659 (6)	46 (3)	24 (1)	342 (12)	-2 (1)	18 (5)	-28 (2)

\* The thermal parameters are expressed in the form

$$T = \exp [ - (\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl) (\times 10^{-4}) ] .$$

pared in the same manner and in the same laboratory, did not show any signs of thymidine. In our experiments, the only difference in treatment was the manner of data collection. The first crystal was exposed to X-rays continuously for long periods of time while exposing the photographic film, whereas the second crystal was exposed to short bursts of the X-ray beam on the diffractometer. The following discussion of the structure will be confined to the dihydrothymidine structure.

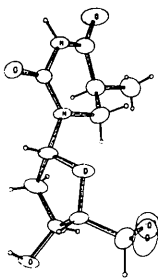
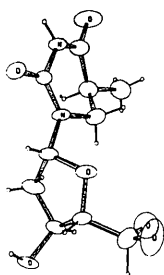


Fig. 3. Stereodiagrams of the dihydrothymidine molecule. (Drawn by a computer program prepared by Johnson, 1965).

Table 5. *Approximate hydrogen fractional coordinates*

	<i>x</i>	<i>y</i>	<i>z</i>
H(N3)	-0.3420	0.1749	0.3100
H(C5)	-0.1000	0.1399	0.7830
H(1C6)	-0.0500	0.0583	0.3660
H(2C6)	0.0670	0.0958	0.5160
H(1C7)	-0.1770	0.0500	0.8420
H(2C7)	-0.0570	0.0417	0.8990
H(3C7)	-0.1670	0.0125	0.7490
H(C1')	0.0840	0.2041	0.0900
H(1C2')	0.1840	0.1808	0.5560
H(2C2')	0.2220	0.2332	0.3730
H(C3')	0.3720	0.1348	0.4230
H(O3')	0.4240	0.2307	0.1570
H(C4')	0.3440	0.1583	-0.0900
H(C5')	0.4890	0.0691	0.0430

The configuration of dihydrothymidine is illustrated in Fig. 3. Bond lengths and angles are shown in Fig. 4. Standard deviations for the bond lengths based on the least-squares fit alone are  $\sim 0.01$  Å for the distances and  $\sim 1^\circ$  for the angles. In the dihydrothymidine moiety the bond lengths and angles are comparable with those found in dihydrothymine (Furberg & Jensen, 1968). The saturation of the C(5)–C(6) bond has caused the

ring to assume a half-chair conformation with the  $\text{CH}_3$  group on C(5) in the equatorial position. The results of the X-ray analysis confirm the stereoconfiguration about the asymmetric C(5) as determined by other means (Kondo & Witkop, 1968).

The atoms in the thymine molecule are coplanar to within  $\pm 0.008 \text{ \AA}$  (Gerdil, 1961). In dihydrothymidine, the ring with the half-chair conformation would be expected to have atoms N(1), C(2), O(2), N(3) and C(4) coplanar. To preserve the planarity of the three bonds to C(4) with the half-chair conformation, there must be a twist about C(4)–C(5) which will cause O(4) to be out of the plane formed by atoms N(1) through C(4). A least-squares plane based on atoms N(1), C(2), N(3) and C(4),

$$0.9400x + 16.8176y + 3.4866z = 3.6332, \quad (3)$$

shows considerable deviation from planarity (Fig. 5). However, the three bonds to C(4) are coplanar to  $\pm 0.01 \text{ \AA}$ , the three bonds to C(2) are coplanar to  $\pm 0.001 \text{ \AA}$ , and those about N(1) are coplanar to  $\pm 0.05 \text{ \AA}$ . These three planar groups are twisted slightly about the bonds connecting them.

Bond lengths in the 2'-deoxyribose moiety are very close to those observed in cytidylic acid (Sundaralingam & Jensen, 1965), 5-fluoro-2'-deoxy- $\beta$ -uridine (Harris & Macintyre, 1964), deoxyadenosine (Watson *et al.*, see Sundaralingam & Jensen, 1965), and adenylic acid (Kraut & Jensen, 1963). The conformation of the furanose ring in these four molecules has four atoms in a plane and either C(2') or C(3') out of the plane by about  $0.55 \text{ \AA}$  (see *e.g.* Sundaralingam & Jensen, 1965). [In adenosine C(4') is displaced by at least  $0.6 \text{ \AA}$  from the best four-atom plane of the furanose ring (Watenpaugh, Dow, Jensen & Furberg, 1968.)] The furanose ring in the present investigation differs in that the four carbon atoms are coplanar and it is O(1') which is  $0.42 \text{ \AA}$  out of the plane of the other four atoms, (Fig. 5). Atom O(1') is *endo* to C(5'). The

equation for least-squares plane of the four carbon atoms is

$$-1.8626x - 17.845y + 3.0167z = -2.7220. \quad (4)$$

These various conformations assumed by the furanose

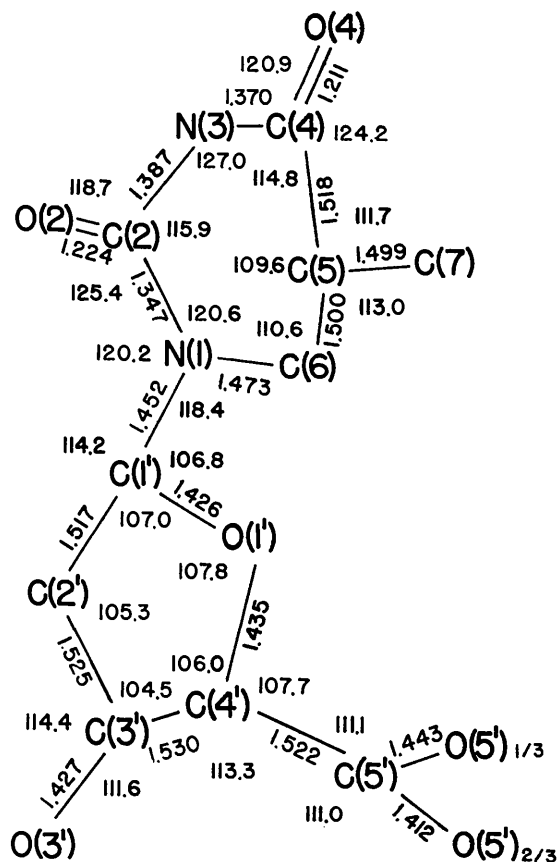


Fig. 4. Bond lengths and angles in dihydrothymidine.

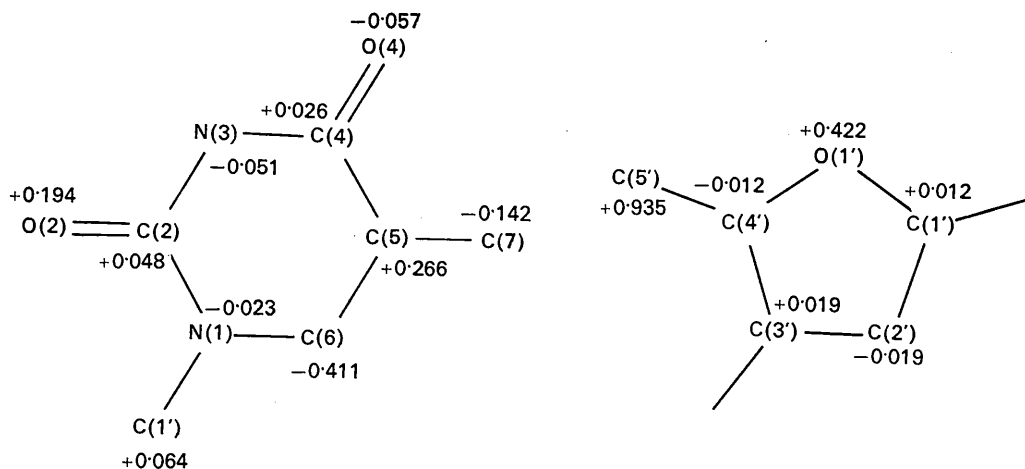


Fig. 5. Deviations (in  $\text{\AA}$  units) from least-squares planes based on (a) atoms N(1), C(2), N(3) and C(4); (b) atoms C(1'), C(2'), C(3') and C(4').

ring in nucleosides and nucleotides complicate nucleic acid model building.

The two rings in dihydrothymidine are twisted with respect to each other. The dihedral angle defined by planes representing the least-squares planes for the two rings, equations (3) and (4), is  $73.2^\circ$ . The angle of twist about the  $C(1')-N(1)$  bond,  $\varphi_{CN}$ , derived by considering the  $O(1')$ ,  $C(1')$ ,  $N(1)$  and  $C(1')$ ,  $N(1)$ ,  $C(2)$  planes is  $-62.9^\circ$ .\*

The packing and hydrogen bonding scheme in dihydrothymidine is illustrated in Fig. 6. Molecules are bonded into double-stranded infinite chains parallel to the  $a$  axis by the  $O(3')H \cdots O(2)$  bond,  $2.78 \text{ \AA}$ , and the

\* Note added in proof: The crystal structure of thymidine has recently been published (Young, Tollin & Wilson, 1969). The compound is not isostructural with dihydrothymidine. Thymidine is orthorhombic in space group  $P2_12_12_1$  with  $a=4.860$ ,  $b=13.91$ ,  $c=16.32 \text{ \AA}$ . Where comparable, the distances and angles agree quite closely with those observed in dihydrothymidine. The conformation of the deoxyribose ring is  $C(3')$  *exo*, with a displacement of  $0.567 \text{ \AA}$  from the least-squares plane through the other four atoms, thus differing from that in dihydrothymidine. The dihedral angle between the base and the best plane of the sugar ring is  $74.3^\circ$  as compared with  $73.2^\circ$  in dihydrothymidine.

The crystal structure of 5-methyluridine has also been determined recently (Hunt & Subramanian, 1969). The conformation of the deoxyribose ring is  $C(3')$  *endo* with a displacement of  $0.58 \text{ \AA}$ . The dihedral angle between the base and the plane segment of the sugar ring is  $72.9^\circ$ .

$N(3)H \cdots O(3')$  bond,  $2.87 \text{ \AA}$ , designated  $A$  and  $B$  in Fig. 6. Two other hydrogen bonds are possible with the disordered  $O(5')H$  group which lies near the screw-axis parallel to the  $c$  axis. Fig. 7 is drawn to show the atoms near this screw axis. Hydrogen bonds can be formed between  $O(5')_{1/3}$  and  $O(5')_{2/3}$  at  $2.59 \text{ \AA}$  and another one at  $3.01 \text{ \AA}$ . The angles involved are reasonable. If the arrangement of  $O(5')_{1/3}$  and  $O(5')_{2/3}$  is ordered along any particular screw axis (parallel to  $c$ ), the hydrogen bonding pattern is consistent with the 2:1 disorder of  $O(5')$ .

The  $O(4) \cdots O(5')_{2/3}$  separation is  $2.93 \text{ \AA}$ , as indicated in Fig. 7, a suitable distance for hydrogen bonding. Although the  $C(4)-O(4) \cdots O(5')_{2/3}$  angle is  $171.7^\circ$  and the  $C(5')-O(5')_{2/3} \cdots O(4)$  angle is  $91.4^\circ$ , hydrogen bonds have been associated with similar geometries (see *e.g.* Donohue, 1968). However, not all possible hydrogen bonds indicated in Fig. 7 may occur simultaneously unless a bifurcated hydrogen bond is assumed.

The shortest intermolecular contacts not involved in hydrogen bonds,  $3.3$  to  $3.4 \text{ \AA}$ , occur between the carbon atoms of the furanose ring and the carbonyl oxygen atoms of the pyrimidine ring.

We are grateful to Dr Bernhard Witkop for providing us with the dihydrothymidine crystals and for helpful discussions.

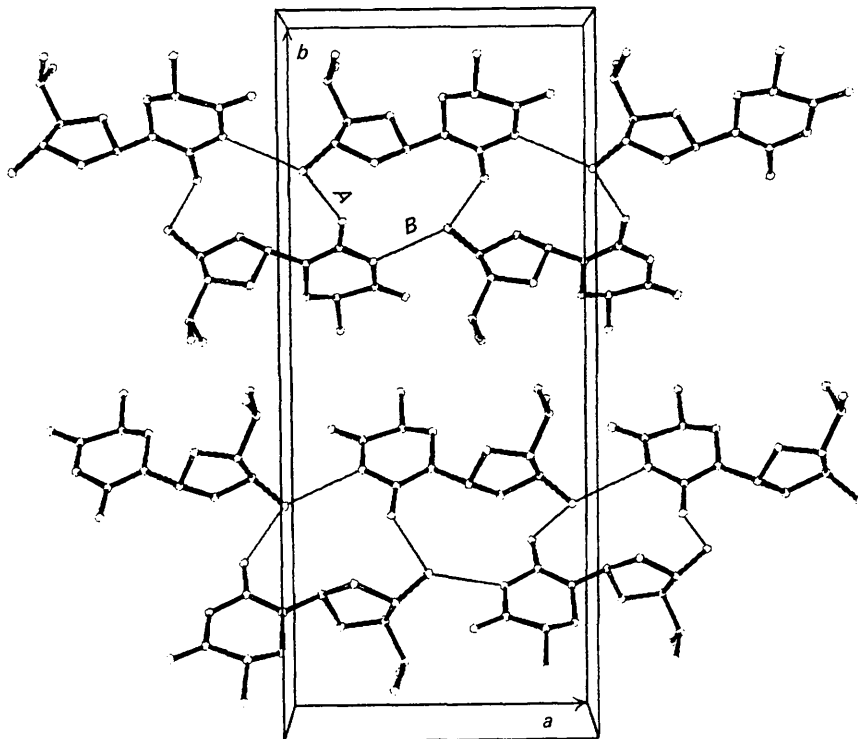


Fig. 6. Packing in dihydrothymidine.  $A$  and  $B$  are two different types of hydrogen bond.



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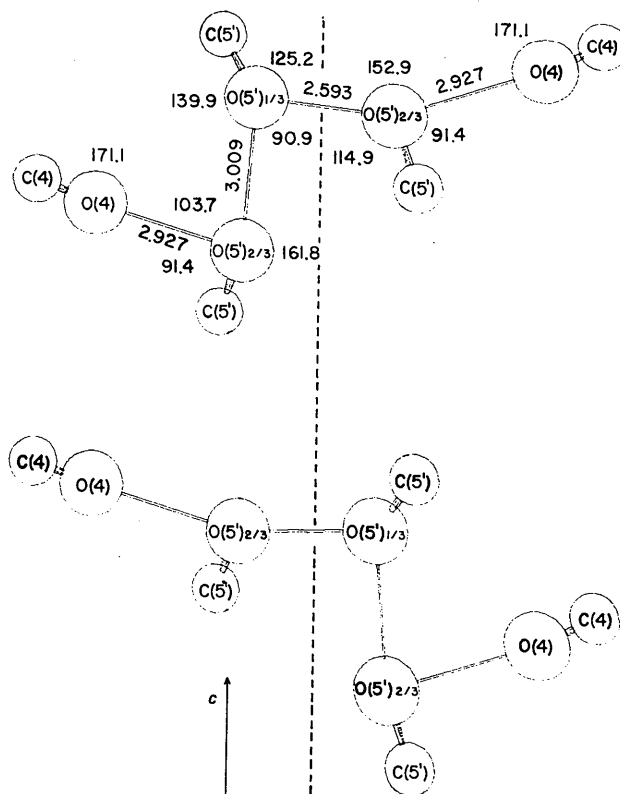


Fig. 7. Atoms near the screw axis parallel to the  $c$  axis. Possible hydrogen-bonding between the disordered  $O(5)_{\frac{1}{3}}$  and  $O(5)_{\frac{2}{3}}$  atoms.

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## The Estimation of Film Intensities

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In the course of the structure investigation of 1-azabicyclo[3.2.0]heptane-1-methyl chloride, very different results were obtained when the intensities were estimated from films by two different techniques. When the intensities were estimated visually by comparison with a scale made up from timed exposures of a given reflection from the crystal, the data refined to a structure which was both crystallographically unsatisfactory and chemically unreasonable. When the intensities were estimated by use of an integrating microdensitometer, the data refined satisfactorily to a value of  $R=0.11$  and produced a chemically reasonable structure. An analysis of both sets of data was carried out. The difficulties with the original visual data have been shown to arise from incorrect scaling of the multiple films within each given zone. Our analysis and conclusions are summarized here as a 'caveat' to other investigators using film techniques.

### Introduction

This paper is concerned with a pitfall that can entrap the unwary investigator during the course of a crystal-

lographic investigation. Having thus been ensnared ourselves, the following is an account summarized here as a 'caveat' to others who might find themselves in similar circumstances. It is a comparison and analysis