Photoreduction of Uridine and Reduction of Dihydrouridine with Sodium Borohydride

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Received August 17, 1967

Abstract: Irradiation of aqueous solutions of uridine (VI) at pH 9.5–10 and 50° with a low-pressure mercury lamp in the presence of a 2.0 *M* excess of sodium borohydride until 65% or less of the absorbance at 260 mμ had disappeared leads to dihydrouridine (VII), which was crystallized for the first time, and only minor amounts of the products X, resulting from photohydration, and VIII, formed by light-independent hydrogenolysis by excess borohydride. The product of the secondary (dark) reaction, N-(β-D-ribofuranosyl)-N-(γ-hydroxypropyl)urea (VIII), was characterized as the penta-*p*-iodobenzoate, mp 248°. The hydrogenolysis product from dihydrouracil and borohydride, γ-ureidopropanol (III), mp 62°, was characterized as the N,O-bis-*p*-nitrobenzoyl derivative. The structure of III was proven by oxidation to γ-ureidopropionic acid (V), identical with the product of basic ring opening of dihydrouracil (II). Pyrolysis of γ-ureidopropanol (III) gave the cyclic urethan IV.

As part of a program on selective chemical modification and degradation of nucleic acids we have previously and briefly reported on the photoreduction of uridine and uridylic acid to their 5,6-dihydro derivatives in the presence of sodium borohydride.[2] This reaction was studied in more detail especially in connection with the unexpected results, which were obtained in the photoreduction of thymidine with sodium borohydride.[3,4] Dihydrothymidine is not found, even chromatographically, among the major products of the photoreduction of thymidine. Dihydrothymine undergoes facile reductive ring opening at a rate which competes with the photoreduction of thymidine. The major photoproduct is the (*S*)-3-ureido-2-methylpropanol-1 derivative resulting from hydrogenolysis of dihydrothymidine, the presumable intermediate.[4]

Similarly dihydrouracil and its derivatives are reduced in a light-independent reaction to the corresponding γ-ureidopropanol derivative after prolonged exposure to sodium borohydride. In the photoreduction of uridine, however, the relation between the rates of the primary formation of 5,6-dihydrouridine and the secondary reductive ring fission is such that dihydrouridine can be obtained as the major product under the following conditions. Uridine is irradiated (2537 Å) at 50° and pH 9.5–10 in the presence of a 2 *M* excess of sodium borohydride. The reaction is stopped by acidification with Dowex 50W-X8 (H^+) at a point where approximately two-thirds or less of the absorbance of uridine at 260 mμ have disappeared. Only traces of N-(ribosyl)-N-(γ-hydroxypropyl)urea (VIII) are detected by thin layer chromatography under these conditions. The elevated temperature is used to favor photoreduction over photohydration of uridine. The product of photohydration, 6-hydroxy-5,6-dihydrouridine, but not the products of photoreduction largely revert to starting material upon heating[5] (Figure 1).

The outlined conditions have proved to be optimal for the formation of 5,6-dihydrouridine and have, for example, been used for the partial reduction of poly U.[6,7] They have been established in a series of experiments in which the reaction was studied as a function of pH (Figure 2) and temperature (Figure 3). The disappearance of the starting material was followed by measuring the absorbance at 260 mμ (pH 1). Due to labilization of the N-glycosidic bond in uridine upon saturation of the 5,6-double bond, the formation of the reduction products can be followed with the orcinol assay for ribose, which is negative for 6-hydroxy-5,6-dihydrouridine, the product of the competing photohydration of uridine.

Dihydrouridine was prepared on a preparative scale by photoreduction of uridine. The compound was purified by column chromatography on silica gel and found to be identical in every respect with the product obtained from the catalytic hydrogenation of uridine in the presence of a rhodium catalyst on alumina. 5,6-Dihydrouridine was for the first time obtained in a crystalline state, mp 101–103° dec, $[\alpha]^{23}D$ −36.8 ± 0.4°.

The light-independent secondary reaction, which occurs after prolonged treatment with sodium borohydride, was studied with dihydrouracil and dihydrouridine as respective starting materials.

When dihydrouracil (II) was reduced with sodium borohydride in water[8] the major product was γ-ureidopropanol (IIIa) which was obtained in 82% yield. The alcohol, a distillable oil, bp 190° (bath temperature) (0.2 mm), which on standing formed crystals, mp 61–62°, had a characteristic ir band at 1656 cm^{-1} (ureido group) and in the nmr spectrum a multiplet, approximating a quartet, at 1.74 ppm (central methylene group), a triplet at 3.23 ppm (methylene group next

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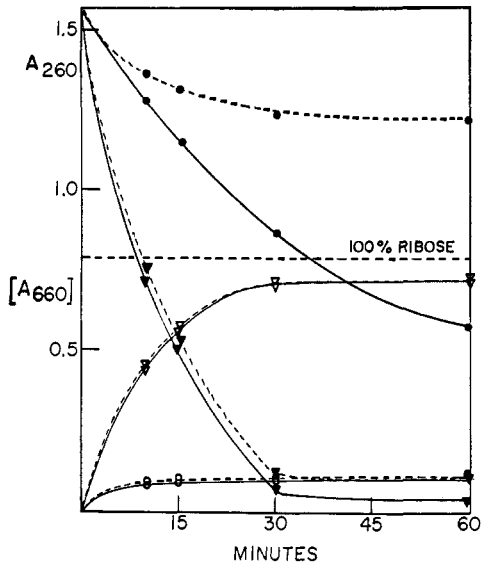


Figure 1. Reversibility of the photohydration and irreversibility of the photoreduction of uridine. Photohydration of uridine at pH 9.0 (irradiation in 0.2 *M* sodium tetraborate–sodium phosphate buffer, 2.6×10^{-3} *M* uridine): —●—, absorbance at 260 mμ (pH 7); --●--, absorbance at 260 mμ (pH 7) after heating the sample for 5 hr at 85°, pH 10; —○— and --○--, orcinol assay before and after heating for 5 hr at 85°, pH 10. Photoreduction of uridine at pH 8.6–9.0 (irradiation in 0.2 *M* sodium tetraborate–sodium phosphate buffer, 2.6×10^{-3} *M* uridine); —▼— and --▼--, absorbance at 260 mμ (pH 7) before and after heating the sample for 5 hr at 85°, pH 10; —▽— and --▽--, orcinol assay before and after heating the sample for 5 hr at 85°, pH 10.

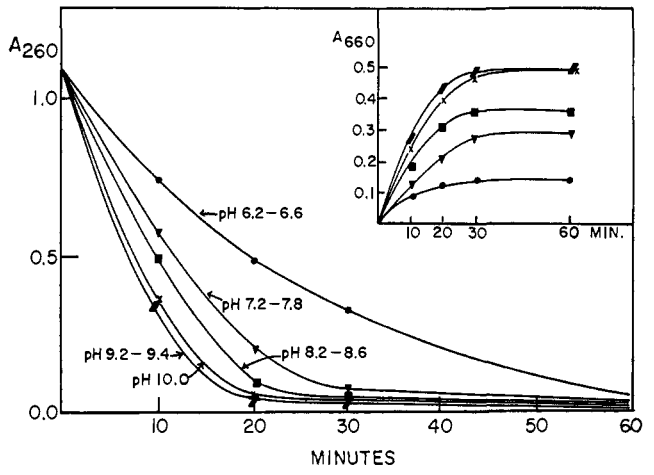


Figure 2. Photoreduction of uridine as a function of pH. Continuous addition of a 2.7×10^{-2} *M* solution of $NaBH_4$ (flow rate 0.17 ml/min) to a 10^{-3} *M* solution of uridine. Aliquots of increasing size were withdrawn as a function of time to compensate for the dilution caused by the addition of the sodium borohydride solution: ●, starting pH 6.2 (0.2 *M* sodium phosphate buffer); ▼, starting pH 7.2; ■, starting pH 8.2 (0.2 *M* sodium tetraborate–sodium phosphate buffer); ◆, starting pH 9.2 and pH 10 (0.1 *M* boric acid–potassium chloride–sodium hydroxide buffer). The pH shifts during the reaction for 0.2–0.6 pH unit due to decomposition of sodium borohydride except for the experiment with a starting pH of 10. Inset: orcinol assay for ribose for each experiment using corresponding symbols.

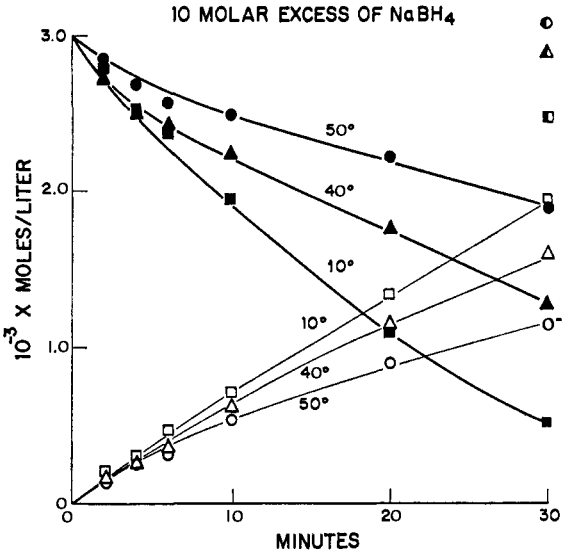


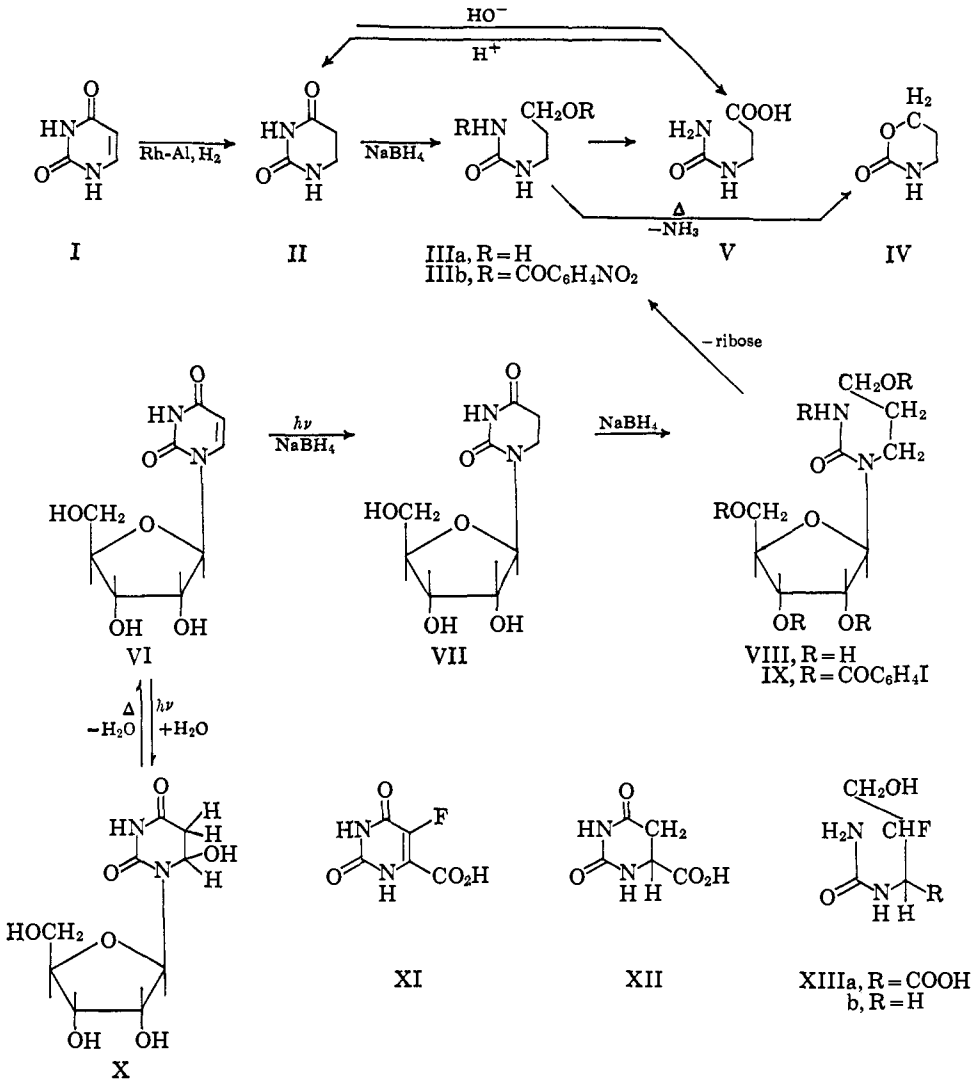
Figure 3. Photoreduction of uridine as a function of temperature. Residual uridine is calculated from the absorbance at 260 mμ (pH 1, ε 9800): ●, 50°; ▲, 40°; ■, 10°. The corresponding open symbols represent the reduction product(s) formed, calculated from the orcinol assay for ribose. The half-open symbols, ◐, ◭, ◨, indicate the sum of the amount of residual uridine and the amount of reduction product formed after 30-min irradiation. The difference between this value and the starting concentration of uridine indicates the extent of photohydration.

to >ND), and a triplet at 3.70 ppm (methylene of primary alcohol). The alcohol IIIa gave a crystalline bis-*p*-nitrobenzoate (IVa), mp 190–191°, whose nmr spectrum showed the α-methylene of the primary alcohol shifted to downfield by approximately 0.8 ppm due to the deshielding effect of the *p*-nitrobenzoyl group.

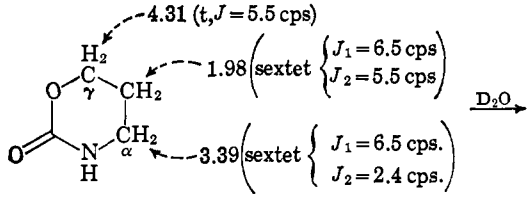
When ureidopropanol (IIIa) was heated to 200–210° for 4 hr, ammonia was liberated (Nessler reagent). The oily reaction product IV showed neither ureido absorption in the ir spectrum nor a color reaction with *p*-dimethylaminocinnamaldehyde (modified Ehrlich reagent). A band at 1692 cm^{-1} (liquid film) suggested the presence of a urethan group. The mass spectrum of IV showed a molecular peak (M^+) at 101. Elemental analysis agreed with the formula $C_4H_7NO_2$. The nmr of IV displayed two sextets and a triplet integrating each two protons, respectively, at 1.98, 3.39, and 4.31 ppm, and a broad signal (1 H) at 7.00 ppm. After exchange with deuterium oxide the sextet at 3.39 ppm changed to a triplet. The triplet at 3.70 ppm in γ-ureidopropanol (IIIa) shifted to 4.31 ppm in the urethan. This proves that the primary alcohol group in III must be involved in esterification in the conversion to the urethan IV. The cyclic urethan IV may arise through an intermediary isocyanate.

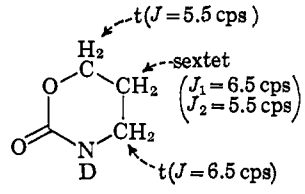
Potassium permanganate oxidized the ureido alcohol III to γ-ureidopropionic acid (V), mp 170–171° dec, and to a small amount of dihydrouracil (II). The nmr spectrum of γ-ureidopropionic acid (V) no longer showed the triplet for the primary alcohol. Instead a new pair of triplets appeared at 2.59 and 3.39 ppm, indicative of the partial structure $>NCH_2CH_2COOH$. Acid V was identical with synthetic γ-ureidopropionic acid obtained by alkaline hydrolysis of dihydrouracil. Both samples readily underwent acid-catalyzed cyclization to dihydrouracil.

When dihydrouridine was treated with sodium borohydride, the starting material disappeared completely within 2 hr. The reduction was more complicated than that of dihydrouracil because the reaction mixture showed at least five spots on thin layer chromatog-



raphy. Chromatography on a column of silica gel gave as the major spot on tlc the alcohol VIII in 20% yield. VIII was identified by a ureido group at 1642 cm^{-1} in the ir and signals at 2.05, 3.37, and 3.9 ppm (ureidopropanol group) and a doublet at 5.84 ppm (C_1





proton of the ribofuranosyl residue) in the nmr spectrum. The amorphous ureido alcohol VIII gave a crystalline pentaiodobenzoate (IX), mp 247.5–248° dec. Mild hydrolysis of the riboside VIII with 0.1 *N* hydrochloric acid afforded γ-ureidopropanol-1 (III).

The characteristic peak of the C-1 proton of the β-D-ribofuranosyl residue shows up near 5.9 ppm (Table I).[9] The presence of a doublet at 5.84 ppm (J = 6 cps) in the nmr spectrum of the reduction product VIII indicates an intact β-D-ribofuranosyl residue.

Table I

Compounds	C_1 proton,[a] ppm (J, cps)
1-(β-D-Ribofuranosyl)uracil (VI)	5.90 (4.0)
1-(β-D-Ribofuranosyl)-5,6-dihydrouracil (VII)	5.94 (5.9)
N-(β-D-Ribofuranosyl)-N-(γ-hydroxypropyl)urea (VIII)	5.84 (6.0)

[a] The solvent is D_2O.

Other reduction products have so far not been isolated or separated in spite of repeated experiments involving work-up by thin layer and column chromatography.[10]

The minor products may result from the easy decomposition of 1-(β-D-ribofuranosyl)-5,6-dihydrouracil (VII) in which the sugar moiety is reductively or hydrolytically cleaved by sodium borohydride. The

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(10) The isolation of "tetrahydrouridine," the carbinolamide, or the ring tautomer of the aldehyde precursor of the ureido alcohol VIII was recently reported: A. R. Hanze, 154th National Meeting of the American Chemical Society, Chicago, Ill., Sept 11–14, 1967, Abstracts P30–33.

photoreduction of 5-fluoroorotic acid (XI) in the presence of sodium borohydride was reported to yield 5,6-dihydroorotic acid (XII).[11] Apparently hydrogenolysis to XIIIa is not observed. However, a major unidentified photoproduct from the reduction of 5-fluorouracil may well be α-ureido-γ-hydroxypropionic acid.

Experimental Section

General Procedures. Melting points are uncorrected and were taken on a Büchi apparatus. Nuclear magnetic resonance spectra were recorded on a Varian A-60 spectrometer. Chemical shifts were measured in ppm either with sodium 3-(trimethylsilyl)-1-propanesulfonate in deuterium oxide or with tetramethylsilane in organic solvents as internal standards. Thin layer chromatography plates were coated with silica gel G (Research Specialties Co.) and were developed by a mixture of chloroform–methanol (7:3). Spots were made visible by exposure to iodine vapor. For irradiation a U-shaped Hanovia low-pressure mercury lamp, No. 87A-45, with an intensity of 4.3 W at 253.7 mμ was used. The light was filtered by a 1-cm layer of methanol. The irradiation apparatus was immersed in a constant temperature bath.

Photoreduction of Uridine to 1-(β-D-Ribofuranosyl)-5,6-dihydrouracil (VII). A solution of 380 mg of uridine in 400 ml of water was irradiated at 50–56° for 45 min with a low-pressure Hg lamp (Hanovia, Type 87A-45) filtered by a 1-cm layer of methanol in the presence of 120 mg of sodium borohydride. A continuous stream of nitrogen was passed through the solution. After termination of the irradiation excess reducing agent was immediately destroyed by the addition of Dowex 50W-X8 (H^+) under vigorous stirring and the pH adjusted to 3. The resin and the acidified solution were then separated. The resin was washed with an additional 200 ml of water and the combined eluates were lyophilized. The residue was dissolved in 50 ml of methanol and the solution evaporated to dryness to remove boric acid as methyl borate. This procedure was repeated three times. The remaining colorless oil was taken up in a small amount of 1-butanol–water, 86:14. The oil did not completely dissolve under these conditions. The suspension was directly applied to a silica gel column (silica gel Merck, 0.2–0.05 mm; 21 × 2.5 cm). A 1-cm layer of silica gel at the column top was stirred in order to obtain a homogeneous mixture of silica gel and compound. The column was then eluted with 1-butanol–water, 86:14, and fractions of 7 ml were collected. The fractions were analyzed by thin layer chromatography (silica gel G, 1-butanol–water, 86:14); spots were developed with the anisaldehyde reagent[12] or with *p*-dimethylaminobenzaldehyde according to Fink.[13] Fractions 15–30 contained 132 mg of unreacted uridine. Fractions 31–54 were pooled and the solvents removed by distillation *in vacuo*. To remove residual traces of 1-butanol the residue was taken up several times in a small amount of water and the solution evaporated to dryness. A colorless glass (178 mg, 47%) was left, which was identified as 1-(β-D-ribofuranosyl)-5,6-dihydrouracil (VII) by its chromatographic and spectroscopic properties and by direct comparison with authentic material obtained from the catalytic reduction of uridine (for a description of the spectroscopic and analytical data of VII, see below). Fractions 96–175 contained a third, minor product (26 mg), which yielded a yellow color with *p*-dimethylaminobenzaldehyde on chromatograms without pretreatment with alkali. The structure of this product is under investigation.

Hydrogenolysis of Dihydrouracil with Sodium Borohydride. To a solution of 1.647 g (1.44×10^{-2} mol) of dihydrouracil in 110 ml of water was added 1.082 g (2.88×10^{-2} mol) of sodium borohydride under mechanical stirring. After stirring for 2 hr at room temperature excess sodium borohydride was destroyed with acetone and the resulting solution passed through a column (20 × 120 mm) of Amberlite IRC-50 (acidic form). Most of the acetone was removed under reduced pressure and the aqueous solution lyophilized. The residue, a colorless powder, was three times codistilled with methanol *in vacuo*. The residual oil was chromatographed on a column (24 × 300 mm) of silica gel followed by elution with chloroform–methanol (8:2). Fractions of 6 ml were taken. Fractions 25–41 showed a negative reaction toward *p*-dimethylaminocinnamaldehyde (modified Ehrlich reagent). Fractions 49–61 gave a positive test with this reagent. Fractions 25–41 were pooled and evaporated. Crystallization from methanol gave unchanged **dihydrouracil** (35 mg) as colorless crystals, mp 280°.

γ-Ureidopropanol-1 (III). Fractions 49–61, which showed a single spot (R_f 0.46) on thin layer chromatography and a positive color test with the modified Ehrlich reagent, were pooled and evaporated *in vacuo* to leave a colorless viscous oil, bp 190° (bath temperature) (0.2 mm), which solidified on standing, mp 61–62°, yield 1.407 g (82.5%).

Anal. Calcd for $C_4H_{10}N_2O_2$: C, 40.66; H, 8.53; N, 23.71. Found: C, 40.60; H, 8.25; N, 23.44.

Spectral results were as follows: ir (liquid film) (cm^{-1}) 3364 ($-NH_2$, >NH, and –OH), 1656 (ureido), 1060 (ν_{C-O}); nmr (D_2O) (ppm) 1.74 (q, J = 6.7 cps) ($-CH_2CH_2CH_2-$), 3.23 (t, J = 7 cps) ($-NH_2CH_2CH_2-$), 3.70 (t, J = 6.7 cps) ($-CH_2CH_2OD$).

Pyrolysis of γ-Ureidopropanol (III). When γ-ureidopropanol (300 mg) was heated in an oil bath to 200–210°, the ir spectrum of aliquots which were taken periodically showed the gradual disappearance of the ureido absorption (1600–1650 cm^{-1}). The ammonia liberated during pyrolysis was trapped in an ice-cooled condenser. The residue was purified by adsorption on a silica gel column (10 × 70 mm) followed by elution with a mixture of chloroform–methanol (9:1). The purified product (230 mg) was a colorless oil, R_f 0.40 (chloroform–methanol, 9:1); modified Ehrlich test negative.

Anal. Calcd for $C_4H_7NO_2$: C, 47.52; H, 6.98; N, 13.86; mol wt, 101. Found: C, 47.30; H, 6.87; N, 13.18; mol wt, 101 (mass spectrum).

Spectral results were as follows: ir (liquid film) (cm^{-1}) 3401 (>NH), 1692 (cyclic urethan), 1075 (ν_{C-O}); nmr ($CDCl_3$) (ppm) 1.98 (sextet, J_1 = 6.5 cps, J_2 = 5.5 cps) ($-NHCH_2CH_2CH_2O-$), 3.39 (sextet, J_1 = 6.5 cps, J_2 = 2.4 cps) ($-NHCH_2CH_2CH_2O-$), 4.31 (triplet, J = 5.5 cps) ($-NH_2CH_2CH_2CH_2O-$), 7.00 (br) ($NHCH_2$); ($CDCl_3$, one drop of D_2O) added 1.98 (sextet) (J_1 = 6.5 cps, J_2 = 5.5 cps) ($-NDCH_2CH_2CH_2O-$), 3.37 (triplet, J = 6.5 cps) ($NDCH_2CH_2CH_2O$), 4.31 (triplet, (J = 5.5 cps) ($-NDCH_2-CH_2CH_2O-$).

N,O-Bis-*p*-nitrobenzoyl-γ-ureidopropanol-1 (IIIa). To a chilled solution of 59 mg of II in dry pyridine was added with stirring 190 mg of *p*-nitrobenzoyl chloride. The reaction mixture which was allowed to stand overnight at room temperature was then poured into ice–water. The crystalline deposit was removed by filtration and dissolved in chloroform. The chloroform solution was washed with aqueous bicarbonate, 5% hydrochloric acid, and water, dried over anhydrous magnesium sulfate, and evaporated to dryness to yield a crystalline residue. Recrystallization from methanol gave the pure *p*-nitrobenzoate IIIa as slightly yellow needles, mp 190–191°.

Anal. Calcd for $C_{18}H_{16}N_4O_8$: C, 51.92; H, 3.87; N, 13.46. Found: C, 52.18; H, 3.65; N, 13.44.

Spectral results were as follows: ir (Nujol) (cm^{-1}) 3401, 3279, and 3195 (>NH), 17.24 (*p*-nitrobenzoyl carbonyl), 1678 (ureido), 1603 (aromatic), 1513, and 1335 (nitro group), 1271 and 1258 (ν_{C-O}), 717 (aromatic); nmr (DMSO-d_6) (ppm) 2.07 (q, J = 7 cps) ($-CH_2CH_2CH_2-$), 3.52 (m) ($-NHCH_2CH_2-$), 4.51 (t, J = 5.5 cps) (CH_2CH_2OH), 8.34 (*p*-nitrobenzoyl ring protons).

γ-Ureidopropionic Acid. A. By Oxidation of γ-Ureidopropanol-1. To a stirred solution of 300 mg of γ-ureidopropanol-1 in 20 ml of water was added 27 ml of an aqueous solution of 1% potassium permanganate in small portions during 2 hr at room temperature. The mixture was then allowed to stand overnight with stirring. Excess permanganate was destroyed with methanol and the precipitate removed by filtration. The clear solution was passed through a column (15 × 200 mm) of Amberlite IRC-50 (acidic form) and lyophilized. The residue was repeatedly recrystallized from methanol to give γ-ureidopropionic acid as colorless rosettes, mp 170–171° dec (lit. mp 170–171°,[14] mp 169–170°[15]).

Anal. Calcd for $C_4H_8N_2O_3$: C, 36.36; H, 6.10; N, 21.20. Found: C, 36.16; H, 5.90; N, 21.09.

Spectral results were as follows: ir (Nujol) (cm^{-1}) 3448 and 3226 ($-NH_2$ and >NH), 2398 (CO_2H and ammonium), 1919 (immonium), 1698 (sh, $-CO_2H$), 1672 and 1637 (ureido), 1577 (carboxyl-

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

ate); nmr (D_2O) (ppm) 2.59 (t, J = 6.0 cps) ($-CH_2CH_2CO_2D$), 3.39 (t, J = 6.0 cps) ($-NDCH_2CH_2$).

The mother liquor from the crystallization of γ-ureidopropionic acid was evaporated *in vacuo*. Chromatography of the residue over a silica gel column (10 × 150 mm) gave on elution with chloroform–methanol (7:3) dihydrouracil which, after recrystallization from water, showed mp 281°. The identity was established by direct comparison with an authentic specimen of dihydrouracil.

B. By Alkaline Ring Opening of Dihydrouracil. An aqueous solution of barium hydroxide was added to dihydrouracil in water. After 3 hr at room temperature the reaction mixture was neutralized with 10% sulfuric acid, and, after removal of the barium sulfate, lyophilized. The residue was crystallized from methanol to afford colorless rosettes, mp 171° dec.

Anal. Calcd for $C_4H_6N_2O_3$: C, 36.36; H, 6.10; N, 21.20. Found: C, 36.25; H, 5.97; N, 21.22.

This product was identical (mixture melting point, ir and nmr spectra) with a sample (mp 170–171° dec) prepared by oxidation of γ-ureidopropanol-1.

Recyclization of γ-Ureidopropionic Acid to Dihydrouracil. A solution of γ-ureidopropionic acid (0.5 g) in 15% hydrochloric acid (20 ml) was heated under reflux for 2 hr. After removal of hydrochloric acid under reduced pressure and addition of water (3 ml), the solution was kept in the ice box. Dihydrouracil slowly crystallized and, after recrystallization from methanol, formed colorless prisms, mp 281.5–282.5° dec.

Anal. Calcd for $C_4H_6N_2O_2$: C, 42.10; H, 5.30; N, 24.55. Found: C, 41.82; H, 5.42; N, 24.13.

Spectral results were as follows: ir (Nujol) (cm^{-1}) 3268 and 3125 (>NH), 1742 (carbonyl), and 1687 (cyclic ureido group).

1-(β-D-Ribofuranosyl)-5,6-dihydrouracil (VII). This compound was prepared according to the method of Cohn and Doherty.[15] A solution of 4.838 g (1.98×10^{-2} mol) of uridine was hydrogenated in water over 1.0 g of 5% rhodium–alumina catalyst under 20-psi pressure. Aliquots were taken at intervals in order to follow the reduction by ultraviolet spectrophotometry. The absorption maximum at 262 mμ completely disappeared during 1.5 hr. The catalyst was removed by filtration through Celite and the filtrate lyophilized to leave an oily residue. This product showed three spots on silica gel thin layer chromatography. The oily product was chromatographed on a column (30 × 350 mm) of silica gel and the column eluted with chloroform–methanol (7:3). The fractions of 6 ml were taken. Fractions 50–129 were pooled and evaporated under reduced pressure to leave colorless needles. Three recrystallizations from methanol gave colorless needles, mp 101–103° dec (lit.[16,17] oil), yield 4.375 g (84.9%), $[\alpha]^{20}D$ −36.8 ± 0.4° (*c* 2.12, water).

Anal. Calcd for $C_9H_{14}N_2O_6 \cdot 0.5CH_3OH$: C, 43.51; H, 6.15; N, 10.68. Found: C, 43.80; H, 5.93; N, 10.58, 10.47, 10.62.

Spectral results were as follows: ir (Nujol) (cm^{-1}) 3413, 3322, 3226, and 3115 (>NH and –OH), 1709 (carbonyl), 1684 (cyclic ureido), 1048, 1034, and 1014 (ν_{C-O}); nmr (D_2O) (ppm) 2.79 (t, J = 6.7 cps) ($-COCH_2CH_2N<$), 3.41 (1.5 *M*) (methanol of crystallization), 3.52 (t, J = 6.7 cps) ($-COCH_2CH_2N<$), 3.77 (d, J = 2.2 cps) (C_5' of ribofuranosyl), 3.83 (C_5'o f ribofuranosyl), 4.0–4.5 (C_2', C_3', and C_4' protons of ribofuranosyl), 5.94 (d, J = 5.9 cps) (C_1' of ribofuranosyl).[18]

Reduction of 1-(β-D-Ribofuranosyl)-5,6-dihydrouracil (VII) with Sodium Borohydride to N-(β-D-Ribofuranosyl)-N-(γ-hydroxypropyl)urea (VIII). To a solution of 2.462 g (10^{-2} mol) of 1-(β-D-ribofuranosyl)-5,6-dihydrouracil in 200 ml of water was added 0.756 g (2×10^{-2} mol) of sodium borohydride under mechanical stirring. The reaction mixture was stirred for 2 hr at room temperature. The resulting mixture was passed through Amberlite IRC-50 (acidic form) and the solution lyophilized. The residue, a colorless powder, was dissolved in methanol and evaporated *in vacuo*. This procedure was repeated three times. The oily residue showed at least five spots on thin layer chromatography. The mixture was chromatographed on a column (24 × 280 mm) of silica gel. The column was eluted with chloroform–methanol (7:3). Fractions of 6 ml were collected: fraction 16, yellow oil (trace); fractions 17–28, crystalline (trace); fractions 29–49, oil.

Fractions 29–49 were pooled and rechromatographed on a column (20 × 240 mm) of silica gel followed by elution with chloroform–methanol (7:3) mixture. Fractions 21–29 which showed a single spot (R_f 0.41) on thin layer chromatography were evaporated *in vacuo* to yield 525 mg of a colorless glassy product (~20% yield).

Spectral results were as follows: ir (film) (cm^{-1}) 3425 (–OH and $-NH_2$), 1642 (ureido), *ca.* 1150–995 (ν_{C-O}); nmr (D_2O) (ppm) 2.05 (unresolved multiplet) ($DOCH_2CH_2CH_2N<$), 3.37 (br) ($>N-CH_2-$), 3.75 (C_5' protons of ribofuranosyl), *ca.* 3.9 ($DOCH_2-$), *ca.* 4.0–4.5 (C_2', C_3', and C_4' protons of ribofuranosyl), 5.84 (d, J = 6 cps) (C_1' of ribofuranosyl).

N-(β-D-Ribofuranosyl)-N-(γ-hydroxypropyl)urea Penta-*p*-iodobenzoate (IX). A solution of 530 mg of N-(β-D-ribofuranosyl)-N-(γ-hydroxypropyl)urea was allowed to react with *p*-iodobenzoyl chloride (2.82 g) in dry pyridine at 50° for 24 hr. The reaction mixture was worked up in the usual manner, and the crude *p*-iodobenzoate chromatographed over a column (20 × 270 mm) of silica gel and eluted with chloroform containing a small amount of methanol. Fractions 24–38 (6 ml each) were collected and evaporated. The residue was rechromatographed over silica gel followed by elution with ethyl acetate–chloroform (4:6). Fractions 7–9 were pooled, evaporated *in vacuo*, and crystallized from a mixture of methylene chloride–ether to give colorless needles, mp 247.5–248° dec.

Anal. Calcd for $C_{44}H_{33}N_2O_{11}I_5 \cdot H_2O$: C, 37.25; H, 2.49; N, 2.00. Found: C, 37.21; H, 2.68; N, 2.02.

Spectral results were as follows: ir (Nujol) (cm^{-1}) 3448 (>NH), 1724 (*p*-iodobenzoyl carbonyl), 1585 (aromatic), 1258, 1117, and 1095 (ν_{C-O}), 751 (aromatic).

Hydrolysis of N-(β-D-Ribofuranosyl)-N-(γ-hydroxylpropyl)urea (VIII) to γ-Ureidopropanol. A solution of 0.5 g of N-(β-D-ribofuranosyl)-N-(γ-hydroxypropyl)urea in 0.1 *N* hydrochloric acid (40 ml) was hydroxylzed by heating for 1 hr in a boiling water bath. The reaction mixture was passed through a column (20 × 140 mm) of Dowex 1-X8 (hydroxy form) and lyophilized. The residual oil was chromatographed on a column (15 × 270 mm) of silica gel followed by elution with chloroform–methanol (8:2). Fractions (each 6.5 ml) 35–46 were collected and evaporated *in vacuo*. The residual oil was identical with the previously obtained γ-ureidopropanol-1 with regard to ir and nmr spectra and thin layer chromatography (R_f 0.47; modified Ehrlich reagent positive).

The bis-*p*-nitrobenzoyl derivative was prepared as described above. Crystallization from methanol gave slightly yellow needles, mp 185–185.5°.

Anal. Calcd for $C_{18}H_{16}N_4O_8$: C, 51.92; H, 3.87; N, 13.46. Found: C, 51.89; H, 3.73; N, 13.44.

This substance was identical with the bis-*p*-nitrobenzoyl derivative IIIa described earlier.

Acknowledgment. P. C. is indebted to the National Institutes of Health for a grant, GM 14090-01, and to the National Science Foundation for support under GB 4894.

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