angle of repose of about 28°, similar to that measured for basalt blocks over collapsed terrestrial lava tubes.

While the above now appears to be a satisfactory account of the origin of Hadley Rille, geologic observations, returned samples from the rille region, and improved photographs and topographic maps from the Apollo 15 mission will undoubtedly enable refinement of this interpretation.

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18. I thank J. F. Kerridge and K. G. Snetsinger for their helpful comments and suggestions on the manuscript. This study was conducted during tenure as a National Academy of Sciences–National Research Council Associate.

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Molecular Conformation of Dihydrouridine: Puckered Base Nucleoside of Transfer RNA

Abstract. *The crystal structure of dihydrouridine hemihydrate has been determined by x-ray diffraction. Crystals of dihydrouridine contain two independent molecules in the asymmetric unit and a molecule of water. The x-ray structure determination has shown that the conformations of both molecules differ in important respects in the saturated base and the ribose. The molecular conformation of dihydrouridine has, for the first time, provided structural evidence that the rare nucleoside can promote "loop" formation in the sugar-phosphate chain.*

X-ray crystallographic studies on the monomers of DNA and RNA have provided considerable insight into their intricate conformational properties (*1*). In addition, the important rule has been established that the preferred conformations of the monomers are the same as those of the monomeric units in the polymer (*1*). In transfer RNA (tRNA), a number of rare nucleotides are present which are distributed mainly in the loop regions of the familiar cloverleaf model. These nucleosides probably have a structural or functional role or both. We now wish to report the molecular conformation of dihydrouridine (Fig. 1) which occurs solely in the dihydrouridine loop of tRNA. The number of dihydrouridines in the loop varies from one to five in the known tRNA's.

Dihydrouridine, $C_9H_{14}N_2O_6$ (Sigma), was crystallized from aqueous ethanol solution by D. Rohrer. Photographic investigation showed that the crystals belong to the orthorhombic system with the space group $P2_12_12_1$. The unit cell dimensions measured on a diffractometer are $a = 8.131$ Å, $b = 11.766$ Å, and $c = 23.016$ Å. Although the experimental density was not available, the calculated density (1.538 g cm^{-3}) for two molecules in the asymmetric unit was in the vicinity of that found for other nucleoside crystals. The crystal

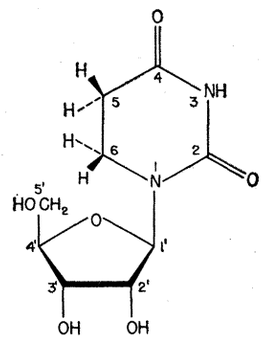

Fig. 1. Chemical structure of dihydrouridine.

structure determination not only confirmed the presence of two molecules of dihydrouridine, but also showed the presence of one water of crystallization in the asymmetric unit. Therefore, there are 35 nonhydrogen atoms and 30 hydrogen atoms in the asymmetric unit.

Approximately 2100 reflections were recorded on a four-circle automated diffractometer operating in the θ-2θ scan mode. Of these about 1900 were considered significantly above background and were used in the structure analysis. The crystal structure was solved by a combination of Patterson search method and the tangent formula (*2*). The structure was refined by the method of full-matrix least-squares to the present agreement index

$$R = \Sigma \, | \, | F_0 | - | F_c | \, | \, / \Sigma \, | F_0 | = 0.056$$

where F_0 and F_c are, respectively, the observed and calculated structure factors.

Dihydrouridine is the only nucleic acid constituent with a puckered base. It is particularly interesting that our study has shown that the conformations of the two molecules (A and B) in the asymmetric unit are different (Fig. 2, left). The bases themselves show approximately opposite (enantiomeric) conformations (Fig. 2, right); atoms C(5) and C(6) demonstrate the greatest deviations, and in opposite directions, from the plane through the six ring atoms. The overall shape of the bases may be described as a twist, half-chair conformation which is similar to that observed for the base (dihydrouracil) itself (*3*).

The glycosidic torsion angle, as defined by Sundaralingam (*1*), is 65.5° in molecule A and 57.1° in molecule B. This angle describes the stereochemical relation of the base with respect to the carbohydrate moiety. Even though the C(5)–C(6) bond is saturated, the conformation is still in the preferred *anti* range found for the common (planar) pyrimidine nucleosides.

The furanoside ring conformations are essentially the same in both molecules. When described as an envelope (E) form, that is, with respect to the best four-atom least-squares plane, the conformations are C(2′)-*endo*. However, the conformation when described with respect to next best four-atom plane is C(1′)-*exo*. This is an unusual conformation for the sugar. Whenever the plane through the "best four-atom plane" shows significant deviations from planarity, the furanoside ring is said to occur in a twist (*T*) form. In describ-

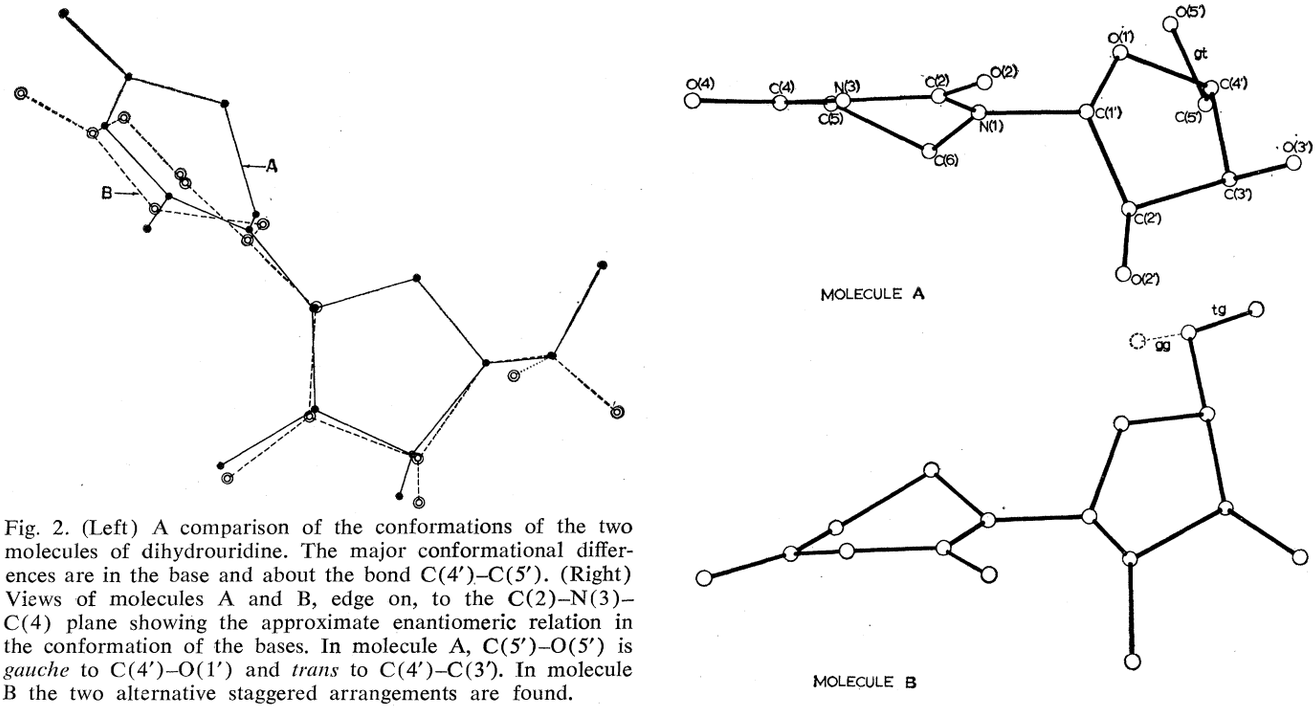

Fig. 2. (Left) A comparison of the conformations of the two molecules of dihydrouridine. The major conformational differences are in the base and about the bond C(4′)–C(5′). (Right) Views of molecules A and B, edge on, to the C(2)–N(3)–C(4) plane showing the approximate enantiomeric relation in the conformation of the bases. In molecule A, C(5′)–O(5′) is *gauche* to C(4′)–O(1′) and *trans* to C(4′)–C(3′). In molecule B the two alternative staggered arrangements are found.

ing the twist form a three-atom plane is calculated. The three atoms are chosen so that they are common both to the best four-atom and next best four-atom planes (*4*). In dihydrouridine the plane is C(3′)–C(4′)–O(1′) for both molecules, and C(2′) and C(1′) are displaced on opposite sides of this plane (Fig. 3). In that the larger of the two deviations is exhibited by C(2′), the conformations of the furanoside rings are abbreviated as $^2T_1$ for C(2′)-*endo*–C(1′)-*exo*. *Endo* here indicates displacement is on the same side of C(5′), while *exo* indicates displacement is on the opposite side.

The $^2T_1$ conformation is rare, in that it has been observed in only two other structures, tubercidin (*5*) and inosine dihydrate (*6*). But what is significant is that only the ribosides have demonstrated this conformation, and no case is yet known for a 2′-deoxyriboside. Although it is premature to draw conclusions from a limited number of known cases, it is worthwhile to point out that the occurrence of the $^2T_1$ conformation may be correlated with the 2′-hydroxy group (*7*). If this is true, then it is germane to the question of conformational differences in the sugar-phosphate backbone of ribonucleic acids on one hand and deoxyribonucleic acids on the other.

Perhaps the most significant aspect of the conformation of dihydrouridine is the torsion angle about the extracyclic

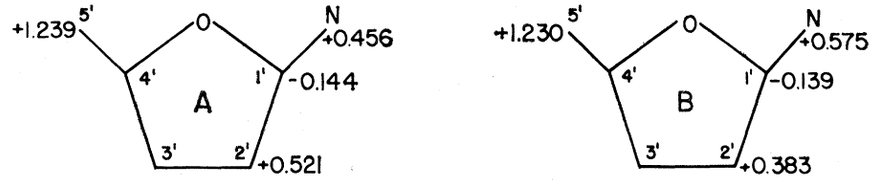

Fig. 3. The deviations of the C(2′) and C(1′) atoms from the three-atom plane C(3′)–C(4′)–O(1′). Although there are some differences in the magnitudes of the deviations, the conformations ($^2T_1$) of the furanoside rings of both molecules are essentially the same.

bond C(4′)–C(5′). In molecule A the conformation about this bond is *gauche-trans* (*gt*), while in molecule B, O(5′) is disordered occupying the two alternative conformations, *trans-gauche* (*tg*) and *gauche-gauche* (*gg*) in the ratio 0.88 : 0.12, respectively. Therefore, between the two independent molecules in the asymmetric unit all three staggered conformations about the C(4′)–C(5′) bond are observed with the following order in the conformation population $gt > tg > gg$. It is noteworthy that this is in contrast to the usual order $gg > gt > tg$ of conformational preference found for the common nucleosides. The *gg* conformation is not only the only conformation exhibited by the known 5′ nucleotides, but it is also the conformation found in all the known double-helical nucleic acids and polynucleotides (*1*).

The apparent reversal of this order in dihydrouridine is possibly contributed by the saturation of the base. If indeed the *gt* or *tg* conformations (*8*) are accommodated by dihydrouridine, then it is possible that it is one of the key nucleosides involved in the formation of the dihydrouridine loop. In fact it has already been pointed out (*1*) from considerations of molecular models that a polynucleotide loop must necessarily have one to three nucleosides in the *gt* or *tg* conformations. Thus it seems that for the first time information has been sought that a rare nucleoside can have an effect on secondary and tertiary structures of tRNA's. To what extent dihydrouridine influences the conformation of the dihydrouridine loop of tRNA's will be seen when the crystal structure of tRNA is determined (*9*).

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7. In a similar fashion, the 2′-deoxyribosides, 5′-deoxycytidine monophosphate monohydrate (M. A. Viswamitra, B. Swaminatha Reddy, G. H. Y. Lin, M. Sundaralingam, *J. Amer. Chem. Soc.*, in press), deoxyadenosine monohydrate [D. G. Watson, D. J. Sutor, P. Tollin, *Acta Cryst.* **19**, 111 (1965)], and thymidine [D. W. Young, P. Tollin, H. R. Wilson, *ibid.* **B25**, 1423 (1969)] exhibit the conformations ${}_3T_2$, ${}_3T^2$, and ${}_3T^2$, respectively. The primary pucker is C(3′)-*exo* in these compounds, and this conformation appears to be unique to the deoxyribosides and relevant to the structure of DNA. Thus, for the first time, evidence for the occurrence of characteristic conformations for the ribosides on one hand and the deoxyribosides on the other has been found.
8. In dihydrothymidine [J. Konnert, I. L. Karle, J. Karle, *Acta Crystallogr.* **B26**, 770 (1970)], which is not a tRNA component, a similar disorder of O(5′) was found, but it occurred between the conformations *gt* and *gg* in the ratio 0.66 : 0.34. Thus, even here the predominant conformer was *gt*, supporting the observations in dihydrouridine. However the 2′-deoxyribose in dihydrothymidine exhibits a novel conformation, ${}^{O}E$ [O(1′)-*endo*] in terms of the best four-atom plane C(1′)–C(2′)–C(3′)–C(4′), and ${}^{O}T_4$ [O(1′)-*endo*–C(4′)-*exo*] in terms of the three-atom plane C(1′)-C(2′)-C(3′).
9. We thank Dr. Douglas Rohrer for growing the crystals. Supported by NIH grant GM-17378.

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Galactosemia: Evidence for a Structural Gene Mutation

Abstract. *Identical immunoprecipitin reactions appeared in double immunodiffusion between rabbit antibodies to human galactose-1-phosphate uridyl transferase and red cell preparations from both normal and galactosemic individuals. The galactosemic erythrocyte preparations quantitatively absorbed the antibody that immunoprecipitates enzymatically active galactose-1-phosphate uridyl transferase.*

Galactosemia is a human autosomal recessive disorder due to a functional deficiency of galactose-1-phosphate uridyl transferase (transferase) (*1*). Every tissue of affected individuals that has been studied is devoid of, or markedly deficient in, transferase activity (*2*). The absence of transferase activity in cells of galactosemic individuals poses the question of whether the genetic defect is an alteration or deletion of the locus that codes for the structural protein, or is an abnormality of the regulatory factors that control the rate of synthesis or degradation of the transferase protein. This report presents evidence that the defect in galactosemia is a "point" mutation in the structural gene which has resulted in the synthesis of a catalytically inactive transferase protein.

Human transferase was purified 1000-fold (specific activity = 1 unit per milligram of protein) from liver by a procedure that includes homogenization, precipitation with protamine sulfate, fractionation with ammonium sulfate, gel filtration, and column electrophoresis in Sephadex G-200 (*3*). Antibody to this purified enzyme was produced in a male New Zealand albino rabbit by four biweekly intramuscular injections of 1 mg of enzyme protein dissolved in 0.5 ml of 50 m*M* glycylglycine, *p*H 8.5, mixed with 0.5 ml of Freund's complete adjuvant. One week after the fourth injection an immunoprecipitin to transferase protein was detected by double diffusion in agar, and 50 ml of blood was collected by ear vein puncture. The serum was collected and fractionated on Sephadex G-200 with 50 m*M* glycylglycine, *p*H 8.5, as the eluting buffer. The fractions containing antibody as determined by double immunodiffusion and precipitation of transferase activity were pooled, lyophilized to dryness and redissolved in water at one-half their concentration in whole serum.

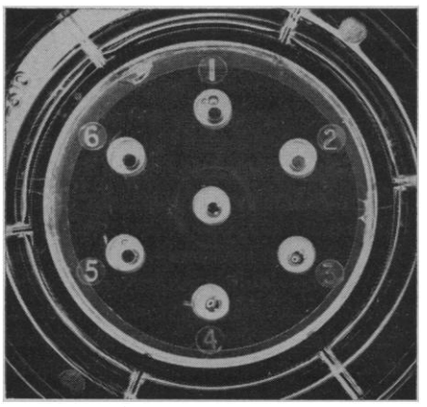

Fig. 1. Double-immunodiffusion cell photographed 24 hours after loading: 25 μl antitransferase after G-200 gel filtration was placed in the center well. Outer wells contained purified human liver transferase preparations (well 1), and the following erythrocyte preparations; 3-month-old male Caucasian galactosemic (well 2), normal (well 3), 3-year-old male Caucasian galactosemic (well 4), normal (well 5), and a 3-year-old female Negro galactosemic (well 6).

Hemoglobin-free red cell preparations were obtained as follows. Red cells from heparinized blood, washed twice with 0.85 percent sodium chloride, were lysed in an equal volume of water and then frozen and thawed. The lysate was diluted with an equal volume of 10 m*M* phosphate buffer, *p*H 7.0, which was then mixed with two volumes of a 50 percent suspension of diethylaminoethyl (DEAE)-cellulose (Whatman DE 52) equilibrated in the same phosphate buffer. The mixture was stirred for 1 hour and poured into a chromatographic column. The hemoglobin fraction was eluted with the same buffer until there was no detectable adsorbance at 280 nm. The fraction containing transferase activity was then eluted with phosphate buffer containing 50 m*M* ammonium sulfate. The first protein peak detected after the addition of buffered ammonium sulfate contains the transferase enzyme and consistently yields 70 to 100 percent recovery of enzyme activity. This fraction was lyophilized to dryness and redissolved in water just before use.

Double immunodiffusion was performed in high-resolution agarose cells (Cordis Laboratories). The wells with the red cell preparations contained 150 μg of protein and the well with the purified transferase enzyme contained 20 μg of protein (Fig. 1). All red cell preparations were assayed for transferase activity by the uridine diphosphoglucose–consumption assay (*4*) before and after fractionation on DEAE-cellulose. The preparations from galactosemic individuals had no detectable transferase activity, and galactokinase activity before fractionation was normal in all preparations regardless of transferase genotype. A single band of identity is present between all red cell preparations which is continuous with that of the purified liver transferase. After several days a second precipitin band appears between the purified liver preparation and the antibody. No additional precipitins appear between red cell preparations and the antibody.

To demonstrate specificity of the antibody for transferase protein, a preparation from a normal individual with a constant amount of normal (Gt^+/Gt^+) transferase activity was mixed with increasing amounts of antibody, incubated at 37°C for 1 hour and then at 4°C for 2 days. After centrifugation, the supernatants were assayed for