Table II

Compd	mmoles recovd	mmoles cor	Yield, %
Methylbutenes	5.2	5.7	30.5
Nitrite	2.7	2.7	14.5
t-Amyl acetate	6.5	8.8	47.0
t-Amyl alcohol	0.5	0.5	2.5
Neopentyl acetate	0.1	0.1	0.5
Total		17.8	95.0

cedure below showed that the more volatile olefin could be recovered from the part of the procedure involving trapping, gas chromatography, and collection in at least 84% yield.

The acetic acid solution was then poured into a mixture of 30 ml of 35% NaOH solution, 40 ml of pentane, and 100 cc of crushed ice. The pentane layer was separated and the aqueous solution extracted with an additional 20 ml of pentane. The combined pentane layers were dried over anhydrous sodium sulfate and the pentane was boiled off through a 1-ft column packed with glass helices. The residue was subjected to gas chromatographic analysis and separation on the column described above at 105° and 150 cc/min. The alcohols, mainly 1-butanol, and the acetates, mainly *t*-amyl acetate, were separated and these mixtures analyzed further on a 10 ft × 0.25 in. column of 15% polyethylene glycol 400 on 80–100 mesh Chromosorb W at 53° and 200 cc/min. (These conditions avoided the slight pyrolysis of *t*-amyl acetate which occurred at higher temperatures.) In the case of the acetates, this procedure allowed accurate analysis for *n*-butyl acetate and although neopentyl acetate was not completely separated under these conditions, its concentration could be bracketed between 1 and 2% of the total acetate fraction. Controls showed that from reaction mixture to gas chromatographically separated product, *t*-amyl acetate recovery was only 74%, a situation which was not appreciably improved by continuous extraction of the aqueous layer with ether. Typical yield data are summarized in Table II for a reaction carried out on 18.7 mmoles.

An attempt was made to isolate the *t*-amyl alcohol produced in the reaction by preparative gas chromatography of alcohol fractions obtained from several reaction mixtures. A very small amount of *t*-amyl alcohol was obtained which was grossly contaminated with water. The sample showed a small positive rotation which was barely outside of a large experimental error. Even after correction for the aqueous impurity, however, it seemed safe to conclude that the rotation was less than half of that reported by Sanderson and Mosher for an impure sample of the same compound. It is possible that some of our *t*-amyl alcohol arises from secondary reactions.

Procedure for the Pyrolysis of *t*-Amyl Acetate.[13] The pyrolysis was carried out by injecting the acetate fraction obtained above onto a 1-ft Pyrex tube packed with Berl saddles at 500°, and blowing the product through the tube and through the trapping arrangement described above with nitrogen. Gas chromatographic analysis and collection were carried out as described. It was found that 6.5% of the volatile fraction was unpyrolyzed *n*-butyl and neopentyl acetates in the same ratio estimated in the original acetate mixture.

Optical Rotations. The rotations were measured using a Rudolph Model 63 polarimeter. Because only small samples of 2-methyl-1-butene-3-*d* were available, it was necessary to use end-filling polarimeter tubes and keep the samples cold. The only practical way to do this seemed to be carrying out the entire operation in a room kept near 0°. An end plate with a small off-center hole was used to facilitate the refilling required after every few readings.

Deuterium Determination. After performance of such measurements as required above, the purity of the samples were checked by gas chromatography (all samples were of greater than 99% purity) and submitted for combustion and falling-drop analyses.[10] Samples were usually composites from several runs.

(13) W. H. Bailey and W. F. Hale, *J. Am. Chem. Soc.*, **81**, 647 (1959).

Nucleic Acids.[1] IV. The Catalytic Reduction of Pyrimidine Nucleosides (Human Liver Deaminase Inhibitors)

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Abstract: The formation of 1-(β-D-ribofurarosyl)-4-aminotetrahydropyrimidin-2(1H)-one [tetrahydrocytidine (III)] and 1-(β-D-ribofuranosyl)tetrahydropyrimidin-2(1H)-one (V) as the major products in the catalytic reduction of cytidine in water over rhodium on alumina is reported. The former compound readily hydrolyzes to give 1-(β-D-ribofuranosyl)-4-hydroxytetrahydropyrimidin-2(1H)-one [tetrahydrouridine (IV)], which is a potent inhibitor of human liver deaminase. The latter compound is also formed by the sodium borohydride reduction of 5,6-dihydrouridine (VI).

The isolation[2] of 5,6-dihydrouridine (VI) as one of the minor nucleosides in tRNA created renewed interest in the chemistry of dihydropyrimidine nucleosides. The earliest synthesis of this type of compound was reported by Levene and LaForge,[3] who prepared 5,6-dihydrouridine (VI) by the reduction of uridine in the presence of colloidal palladium. Subsequent syntheses of dihydropyrimidinenucleosides include catalytic reduction of the parent nucleoside over rhodium on alumina[4] and reduction with sodium in liquid ammonia containing ethanol.[5] More recently Cerutti, *et al.*,[6] reported the sodium borohydride reduction of uridine and uridylic acid in their photoexcited stage to give 5,6-dihydro products.

In the course of work on the synthesis of nucleic

(1) Paper III of this series: H. E. Renis, C. A. Hollowell, and G. E. Underwood, *J. Med. Chem.*, 10, 777 (1967).
(2) J. T. Madison and R. W. Holley, *Biochem. Biophys. Res. Commun.*, **18**, 153 (1965); R. W. Holley, G. A. Everett, J. T. Madison, M. Marquisee, S. H. Merrill, J. P. Penswick, and A. Zamir, *Science*, **147**, 1462 (1965); G. F. Zachau, D. Dütting, and H. Feldman, *Angew. Chem.*, **77**, 1043 (1965).
(3) P. A. Levene and F. B. LaForge, *Chem. Ber.*, **45**, 608 (1912).
(4) (a) W. E. Cohn and D. G. Doherty, *J. Am. Chem. Soc.*, **78**, 2863 (1956); (b) M. Green and S. S. Cohen, *J. Biol. Chem.*, **225**, 397; **228**, 601 (1957).
(5) D. C. Burke, *Chem. Ind.* (London), 1393 (1954); *J. Org. Chem.*, **20**, 643 (1955).
(6) P. Cerutti, K. Ikeda, and B. Witkop, *J. Am. Chem. Soc.*, **87**, 2505 (1965).

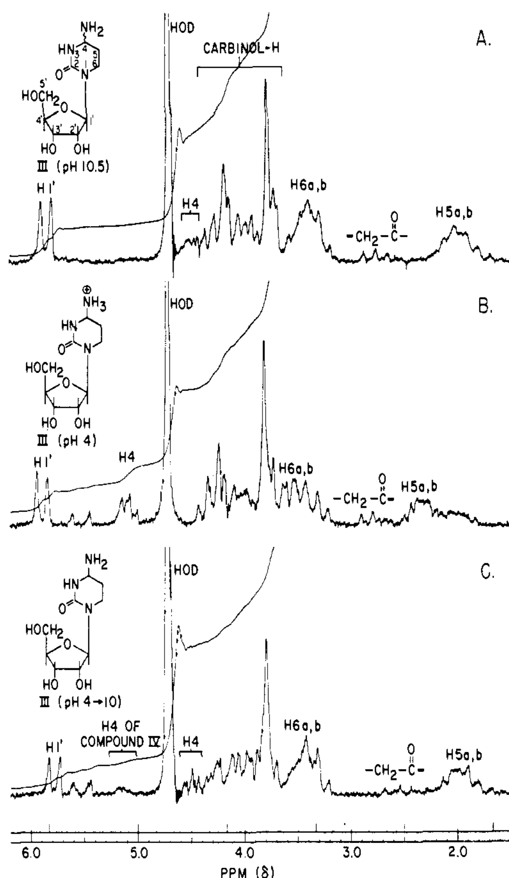


Figure 1. Nmr spectra (60 Mc) of crude tetrahydrocytidine III in D_2O. All designated protons refer to tetrahydrocytidine except where noted. A, crude tetrahydrocytidine at pH 10.5; B, nmr solution adjusted to pH 4; C, nmr solution readjusted to pH 10.

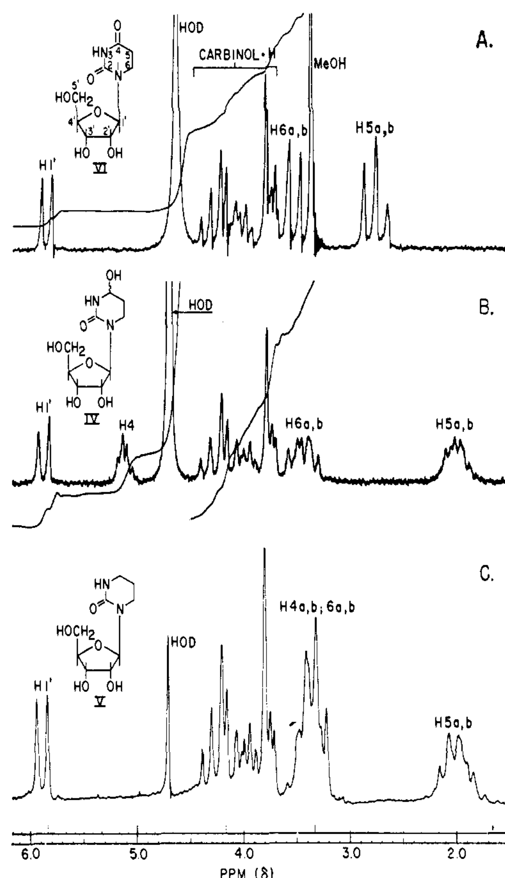


Figure 2. Nmr spectra (60 Mc) in D_2O: A, 5,6-dihydrouridine (VI); B, 1-(β-D-ribofuranosyl)-4-hydroxytetrahydropyrimidine-2(1H)-one (IV); C, 1-(β-D-ribofuranosyl)tetrahydropyrimidin-2(1H)-one (V).

acid analogs we prepared a group of dihydropyrimidine nucleosides by the rhodium on alumina reduction.[4a] The reduction of uridine and thymidine stopped after the absorption of 1 mole of hydrogen and the reduction mixtures yielded crystalline dihydrouridine and dihydrothymidine, although the presence of other products could be detected by thin layer chromatography (tlc) of the mother liquors. On the other hand, the uptake of hydrogen during the reduction of cytidine did not level off at 1 mole of hydrogen but continued until approximately 2 moles was absorbed over an 18–20-hr period. In this case, no dihydrocytidine and little dihydrouridine were found in the reaction mixture. This "overreduction" of cytosine and cytidine had been noted earlier by Green and Cohen.[4b]

Since the first step in the catalytic reduction of pyrimidine nucleosides appears to be the saturation of the 5,6-double bond, none of the products can be detected on papergrams or tlc plates by ultraviolet irradiation. Earlier workers[4] detected dihydropyrimidine nucleosides by a method developed by Fink, *et al.*[7] In this procedure the dihydropyrimidine nucleoside on a papergram was first sprayed with alkali which opened the dihydronucleoside to a ureidopropionic acid derivative which gave a yellow color when subsequently sprayed with *p*-dimethylaminobenzaldehyde (Ehrlich's reagent). When our crude reduction mixture of cytidine was chromatographed on paper using system A, and the paper was sprayed with Ehrlich's reagent, omitting the alkali spray, two bright pink to red spots with R_f's 0.32, 0.57, a yellow spot with R_f 0.4, and an orange spot with R_f 0.45 were observed. When the paper was first sprayed with alkali and then Ehrlich's, dihydrouridine was detected as an additional spot (R_f 0.53) just behind the fastest moving red spot. Tlc on silica gel (50% MeOH–$CHCl_3$) usually showed three to four spots after treatment with sulfuric acid followed by heat.

Although papergrams and thin layer chromatography showed that the crude reduction product was a mixture of four or five components, nmr spectroscopy (Figure 1) proved fruitful. The crude mixture (pH 10.5) was concentrated, lyophilized, dissolved in deuterium oxide, and relyophilized. The latter operation was repeated. A well-defined nmr spectrum in D_2O was then obtained. On the basis of the spectra of common nucleosides, a doublet at 5.83 ppm was assigned to the anomeric proton on C-1 of the sugar. In similar fashion absorptions in the range of *3.6* to *4.4* were assigned to the other protons on the sugar portion. In addition to the above, there remained three major regions of absorption (multiplets centered at 2.0, 3.5, and 4.5 ppm) and a minor peak (triplet centered at 2.75) to be explained. From the known nmr spectrum of dihydrouridine (Figure 2) the minor peak centered at 2.75 was assigned to the C-5 protons (CH_2—C=O) of a small amount of dihydrouridine in the product. The multiplet centered at 2.0 ppm was tentatively assigned to the C-5 protons

(7) R. W. Fink, R. E. Cline, C. McGaughey, and K. Fink, *Anal. Chem.*, 28, 4 (1956).

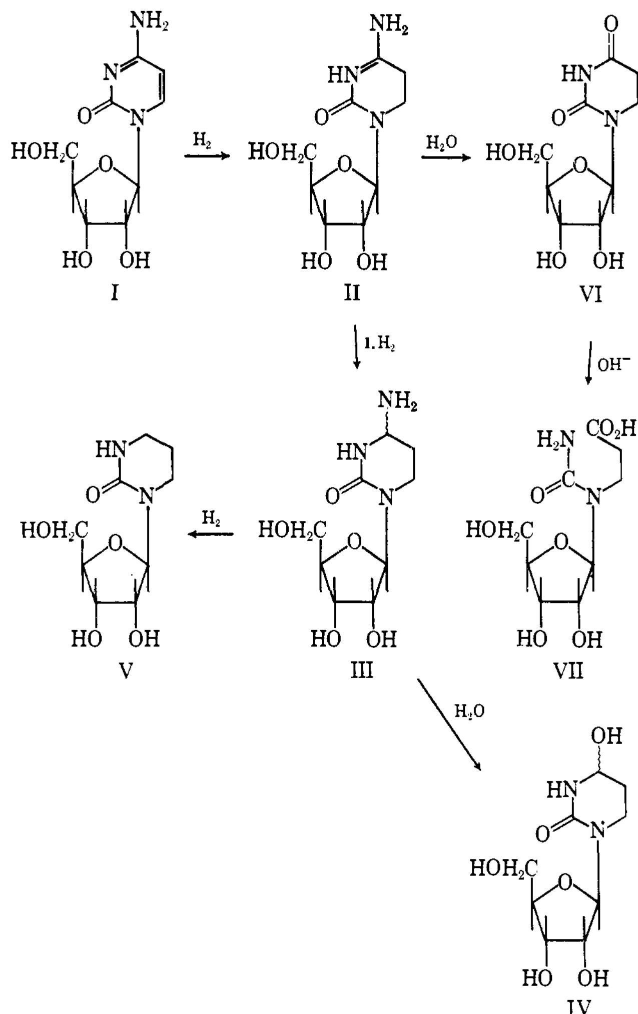


Figure 3.

of a reduced pyrimidine species containing a $>CH_2$ at C-6 and a $>CHX$ at C-4 where X = NH_2 or OH. The multiplet at 3.5 ppm was initially assumed to be due only to the protons at C-6 in a 5,6-dihydropyrimidine ($CH_2N<$). The multiplet at 4.5 ppm (just above the water line at 4.7 ppm) was the most informative. It is in the region where $>NCHN<$ would be expected to absorb. When the pH of the solution was adjusted to 3–5 with DCl and the nmr spectrum rerun quickly, two shifts occurred. The multiplet at 4.5 moved approximately 0.6 ppm downfield to 5.08 ppm and a part of the multiplet at 2.0 ppm moved *ca.* 0.2 ppm downfield to 2.2. When the solution was quickly made basic (pH 10) with NaOD, the displaced peaks returned to their original positions. On the basis of these shifts the peak at 5.08 ppm in the acidic medium was assigned to a proton on a carbon attached to two nitrogen atoms, one of which was protonated ($>NCHN^{+}H<$). With the knowledge that 2 moles of hydrogen was absorbed during the reduction and evidence to be presented later, the peak was assigned to the proton on C-4 of 1-(β-D-ribofuranosyl)-4-aminotetrahydropyrimidin-2(1H)-one [tetrahydrocytidine (III)] (Figure 3). The portion of the absorption at 2.0 ppm which moved downfield upon acidification was assigned to the protons on C-5 of tetrahydrocytidine by similar reasoning. Since some of the peak remained at 2.0 ppm upon acidification, it was obvious that this represented absorption due to a compound other than III.

When the acidified solution was allowed to remain at room temperature for some time before being made basic (30–40 min), the peak at 5.08 ppm broadened somewhat and upon basification only a portion of this peak moved back upfield to 4.5 ppm, leaving a peak at *ca.* 5.12 ppm. When the time at acid pH was extended, none of the downfield peak at 5.12 ppm moved upfield upon basification. This presumably represented a hydrolysis of the amino group of tetrahydrocytidine (III) to give the 4-hydroxyl of 1-(β-D-ribofuranosyl)-4-hydroxytetrahydropyrimidin-2(1H)-one [tetrahydrouridine (IV)] (Figure 3). Since the hydrolysis of III to give IV occurs even in neutral solution, although slowly, III is converted to IV in the process of purification, which in all cases involved aqueous media. To assure complete conversion of III to IV, the crude reduction mixture was adjusted to pH 6 before purification. Neither chromatography of the crude reduction mixture on silica gel or cellulose nor continuous flow electrophoresis was able to effect a satisfactory separation; however, countercurrent distribution using 2-butanol–water provided a separation after 2500 transfers into two major peaks and several minor ones. The slower moving major peak had an R_f of 0.57 in system A and gave a bright red color when sprayed with Ehrlich's reagent. This compound was also obtained in a high state of purity by partition chromatography of the crude reduction mixture on acid-washed Celite or Dicalite using 1-butanol saturated with water as the developing solution. The initial determination of the structure of this product as 1-(β-D-ribofuranosyl)-4-hydroxytetrahydropyrimidin-2(1H)-one [tetrahydrouridine (IV)] was based on its nmr spectrum in D_2O (Figure 2). The nmr spectrum of compound IV shows a multiplet at 5.12 ppm corresponding to one proton and multiplets at 3.45 and 2.00 ppm, each corresponding to two protons. The multiplet at 2.00 ppm was assigned to the protons on C-5 and that at 3.45 ppm to those at C-6. The peak at 5.12 ppm corresponding to one proton was assigned to that on C-4 of tetrahydrouridine because it is in the region in which a proton attached to a carbon bonded to an amide nitrogen and oxygen atom would be expected to absorb and it is not shifted upon the addition of acid or base. The major peaks in the mass spectrum of IV are given in Table I. Biemann and McCloskey[8] have shown that four major peaks of ribonucleosides are at M − 89, B + 30, B + 2, and B + 1. Uridine, for example, shows peaks (Table I) at 155 (M − 89), 141 (B + 30), 113 (B + 2), and 112 (B + 1). By contrast, the mass spectrum of compound IV shows the expected peaks, minus 18 units in each case, indicating that the compound readily dehydrates in the mass spectrometer.

Attempts to crystallize IV or to obtain crystalline derivatives have been unsuccessful. This is due, presumably, to the presence of a mixture of C-4 hydroxy isomers in the product. However, a satisfactory analysis was obtained on a sample purified first by partition chromatography and then by paper chromatography. Additional proof of the structure of IV

(8) K. Biemann and J. A. McCloskey, *J. Am. Chem. Soc.*, **84**, 2005 (1962).

Table I. Mass Spectra Peaks of Cytidine Reduction Products

Uridine		5,6-Dihydrouridine (VI)		1-(β-D-Ribofuranosyl)-4-hydroxy-tetrahydropyrimidin-2(1H)-one (IV)		1-(β-D-Ribofuranosyl)tetra-hydropyrimidin-2(1H)-one (V)	
m/e	Ion	*m/e*	Ion	*m/e*	Ion	*m/e*	Ion
155	M − 89	157	M − 89	141	(M − 18) −89	143	M − 89
141	B + 30	143	B + 30	127	(B − 18) + 30	129	B + 30
113	B + 2	115	B + 2	99	(B − 18) +2	101	B + 2
112	B + 1	114	B + 1	98	(B − 18) +1	100	B + 1

is furnished by its ready oxidation to 5,6-dihydrouridine (VI) by oxygen in the presence of platinum black.

The structure of the faster moving of the major peaks from the countercurrent distribution was also determined initially by its nmr spectrum (Figure 2). Since this compound gave no appreciable color with Ehrlich's reagent, it was detected on tlc plates by spraying with sulfuric acid. The absence of absorption at 5.12 ppm and the presence of absorption of four protons at C-4 and C-6 (multiplet at 3.23 ppm) and two protons at C-5 (multiplet at 2.00) in the nmr identified this compound as 1-(β-D-ribofuranosyl)tetrahydropyrimidin-2(1H)-one (V). The presence of some 5,6-dihydrouridine (VI) in this product was evident by a small amount of absorption at 2.75 ppm corresponding to the two protons at C-5 adjacent to the C-4 carbonyl (Figure 2) of dihydrouridine.

The structure of compound V was confirmed by comparison of its nmr spectrum with that of a known sample prepared by the catalytic reduction of cytidine in the presence of Adam's catalyst according to Iwasaki,[9] and by its mass spectrum which showed the expected peaks (Table I).

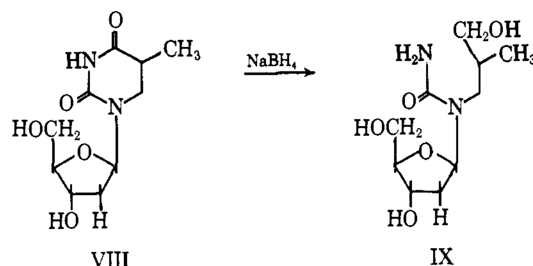
The presence of N^3-ribofuranosylureidopropionic acid (VII) in one of the minor peaks in the countercurrent extraction was shown by comparison with an authentic sample[4] on tlc and by its formation of a yellow color with Ehrlich's reagent.

The proposed reaction pathway by which the above products are formed during the reduction of cytidine is given in Figure 3.

As noted earlier, uridine is readily reduced in aqueous solution to give 5,6-dihydrouridine. Further reduction does not take place to any extent. This is not surprising since this would involve reduction of an amide carbonyl at C-4 or C-2. Cytidine, on the other hand, is known to exist in the amino form. Thus, the probable initial product of reduction, 5,6-dihydrocytidine (II), contains an imino bond at the 3,4 position. This should readily reduce under the reaction conditions to yield 3,4,5,6-tetrahydrocytidine (III) which can then undergo hydrogenolysis of the amino group at C-4 to give compound V or hydrolyze to give compound IV as discussed earlier. The formation of dihydrouridine, VI, and N^3-ribosylureidopropionic acid (VII) presumably occurs by hydrolysis of dihydrocytidine II to give VI followed by ring opening of VI in the basic medium.

As noted above, cytidine is reduced to the tetrahydro stage because it exists mainly in the amino form. Reduction of uridine, on the other hand, stops at the dihydro stage since it exists mainly in the 4-keto form. If, however, uridine were reduced in dilute base, in which it would exist as the enolic anion, it should give tetrahydrouridine, if ring opening of dihydrouridine is not too rapid. To test this hypothesis, uridine was reduced in dilute sodium hydroxide in the presence of 5% rhodium on alumina. Integration of the nmr of the crude reduction mixture indicated the formation of tetrahydrouridine to the extent of 30–40%. Similar results were obtained in dilute ammonium hydroxide.

An alternative method of preparing IV is the chemical reduction of dihydrouridine VI using 1 mole of sodium borohydride per mole of VI. The main product is IV on the basis of nmr. Purification of the product is complicated by the instability of IV to the acidic conditions necessary to remove borate from the crude product. Balle, *et al.*,[10] recently reported on the formation of the open-ring alcohol IX on reduction of 5,6-dihydrothymidine (VIII) with sodium borohydride.



We have seen no evidence for a compound corresponding to IX in our catalytic reduction products of cytidine. However, the borohydride reduction product of dihydrouridine shows some material with an R_f slightly faster than that of IV which gives a yellow color with Ehrlich's reagent.[11] A striking difference in the sodium borohydride reduction products of dihydrouridine and dihydrothymidine can be seen by a comparison of the nmr spectra of the products formed under identical reduction conditions. The dihydrouridine reduction products show absorption at 2.0, 3.4, and 5.1 ppm corresponding to IV (Figure 2). The dihydrothymidine reduction product, on the other hand, showed a much more poorly defined nmr with peaks at *ca.* 0.9, 2.0, 3.3, 3.75, and 4.8 ppm.

Biological Activity. Work in this area and on related nucleosides and nucleotides was greatly stimulated by the finding by Dr. G. W. Camiener of these laboratories that 1-(β-D-ribofuranosyl)-4-hydroxytetrahydropyrimidin-2(1H)-one (IV) is a potent inhibitor of the deaminase present in human liver as well as deaminases present in other animals and in microorganisms. Preparations

(9) H. Iwasaki, *Yakugaku Zasshi*, **82**, 1358 (1962).

(10) G. Balle, P. Cerutti, and B. Witkop, *J. Am. Chem. Soc.*, **88**, 3946 (1966).

(11) This is presumably the ring-opened alcohol corresponding to IX (lacking the methyl group) which P. Cerutti and N. Miller, *J. Mol. Biol.*, **26**, 55 (1967), report as the main borohydride reduction product of dihydrouridine in a reference published subsequent to submission of our manuscript. The difference in our results is undoubtedly due to their using a much greater excess of sodium brohydride.

with a competitive efficacy[12] as high as 7400 have been obtained by countercurrent extraction or partition column chromatographic purification of crude cytidine reduction mixtures. The enzymatic and biological data will be published in a forthcoming communication.[13]

Experimental Section

Methods and Materials. Paper chromatograms were run on Whatman 3MM paper using system A (*i*-PrOH–concentrated NH_4OH–H_2O, 7:1:2). For development they were sprayed with Ehrlich's reagent which is made by dissolving 1 g of *p*-dimethylaminobenzaldehyde in 10 ml of concentrated hydrochloric acid and 100 ml of ethyl alcohol. Thin layer chromatography was run on microscope slides coated with Camag Kieselgel DF-5 and were then sprayed with sulfuric acid and heated on a hot plate. The nmr spectra were recorded on a Varian A-60A nmr spectrometer. Mass spectra were run on an Atlas CH 4 instrument equipped with a TO4 source; ionizing voltage was 70 ev.

Dicalite, a diatomaceous earth, was purchased from Gresco Inc., a division of General Refractories Co., suspended in dilute hydrochloric acid, filtered, washed with dilute hydrochloric acid and then water until neutral, and dried at 85–90° overnight.

Reduction of Cytidine. A solution of 17.5 g (72 mmoles) of cytidine in 540 ml of water was hydrogenated in the presence of 3.6 g of 5% rhodium on alumina catalyst (Engelhard) in a Parr shaker at 30 psi of hydrogen. The hydrogen uptake overnight (18–19 hr) was approximately 2 moles. The solution (pH 10.5) was filtered through a bed of Celite. A small sample was lyophilized and relyophilized several times in D_2O; nmr (D_2O), doublet at 5.83 ppm (anomeric proton), multiplet at 4.50 (>NCHN<), multiplets at 3.45 (—CH_2N<) and 2.00 (CCH_2C), and a small amount of absorption at 2.75 (—CH_2C=O)—). Upon acidification, the 4.50 multiplet moved downfield to 5.08 ppm (>$NCHN^+$—) and part of the peak at 2.00 moved slightly downfield to 2.2. Upon making the solution basic the downfield shifts returned to their original positions.

The filtered solution was adjusted to pH 7.5–8.00 and rereduced in the presence of fresh catalyst (3.6 g) overnight. An additional 0.1–0.3 mole of hydrogen was absorbed. The solution was filtered through Celite, the pH adjusted to 5.5–6.0 with acetic acid, and the mixture stirred overnight at room temperature. The solution was concentrated under reduced pressure on a 40° bath and finally lyophilized to yield 16.5 g of clear syrup.

Paper chromatographic analysis of the crude material showed two pink to red spots with R_f 0.32 and 0.57, a yellow spot with R_f 0.4, and an orange spot with R_f 0.45 when sprayed with Ehrlich's reagent. When the developed papergram was first sprayed with 0.5 *N* sodium hydroxide, allowed to stand 30 min, and then sprayed with Ehrlich's reagent, an additional yellow spot corresponding to dihydrouridine (VI) with R_f 0.53 developed. Thin layer chromatography using methanol chloroform (1:1) showed four spots.

1-(β-D-Ribofuranosyl)-4-hydroxytetrahydropyrimidin-2(1H)-one (IV) and 1-(β-D-Ribofuranosyl)tetrahydropyrimidine-2(1H)-one (V). 1. By Craig Countercurrent Extraction. The crude reduction product of cytidine (5 g) was introduced into a 500-tube Craig countercurrent machine containing 10 ml each of upper and lower phases. The system used was 2-butanol-water. After 2500 transfers the crude material had separated into two major peaks and several minor peaks. The compound peaking at tube 380 (tubes 310–400) was isolated by concentration under reduced pressure in a 40° bath followed by solution in water, clarification, and lyophilization to yield 1.5 g of a clear syrup. This material IV showed one major spot on tlc and papergram (system A) with only a trace of impurity. The nmr spectrum showed multiplets at 5.1 ppm (C-4 proton), 2.0 ppm (C-5 proton), and 3.45 ppm (C-6 proton). The mass spectrum (Table I) supported the proposed structure.

The material V peaking at tube 460 (tubes 460–500) was isolated in the manner given above to yield 590 mg of a syrup moving slightly faster than the above-described IV on tlc. The presence of a small amount of 5,6-dihydrouridine was also discernible on tlc. V was identified by its nmr spectra (D_2O); multiplets at 3.23 ppm, area 4 (C-4 and C-6 protons), and at 2.0 ppm, area 2 (C-5 protons). The structure was substantiated by its mass spectrum (Table I) and by comparison of its nmr with a sample prepared by catalytic reduction of cytidine with Adams' catalyst.[9]

2. By Partition Column Chromatography. A solvent mixture of 100 gal. of 1-butanol and *ca.* 30 gal. of water was equilibrated at room temperature. Acid-washed Dicalite 4200 (15.0 kg) was suspended in 66 l. of the upper phase, 5.6 l. of lower phase was added with good stirring, and the mixture was then poured to make a 6 × 96 in. column. Crude reduced cytidine (see above), 78 g, was dissolved in 275 ml of lower phase and to this was added 100 g of Dicalite and 100 ml of upper phase. The mixture was concentrated to remove solvent and the resulting solid poured on top of the column using upper phase for the transfer. The column was washed with 16.5 gal. of upper phase and then 1-gal. fractions were taken at a flow rate of 1 gal./48 min. The column was followed by concentrating 100-ml aliquots and running tlc and papergram, when needed. The first major peak came off in fractions 21–23 and contained approximately 6 g of 1-(β-D-ribofuranosyl)tetrahydropyrimidin-2(1H)-one (V). The next compound off was 5,6-dihydrouridine (VI) in fractions 24–26 (6 g). The main product, 1-(β-D-ribofuranosyl)-4-hydroxytetrahydropyrimidin-2(1H)-one (IV), came off in fractions 31–57 in a high state of purity (22 g). An additional 8 g in a slightly less pure state came off in fractions 57–87. The above products were identified as in method 1.

An analytically pure sample of IV was obtained by preparative paper chromatography. The above purified IV (400 mg) was streaked on eight sheets of 3MM paper and developed in system A. The location of the material was determined by spraying the edges of the sheets with Ehrlich's reagent. The bars representing pure IV were cut out and eluted with water and the solution was lyophilized to yield 300 mg of IV. The sample was not pure by papergram so the preparative paper chromatography was repeated to give 100 mg of pure product.

The syrup is extremely hygroscopic and special handling and drying (120 hr under high vacuum over phosphorus pentoxide) were required to obtain a satisfactory analysis.

Anal. Calcd for $C_9H_{16}N_2O_6$ (248.24): C, 43.54; H, 6.49; N, 11.29. Found: C, 43.70; H, 6.38; N, 11.24.

1-(β-D-Ribofuranosyl)tetrahydropyrimidin-2(1H)-one (V) by Reduction of Cytidine with Adams' Catalyst. A mixture of 1.7 g (7 mmoles) of cytidine and 0.75 g of platinum oxide in 10 ml of water was reduced on a Parr hydrogenator overnight at 30–40 psi.[9] The mixture was filtered through Celite and lyophilized to yield a syrup. Its nmr spectrum in D_2O is identical with that of V isolated from the rhodium on alumina reduction of cytidine. The material shows one spot on tlc (50% MeOH–$CHCl_3$) with R_f *ca.* 0.6 and is identical with V.

Anal. Calcd for $C_9H_{16}N_2O_5$ (232.24): C, 46.60; H, 6.94; N, 12.05. Found: C, 46.24; H, 7.11; N, 12.04.

5,6-Dihydrouridine. 1. By Reduction of Uridine. A solution of 2.44 g of uridine in 75 ml of water was reduced under 40 psi of hydrogen in the presence of 0.5 g of 5% rhodium on alumina. The theoretical amount of hydrogen was adsorbed in 24 hr. The mixture was filtered through a bed of Celite and concentrated to dryness. By tlc (25% methanol in chloroform) the material is mainly dihydrouridine.

The crude material was crystallized from 40 ml of methanol to give 1.87 g of dihydrouridine (two crops) melting at 106–108°; $\lambda_{max}^{H_2O}$ 208 (sh) mμ (ε 6600).

Anal. Calcd for $C_9H_{14}N_2O_6 \cdot 0.5CH_3OH$ (262.24): C, 43.51; H, 6.15; N, 10.68. Found: C, 43.39; H, 6.15; N, 10.89.

The nmr spectrum[14] shows absorption at 2.75 ppm (C-5 protons) and at 3.57 ppm (C-6 protons) (Figure 2). The mass spectrum shows the expected peaks at 157 (M − 89), 143 (B + 30), 115 (B + 2), and 114 (B + 1) (Table I).

2. By Oxidation of IV. Oxygen was bubbled through a solution of 87 mg of 1-(β-D-ribofuranosyl)-4-hydroxytetrahydropyrimidin-2(1H)-one in 10 ml of water in the presence of 200 mg of platinum black. The reaction was followed by spotting the reaction mixture on silica gel thin layer chromatographic plates and developing in methanol–chloroform (1:1). After 25 min the reaction mixture appeared to be a 1:1 mixture of starting material and a less polar material. After 1 hr, all starting material was gone and there was only one product by tlc. The mixture was cooled

(12) Competitive efficacy is a relative measure of the ability of a test compound to inhibit the enzymatic deamination of 1-(β-D-arabinofuranosyl)cytosine.

(13) G. W. Camiener, presented in part at the 154th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1967.

(14) These nmr values agree with those recently published by R. J. Cushley, K. A. Watanabe, and J. J. Fox, *J. Am. Chem. Soc.*, 89, 394 (1967).

and centrifuged and the clear liquid lyophilized to yield 75 mg of product. This was crystallized from methanol to yield 40 mg (two crops). Recrystallization from methanol gave pure material; 24 mg, mp 105–107°; identical with authentic 5,6-dihydrouridine by mixture melting point and nmr.

5,6-Dihydrothymidine. Catalytic reduction of 2.42 g of thymidine in water in the presence of 0.5 g of 5% rhodium on alumina catalyst in the manner given for uridine above gave, after crystallization of the crude product from isopropyl alcohol, 750 mg of 5,6-dihydrothymidine, mp 123.5–124.5° (lit.[4b] mp 152–153°, presumably a polymorph). The structure was supported by infrared, nmr, and mass spectroscopy.

Anal. Calcd for $C_{10}H_{16}N_2O_5$ (244.2): C, 49.18; H, 6.60; N, 11.47. Found: C, 49.23; H, 6.59; N, 11.74.

1-(β-D-Ribofuranosyl)-4-hydroxytetrahydropyrimidin-2(1H)-one (IV) by Catalytic Reduction of Uridine. A solution of 2.44 g (10 mmoles) of uridine in 75 ml of 0.01 *N* sodium hydroxide was hydrogenated in the presence of 0.5 g of 5% rhodium on alumina catalyst overnight at 50 psi of hydrogen. The hydrogen uptake corresponded to 1.0 mole of hydrogen. An additional 0.5 g of catalyst was added and the reduction continued for 4 hr during which time 0.25 mole of hydrogen was adsorbed. The solution was filtered through a bed of Celite, 1.0 g of fresh catalyst was added, and the solution was reduced an additional 20 hr. The hydrogen uptake during this time was 0.25 mole, making a total uptake of 1.5 moles of hydrogen. The solution was filtered through a bed of Celite (the pH then adjusted to 5.5), concentrated under reduced pressure in a 40° bath, and finally lyophilized. The amorphous solid was dissolved in deuterium oxide and relyophilized and this procedure was repeated. Assuming the anomeric absorption at 5.8 ppm to represent one proton, nmr indicated that there was approximately 45% of dihydrouridine (VI) and 35% of tetrahydrouridine (IV) in the crude product.

Sodium Borohydride Reduction of Dihydrouridine. To an ice-cold solution of 246.2 mg (1.0 mmole) of 5,6-dihydrouridine in 10 ml of water at pH 7.5–8 was added 37.8 mg (1.0 mmole, 4.0 equiv) of sodium borohydride. After 35 min, the reaction mixture was set in the freezer overnight and put in the ice bath the next morning. By tlc (25% MeOH in chloroform) there were two spots moving slower than starting material but all starting material was gone. The excess sodium borohydride was destroyed with acetic acid and the mixture was lyophilized. The crude material was mainly tetrahydrouridine (IV) by nmr and tlc.

Reduction of thymidine under our conditions gave a poorly defined nmr spectrum with absorption at 0.9, 2.0, 3.3, 3.75, and 4.8 ppm.

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The Alkaline Hydrolysis of Aromatic Esters of Phosphoric Acid

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Abstract: The five-membered cyclic aromatic phosphate, *o*-phenylene phosphate (I), undergoes alkaline hydrolysis 6×10^6 times faster than does its open-chain analog, diphenyl phosphate (II). This rate acceleration is comparable to those observed in the hydrolyses of five-membered cyclic aliphatic phosphates, five-membered cyclic aromatic sulfates, and five-membered cyclic aromatic sulfonates.

In recent publications we have reported that various five-membered cyclic aromatic esters of sulfur-containing acids hydrolyze in alkaline solution at rates which are far greater than those for their acyclic and larger ring analogs. The five-membered cyclic aromatic sulfate, catechol cyclic sulfate, hydrolyzes in alkali with a rate enhancement of 2×10^7 when compared to its open-chain analog, diphenyl sulfate.[1] Furthermore, the five-membered cyclic sulfonate, *o*-hydroxy-α-toluenesulfonic acid sultone, reacts in alkali at a rate which is more than 10^4 times faster than that of the corresponding six-membered compound,[2] β-*o*-hydroxyphenylethanesulfonic acid sultone, and 7×10^5 times faster than that of the open-chain analog, phenyl α-toluenesulfonate.[3] These large rate accelerations are comparable to those described earlier by Westheimer for the alkaline hydrolyses of five-membered cyclic aliphatic esters of phosphorus-containing acids such as potassium ethylene phosphate,[4] methyl ethylene phosphate,[5] and lithium propylphostonate.[6] However, the second-order rate constant for attack by hydroxide ion in the case of the five-membered cyclic aliphatic sulfate, ethylene sulfate, is greater than that for its acyclic analog, dimethyl sulfate, by only a small amount, a factor of about 20.[7]

In view of the contrast between the observations made for the hydrolyses of the five-membered cyclic aliphatic and aromatic sulfates, it is of considerable interest to establish what the lability of a five-membered cyclic aromatic phosphate is relative to its acyclic analog. Accordingly, we have undertaken an investigation of the alkaline hydrolysis of *o*-phenylene phosphate (I) and the open-chain compound, diphenyl phosphate (II), and we report our findings here.

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