Anal. Calcd for $C_{22}H_{24}N_2O_8$: C, 59.46; H, 5.40; N, 6.30. Found: C, 59.44; H, 5.58; N, 6.43.

β-N-Aspartyl-L-serine. A solution of benzyl-N-carbobenzoxy-L-aspartyl-L-serine (2 g) in aqueous methanol (70:30 v/v) was hydrogenated at room temperature over 10% palladium on charcoal (1 g) for 7 hr. The catalyst was removed by filtration, the solution was evaporated under reduced pressure, and the residue (940 mg) recrystallized from aqueous methanol, mp 89–91°. Thin layer chromatography on silica gel G with *n*-propyl alcohol–water (70:30 v/v) gives a brownish spot, R_f 0.15, with ninhydrin (1% in acetone).

Anal. Calcd for $C_7H_{12}N_2O_6$: C, 38.18; H, 5.45; N, 12.72. Found: C, 37.80; H, 5.36; N, 12.56.

***t*-Butyl-N-carbobenzoxy-α-L-aspartyl-O-*t*-butyl-L-serine *t*-Butyl Ester.** To a solution of O-*t*-butyl-L-serine *t*-butyl ester (4.34 g, 20 mmoles) and β-*t*-butyl N-carbobenzoxyaspartate (6.46 g, 20 mmoles) in 50 ml of methylene chloride was added $EtN{=}C{=}N(CH_2)_3N(CH_3)_2{\cdot}HCl$ (4.22 g, 22 mmoles). After storage overnight at room temperature the solution was concentrated. The residual oil was dissolved in ethyl acetate. The solution was washed successively with citric acid, water, 5% sodium bicarbonate, and water. The dried solution was filtered and evaporated to an oil (9.95 g, 95%). Thin layer chromatography on silica gel G with *n*-propyl alcohol–water (70:30 v/v) gives one spot, R_f 0.74.

N-Carbobenzoxy-α-L-aspartyl-L-serine. *t*-Butyl-N-carbobenzoxy-α-L-aspartyl-O-*t*-butyl-L-serine *t*-butyl ester (9.85 g) was dissolved in 50 ml of dry trifluoroacetic acid. After 45 min the trifluoroacetic acid was evaporated under reduced pressure at 40°. The remaining oil solidified upon addition of ether (350 ml): yield, 3.64 g (54%); mp 155° after recrystallization from ethyl acetate–petroleum ether. Thin layer chromatography of silica gel G with *n*-propyl alcohol–water (70:30 v/v) gave one spot.

Anal. Calcd for $C_{15}H_{18}N_2O_8$: C, 50.84; H, 5.08; N, 7.90. Found: C, 50.58; H, 5.29; N, 7.52.

α-L-Aspartyl-L-serine. A solution of N-carbobenzoxy-α-L-aspartyl-L-serine (2 g) in aqueous methanol (70:30) was hydrogenated over palladium on charcoal (1 g) for 7 hr at room temperature (1 atm). The catalyst was removed and the solution evaporated: yield, 1.12 g (90.3%); mp 175° dec after one recrystallization from aqueous methanol. Thin layer chromatography on silica gel G with *n*-propyl alcohol–water (70:30 v/v) gave one spot, R_f 0.17. Spraying with ninhydrin gave a blue-violet color.

Anal. Calcd for $C_7H_{12}N_2O_6$: C, 38.18; H, 5.45; N, 12.72. Found: C, 38.32; H, 5.82; N, 12.51.

Comparison of the Dipeptide (Asp-Ser) Degradation Product from Telomycin with Synthetic Samples of α-L-Aspartyl-L-serine and β-L-Aspartyl-L-serine. The respective samples were applied to Whatman No. 3MM paper (pH 6.2, 3 kv, 5 hr). The paper was dried and sprayed with ninhydrin (1% solution in acetone). β-L-Aspartyl-L-serine was in ninhydrin color (brown) and in mobility on electrophoresis (R_f 0.77; Asp = 1 taken as unity) identical with dipeptide Asp-Ser isolated from a partial basic hydrolysate of Telomycin.

Acknowledgments. This work was aided by a Grant from the U. S. Public Health Service, National Institutes of Health (No. C-2239). We are indebted to Bristol Laboratories, Syracuse, N. Y., for our supply of Telomycin. We are grateful to Professor George Büchi for helpful discussions concerning the ultraviolet chromophore, to Drs. Wolfgang Steglich and Istvan Lengyel for suggestions concerning the mechanism of breakdown of the chromophore, to Dr. Istvan Lengyel for his assistance in preparation of this manuscript, and to Professor Kalus Biemann for his help in establishing the structure of the β-methyltryptophan.

The Crystal and Molecular Structure of Dihydrothymine

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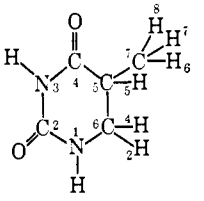
Contribution from the Department of Biological Structure. University of Washington, Seattle, Washington 98105. *Received June 21, 1967*

Abstract: The crystal structure of dihydrothymine has been solved from three-dimensional X-ray diffraction data collected by counter and photographic measurements and refined to $R = 0.048$. The crystals are orthorhombic with unit cell parameters $a = 7.336$ Å, $b = 23.474$ Å, $c = 7.034$ Å. The hydrogenated carbon atoms are out of the plane of the other ring atoms by about 0.35 Å and the methyl group is equatorial. Hydrogenation also causes significant changes in the nonhydrogenated part of the molecule. The structure is disordered, the two enantiomorphs occupying all sites in the space group Pbca in the ratio 3:2.

The dihydropyrimidines are interesting compounds both from a biochemical and a structural point of view. They are implicated in the catabolism of pyrimidine bases, and dihydrouracil occurs in alanine-RNA, possibly playing the role of "insulating" the coding triplet from neighboring nucleotides.[2] Structurally, the molecules consist of a π-electron system closed by two saturated carbon atoms, thus containing features favoring both a planar and a partly nonplanar ring. Little appears to be known about the chemistry of dihydropyrimidines, but the observation has been made that when a pyrimidine nucleoside is hydrogenated at the 5,6 double bond, the sugar can be removed from it under hydrolytic conditions much gentler than normally possible.[3]



dihydrothymine

No dihydrogenated π-electron system of this type appears to have been subjected to detailed structure analysis. The present paper describes the crystal and molecular structure of dihydrothymine.

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(2) R. W. Holley, *et al.*, *Science*, **147**, 1462 (1965).

(3) P. A. Levene and F. B. LaForge, *Ber.*, **45**, 608 (1912).

Experimental Section

A sample of dihydrothymine was supplied by Sigma Chemical Co. and recrystallization from water or alcohol yielded crystals of suitable size. They were elongated along c (occasionally along a) with (010) as the predominant face. Weissenberg photographs showed the crystals to be orthorhombic, and the space group to be Pbca. The unit cell dimensions, derived from diffractometer measurements, are $a = 7.336 \pm 0.002$ Å, $b = 23.474 \pm 0.005$ Å, $c = 7.034 \pm 0.001$ Å (based on λ 0.71069 Å for Mo Kα radiation). There are eight molecules in the unit cell, corresponding to a density of 1.40 g/cm^3.

The crystals have a pronounced tendency to crack, cleaving very easily along planes (100) and (010), and no completely satisfactory crystal big enough for efficient diffractometer work was found. The intensity measurements were made on a slightly split crystal of size 0.20 × 0.18 × 0.15 mm mounted along a, using a Picker manual diffractometer and Mo Kα radiation. By varying the experimental conditions and selecting the best among equivalent reflections, the effect of crystal splitting on the intensity was minimized. However, for χ near 90° the effect was very marked in a certain range of φ. An empirical correction (up to 50%) was applied to 30 reflections of this type, using a correction graph derived by measuring the intensities of reflections $h00$ as a function of φ, and also comparing the intensities of selected equivalent reflections. The corrections were later checked by photographic measurements. Reflections out to $2\theta \approx 53°$ were measured, in all 1190, but only 680 of these were observed, using an observed–unobserved cutoff at $1.7\sigma(I)$. Reflections $hk0$–$hk4$ were therefore recorded on unidimensionally integrated Weissenberg photographs, using Cu Kα radiation and a slightly bigger crystal. A scanning densitometer was used to measure 530 reflections, including all the weak ones, and scaled with the diffractometer data to give the final set of data, consisting of 1190 reflections, out of which 930 were observed (the strongest two-thirds from diffractometer, the weakest third from photographic measurements). No correction for absorption or extinction was made.

The standard deviations $\sigma(I)$ in the intensities of the reflections was assumed to be $\sigma(I) = [C_T + (0.02C_N)^2]^{1/2}$ where C_T is the total number of counts, peak plus backgrounds, and C_N is the net count, peak minus background. The least-squares weights of the reflections measured on the diffractometer were calculated on the basis of this formula. The others (weak, far-out reflections) were given a constant, reasonable weight.

Structure Determination

The structure was solved by inspection of the sharpened, origin-removed Patterson map, which was remarkably clear and showed the orientation and location of the molecule in the unit cell. The first postulated structure gave an R of 0.30, which decreased to 0.18 in two cycles of least-squares refinement with individual isotropic temperature factors and to 0.073 in two cycles with individual anisotropic temperature factors, including hydrogen atoms in positions derived from a difference electron density map. At this stage the molecular structure appeared to be nearly planar, the atoms C(5), C(6), and C(7) being only 0.14 Å, −0.06 Å, and 0.06 Å away from the plane of the other atoms. However, the bond C(5)–C(6) was unreasonably short, 1.379 ± 0.006 Å. Furthermore, while the vibrational anisotropy was large for all atoms, it was huge for atoms C(5) and C(6). For these atoms the B_{11} components of the conventional temperature factors were 15 and 23, respectively, corresponding to root mean square amplitudes of 0.44 and 0.54 Å. The values of B_{11} for the other atoms ranged from 5 to 10.

These unreasonable results indicate that the structural model used in the refinement was incorrect and suggest that the structure is disordered. Assuming the compound to be pure, two types of disorder may be conceived for a molecule of this type, either D and L molecules at the same crystal site, or the two interconversion forms of one enantiomorph in the same site. However, interconversion at C(5)–C(6) radically changes the position of the methyl group at C(5), making it "axial." The low R factor and the lack of any corresponding peak in the difference map rule out this possibility. Subsequent refinements were therefore based on the assumption that both enantiomorphs are present at each crystal site. This is qualitatively in agreement with the results of the refinement of the ordered model, because all the atoms of a D and a L molecule (including C(7)) may be brought to coincidence, except for the two grossly disordered atoms C(5) and C(6). These two atoms were then substituted by two half-atoms of carbon 0.6 Å apart on either side of the molecular plane. In the course of refining this model, it became evident that the disordered atoms in each enantiomorph had different weights and the occupancies of each site were therefore also refined for atoms C(5), C(5′), C(6), and C(6′). The final value of R was 0.048 for observed reflections and 0.050 for all reflections, considerably lower than that for the ordered model.[4] The bond lengths and bond angles are all reasonable within the limits of error. Furthermore, the weights of the atoms refined to consistent values, being 0.64, 0.37, 0.56, and 0.41 for C(5), C(5′), C(6), and C(6′), respectively, with esd in the range 0.02–0.11, a fact which lends confidence to the model. Indeed, the hypothesis of the ordered model can be tested against the disordered model by Hamilton's R ratio.[5] In this case the number of degrees of freedom is 31, the difference between the number of parameters in the two models, and the number of observations can be taken as the number of observed reflections, 930. Interpolation in Hamilton's table for $\alpha = 0.005$ gives

$$R_{31,930,0.005} = 1.029$$

i.e., if the ratio of the R's for the ordered to the disordered structure is 1.029, the hypothesis that the structure is ordered is significant at the 0.005 level. Since in the present instance the ratio

$$R_{\text{ordered}}/R_{\text{disordered}} = 7.3/4.8 = 1.52$$

so greatly exceeds 1.029, it is essentially certain that the ordered model is not correct. The disordered model is obviously preferable.

The temperature factors of the disordered atoms refined to reasonable values (Table I). There are strong correlations among the partial atoms in the least-squares refinement, and it was found essential to adjust the weights of the hydrogen atoms to those of the corresponding carbon atoms as the refinement proceeded. The hydrogen atoms appear on the difference map with peak heights in general agreement with their weights. Those of the two superimposed methyl groups were not resolved and were represented by a single set of three atoms with unit weight. The hydrogen atoms were assigned the same anisotropic temperature factor as the atom to which they were attached, and only their positions were refined.

In this model it has been assumed that only the positions of atoms C(5) and C(6) (and attached hydrogen atoms) differ in the two molecules. This is probably not quite true, especially not for the methyl carbon

(4) A list of F_o and F_c will be supplied by the authors on request.
(5) W. C. Hamilton, *Acta Cryst.*, 18, 502 (1965).

Table I. Positional and Thermal Parameters ($b_{ij} \times 10^4$)[a]

Atom	x/a	y/b	z/c	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
C(2)	0.3118 (3)	0.04134 (8)	0.0258 (2)	231 (5)	16 (0)	131 (4)	−1 (1)	−4 (4)	−3 (1)
C(4)	0.4318 (3)	0.12059 (8)	0.2136 (3)	272 (6)	18 (1)	150 (5)	−11 (1)	−13 (5)	−0 (1)
C(7)	0.5401 (5)	0.20904 (10)	0.0452 (4)	487 (10)	19 (0)	200 (5)	−30 (2)	−47 (7)	4 (1)
N(3)	0.3577 (3)	0.06786 (6)	0.1947 (2)	255 (5)	15 (0)	109 (3)	−8 (1)	−3 (3)	2 (1)
N(1)	0.3388 (3)	0.07098 (7)	−0.1323 (2)	315 (5)	17 (0)	107 (3)	−13 (1)	−7 (4)	2 (1)
O(2)	0.2496 (2)	−0.00755 (5)	0.0290 (2)	346 (4)	16 (0)	144 (3)	−17 (1)	−8 (3)	−1 (1)
O(4)	0.4633 (2)	0.14052 (6)	0.3691 (2)	352 (5)	21 (0)	148 (3)	−19 (1)	−31 (3)	−11 (1)
C(6)	0.3733 (76)	0.13178 (74)	−0.1296 (17)	233 (54)	15 (1)	130 (12)	−6 (6)	−9 (17)	10 (3)
C(5)	0.5087 (15)	0.14445 (27)	0.0277 (6)	239 (23)	17 (1)	169 (9)	−5 (4)	12 (9)	5 (2)
C(6′)	0.4540 (108)	0.12234 (165)	−0.1314 (22)	230 (82)	18 (2)	164 (19)	−12 (13)	19 (24)	7 (4)
C(5′)	0.4034 (24)	0.15961 (37)	0.0375 (11)	198 (32)	15 (1)	190 (14)	10 (5)	−25 (16)	1 (3)
H(3)	0.334 (3)	0.0475 (7)	0.298 (2)						
H(1)	0.316 (3)	0.0523 (7)	−0.244 (3)						
H(2)	0.420 (7)	0.1461 (14)	−0.244 (6)						
H(4)	0.267 (6)	0.1507 (18)	−0.098 (6)						
H(5)	0.636 (6)	0.1237 (16)	0.001 (6)						
H(6)	0.466 (4)	0.2293 (9)	0.116 (3)						
H(7)	0.636 (3)	0.2125 (9)	0.139 (3)						
H(8)	0.555 (3)	0.2302 (9)	−0.071 (3)						
H(2′)	0.458 (11)	0.1364 (20)	−0.247 (11)						
H(4′)	0.578 (12)	0.1134 (22)	−0.102 (9)						
H(5′)	0.303 (9)	0.1708 (21)	0.027 (9)						

[a] The b_{ij}'s are the thermal parameters in the expression: $\exp[-(b_{11}h^2 + b_{22}k^2 + b_{33}l^2 + b_{12}hk + b_{13}hl + b_{23}kl)]$. Estimated standard deviations are given in parentheses and may not be realistic because of disorder.

atom, but no attempt was made to refine a more complicated model due to the limited amount of data available.

The final atomic parameters are given in Table I. All refinements were done by least squares using the full matrix of the normal equations. The scattering factors used were those of Berghuis, *et al.*,[6] for carbon and nitrogen atoms and of Stewart, *et al.*,[7] for hydrogen atoms. In all calculations the programs in the X-ray 63 system were used.[8]

Results and Discussion

Molecular Structure. The bond lengths and angles and their estimated standard deviations are given in Table II. In Figure 1 the "mean" molecular structure is shown, the parameters involving the disordered atoms being taken as the weighted mean of the two values. No corrections for the effect of thermal vibra-

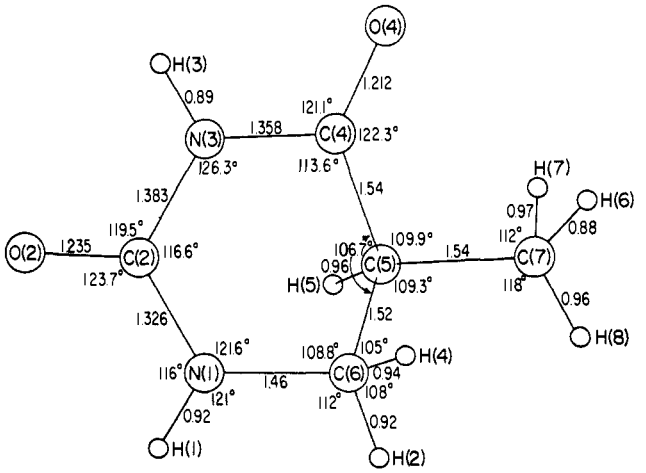

Figure 1. Bond lengths in ångström units and bond angles in degrees in dihydrothymine.

Table II. Bond Lengths and Angles in Dihydrothymine and Thymine Monohydrate[a]

	Dihydrothymine		Thymine monohydrate
	Bond Lengths		
N(1)–C(2)	1.326 (2)		1.355
C(2)–N(3)	1.383 (2)		1.361
N(3)–C(4)	1.358 (3)		1.391
C(4)–C(5)	1.531 (6)	[1.555 (8)]	1.447
C(5)–C(6)	1.516 (38)	[1.521 (33)]	1.349
C(6)–N(1)	1.450 (20)	[1.472 (56)]	1.382
C(5)–C(7)	1.538 (7)	[1.535 (14)]	1.503
C(2)–O(2)	1.235 (2)		1.234
C(4)–O(4)	1.212 (2)		1.231
N(1)–H(1)	0.92 (2)		
N(3)–H(3)	0.89 (2)		
C(6)–H(2)	0.94 (5)	[0.88 (8)]	
C(6)–H(4)	0.93 (7)	[0.96 (11)]	
C(5)–H(5)	1.07 (4)	[0.79 (7)]	
C(7)–H(6)	0.88 (2)		
C(7)–H(7)	0.97 (2)		
C(7)–H(8)	0.96 (2)		
	Bond Angles		
N(1)C(2)N(3)	116.6 (0.2)		115.2
N(1)C(2)O(2)	123.9 (0.2)		122.7
N(3)C(2)O(2)	119.5 (0.2)		122.1
C(2)N(3)C(4)	126.3 (0.2)		126.3
N(3)C(4)C(5)	113.4 (0.3)	[113.9 (0.4)]	115.6
N(3)C(4)O(4)	121.1 (0.2)		118.3
O(4)C(4)C(5)	124.1 (0.3)	[121.1 (0.4)]	126.1
C(2)N(1)C(6)	122.1 (0.5)	[120.8 (0.8)]	122.8
C(4)C(5)C(6)	108.1 (1.5)	[104.5 (1.4)]	118.2
C(4)C(5)C(7)	110.3 (0.4)	[109.2 (0.8)]	119.0
C(6)C(5)C(7)	110.5 (0.8)	[107.6 (2.6)]	122.8

[a] Bond lengths in ångström units and angles in degrees. The values in square brackets are those involving disorder atoms of lower weight. The standard deviations are given in parentheses as the number of units in the last place of the reported bond lengths and angles. The standard deviations in thymine monohydrate are 0.003–0.005 Å in bond lengths and 0.3–0.5° in bond angles.

(6) F. Berghuis, *et al.*, *Acta Cryst.*, 8, 478 (1955).
(7) R. F. Stewart, E. Davidson, and W. Simpson, *J. Chem. Phys.*, 42, 3175 (1965).
(8) J. M. Stewart, *et al.*, "Crystal Structure Calculations System," Computer Science Center, University of Maryland, 1964.

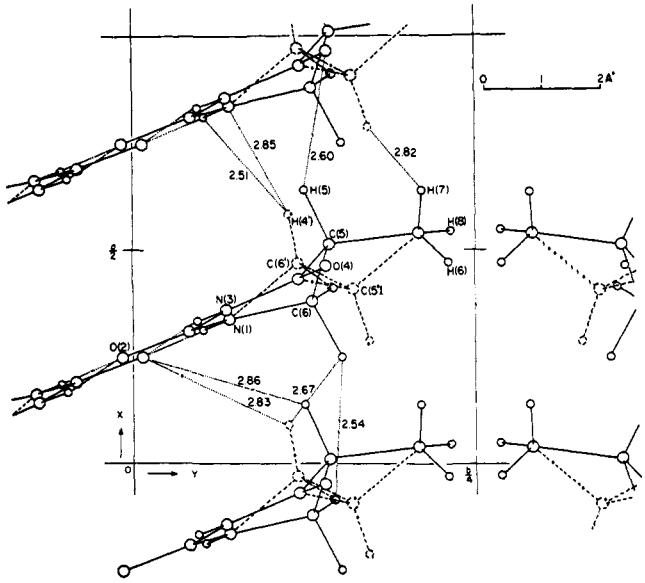

Figure 2. View of crystal structure in z direction. One enantiomorph in a given site is shown in broken lines. Dotted lines indicate short intermolecules distances in ångström units.

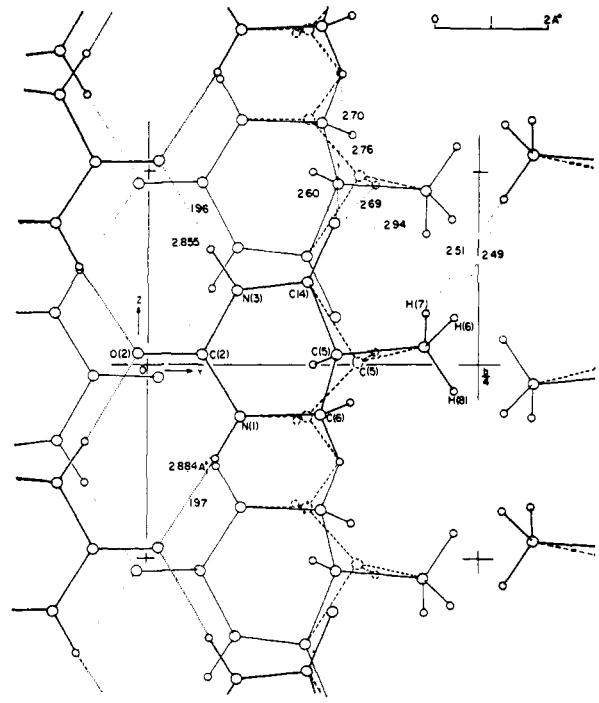

Figure 3. View of crystal structure in x direction.

tions on the bond lengths have been made. The main molecular vibration is, however, translational and the corrections are estimated to be smaller than ~0.01 Å.

In Table III the distances from a least-squares plane defined by all nonhydrogen atoms except C(5), C(6), and C(7) are given. C(5) and C(6) are out of the plane by 0.42 and 0.31 Å in opposite directions, whereas C(7) is displaced from it by only 0.06 Å. The atoms C(4) and O(4) are also slightly, but significantly, out of the plane, probably a consequence of the large displacement of C(5) from it. In the planar, presumably ordered part of the structure, the estimated standard deviations are low, about 0.0025 Å in bond lengths and 0.17° in the angles. In the saturated, disordered part these values increase to 0.01–0.06 Å and 1–3°. Within these limits the bond lengths and angles are all found to be normal. Whether or not the bonds N(1)–C(6) and C(4)–C(5) adjacent to the π-electron system are different from normal (sp^2)–(sp^3) bonds cannot be deduced from the present work because of the large errors in these bond lengths. However, the π-electron system is essentially limited to the planar NHCO-NHCO part of the molecule.

Table III. Distance from the Least-Squares Plane Defined by the First Six Atoms

Atom	Deviation, Å	Atom	Deviation, Å
C(2)	−0.003	C(7)	0.057
C(4)	0.023	C(6)	−0.310
N(3)	−0.006	C(5)	0.422
N(1)	−0.006	C(6′)	0.322
O(2)	0.005	C(5′)	−0.430
O(4)	−0.013	H(3)	−0.037
		H(1)	0.059

The two C=O bonds differ significantly in length (by 0.023 Å). This may possibly be due to the hydrogen bonding, O(2) being engaged in two bonds, O(4) in none, but, on the other hand, no corrections for thermal effects have been made, and the bond order of the two bonds may also be different. Similar differences have been found in related structures, *e.g.*, uracil[9] and methylthymine.[10]

It would appear to be of interest to compare the structure of dihydrothymine with that of thymine itself, which has been determined by Gerdil by X-ray analysis of thymine monohydrate.[11] The results are included in Table II. It is seen that hydrogenation, besides radically changing the stereochemistry at C(5) and C(6), also leads to a redistribution of the bonding electrons in the planar part of the molecule. The middle bond, N(3)–C(2), becomes longer, the two adjacent bonds shorter. The differences are 0.02–0.03 Å and therefore probably significant. Some of the angles in the planar part of the molecule are also significantly different, especially those at C(2). This is probably also, in part, a consequence of the hydrogenation.

In the nucleic acids the dihydropyrimidines are linked to the sugar at N(1) and the stereochemistry at this atom is therefore of interest. The bond orders of the bonds at N(1) are changed by hydrogenation and this is probably related to the changes in chemical properties. However, the N(1)–sugar bond is likely to lie in the π-electron system plane, as N(1)–C(2) has strong double bond character and H(1) also is found to lie approximately in this plane. This would seem to imply that the relative orientation of base and sugar should be expected to be essentially the same in dihydronucleosides as in nucleosides.

Molecular Packing

There are only two intermolecular distances shorter than normal van der Waals separations, namely, H(1)–O(2) and H(3)–O(3), both of length 1.97 Å. These

(9) R. F. Stewart and L. H. Jensen, *Acta Cryst.*, in press.
(10) K. Hoogsteen, *ibid.*, 16, 28 (1963).
(11) R. Gerdil, *ibid.*, 14, 333 (1961).

interactions clearly correspond to hydrogen bonds N(1)–H(1)···O(2) and N(3)–H(3)···O(2), the distances N(1)–O(2) and N(3)–O(2) being 2.884 and 2.855 Å, respectively. The bonds are nearly linear and coplanar with the planar part of the molecule (the angles at H(1) and H(3) being 174.4 and 172.6°) and hold the molecules together in approximately planar infinite ribbons running in the z direction (Figures 2 and 3). In the x direction there is π-electron contact between successive ribbons, which are about 3.3 Å apart, and in the y direction between the methyl groups (H–H distances of 2.49 and 2.51 Å). The strong bonding in only one direction explains the cleavage properties of the crystal and also the vibrational anisotropy of the atoms. The O(4) is not taking part in hydrogen bonds, its shortest separation from a hydrogen atom in a neighboring molecule (H(5)) being 2.60 Å. It is noteworthy that both D and L molecules can be accommodated in the same site without coming too close to any atom in either D or L molecules in neighboring sites. However, the occupancies differ significantly from 0.50, being close to 0.60 and 0.40, and the packing of D and L molecules is therefore not random. It is more favorable for a molecule to obey the symmetry operations of the crystal than not, although the energy differences must be small (the Boltzmann equation gives an energy difference of 0.2 kcal/mol for 3:2 occupancies at room temperature).

The crystals of dihydrothymine represent an unusual and complicated form of racemate, in which both enantiomorphs occupy all sites in a centrosymmetrical space group. It can probably only occur when the difference in space requirement between the enantiomorphs is small and the packing mainly is governed by the common, "nonenantiomorph," part of the molecule.

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Reaction of Ninhydrin with Cytosine Derivatives

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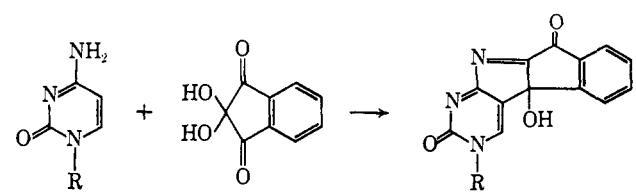
Abstract: Ninhydrin (1,2,3-indantrione monohydrate) reacted with cytosine, cytidine, and cytidine nucleotides to form products (**3a–d**) in which the amino nitrogen and 5-carbon of cytosine had formed bonds with two adjacent carbons of the five-membered ring of ninhydrin. The use of this reaction is suggested for the modification of cytosine residues of nucleic acids. Bovine pancreatic ribonuclease cleaved the reaction product of ninhydrin and cytidine 2′,3′-cyclic phosphate (**3c**) to the corresponding open 2′(3′)-phosphate **3d**. The structure of **3a**, the adduct of ninhydrin and cytosine, was deduced from its spectroscopic properties and from its conversion to a number of degradation products. Compound **3a** was ultimately converted to 5-(3-phthalamidyl)uracil (**12a**). The properties of the transformation products of **3a** and the mechanisms of the reactions are discussed.

There appears to be a considerable need for organic reagents for the modification of nucleic acids, especially those with specificity for a particular heterocyclic component.[1] Such reagents are of value as mutagens,[2] as markers for electron microscope studies,[3] as aids to the determination of the structure[4] and biological function[5] of nucleic acids, and for the purpose of increasing the specificity of enzymatic cleavage of nucleic acids.[6]

The color reaction of ninhydrin (1,2,3-indantrione monohydrate) with amino acids and peptides has been known for more than half a century,[7] and it has been termed "one of the most valuable of all biochemical reagents."[8] Virtually nothing is known, however, of its reactions with aromatic and heterocyclic amines. We recently reported a reaction between ninhydrin and guanine derivatives in which a five-membered ring was formed by addition of the 1 and N-2 positions of guanine across two adjacent carbonyl groups of ninhydrin.[9] This reaction was also observed with glyoxal and guanine derivatives. The adduct of ninhydrin and guanine was labile, and decomposed into its components under mildly alkaline conditions. We wish to report now that ninhydrin (**2**), unlike glyoxal, also reacts with cytosine derivatives (**1**), to form adducts of structure **3**. Adenine and uracil derivatives do not



1a, R = H **2** **3**
b, R = β-D-ribofuranosyl
c, R = β-D-ribofuranosyl 2′,3′-cyclic phosphate
d, R = β-D-ribofuranosyl 2′(3′)-phosphate

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