The Stereochemistry of the Catalytic and Light-Induced Reduction of Thymidine to Dihydrothymidine and Ureido Alcohols

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Abstract: Only one diastereoisomer (II) of 1-dihydrothymidine was obtained by catalytic reduction of thymidine (I). This 1-dihydrothymidine (II) on acid hydrolysis yielded (*S*)-(−)-dihydrothymine (IV) which was hydrolyzed to (*S*)-(+)-β-ureidoisobutyric acid (V), the antipode of the analogous thymidine metabolite (*R*)-(−)-β-aminoisobutyric acid. II was hydrogenolyzed by sodium borohydride in aqueous solution at room temperature to (*S*)-(−)-3-ureido-2-methylpropanol-1 (IVa), characterized as the N,O-diacetate (VIb) and N,O-di-*p*-iodobenzoate (VIc). Direct hydrogenolysis of 1-dihydrothymidine (II) with sodium borohydride gave N^4-deoxyribosyl-(*S*)-3-ureido-2-methylpropanol-1 (IIIa), characterized as the tetra-*p*-nitrobenzoyl (IIIb) and the tetra-*p*-iodobenzoyl (IIIc) derivatives. A by-product of the hydrogenolysis of 1-dihydrothymidine was isolated as the methyl ether VII of the carbinolamide. On hydrolysis the racemic 2-oxo-4-methoxy-5-methyl-5,6-hexahydropyrimidine was obtained which on the basis of nmr data was assigned the *cis* configuration IX. The photochemical reduction of thymidine, after acid hydrolysis and column chromatography over silica gel, led to 3-ureido-2-methylpropanol-1, characterized as the diacetate, identical with the product having the *S* configuration obtained *via* catalytic hydrogenation. A product of the photoreduction is N-deoxyribosylurea (VIII) which must be formed *via* photohydration of thymidine.

Previous reports from this laboratory have demonstrated that the photoreduction of uridine[2] in the presence of sodium borohydride proceeds in two stages. First the 5,6 double bond is hydrogenated. Subsequently the resulting dihydrouridine is hydrogenolyzed to a β-ureido alcohol in a light-independent reaction in analogy to the reduction of dihydrothymine.[3]

When thymidine (I) was hydrogenated in the presence of 5% rhodium on alumina, (−)-dihydrothymidine (II), $[\alpha]^{23}D$ −20.5°, was obtained (Chart I). It was originally thought that the dihydrothymidine obtained by catalytic reduction of thymidine on rhodium–alumina in 0.001 *N* hydrochloric acid was the C-5 diastereoisomer of II.[4] We find that under these conditions hydrogenation is slow[5] and accompanied by cleavage of the N-glycoside bond.[6] The resulting mixture of (−)-dihydrothymidine (II) and (−)-dihydrothymine (IV) was erroneously taken for a new diastereoisomer of III.[4]

Acid hydrolysis of pure (−)-dihydrothymidine (II) gave (−)-dihydrothymine (IV), $[\alpha]^{23}D$ −6.5° (H_2O), $[\alpha]^{20}D$ −11.3° (pyridine).

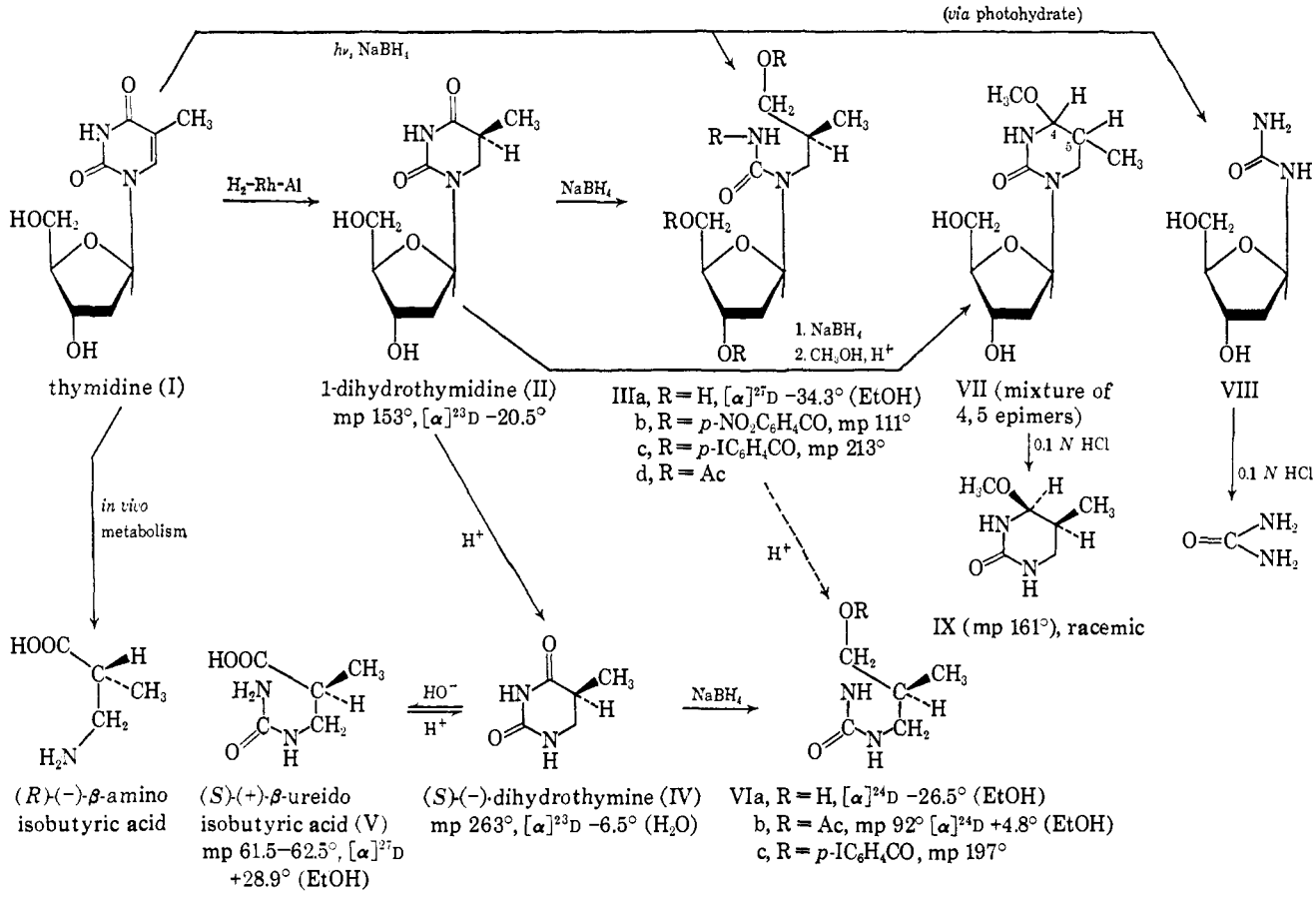
When (−)-dihydrothymidine (II) was treated with sodium borohydride in water a major and a minor product was obtained after separation by silica gel column chromatography. The major product (IIIa) showed the presence of a ureido group by the positive color test with *p*-dimethylaminocinnamaldehyde and by the infrared absorption bands at 1692 and 1639 cm^{-1}. Compound IIIa was characterized by a crystalline tetra-*p*-nitrobenzoate (IIIb, mp 111° dec) and a tetra-*p*-iodobenzoate (IIIc, mp 212–213° dec). Mild hydrolysis of IIIa with 0.1 *N* hydrochloric acid afforded (−)-β-ureido-2-methylpropanol-1 (VIa) identical with respect to tlc and spectra with an authentic (racemic) specimen provided by the reduction of dihydrothymine (IV). Nmr spectra of IIIa, IIIc, and the tetraacetate IIId showed the triplet [6.28 ppm (J = 7.5 cps), 6.40 ppm (J = 7 cps), and 6.33 ppm (J = 8 cps)] characteristic of the C-1′ proton of the deoxyribosyl residue. These observations suggest that N^3-(2-deoxy-β-D-*erythro*-pentafuranosyl)-3-ureido-2-methylpropanol-1 (IIIa) represents the structure of the reduction product of dihydrothymidine.

In spite of apparent homogeneity by the criteria of tlc, the splitting pattern of the methyl group in the nmr spectrum of IIIa raises the possibility of the presence of another isomer. However, the nmr spectra of the acetate IIId and the *p*-iodobenzoate IIIc showed the 5-methyl group as a doublet. The photolysis product of thymidine has exactly the same nmr spectrum as IIIa. We have commented previously on this splitting pattern and attributed it to the two nonequivalent C-5 methyl groups of the two diastereoisomers of dihydrothymidine. However, hydrogenolysis of sterically pure dihydrothymidine with sodium borohydride does not involve the C-5 position. By this reasoning and by the optical properties of the hydrolysis product VIa we consider IIIa to be optically pure.

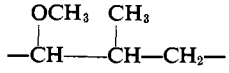
The minor component VII was obtained from subsequent elutions from the silica gel column. The substance was homogeneous but could not be crystallized. The presence of a deoxyribosyl residue was shown by a positive diphenylamine reaction. Mild hydrolysis of VII with 0.1 *N* hydrochloric acid and purification by silica gel column chromatography gave colorless needles (IX, mp 159–161°) which were optically inactive. The ir spectrum of IX had a single band at 1689 cm^{-1} indicative of the cyclic ureido group and strong C–O stretching vibrational absorption at 1072 cm^{-1}. The nmr spectrum of IX contained the typical signal of a methyl group attached to a tertiary carbon atom as a doublet at 0.93 ppm (J = 6.3 cps) and a multiplet at 1.9 ppm. Sur-

(1) Associate in the Visiting Program of the U. S. Public Health Service, 1966–1968.
(2) P. Cerutti, Y. Kondo, W. Landis, and B. Witkop, *J. Amer. Chem. Soc.*, 90, 771 (1968).
(3) G. Ballé, P. Cerutti, and B. Witkop, *ibid.*, 88, 3946 (1966).
(4) H. T. Miles, *Biochim. Biophys. Acta*, 27, 46 (1958).
(5) Hydrochloric acid is known to poison the rhodium catalyst: M. Freifelder, *J. Org. Chem.*, 26, 1835 (1961).
(6) H. A. Smith and R. G. Thompson, *Advan. Catalysis*, 9, 727 (1957).

Chart I. Stereochemistry of the Catalytic and Light-Induced Reduction of Thymidine and of Hydrogenolysis of Dihydrothymidine with Sodium Borohydride

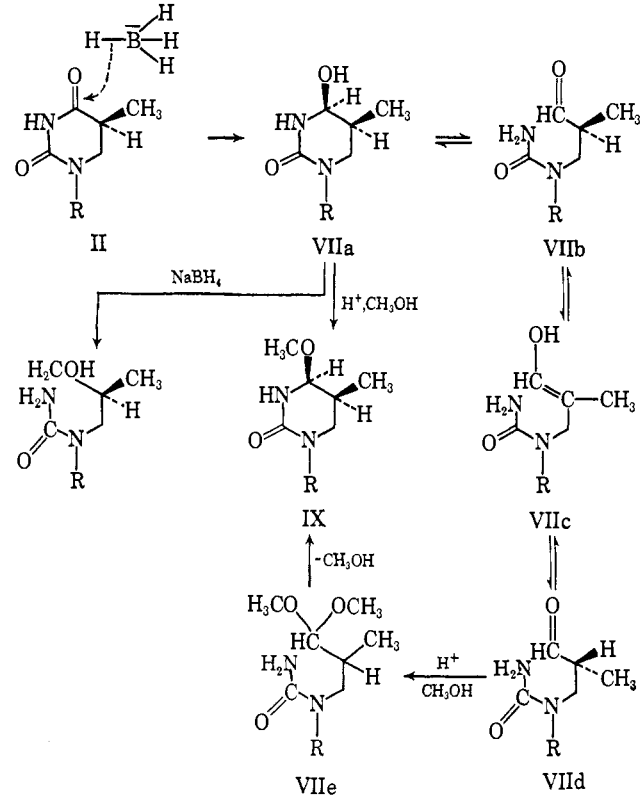


prisingly, IX showed a sharp singlet at 3.13 ppm (3 H) and a doublet at 3.97 ppm (1 H, J = 2.6 cps) due to a methoxy group attached to >C–H. The AB_2 type pattern of a methylene at 2.88 ppm proves the partial structure



(Figure 1). This information together with the analytical data establishes the structure of IX as 2-oxo-4-methoxy-5-methylhexahydropyrimidine, and the precursor VII as N^1-deoxyribosyl-2-oxo-4-methoxy-5-methylhexahydropyrimidine (VII). The formation of VII proceeds probably through the carbinolamide VIIa[7,8] which is in ring–chain tautomerism with the open aldehyde VIIb. Epimerization of the aldehyde through the enol VIIc should lead to two epimeric ribosides (VIIb and VIId). When in the hydrogenolysis of II → VII boron is removed by repeated distillation with acidic methanol, the aldehyde is converted to the acetal VIIe. Loss of methanol from the acetal VIIe, or direct O-methylation of the carbinolamide VIIa, would lead to VII, possibly a mixture of epimers, from which acid liberates the racemic IX.

The conformation of IX was determined by application of the Karplus equation[9] which defines the coupling constants of protons bonded to vicinal carbon atoms in terms of the dihedral angle. Evaluations by this method have to be made with caution because the vicinal coupling constant depends not only on the dihedral angle, but also on other factors, such as the electronegativity of the substituents.[10] If both substituents



(7) H. C. Brown, O. H. Wheeler, and K. Ichikawa, *Tetrahedron*, **1**, 221 (1957).
(8) K. Bowden and M. Hardy, *ibid.*, **22**, 1169 (1966).
(9) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); *J. Amer. Chem. Soc.*, **85**, 2870 (1963).

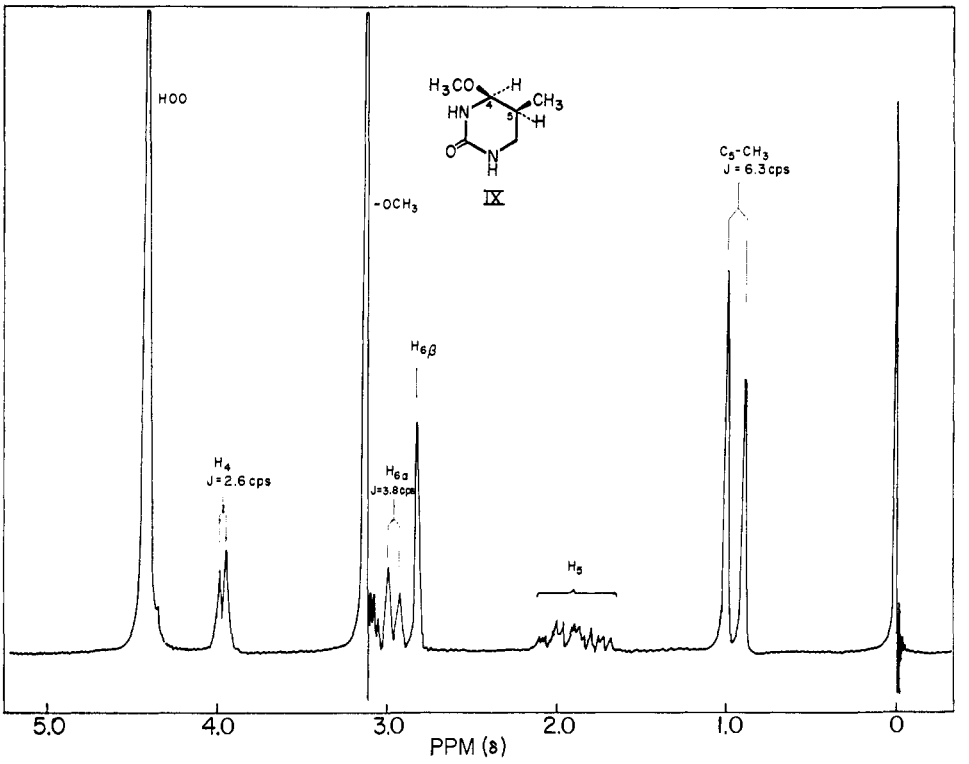
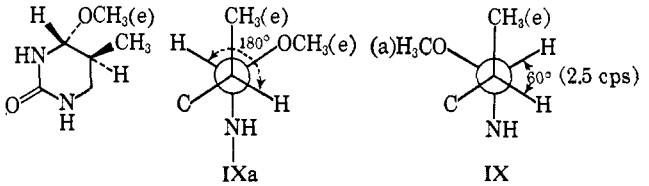


Figure 1. Nmr spectrum of racemic *cis*-2-oxo-4-methoxy-5-methylhexahydropyrimidine (IX) in deuteriomethanol-d_4.

were diequatorial and *trans* as in IXa, the dihedral angle between the vicinal protons should be approximately 180°. In that case the coupling constant should be large, about 10 cps. The observed coupling constant is 2.5 cps (Figure 1). The diequatorial *trans* conformation IXa is therefore ruled out in favor of the *cis* structure IX.



IXa (*trans*, diequatorial) IX (*cis*, equatorial–axial)

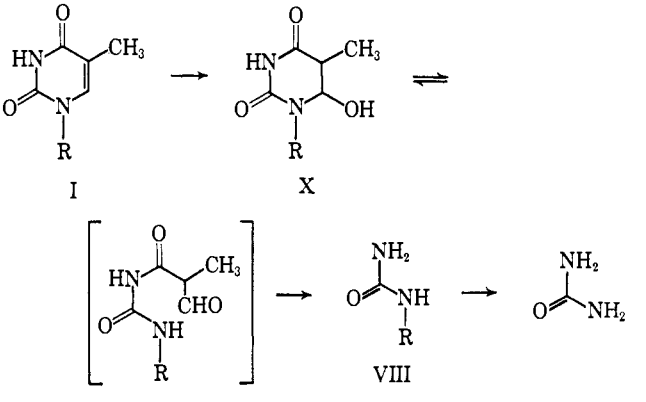
When thymidine was photoreduced in the presence of a tenfold excess of sodium borohydride, the starting material had disappeared completely after 2.5–3.5 hr. The reaction mixture showed at least seven products on thin layer chromatography. Repeated purification by silica gel column chromatography gave three compounds. The major product, which had the same R_f value (0.60) as N^3-(2-deoxy-β-D-*erythro*-pentafuranosyl)-3-ureido-2-methylpropanol-1 (IIIa), was obtained as a homogeneous oil, $[\alpha]^{27}D$ −34°. Its nmr spectrum was identical with that of IIIa obtained by hydrogenolysis of dihydrothymidine (II) with sodium borohydride.

The second product after hydrolysis yielded 2-oxo-4-methoxy-5-methylhexahydropyrimidine (IX) and must therefore be N^1-deoxyribosyl-2-oxo-4-methoxy-5-methylhexahydropyrimidine (VII). Compound VII arises from thymidine *via* dihydrothymidine.

(10) K. L. Williamson, *J. Am. Chem. Soc.*, **85**, 516 (1963); P. Laszlo and P. von R. Schleyer, *ibid.*, **85**, 2709 (1963); R. J. Abraham and K. G. R. Pachler, *Mol. Phys.*, 7, 165 (1963); K. L. Williamson, C. A. Lanford, and C. R. Nicholson, *J. Amer. Chem. Soc.*, **86**, 762 (1964); D. H. Williams and N. S. Bhacca, *ibid.*, **86**, 2742 (1964).

The third product was obtained as a colorless amorphous powder and was eluted last from the silica column. It was a weak base. This compound was repeatedly purified by precipitation from a solution in methanol by the addition of acetone. Complete purification or crystallization could not be achieved. The purified material displayed the typical ir absorption band of the ureido group at 1642 cm^{-1} and also gave a positive color test with *p*-dimethylaminocinnamaldehyde. After hydrolysis with dilute hydrochloric acid urea was obtained. On the basis of these observations this material is considered to be N-deoxyribosylurea which might arise *via photohydration of thymidine* followed by hydrogenolysis with sodium borohydride.

In solution, 2-deoxyribosylurea is present in an equibrium containing mostly 2-deoxy-β-D-ribopyranosylurea, some of the α-pyranoside, and possibly 2-deoxy-β-D-ribofuranosylurea (VIII).[11]



(11) *Cf.* W. E. Jenson, A. S. Jones, and G. W. Ross, *J. Chem. Soc.*, 2463 (1965).

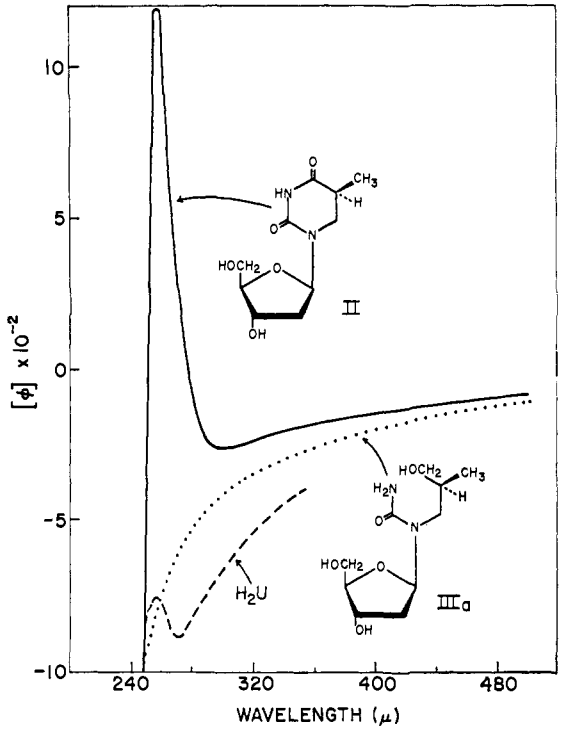


Figure 2. ORD curves for 5,6-dihydrouridine (H_2U) - - - -, 5,6-dihydrothymidine (II) ———, and N_3-(2-deoxy-β-D-*erythro*-pentafuranosyl)-3-ureido-(*S*)-(−)-2-methylpropanol-1 (IIIa) ·····.

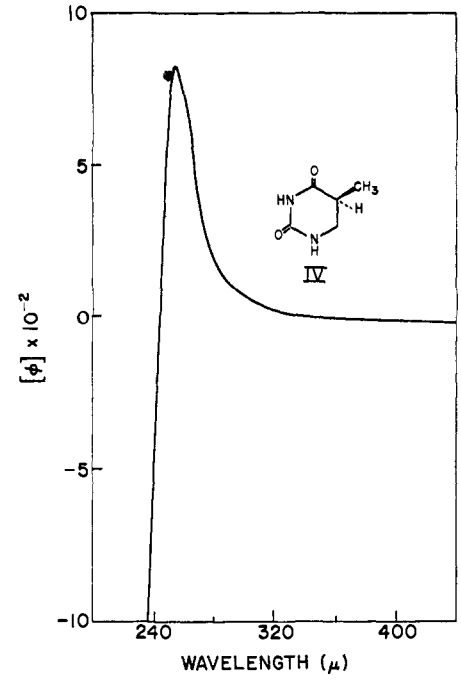
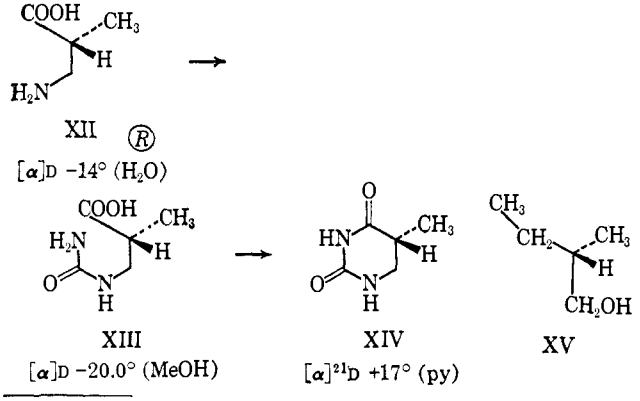


Figure 3. ORD curve for (−)-dihydrothymine (IV) obtained by acid hydrolysis of (−)-dihydrothymidine. The positive Cotton effect (257 mμ) supports the *S* configuration.

Absolute Configuration

The reduction of thymidine in the presence of 5% rhodium catalyst on alumina yields levorotatory dihydrothymidine II. The specific rotation of dihydrothymidine depended to some extent on the quality of the rhodium catalyst. Acid hydrolysis of (−)-dihydrothymidine (II) gave(−)-dihydrothymine (IVa).

Recently, (*R*)-(+)-dihydrothymine (XIV) has been synthesized[12] *via* (*R*)-(−)-β-ureidoisobutyric acid (XIII) which was prepared by the condensation of β-aminoisobutyric acid (XII) with potassium cyanate. (−)-β-Aminoisobutyric acid (XII) has been isolated from human urine[13] as the metabolic breakdown product of thymidine[14] and from bulbs of *Iris tingitana* var. Wedgewood.[15] (−)-β-Aminoisobutyric acid (XII) has the



(12) K. Balenović and W. Bregant, *Croat. Chem. Acta*, **32**, 193 (1960).
(13) H. R. Crumpler, C. E. Dent, H. Harris, and R. G. Westall, *Nature*, **167**, 307 (1951).
(14) R. M. Fink, C. McGaughey, R. E. Cline, and K. Fink, *J. Biol. Chem.*, **218**, 1 (1956).
(15) S. Asen, J. E. Thompson, C. J. Morris, and F. Irreverre, *ibid.*, 234, 343 (1959).

R configuration[16] on the basis of its correlation with the natural (*R*)-(−)-2-methylbutanol (active amyl alcohol).[17]

Dihydrothymine (IV) from the hydrolysis of (−)-dihydrothymidine (II) is levorotatory and must have the *S* configuration in the same way as its precursor II. The ORD curves of II (Figure 2) and IV (Figure 3) showed a positive Cotton effect at 257 and 256 mμ and a negative Cotton effect at 227.5 and 224 mμ due to n−π* transition at $C^4{=}O$ and $C^2{=}O$, respectively. β-Ureido derivatives IIIa (Figure 2) and V and VIa (Figure 4) had plain ORD curves. The positive Cotton effect at 257 mμ supports the *S* configuration with the absolute configuration at the C-5 methyl group as shown for compounds II and IV. This configuration has been confirmed by independent X-ray analysis carried out in the U. S. Naval Research Laboratory by Mrs. I. L. Karle.

(*S*)-(−)-Dihydrothymine (IV) was hydrolyzed with aqueous barium hydroxide to (*S*)-(+)-β-ureidoisobutyric acid (V), which was obtained crystalline for the first time (mp 61.5–62.5°, $[\alpha]^{27}D$ +28.9°). Ring opening of IV → V reverses the sign of rotation in analogy to the behavior of the antipods XIV → XIII.

Both N^3-[2-deoxy-β-D-*erythro*-pentafuranosyl]-3-ureido-2-methylpropanol-1 (IIIa) and its hydrolysis product, 3-ureido-2-methylpropanol-1 (VIa), showed negative plain ORD curves (Figures 2 and 4), which supports retention of the *S* conformation at the C-5 position.

In the same way IIIa obtained by photoreduction of thymidine and its hydrolysis product VIa had negative plain ORD curves. These observations suggested that

(16) K. Balenović and N. Bregant, *Chem. Ind.* (London), 2173 (1957); *J. Chem. Soc.*, 5131 (1965).
(17) (a) J. A. Mills and W. Klyne in "Progress in Stereochemistry," W. Klyne, Ed., Butterworth & Co. (Publishers) Ltd., 1954, p 188; (b) L. Crombie and S. H. Harper, *J. Chem. Soc.*, 2685 (1950).

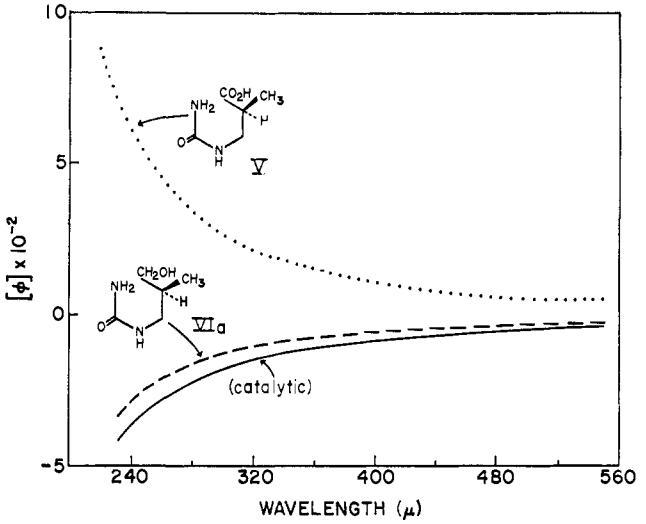


Figure 4. ORD spectra of (+)-β-ureidoisobutyric acid (V) ·····, and (−)-3-ureido-2-methylpropanol-1 (VIa) obtained from thymidine by photoreduction in the presence of $NaBH_4$ - - - -, and obtained from catalytically reduced dihydrothymidine by acid cleavage ———. The rotational data support the *S* configuration for both compounds V and VIa.

the photoreduction of thymidine to IIIa proceeds *via* dihydrothymine II.

Experimental Section

General Procedures. Melting points are uncorrected and were taken on a Büchi apparatus. A Perkin-Elmer Infracord spectrophotometer, Model 137B, was used for infrared spectra. Nuclear magnetic resonance spectra were obtained with a Varian A-60 spectrometer. Chemical shifts in deuterated solvents are given as ppm with tetramethylsilane (TMS) as an internal standard. For spectra in D_2O the sodium salt of 3-(trimethylsilyl)-1-propanesulfonic acid served as an internal reference. Rotations were measured with a Rudolf spectropolarimeter. Optical rotatory dispersion curves were taken on a Cary Model 60 spectrometer at 27°. Thin layer chromatograms coated with silica gel G (Research Specialties Co.) were developed with the following solvent systems: solvent A, chloroform–methanol 9:1; solvent B, chloroform–methanol 7:3. The spots were made visible by exposure to iodine vapor or by spraying with *p*-dimethylaminocinnamaldehyde (modified Ehrlich reagent).

1-Dihydrothymidine (II). Thymidine was reduced catalytically by the method of Cohn, *et al.*[18] Crystallization from ethanol yielded colorless plates, mp 155–156° (lit.[19] mp 152–153°).

Anal. Calcd for $C_{10}H_{16}N_2O_5$: C, 49.17; H, 6.60; N, 11.47. Found: C, 49.30; H, 6.78; N, 11.24.

The following data were also obtained: $[\alpha]^{23}D$ −20.5 ± 0.3° (*c* 1.11, H_2O); ir (Nujol) (cm^{-1}) 3610, 3460, 3226, and 3115 (-OH and >NH), 1686 (ureido), and 1075–1001 (ν_{C-O}); nmr (D_2O) (ppm) 1.21 (d, *J* = 7 cps) (>CHCH_3), 2.23 (C_2' protons of deoxyribosyl), 2.27 (m) (>CHCH$_3$), 3.37 (-CH_2N<), 3.70 (C_5' protons of deoxyribosyl), 3.86 (C_4' protons of deoxyribosyl), 4.37 (C_3' protons of deoxyribosyl), 6.27 (t, *J* = 7.2 cps, C_1' protons of deoxyribosyl); ORD (*c* 0.101, EtOH) $[\phi]_{500}$ −71.94°, $[\phi]_{475}$ −88.60°, $[\phi]_{450}$ −103.79°, $[\phi]_{425}$ −120.40°, $[\phi]_{400}$ −144.63°, $[\phi]_{375}$ −165.84°, $[\phi]_{350}$ −194.98°, $[\phi]_{325}$ −222.92°, $[\phi]_{300}$ −257.45°, $[\phi]_{290}$ −223.38°, $[\phi]_{280}$ −109.80°, $[\phi]_{270}$ +299.10°, $[\phi]_{265}$ +672.99°, $[\phi]_{260}$ +1115.98°, $[\phi]_{257}$ +1196.43°, $[\phi]_{255}$ +1120.70°, $[\phi]_{250}$ +216.96°, $[\phi]_{245}$ −2319.0°, $[\phi]_{240}$ −5631.95°, $[\phi]_{235}$ −9228.82°, $[\phi]_{230}$ −12068.5°, $[\phi]_{227.5}$ −12588.7°, $[\phi]_{220}$ −7193.5°.

(*S*)-(−)-Dihydrothymine (IV). A. By Acid Hydrolysis of Dihydrothymidine. A solution of 248.8 mg of 1-dihydrothymidine (II) in 25 ml of 0.1 *N* HCl was heated on the steam bath for 1 hr. The reaction mixture was neutralized with silver carbonate and the deposited silver chloride removed by filtration. The aqueous filtrate was saturated with hydrogen sulfide and silver sulfide removed by filtration over Celite. The filtrate was lyophilized and the residue crystallized from methanol to give a quantitative yield of colorless needles, mp 262.5–263°.

Anal. Calcd for $C_5H_8N_2O_2$: C, 46.87; H, 6.29; N, 21.87. Found: C, 46.88; H, 6.21; N, 21.70.

The following data were obtained: $[\alpha]^{23}D$ −6.5 ± 2.0° (*c* 0.16, H_2O), $[\alpha]^{20}D$ −11.3 ± 1.0° (*c* 0.49, pyridine); ir (Nujol) (cm^{-1}) 3289 and 3115 (>NH), 1754 (sh), 1733 and 1712 (C=O), 1669 (ureido group); nmr (DMSO-d_6) (ppm) 1.07 (d, *J* = 6.7 cps) (>CHCH_3), 2.67 (m) (>CHCH$_3$), 3.14 (m) (-NHCH_2CHCH$_3$), 7.47 (br) (=NH); ORD (*c* 0.098, EtOH) $[\phi]_{400}$ −16.88°, $[\phi]_{375}$ −14.33°, $[\phi]_{350}$ −5.12°, $[\phi]_{325}$ +13.80, $[\phi]_{300}$ +63.89°, $[\phi]_{290}$ +108.49°, $[\phi]_{280}$ +196.90°, $[\phi]_{270}$ +419.66°, $[\phi]_{265}$ +595.17°, $[\phi]_{260}$ +766.59°, $[\phi]_{256}$ +822.26°, $[\phi]_{255}$ +822.26°, $[\phi]_{250}$ +524.18°, $[\phi]_{245}$ +197.03°, $[\phi]_{240}$ −55.26°, $[\phi]_{235}$ −1356.13°, $[\phi]_{230}$ −2302.55°, $[\phi]_{224}$ −2852.81°, $[\phi]_{220}$ −2198.42°.

B. By Reduction and Hydrogenolysis of Thymidine. A solution of 200 mg of thymidine in 40 ml of 0.001 *N* HCl was hydrogenated at 22 psi for 3 hr in the presence of 70 mg of 5% rhodium on alumina. The catalyst was removed by filtration and the filtrate lyophilized. The residual oil was dissolved in methanol. This solution on standing deposited colorless crystals. Recrystallization from methanol or water yielded (*S*)-(−)-dihydrothymine (IV) as colorless prisms, mp 259.5–260°.

Anal. Calcd for $C_5H_8N_2O_2$: C, 46.87; H, 6.29; N, 21.87. Found: C, 46.73; H, 6.35; N, 21.78.

C. By Catalytic Reduction of Thymidine by the Method of Miles[4] and Acid Hydrolysis. Thymidine was reduced catalytically at atmospheric pressure for 12–24 hr as described by Miles.[4] The reduction product showed three to four spots on tlc in chloroform–methanol (7:3). The mixture was hydrolyzed with 0.1 *N* HCl on the steam bath and the hydrolysate worked up as described above. Crystallization from methanol afforded colorless prisms, mp 261–262°.

Anal. Calcd for $C_5H_8N_2O_2$: C, 46.87; H, 6.29; N, 21.87. Found: C, 47.08; H, 6.10; N, 21.84; $[\alpha]^{21}D$ −7.8 ± 1.0° (*c* 0.683, H_2O).

The melting point of this material was undepressed on admixture with an authentic specimen of (*S*)-(−)-dihydrothymine (IV), and both compounds were identical with regard to infrared spectra and R_f values on tlc in solvent B.

(*S*)-3-Ureido-2-methylpropanol-1 (VIa) by Reductive Cleavage of 1-Dihydrothymine (II) with Sodium Borohydride. The hydrogenolysis was carried out as described by Ballé, *et al.*[3] The reduction product was purified by chromatography on a column of silica gel (chloroform–methanol, 7:3). In this way a colorless homogeneous oil was obtained which showed a single spot on tlc in solvent system B.

N,O-Diacetyl-(*S*)-(−)-3-ureido-2-methylpropanol-1 (VIb). This diacetate was prepared by following the published procedure for acetylation.[5] Crystallization from aqueous methanol gave colorless plates, mp 90.5–92°, $[\alpha]^{24}D$ +4.8 ± 1.5° (*c* 0.137, EtOH).

Anal. Calcd for $C_9H_{16}N_2O_4$: C, 49.99; H, 7.46; N, 12.96. Found: C, 50.08; H, 7.53; N, 13.20.

The following data were obtained: ir (Nujol) (cm^{-1}) 3378, 3322, 3165 (>NH), 1748 and 1704 (sh, acetyl), 1669 (ureido), 1259 and 1236 (acetyl), 1031 (ν_{C-O}); nmr ($CDCl_3$) (ppm) 1.00 (d, *J* = 7.1 cps) (CH_3CH<), 2.1 (m, CH_3CH<), 2.16 (methyl of acetyl), 3.34 (t, *J* = 6 cps) (-NHCH_2CH<), 4.06 (d, *J* = 6 cps) (>CHCH_2OAc), 8.74 (t, *J* = 6 cps) (-CONHCH$_2$-), 10.31 (s, AcNHCO-).

N,O-Bis-*p*-iodobenzoyl-DL-3-ureido-2-methylpropanol-1. To a chilled solution of 1.28 g of DL-3-ureido-2-methylpropanol-1 in dry pyridine (20 ml) was added 5.0 g of *p*-iodobenzoyl chloride. The reaction mixture was allowed to stand overnight at room temperature and then poured into ice water. The crystalline deposit was dissolved in methylene chloride and the solution washed with dilute alkali, acid, and water. The solvent was removed and the residue crystallized from a mixture of methylene chloride and methanol to yield the pure bis-*p*-iodobenzoate as colorless needles, mp 199–200°.

Anal. Calcd for $C_{19}H_{18}N_2O_4I_2$: C, 38.53, H, 3.06; N, 4.79. Found: C, 38.61; H, 2.82; N, 4.62.

The following data were obtained: ir (Nujol) (cm^{-1}) 3322 and 3135 (>NH), 1718 and 1692 (carbonyl of *p*-iodobenzoyl), 1664 (ureido), 1585 (aromatic), 1276 and 1261 (ν_{C-O}), 1111 and 1099 (ν_{C-O}), 750 (aromatic).

O-*p*-Iodobenzoyl-DL-3-ureido-2-methylpropanol-1. When 66 mg (5×10^{-4} mol) of DL-3-ureido-2-methylpropanol-1 was acylated as above with an equimolar quantity, namely, 134 mg (5×10^{-4} mol) of *p*-iodobenzoyl chloride, the O-mono-*p*-iodobenzoate was

(18) W. E. Cohn and D. G. Doherty, *J. Amer. Chem. Soc.*, 78, 2863 (1956).

(19) M. Green and S. S. Cohen, *J. Biol. Chem.*, 225, 397 (1957).

obtained. On recrystallization from ether it formed colorless prisms, mp 131° dec; modified Ehrlich test positive.

Anal. Calcd for $C_{12}H_{15}N_2O_3I$: C, 39.79; H, 4.17; N, 7.73. Found: C, 39.98; H, 4.18; N, 7.76.

The following data were obtained: ir (Nujol) (cm^{-1}) 3472, 3378 and 3236 (NH_2 and >NH), 1712 (*p*-iodobenzoyl carbonyl), 1639 (ureido), 1597 (aromatic), 1272 (sh), 1264, 1114 and 1101 (ν_{C-O}), 750 (aromatic).

O,N-Bis-*p*-iodobenzyl-(*S*)-(−)-3-ureido-2-methylpropanol-1 (VIc). The bis-*p*-iodobenzoate VIc of the optically active ureido alcohol VIa was prepared by the procedure described for the racemic bis-*p*-iodobenzoate. Crystallization from a mixture of methylene chloride–ether gave colorless needles, mp 196.5–197° dec.

Anal. Calcd for $C_{19}H_{18}N_2O_4I_2$: C, 38.53; H, 3.06; N, 4.79. Found: C, 38.29; H, 2.92; N, 4.63.

The following data were obtained: ir (Nujol) (cm^{-1}) 3344, 3300 and 3195 (>NH), 1718 and 1669 (*p*-iodobenzoyl carbonyl), 1664 (-NH*CO*NH-), 1585 (aromatic), 1277, 1263, 1112 and 1099 (ν_{C-O}), 751 (aromatic).

(*S*)-(+)-3-Ureidoisobutyric Acid. The hydrolysis of 200 mg of (*S*)-(−)-dihydrothymine was carried out in the cold with baryta as described for the racemic compound. The resulting hydrolysate, which showed two spots on tlc, was purified by silica gel column chromatography. The column (15 × 300 mm) was eluted with a mixture of chloroform–methanol (7:3). Fractions of 6.5 ml were collected. Fractions 8–14 on evaporation gave the free acid V as a pale yellow oil which solidified on standing, mp 61.5–62.5°, $[\alpha]^{27}D$ +28.9° (*c* 0.1, EtOH).

Anal. Calcd for $C_5H_{10}N_2O_3$: C, 41.09; H, 6.90; N, 19.17. Found: C, 41.11; H, 6.85; N, 19.13.

The following data were obtained: ir (Nujol) (cm^{-1}) 3448 and 3279 (NH_2, >NH, and $-CO_2H$), 1721 (CO_2H), 1639 and 1613 (ureido); nmr ($CDCl_3$) (ppm) 1.16 (d, J = 7 cps) (>CHCH_3), 2.69 (sextet, J = 7 cps) (>CHCH$_3$), 3.31 (br t, J = 6 cps) (-NH-CH_2CHCH$_3$), 7.69 (CO_2H), 4.99 (br) (-NH_2), 6.07 (br t) (-NHCH$_2$-); ($CDCl_3$ + one drop D_2O) 1.16 (d, J = 7 cps) (>CHCH_3), 2.68 (sextet) (>CHCH$_3$), 3.31 (d, J = 6.4 cps) (-NDCH_2CHCH$_3$); ORD (*c* 0.104, EtOH) $[\phi]_{600}$ +47.34°, $[\phi]_{575}$ +47.34°, $[\phi]_{550}$ +49.08°, $[\phi]_{525}$ +54.80°, $[\phi]_{500}$ +61.29°, $[\phi]_{475}$ +74.75°, $[\phi]_{450}$ +84.47°, $[\phi]_{425}$ +97.17°, $[\phi]_{400}$ +117.10°, $[\phi]_{375}$ +137.04°, $[\phi]_{350}$ +165.69°, $[\phi]_{325}$ +204.31°, $[\phi]_{300}$ +259.62°, $[\phi]_{290}$ +289.02°, $[\phi]_{280}$ +329.64°, $[\phi]_{270}$ +378.72°, $[\phi]_{260}$ +440.26°, $[\phi]_{250}$ +478.14°, $[\phi]_{240}$ +609.40°, $[\phi]_{230}$ +733.39°, $[\phi]_{220}$ + 864.25°.

Reclosure of 3-Ureido-2-methylpropionic Acid (XIII) to Dihydrothymine (II). A solution of 0.5 g of 3-ureido-2-methylpropionic acid in 6.0 *N* hydrochloric acid was refluxed for 9 hr and then evaporated to dryness. Crystallization and recrystallization from methanol gave homogeneous dihydrothymine as colorless plates (240 mg), mp 264.5–265°, identical with an authentic sample by ir and mixture melting point.

Anal. Calcd for $C_5H_8N_2O_3$: C, 46.87; H, 6.29; N, 21.87. Found: C, 47.07; H, 6.07; N, 22.11.

N^3-(2-Deoxy-β-D-*erythro*-pentafuranosyl)-3-ureido-(*S*)-2-methylpropanol-1 (IIIa). Reductive Ring Opening of 1-Dihydrothymidine (II) with Sodium Borohydride. To a solution of 2.0 g (8.18 × 10^{-3} mol) of 1-dihydrothymidine (II) in 200 ml of water was added 0.3 g (8.0 × 10^{-3} mol) of sodium borohydride. Stirring was continued for 30 min. Then another 0.3 g of sodium borohydride was added and the solution stirred for another 30 min. The resulting solution was passed through a column (10 × 300 mm) of exchange resin IRC-50 (H^+ form). The aqueous eluate (pH 6–7) was lyophilized. The amorphous colorless solid residue was dissolved in methanol and the solvent removed *in vacuo*. This process was repeated three times. The resulting colorless viscous oil showed three spots on tlc in chloroform–methanol (7:3). The oil was dissolved in chloroform and put on a column of silica gel (24 × 300 mm, 0.05–0.20 mm). The column was eluted with a mixture of chloroform–methanol (7:3). Fractions were collected for every 5 ml: fractions 10 and 11, colorless crystals (trace); fractions 12–20, oil (R_f 0.60) (1.554 g); fractions 21–83, oil (three spots, rechromatographed).

Fractions 12–20 were pooled and rechromatographed on a column of silica gel in the same solvent system. The resulting colorless homogeneous oil (1.554 g, 76.4%) showed a single spot (R_f 0.6) on tlc (solvent B).

The following data were obtained: ir (liquid film) (cm^{-1}) 3448 (OH, NH_2), 1692 and 1639 (ureido), 1075–1004 (ν_{C-O}); nmr (D_2O) (ppm)[20] 0.83–1.31 (m) (>CHCH_3), 2.20 (m) (C_2' protons of deoxyribosyl), 3.77 (C_5' proton of deoxyribosyl), 6.28 (t, J = 7.5 cps) (C_1' proton of deoxyribosyl). Other protons could not be assigned owing to overlap.

Tetra-*p*-nitrobenzoate IIIb. To a chilled solution of 124 mg (5 × 10^{-4} mol) of IIIa in dry pyridine (5 ml) was added with stirring 371 mg (2 × 10^{-3} mol) of *p*-nitrobenzoyl chloride. The reaction mixture was kept overnight at 35–40° and then poured into ice–water. The precipitate was collected, washed with water, and dissolved in chloroform. The chloroform solution was washed with 10% aqueous soda solution and water. The solvent was removed under a stream of nitrogen and the residue recrystallized from ethanol to yield yellow crystals, mp 111° dec.

Anal. Calcd for $C_{38}H_{32}N_6O_{17}\cdot H_2O$: C, 52.90; H, 3.76; N, 9.74. Found: C, 52.63; H, 4.05; N, 9.45.

The following data were obtained: ir (Nujol) (cm^{-1}) 3165 (>NH), 1724 (*p*-nitrobenzoyl carbonyl), 1684 (ureido carbonyl), 1605 (aromatic), 1520 and 1339 (NO_2), 1261 and 1098 (ν_{C-O}), 718 (aromatic).

Tetra-*p*-iodobenzoate IIIc. To a solution of 0.206 g (8.3 × 10^{-4} mol) of IIIa in 15 ml of dry pyridine was added 0.9 g (4 equiv) of *p*-iodobenzoyl chloride. The mixture was kept at 50° for 48 hr and then worked up in the usual manner. The crude crystalline product was recrystallized from methanol to yield slightly yellow needles, mp 212–213° dec.

Anal. Calcd for $C_{38}H_{32}N_2O_9I_4$: C, 39.06; H, 2.76; N, 2.39. Found: C, 38.86; H, 2.61; N, 2.31.

The following data were obtained: ir (Nujol) (cm^{-1}) 3484 and 3311 (>NH), 1718, 1686, and 1664 (*p*-iodobenzoylic carbonyl and ureido), 1585 (aromatic), 1267, 1114, and 1103 (ν_{C-O}), 756 (aromatic); nmr ($CDCl_3$ + CD_3OD) (ppm) 1.11 (d, J = 7 cps) (>CHCH_3), 2.37 (m) (C_2' protons of deoxyribosyl), 3.45 (C_5' protons of deoxyribosyl), 5.51 (C_3' protons of deoxyribosyl), 6.40 (t, J = 7 cps) (C_1' protons of deoxyribosyl), 7.79 (*p*-iodobenzoyl ring protons). Other protons could not be assigned owing to overlap.

Tetraacetate IIId. To a solution of 750 mg of IIIa in dry pyridine (8 ml) was added 5 ml of acetyl anhydride. The reaction mixture was allowed to stand overnight at room temperature. Excess reagents were removed *in vacuo* and poured into ice–water. The deposit was extracted with chloroform and purified on a silica gel column (15 × 200 mm, chloroform). The fractions which showed R_f 0.64 were collected and evaporated *in vacuo* to yield a colorless viscous oil.

Anal. Calcd for $C_{15}H_{28}N_2O_9$: C, 51.91; H, 6.78; N, 6.73. Found: C, 51.89; H, 6.59; N, 6.81.

The following data were obtained: ir (liquid film) (cm^{-1}) 3311 (>NH), 1739–1692 (acetyl and ureido), 1225 (acetyl), 1053 and 1020 (ν_{C-O}); nmr ($CDCl_3$ + CD_3OD) (ppm) 1.27 (d, J = 6.9 cps) (>CHCH_3), 2.09 (acetyl), 2.15 (m) (C_2' protons of deoxyribosyl), 2.65 (m) (>CHCH$_3$), 3.33 (m) (>NCH_2CHCH$_3$), 4.10 and 4.22 (C_5' protons of deoxyribosyl), 4.27 (C_4' proton of deoxyribosyl), 5.14 (m) (C_3' protons of deoxyribosyl), 6.33 (t, J = 8 cps) (C_1' protons of deoxyribosyl).

Hydrolysis of N^3-(2-Deoxy-β-D-*erythro*-pentafuranosyl)-3-ureido-2-methylpropanol-1 (IIIa) to (*S*)-(−)-β-Ureido-2-butanol (VIa). A solution of 0.5 g of N^3-(2-deoxy-β-D-*erythro*-pentafuranosyl)-3-ureido-(*S*)-2-methylpropanol-1 was hydrolyzed by heating in 0.1 *N* hydrochloric acid (50 ml) for 1 hr in a boiling water bath. The reaction mixture was then passed over a column (20 × 150 mm) of Dowex 1-X8 (hydroxyl form) and lyophilized. The residue was purified on a silica gel column (15 × 250 mm) and the column eluted with a mixture of chloroform–methanol (8:2). Fractions of 6 ml each were collected. Fractions 15–22 were pooled and evaporated *in vacuo*. This fraction showed a homogeneous spot (R_f 0.58) on tlc in solvent B; modified Ehrlich test positive. This substance was identical with (*S*)-(−)-β-ureido-2-butanol (VIa) by ir spectra and R_f on tlc.

The following data were obtained: ORD (*c* 0.10, MeOH) $[\phi]_{600}$ −33.12°, $[\phi]_{575}$ −35.92°, $[\phi]_{550}$ −39.95°, $[\phi]_{525}$ −43.32°, $[\phi]_{500}$ −48.74°, $[\phi]_{475}$ −55.32°, $[\phi]_{450}$ −62.49°, $[\phi]_{425}$ −72.11°, $[\phi]_{400}$ −84.86°, $[\phi]_{375}$ −99.53°, $[\phi]_{350}$ −117.48°, $[\phi]_{325}$ −142.06°, $[\phi]_{300}$ −179.23°, $[\phi]_{275}$ −234.85°, $[\phi]_{250}$ −319.94°, $[\phi]_{240}$ −368.71°.

Hydrolysis of N^1-Deoxyribosyl-2-oxo-4-methoxy-5-methylhexahydropyrimidine (VII) to Racemic 2-Oxo-4-methoxy-5-methylhexahydropyrimidine (IX). The combined fractions 21–83 were rechromatographed on a silica gel column followed by elution with chloroform–methanol (8:2). The fractions with the same R_f value (0.35) in solvent B were collected to yield a viscous oil which showed the presence of the 2-deoxyribosyl residue by the diphenyl-

(20) From TMS as external standard.

amine assay.[21] A solution of 200 mg of N¹-deoxyribosyl-2-oxo-4-methoxy-5-methylhexahydropyrimidine was heated in 30 ml of 0.1 *N* hydrochloric acid in a boiling water bath. After passing through a Dowex 1-X8 (hydroxyl form) column (15 × 150 mm) the solution was evaporated at 40° under reduced pressure. The residual oil was purified by column chromatography on silica gel (15 × 300 mm) followed by elution with chloroform–methanol (9:1). Fractions of 6 ml were collected. Fractions 9–14 were crystalline and showed a homogeneous spot (R_f 0.73) on tlc in solvent B. Crystallization from methanol–ether gave colorless needles, mp 159–161°; modified Ehrlich test positive.

Anal. Calcd for $C_6H_{12}N_2O_2$: C, 49.98; H, 8.39; N, 19.43. Found: C, 49.86; H, 8.17; N, 19.28; $[\alpha]^{27}D \pm 0.0°$ (*c* 1.0, EtOH).

The following data were obtained: ir (Nujol) (cm^{-1}) 3401 (sh), 3311 and 3135 (>NH), 1689 (cyclic ureido group), 1072 (ν_{C-O}); nmr (CD_3OD) (ppm) 0.93 (d, J = 6.3 cps) (>CHCH_3), 1.9 (m) (>CHCH$_3$), 2.81 (s) (>NDCH^{β}H–), 2.95 (d, J = 3.8 cps) (>NDCHH^{α}–), 3.13 (>CHOCH_3), 3.97 (d, J = 2.6 cps) (–NDCH(OCH$_3$)-CHCH$_3$).

Photoreduction of Thymidine. An aqueous solution of thymidine (2×10^{-3} mol) and sodium borohydride (2×10^{-2} mol) were irradiated with a U-shaped Hanovia low-pressure mercury lamp, 687A-45, with an intensity of 4.3 W at 253.7 mμ at room temperature where nitrogen was bubbled slowly through the reaction mixture. Irradiation was continued until after 2.5–3.5 hr the uv absorption maximum at 267 mμ of thymidine had disappeared. A total of 1.0 g of thymidine was irradiated. The irradiated mixture was then passed over a column of Amberlite IRC-50 (H^+ form). The eluted solution was lyophilized. The residue was codistilled twice with methanol *in vacuo*. Tlc (silica gel G + AcONa; solvent B) of the residue, a colorless viscous oil, 1.0 g, showed the presence of four major and at least an additional three to four minor products. The photoreduced products were chromatographed on a column (24 × 280 mm) of silica gel and eluted with chloroform–methanol (9:1). Fractions of 6 ml each were collected: fractions 33–47, colorless crystals (3 mg); fractions 48–60, oil (compound IIIa); fractions 74–92, oil (trace); fractions 93–139, oil; fractions 145–165, oil; fractions 166–251, amorphous powder.

N³-(2-Deoxy-β-D-*erythro*-pentafuranosyl)-3-ureido-(*S*)-2-methylpropanol-1 (IIIa). Fractions 48–60 were pooled (yield 0.213 g), rechromatographed on a column (15 × 290 mm), and eluted with a mixture of chloroform–methanol (9:1). The colorless homogeneous oil was identical with N³-(2-deoxy-β-D-*erythro*-pentafuranosyl)-3-ureido-(*S*)-methylpropanol-1 (IIIa) from the hydrogenolysis of dihydrothymidine by sodium borohydride with regard to nmr spectrum and R_f value (0.60) on tlc in solvent B; $[\alpha]^{27}D$ −34.35° (*c* 0.08, EtOH).

The following data were obtained: ORD (*c* 0.08, EtOH) $[\phi]_{600}$ −82.54°, $[\phi]_{575}$ −87.49°, $[\phi]_{550}$ −89.14°, $[\phi]_{525}$ −107.30°, $[\phi]_{500}$ −110.05°, $[\phi]_{475}$ +24.06°, $[\phi]_{450}$ −140.86°, $[\phi]_{425}$ −162.32°, $[\phi]_{400}$ −187.09°, $[\phi]_{375}$ −220.10°, $[\phi]_{350}$ −266.97°, $[\phi]_{325}$ −330.15°, $[\phi]_{300}$ −405.48°, $[\phi]_{290}$ −433.60°, $[\phi]_{280}$ −519.99°, $[\phi]_{270}$ −642.69°, $[\phi]_{260}$ −745.59°, $[\phi]_{250}$ −938.49°, $[\phi]_{240}$ −1334.81, $[\phi]_{230}$ −1926.53°.

(*S*)-(−)-3-Ureido-2-methylpropanol-1 (VIa). N³-(2-Deoxy-β-D-*erythro*-pentafuranosyl)-3-ureido-(*S*)-2-methylpropanol-1 (IIIa), obtained by photoreduction of thymidine, was hydrolyzed in the same manner as described above. The resulting hydrolysate was purified by silica gel column chromatography. The homogeneous oil was identical with an authentic specimen of (*S*)-(−)-β-ureido-2-methylpropanol-1 (VIa).

The following data were obtained: ORD (*c* 0.1, MeOH) $[\phi]_{600}$ −22.16°, $[\phi]_{575}$ −25.12°, $[\phi]_{550}$ −27.41°, $[\phi]_{525}$ −30.21°, $[\phi]_{500}$ −33.62°, $[\phi]_{475}$ −37.67°, $[\phi]_{450}$ −42.69°, $[\phi]_{425}$ −49.75°, $[\phi]_{400}$ −55.98°, $[\phi]_{375}$ −68.72°, $[\phi]_{350}$ −81.76°, $[\phi]_{325}$ −101.55°, $[\phi]_{300}$ −128.78°, $[\phi]_{275}$ −175.70°, $[\phi]_{250}$ −245.90°, $[\phi]_{240}$ −287.03°.

Identification of 2-Oxo-4-methoxy-5-methylhexahydropyrimidine (IX) as a Product of Photoreduction. The combined fractions 93–139 left a viscous oil which showed several spots on tlc. The oil was hydrolyzed with 0.1 *N* hydrochloric acid in a boiling water bath. The resulting hydrolysate was worked up as usual and purified on a column of silica gel followed by elution with a mixture of chloroform–methanol (9:1). Fractions 13 and 14 were crystalline. Recrystallization from methanol–ether gave colorless needles, mp 160°, identical with a specimen of 2-oxo-4-methoxy-5-methylhexahydropyrimidine with regard to infrared spectrum, mixture melting point, and R_f value on tlc.

N-Deoxyribosylurea and Hydrolysis to Urea. Fractions 166–251 were pooled and evaporated *in vacuo*. The amorphous powder was dissolved in a small amount of methanol and acetone added until the solution became turbid. On standing a microcrystalline powder deposited. Although this process was repeated several times, tlc showed that this substance was not completely homogeneous; yield 220 mg (30.26%); ir (Nujol) (cm^{-1}) 3425 (–NH_2, >NH, and –OH), 1642, (ureido group), and 1073 (ν_{C-O}).

For hydrolysis 210 mg of N-β-deoxyribosylurea was heated with 35 ml of 0.1 *N* hydrochloric acid at 80° for 5 hr. The resulting solution was passed through a column (15 × 160 mm) of Dowex 1-X8 (hydroxyl form) exchange resin and the column thoroughly washed with water. The combined eluate was lyophilized. The residue was chromatographed on a column (10 × 250 mm) of silica gel followed by elution with a mixture of chloroform–methanol (7:3). Fractions of 6 ml were collected. Fractions 25–35 were pooled and evaporated *in vacuo*. The residue crystallized from a mixture of chloroform–methanol to yield colorless needles, mp 131–132°, yield 10 mg, identical with an authentic specimen of urea with regard to infrared spectrum, mixture melting point, and R_f value (0.35) on tlc in solvent B.

ORD of dihydrouridine (*c* 0.102, EtOH, 27°), mp 101–103°, was as follows: $[\phi]_{550}$ −396.12°, $[\phi]_{325}$ −496.86°, $[\phi]_{300}$ −651.39° $[\phi]_{290}$ −732.53°, $[\phi]_{280}$ −819.13°, $[\phi]_{270}$ −883.85°, $[\phi]_{265}$ −854.22° $[\phi]_{260}$ −770.36°, $[\phi]_{258.5}$ −755.75°, $[\phi]_{255}$ −813.21°, $[\phi]_{250}$ −1308.69° $[\phi]_{245}$ −1566.32°, $[\phi]_{240}$ −4056.02°, $[\phi]_{235}$ −7070.63°, $[\phi]_{23}$ −9245.18°, $[\phi]_{227.5}$ −9404.78°, $[\phi]_{225}$ −8937.38°, $[\phi]_{220}$ −5129.88°.

(21) E. Volkin and W. Cohn, *Methods Biochem. Anal.*, **1**, 287 (1954).