this compound was crystallized directly by dissolving the reaction mixture in petroleum ether and allowing it to stand overnight at room temperatures; yields in both cases were comparable; $[\alpha]^{25}D$ −42.20° (*c* 0.40, chloroform).

*Anal.* Calcd for $C_{29}H_{34}O_7N_2$: C, 66.30; H, 6.51; N, 5.37. Found: C, 66.50; H, 6.53; N, 5.26.

**5-β-D-Xylofuranosyluracil (VIII).**—The protective groups of compound VII were cleaved using exactly the same reaction conditions employed for the preparation of 5-α-D-arabinitoluracil. Fractional crystallization from methanol–ethanol afforded 5-β-D-xylofuranosyluracil (1.702 g, 78% yield, 23% yield from diisopropylidene-*aldehydo*-D-xylose): mp 202–204°; $[\alpha]^{25}D$ −27.77° (*c* 0.54, 0.1 *N* NaOH); $\lambda_{max}^{MeOH}$ 262 mμ (ε 6720, pH 7), 289 mμ (ε 6610, pH 12).

*Anal.* Calcd for $C_9H_{12}O_6N_2$: C, 44.30; H, 4.92; N, 11.48. Found: C, 44.04; H, 5.07; N, 11.44.

**5-α-D-Ribitoluracil (XI).**—Diisopropylidene-*aldehydo*-D-ribose (IX, 3.317 g, 14.5 mmoles), prepared by the same sequence of reactions shown for compound VI, was condensed with 2,4-dibenzyloxy-5-lithiopyrimidine (16 mmoles). The condensed product was chromatographed on alumina (Merck acid washed, 200 g), and the column was eluted with benzene (200 ml), benzene–chloroform (5:5, 200 ml), and chloroform (600 ml). The desired compound was eluted with chloroform. After cleaving the protective groups and fractional crystallization from ethanol, 5-α-D-ribitoluracil was obtained in 10.3% yield: mp 195–197°; $[\alpha]^{25}D$ −19.7° (*c* 0.37, 0.1 *N* NaOH); $\lambda_{max}^{H_2O}$ 262 mμ (ε 5610, pH 7), 287 mμ (ε 3740, pH 12).

*Anal.* Calcd for $C_9H_{14}O_7N_2$: C, 41.30; H, 5.35; N, 10.69. Found: C, 41.50; H, 5.61; N, 10.16.

**Micromethod for the Determination of Ring Size of Sugars in Nucleosides.**[16]—The same procedure was used to determine the ring size of 5-β-D-arabinofuranosyluracil and 5-β-D-xylofuranosyluracil. The compound (40 μmoles) and sodium periodate (80 μmoles) were dissolved in 0.8 ml of water. After 4 hr, 16 mg of sodium borohydride in 0.8 ml of water was added and the solution was allowed to stand overnight. The solution was acidified with 0.8 ml of 2 *N* HCl and heated in a steam bath for 30 min. The solution was evaporated to semidryness under vacuum and the residue was extracted with ethanol. The ethanolic extract was concentrated and spotted on Whatman No. 1 filter paper (6 × 22 in.). Ethylene glycol and glycerol were used as reference compounds. The chromatograms were developed for 8 hr with a solvent mixture of ethyl acetate, pyridine, and water (70:20:10). The papers were dried and sprayed with a 0.5% solution of sodium periodate. After 3 min they were sprayed with a 5% solution of benzidine in a ethyl acetate–ethanol mixture (2:8). The compounds appeared as white spots against a blue background. Both sugar nucleosides gave single spots which had $R_f$ values identical with that of glycerol.

# The Acid-Catalyzed Solvolysis of Pyrimidine Nucleosides[1]

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The kinetics of acid-catalyzed solvolysis of various pyrimidine nucleosides have been studied at various temperatures and specific acid catalysis was established for all compounds except 5-hydroxyuridine which exhibited pH-independent solvolysis. Acid stability is markedly affected by the number of hydroxyl groups in the sugar ring with complete hydroxylation of the sugar preventing degradation under the conditions used. The stereochemistry of the 3′-hydroxyl exerted a significant effect on the rate of solvolysis of 2′-deoxyribosides. Both electronegative and electropositive substituents in the 5 position of the pyrimidine ring increased the rate of acid-catalysed solvolysis relative to the unsubstituted 2′-deoxyuridine. The nucleosides solvolyzed at the glycoside link yielding the corresponding uracil and degraded sugar as products. An exception was 5-iodouridine which was transformed to uridine. The mechanism of N-glycoside hydrolysis in acid media is not clear; however, the substituent effects observed can be explained in terms of reactivity of various proposed intermediates in the degradation sequence.

The varying sensitivity of pyrimidine and purine nucleosides to hydrolysis is qualitatively well known.[2,3] It depends on both the nature of the nitrogen moiety, the nature and position of its substituents, and the structure of the sugar moiety.[4-9] Purine nucleosides are less stable to acid hydrolysis than pyrimidine nucleosides[4,5] and isocytidine is less stable than cytidine.[2] Hydrogenation of the 4,5 double bond in pyrimidine nucleosides causes an enormous increase in instability,[8b,10] a very important fact in the determination and characterization of the sugar moiety in different biological systems.

Some biological data can be interpreted by hypothesizing exchange of sugar moieties. The blocking of deoxyribonucleic acid (DNA) synthesis by 5-fluoro-2′-deoxyuridine (FDU) can be inhibited by 5-bromo-2′-deoxyuridine (BDU), thymidine, or by thymine and 5-bromouracil (BU) with the special purine nucleosides, deoxyguanosine or adenosine.[11] This could be explained by sugar transfer in biological systems since the rate of hydrolysis of purine nucleosides is known to be relatively fast.

The importance of nucleosides in biological systems has been well demonstrated by the discovery of their antitumor[12] and antiviral[13] properties. Quantitative studies on the stability of nucleosides and the effects of structure and substituents should provide insight into their metabolic transformations and are of pharmaceutical importance for the estimation of maximum stability and the inhibition of the formation of toxic side products.[14] The number of quantitative kinetic studies on nucleosides with determination of thermodynamic

(1) (a) Presented in part at the 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965; (b) supported by U. S. Public Health Service Grant GM-09864-03,04, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

(2) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press Inc., New York, N. Y., 1963, p 26.

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(10) P. A. Levene and F. B. La Forge, *Chem. Ber.*, **45**, 608 (1912).

(11) P. Langen and E. Liss, *Biochem. Z.*, **336**, 139 (1962).

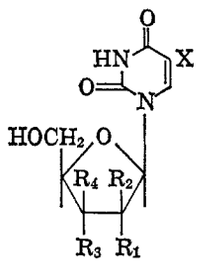
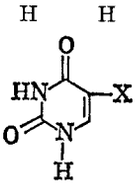
(12) R. Papac, E. Jacobs, C. Wong, W. Silliphant, and D. A. Wood, *Proc. Am. Assoc. Cancer Res.*, **3**, 257 (1961).

(13) H. E. Kaufman, E. L. Martola, and C. Dohlman, *Arch. Opthalmol.*, **68**, 235 (1962).

(14) E. R. Garrett, T. Suzuki, and D. J. Weber, *J. Am. Chem. Soc.*, **86**, 4460 (1964).

TABLE I

THE COMPOUNDS USED IN THIS STUDY AND THEIR ULTRAVIOLET SPECTRAL DATA

| Name | X | $R_1$ | $R_2$ | $R_3$ | $R_4$ | 0.1 *N* HCl $\lambda_{max}$, mμ | 0.1 *N* HCl $\epsilon_{max}$ | 0.01 *N* NaOH $\lambda_{max}$, mμ | 0.01 *N* NaOH $\epsilon_{max}$ |
|---|---|---|---|---|---|---|---|---|---|
|  | | | | | | | | | |
| 2′-Deoxyuridine (DU) | H | H | H | OH | H | 262 | 9,700 | 261 | 7,300 |
| Uridine (UD) | H | OH | H | OH | H | 262 | 9,900 | 261 | 7,200 |
| 1-(2′-Deoxy-β-D-lyxofuranosyl)uracil (DLU) | H | H | H | H | OH | 262 | 9,450 | 261 | 7,250 |
| 5-Iodouridine (IUD) | I | OH | H | OH | H | 288 | 7,100 | 278 | 5,550 |
| 5-Bromo-2′-deoxyuridine (BDU) | Br | H | H | OH | H | 279 | 10,250 | 276 | 6,600 |
| 5-Bromouridine (BUD) | Br | OH | H | OH | H | 279 | 9,800 | 276 | 6,800 |
| 5-Chloro-2′-deoxyuridine (CDU) | Cl | H | H | OH | H | 277 | 9,200 | 274 | 6,500 |
| 5-Chlorouridine (CUD) | Cl | OH | H | OH | H | 277 | 9,150 | 274 | 6,600 |
| 5-Fluoro-2′-deoxyuridine (FDU) | F | H | H | OH | H | 268 | 8,900 | 267 | 7,020 |
| 1-(β-D-Lyxofuranosyl)-5-fluorouracil (FUL) | F | H | OH | H | OH | 268 | 9,400 | 268 | 7,400 |
| 1-(β-D-Arabinofuranosyl)-5-fluorouracil (FUA) | F | H | OH | OH | H | 268 | 9,000 | 268 | 7,400 |
| 5-Hydroxy-2′-deoxyuridine (OHDU) | OH | H | H | OH | H | 280 | 7,000 | 304 | 6,300 |
| 5-Hydroxyuridine (OHUD) | OH | OH | H | OH | H | 280 | 8,150 | 305 | 7,000 |
| | | | | | | | | 218 | 14,750 |
| 5-Methyl-2′-deoxyuridine(thymidine) (MDU) | $CH_3$ | H | H | OH | H | 267 | 9,300 | 266 | 7,300 |
| 2′,3′-Dideoxy-3′-iodouridine (DIU) | H | H | H | I | H | 263 | 11,250 | 263 | 8,700 |
|  | | | | | | | | | |
| Uracil (U) | H | | | | | 259 | 8,100 | 283 | 6,200 |
| 5-Bromouracil (BU) | Br | | | | | 275 | 7,000 | 289 | 6,500 |
| 5-Chlorouracil (CU) | Cl | | | | | 272 | 7,400 | 287 | 6,700 |
| 5-Fluorouracil (FU) | F | | | | | 265 | 5,900 | 282 | 4,000 |
| 5-Hydroxyuracil(isobarbituric acid) (OHU) | OH | | | | | 285 | 4,200 | 313 | 3,800 |
| 5-Methyluracil(thymine) (MU) | $CH_3$ | | | | | 264 | 7,800 | 288 | 5,400 |

parameters is limited. Examples are the studies on psicofuranine[15,16] and iododeoxyuridine.[14,17]

Recently some initial observations on the acidic hydrolyses of uridine, deoxyuridine, thymidine, and dideoxyuridine have been made by Pfitzner and Moffatt[18] and Garrett, *et al.*[14] It was shown that in 1 *N* HCl at 100° uridine is stable, whereas the dideoxyuridine is more unstable than deoxyuridine and thymidine. Optical rotation has been used in the quantitative studies of the kinetics of acid hydrolysis of N- and O-glycosides of the anilides.[19] This technique has the drawback of not being specific for parallel and sequential reactions.

The purpose of this paper is to study the kinetics of acidic solvolysis of pyrmidine nucleosides as a function of variation of 5 substituents in the uracil ring and as a function of the stereochemistry and degree of hydroxylation of the sugar moiety. The nucleosides and substituted uracils studied, with the abbreviations used in this paper, are given in Table I.

(15) E. R. Garrett, *J. Am. Chem. Soc.*, **82**, 827 (1960).
(16) E. R. Garrett and L. J. Hanka, *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 526 (1960).
(17) E. R. Garrett, P. B. Chemburkar, and T. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **13**, 1113 (1965).
(18) K. E. Pfitzner and J. G. Moffatt, *J. Org. Chem.*, **29**, 1508 (1964).
(19) K. Butler, S. Laland, W. G. Overend, and M. Stacey, *J. Chem. Soc.*, 1433 (1950).

## Results

**Solvolysis of Uridines.**—Uridine (UD), itself, showed no spectrophotometric or chromatographic indications of solvolysis under vigorous conditions (80° and 1 *N* HCl for 1 month) in agreement with Pfitzner and Moffatt[18] and our prior observations.[14]

The stability of halogenated uridines to acid-catalyzed solvolysis was established in our studies. The compounds, 5-bromouridine (BUD), 5-chlorouridine (CUD), 1-(β-D-lyxofuranosyl)-5-fluorouracil (FUL), and 1-(β-D-arabinofuranosyl)-5-fluorouracil (FUA), were as resistant to acid hydrolysis as uridine.

5-Iodouridine (IUD) and 5-hydroxyuridine (OHUD) were exceptions. They do react under acidic conditions. The IUD ($\lambda_{max}$ 288 mμ) degrades to uridine ($\lambda_{max}$ 262 mμ) maintaining a colorless solution and an isobestic point at 274 mμ, indicative of 1:1 transformation (Figure 1). This is consistent with the interaction of hydrochloric acid and iodinated pyrimidines observed in the IDU and IU cases[14] and can be explained by the equilibria between IUD and UD and ICl where the latter two compounds are favored. The final absorbance at 262 mμ corresponds to an almost stoichiometric transformation to UD (see dashed curve in Figure 1). Thin layer chromatography confirmed this transforma-

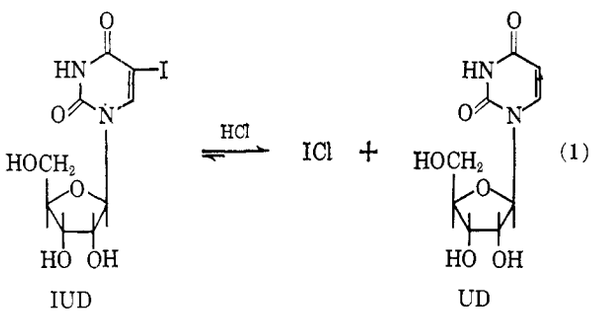
TABLE II

CONDITIONS AND APPARENT FIRST-ORDER RATE CONSTANTS AS $10^6$ $k$ $SEC^{-1}$ FOR THE SOLVOLYSIS OF SUBSTITUTED PYRIMIDINE NUCLEOSIDES IN 1.100 $N$ HCl

| °C | OHDU[a] | IDU[a,b] | BDU[a] | CDU[a] | FDU[a] | DLU[a] | MDU[a] | DU[a] | DIU[a] | IUD[c] | OHUD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 80.0 | 154[d] | 39.5 | 48.0 | 44.2 | 46.5 | 43.8 | 23.6[d] | 13.8[e] | 6.4 | 2.41 | 0.57 |
| 76.0 | 122[d] | | | | | | 16.4[d] | | | | |
| 75.0 | | | 24.7 | 24.9 | | 22.2 | | 10.4[e] | 3.8 | 1.34 | 0.36 |
| 72.0 | 75.0[d] | | | | | | 11.3[d] | | | 1.03 | |
| 70.0 | | 11.9 | 10.6 | 12.4 | 12.3 | 12.6 | | 3.97[e] | 2.2 | | 0.27 |
| 60.0 | 29.7[d] | 2.90 | 3.00 | 3.28 | 3.14 | 3.46 | 2.82[d] | 1.07[e] | | 0.27 | 0.08 |

[a] Solvolysis to the pertinent uracil and deoxyribose; see text. [b] See ref 14. [c] Solvolyzed to uridine and ICl. [d] In 1.0 $N$ HCl. [e] Estimated at 1.100 $N$ HCl from plots of the data in Table IV.

tion. The rate constants obtained are listed in Table II for several temperatures.




The absorbance of OHUD ($\lambda_{max}$ 280 m$\mu$) in acid solution after the chloroform extraction of degraded ribose ($\lambda_{max}$ 276 m$\mu$) disappears with time (Figure 2). This is indicative of the solvolysis of the 5-hydroxyuridine to ribose and 5-hydroxyuracil (isobarbituric acid) ($\lambda_{max}$ 285 m$\mu$). Under these conditions isobarbituric acid has the same magnitude of rate of solvolysis, $k$ = $2.8 \times 10^{-7}$ $sec^{-1}$ in 1.0 $N$ HCl at 80° for $10^{-4}M$ solutions.

Thin layer chromatography of samples of the degrading mixture showed the disappearance with time of the spot corresponding to OHUD together with the appearance and subsequent disappearance of a spot corresponding to OHU. The apparent first-order loss of the 280-m$\mu$ maximum (Figures 2 and 3) intimates a specific relation between the rates of OHUD solvolysis, OHU decomposition, and their absorptivities at 280 m$\mu$ in accordance with the development given previously[14] for a similar phenomenon occurring in the acid-catalyzed sequence IDU → IU → U. One point that is inconsistent with this explanation is the fact that toward the end of the reaction the $\lambda_{max}$ of the residual chromophore tends toward 278 m$\mu$ rather than the expected 285 m$\mu$. The possibility of a small yield of CUD ($\lambda_{max}$ 277 m$\mu$) arising by exchange of chlorine from HCl is indicated. The apparent first-order rate constants at several temperatures are given in Table II.

Uracil and its 5-fluoro, 5-chloro, 5-bromo, and 5-methyl (thymine) derivatives were stable under these acidic conditions and in the presence of acid degraded deoxyribose. Only the iodouracil, which has been previously reported[14] to react in the presence of acid-degraded deoxyribose, and the isobarbituric acid discussed above were unstable.

**Solvolysis of Deoxyuridines.**—The acidic solvolysis of deoxyuridine (DU) was followed spectrophotometrically in acidic ($\lambda_{max}$ 262 m$\mu$) and alkaline ($\lambda_{max}$ 261 m$\mu$) solutions. The absorbance at 262 m$\mu$ in acidic solution initially decreased and then subsequently

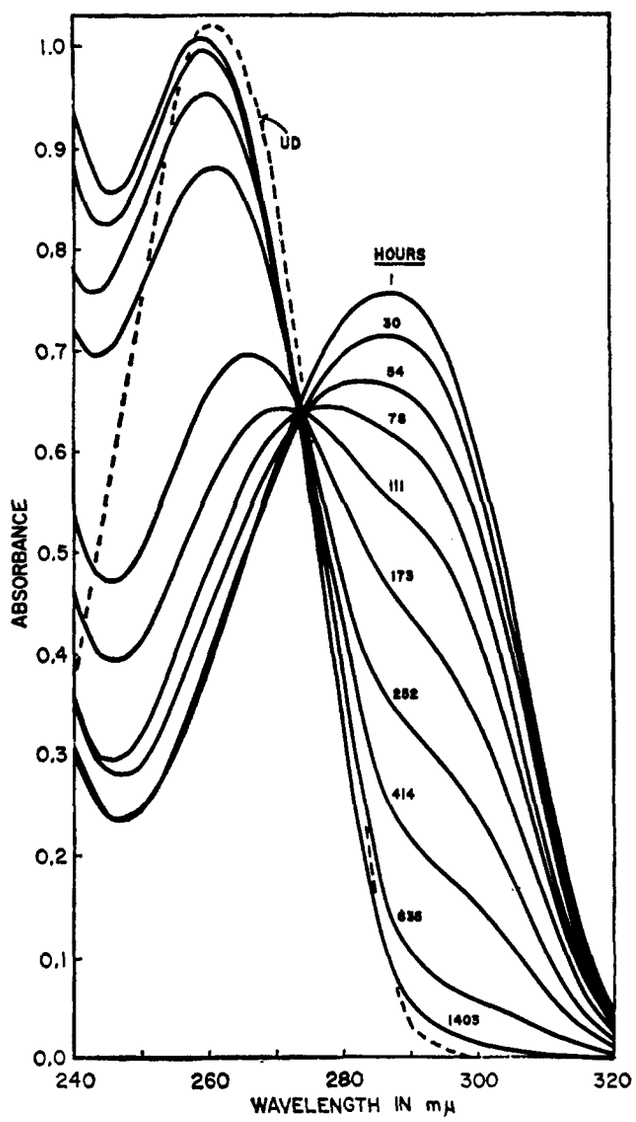



Figure 1.—Typical curves of the spectral changes of acid-degrading 5-iodouridine (IUD). The solution was treated at 72°, 1.0 $N$ HCl with an initial concentration of IUD of $10^{-4}$ $M$, and read at this concentration. Each curve is labeled as to the number of hours after the start of the degradation. The dashed curve is for an equimolar concentration of uridine (UD).

increased, while in alkaline solutions the increase at 283 m$\mu$ was greater than could be assigned to a uracil product (uracil $\lambda_{max}$ in alkali is 283 m$\mu$) when measured quickly. These phenomena result from the decomposition of DU to give deoxyribose which further degrades under acidic conditions to give a chromophore ($\lambda_{max}$ 261 m$\mu$) which is stable in acid.[20] Under alkaline condi-

(20) J. K. Seydel and E. R. Garrett, *Anal. Chem.*, **37**, 271 (1965).

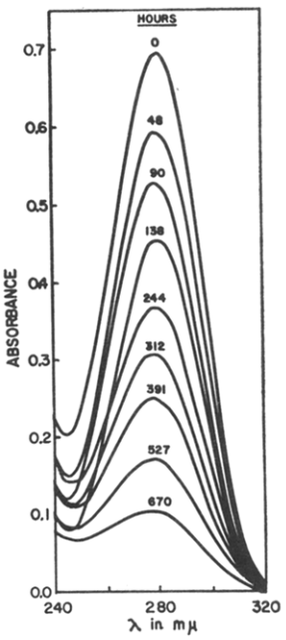



Figure 2.—Typical curves of the spectral changes of acid-degrading 5-hydroxyuridine (OHUD) after chloroform extraction to remove the interfering 276-mμ chromophore of acid-degraded ribose. The solution was treated at 80°, 0.50 *N* HCl with an initial concentration of OHUD of $10^{-4}$ *M*, and read at this concentration. Each curve is labeled as to the number of hours after the start of the degradation.

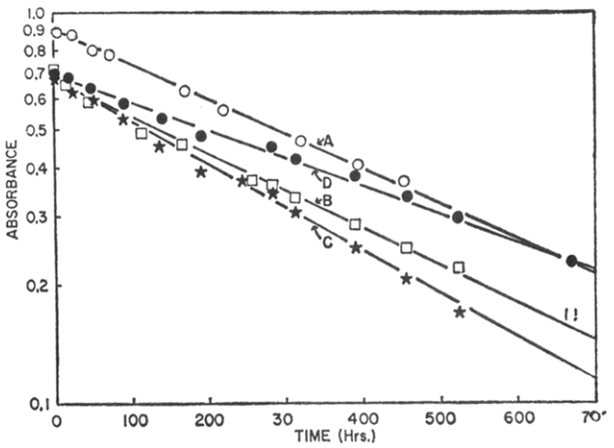



Figure 3.—Apparent first-order plots for the decrease in absorbance (A) at 280 mμ for 5-hydroxyuridine (OHUD) at 80°. The initial concentration of OHUD was $10^{-4}$ *M*. The [HCl] for the various curves are 1.0, A; 0.75, B; 0.50, C; 0.25, D.

tions the absorption maximum of this chromophore shifts to 293 mμ and then decreases rapidly.

Confirmatory evidence of the degraded deoxyribose chromophore interferences with nucleoside and pyrimidine product chromophores (nucleoside → pyrimidine + sugar) were obtained by following the progress of the reaction by thin layer chromatography. All deoxynucleosides were similar to deoxyuridine in that they degraded with time with the concomitant appearance of two chromatographic spots, one of the same $R_f$ value as the corresponding uracil and the other of the same $R_f$ value as degraded deoxyribose (DDR)[20] (Figure 4). In all cases of acid solvolysis studied, the deoxynucleosides were separable from their pyrimidine products by thin layer chromatography and no other product

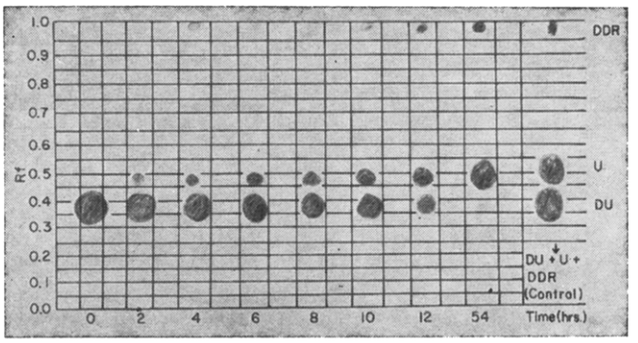



Figure 4.—Typical thin layer chromatogram for monitoring pyrimidine deoxynucleoside decomposition in acid solution. The particular example is for 2′-deoxyuridine in 1 *N* HCl at 80° developed by chloroform–isopropyl alcohol (2:8) and shows the disappearance of 2′-deoxyuridine (DU) ($R_f$ 0.37) concomitant with the appearance of uracil (U) ($R_f$ 0.48) and degraded deoxyribose (DDR) ($R_f$ 0.98). The right-hand column demonstrates the chromatographic separation of a prepared mixture.

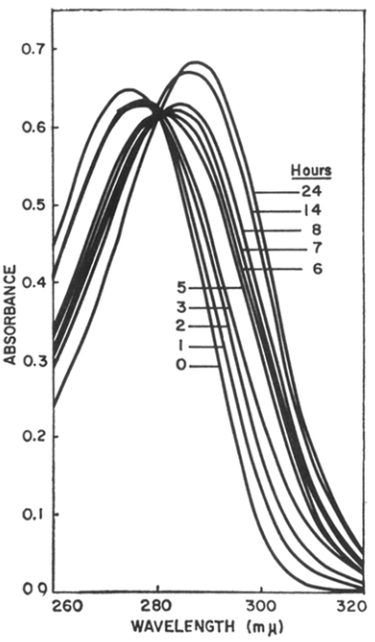



Figure 5.—The alkaline spectra of 5-chloro-2′-deoxyuridine hydrolyzed in 1 *N* HCl at 80° and after alkaline decomposition of degraded deoxyribose.

than DDR could be detected. The derived pyrimidines, except for isobarbituric acid and 5-iodouracil[14] which have been previously discussed, were stable under the assay conditions used.

These facts permitted the development of specific procedures for kinetic studies of the deoxynucleosides in acidic regions. The acidic aliquots were made alkaline and allowed to stand 24 hr at room temperature to remove the interfering spectra of degraded deoxyribose.[20] *E.g.*, the 274-mμ maximum absorbance of CDU after alkaline treatment decreased with time by a first-order process and a new absorbance maximum appeared at 287 mμ with retention of an isobestic point. This is a strong indication of a 1:1 transformation (Figure 5). Typical examples of the first-order nature of such plots for acidic solvolysis are given in Figure 6 for 1-(2′-deoxy-β-D-lyxofuranosyl)uracil analyzed in this manner. The only exception to this technique of following the kinetics was the case of 5-hydroxy-2′-deoxyuridine (OHDU), where an apparently different situation

TABLE III

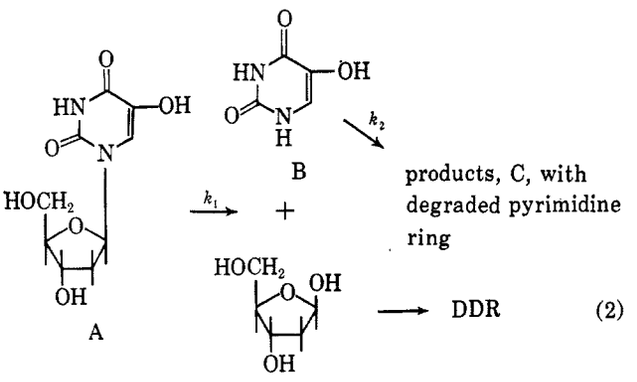
CONDITIONS AND APPARENT FIRST-ORDER RATE CONSTANTS AS $10^6$ $k$ $sec^{-1}$ FOR THE HYDROLYSIS OF SUBSTITUTED PYRIMIDINE NUCLEOSIDES AT 80°

| [HCl] | OHDU | FDU | CDU | BDU | DLU | MDU | OHUD[a] | IUD[b] |
|---|---|---|---|---|---|---|---|---|
| 1.10 | 154[c] | 46.5 | 44.2 | 48.0 | 43.8 | 23.6[c] | 0.57[c] | 2.41[c] |
| 0.75 | 120 | 33.5 | 31.0 | 30.9 | 27.0 | 18.3 | 0.61 | 1.62 |
| 0.50 | 79.5 | 22.7 | 24.5[d] | 20.6 | ... | 12.5[d] | 0.70 | 0.97 |
| 0.40 | 61.7 | | | | | | | |
| 0.27 | 39.2[e] | 9.95 | 12.2[f] | 10.7 | 8.9 | 6.58 | 0.53[e] | 0.47[e] |
| 0.2 | 30.1 | | | | | | | |
| 0.1 | 19.8 | | | | | | | |

[a] At pH 3.4 in acetate buffer, a rate of $0.41 \times 10^{-6}$ $sec^{-1}$ was observed. [b] Solvolyzed to uridine and ICl, whereas others are solvolyzed to pertinent uracil and deoxyribose. [c] At 0.98 $N$ HCl. [d] At 0.565 $N$ HCl. [e] At 0.25 $N$ HCl. [f] At 0.283 $N$ HCl.

was encountered. Since the absorption maximum for OHDU occurred at 280 mμ, the chromophore for degraded deoxyribose (261 mμ) should not interfere. However, an apparent first-order plot for the loss of the 280 mμ chromophore showed two sequential rates $A \xrightarrow{k_1} B \xrightarrow{k_2} C$ *i.e.* (curve A in Figure 7) and the OHDU chromophore showed a shift to 275 mμ, the loss of which, *i.e.*, $k_2$, is the second rate of the sequence (Figure 8).

When the acid samples were made slightly alkaline and allowed to stand for 6 hr at room temperature, the first-order plot of the spectra of these samples at the absorption maximum (304 mμ), which corresponds to the chromophore of OHDU in alkali, is nicely linear (Figure 7, curve B). These times and conditions were shown to be sufficient to degrade all isobarbituric acid but not to affect the absorbance of OHDU. When an absorbance asymptote, $A_\infty$ at 280 mμ, is estimated for the transformation A → B ($k_1$) and the log ($A - A_\infty$) *vs.* time is plotted for the samples in acid solution, the rate constant for the A → B ($k_1$) transformation (curve C, Figure 7) is coincident with that obtained for the loss of the chromophore for the alkali treated samples. This demonstrates that this A → B transformation represents the solvolysis of OHDU to isobarbituric acid and deoxyribose. The slow subsequent transformation B → C can be assigned to the acid-catalyzed degradation of the product B, isobarbituric acid as shown.




The apparent rate constant, $k_2$, for the B → C transformation starting with OHDU is $6 \times 10^{-7}$ $sec^{-1}$ in 1 $N$ HCl at 80° and that for the degradation of isobarbituric acid under the same conditions was similar.

The apparent first-order rate constants for the various nucleosides in 1.0 $N$ HCl are given in Table II for the several temperatures studied. The rate constants obtained at 80° and various hydrochloric acid concentrations are given in Table III for the several nucleosides. The Table IV gives such dependencies for deoxyuridine at several temperatures.

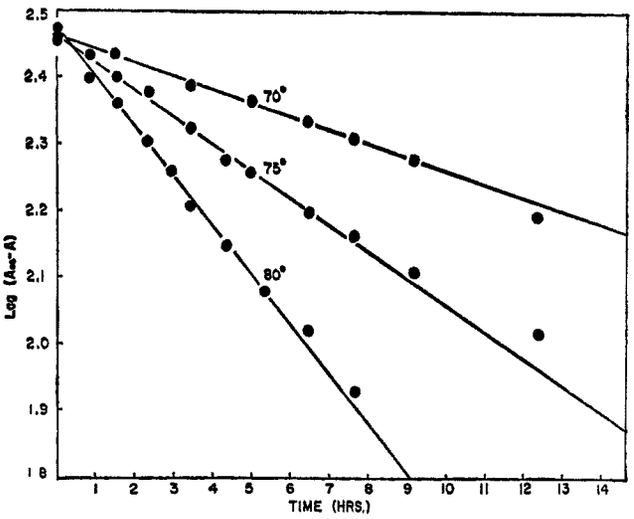



Figure 6.—Apparent first-order plots for the acid-catalyzed solvolysis of 1-(2′-deoxy-β-D-lyxofuranosyl)uracil at several temperatures at 1.0 $N$ HCl. The data was obtained at 295 mμ in alkaline solution after the alkaline decomposition of interfering degraded deoxyribose.

TABLE IV

CONDITIONS AND APPARENT FIRST-ORDER RATE CONSTANTS AS $10^6$ $k$ $sec^{-1}$ FOR THE HYDROLYSIS OF 2-DEOXYURIDINE

| [HCl] | 80° | 75° | 70° | 60° |
|---|---|---|---|---|
| 0.928 | 11.6 | 8.77 | 3.34 | 1.07 |
| 0.743 | ... | 5.67 | 2.48 | 0.673 |
| 0.459 | 5.33 | 3.20 | 1.75 | 0.417 |
| 0.244 | 3.35 | 1.85 | 0.914 | 0.210 |
| Av $10^6$ $k_{HCl}$ = $10^6$ $k$/[HCl] | 12.2 | 7.20 | 3.58 | 0.93 |

Except in the case of OHDU where the solvolysis appeared to be independent of acid concentration (Figure 3), plots of first-order rate constants (Tables II and III) *vs.* hydrochloric acid concentration were linear and passed through the origin (Figure 9) thus indicating specific hydrogen ion catalyzed solvolysis. Spontaneous decomposition or pH-independent effects for all the studied nucleosides except OHUD and the previously reported IDU[14] were small with respect to specific acid catalysis, but this does not mean that such effects are nonexistent.[14]

**Temperature Effects.**—The Arrhenius parameters for the various specific rate constants of the deoxynucleosides were derived from the slopes and intercepts

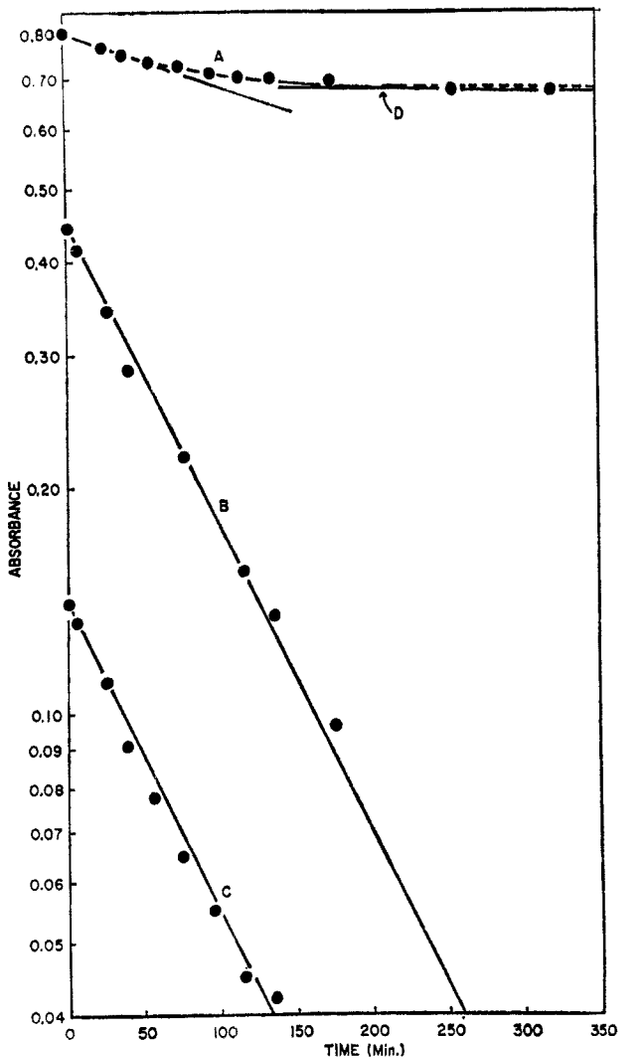



Figure 7.—Apparent first-order plots for the solvolysis of 5-hydroxy-2′-deoxyuridine at 80° in 1 *N* HCl. Curve A represents the plot for the spectrophotometric absorbance of the samples in acid solution. Curve B represents the plot for the loss of absorbance of alkali-treated samples. Curve C represents the difference of the absorbance of the samples in acid solution and the absorbance attributed to a degrading intermediate which is assigned to isobarbituric acid and represented by D in the above figure.

of plots of the logarithms of the rate constants *vs.* the reciprocals of the absolute temperature (Figure 10) in accordance with eq 3.

$$\log k = -(\Delta H_a/2.303R)(1/T) + \log P \qquad (3)$$

Except for 2′-deoxyuridine where the specific catalytic rate constant $k_{H^+}$ was used, the plots were derived from the apparent first-order rate constants at 80° corrected to 1.0 *N* hydrochloric acid. These plots were consistent with heats of activation of $\Delta H_a$ = 31.0 kcal/mole for BDU, CDU, DLU, DU, FDU, and DIU. The $\Delta H_a$ values for MDU and DIU were 25.1 and 19.2 kcal/mole for OHDU.

The log *P* values for Arrhenius plots of apparent first-order rate constants in 1.0 *N* hydrochloric acid where solvent effects are negligible are equivalent to the log *P* values for hydrogen ion attack on the deoxynucleoside.

$$\log P_{k_{H^+}} = \log P_k - \log [H^+] = \log P_k - \log 1.0 = \log P_k \qquad (4)$$

These log *P* values for the various compounds were 14.8 for BDU, CDU, DLU, and FDU, 10.0 for MDU, 8.1 for OHDU, 10.4 for DIU, and 14.2 for DU.

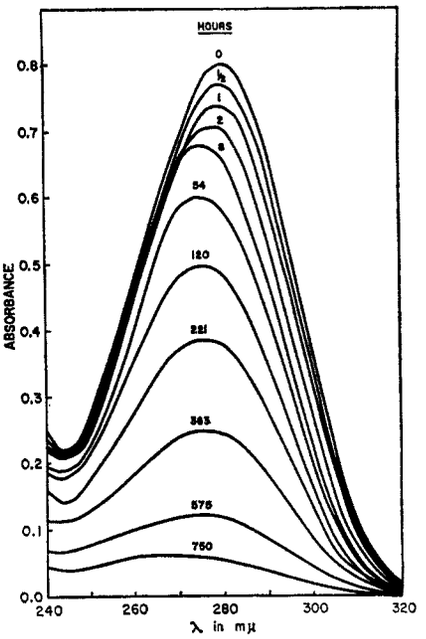



Figure 8.—Spectral absorbance of 5-hydroxy-2′-deoxyuridine as a function of time in 1.0 *N* HCl at 80°. Each curve is labeled as to the number of hours after the start of the degradation.

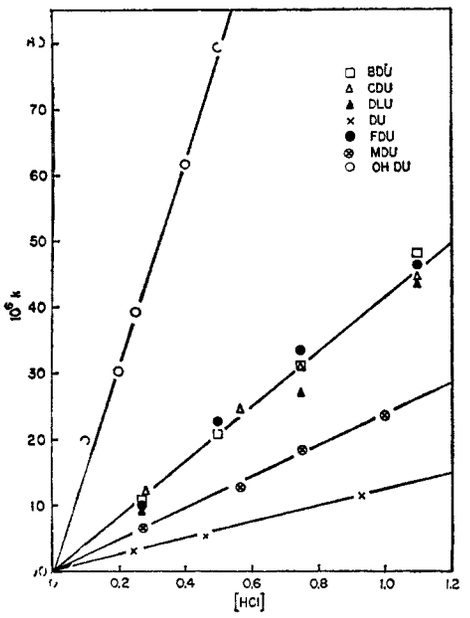



Figure 9.—Dependence of the rate constants for the solvolysis of deoxynucleosides on HCl concentration at 80°. No significant solvent effect is apparent for these compounds.

The two acid-degradable uridines had $\Delta H_a$ values of 24.4 and 20.0 kcal/mole for IUD and OHUD, respectively. The log *P* value for the specific acid catalytic rate constant of IUD was 9.5. The degradation of OHUD was independent of acid concentration and its log *P* value for apparent first-order solvolysis in 1.0 *N* hydrochloric acid was 6.1. This figure should hold for the solvolysis of the undissociated OHUD at all pH values.

## Discussion

The pyrimidine deoxynucleosides investigated were solvolyzed by acid to the respective pyrimidines and sugars. The sugar then degraded to give a chromophore which frequently interfered with the spectro-

photometric assay of the reaction mixture. This interference could be overcome by a mild alkaline treatment.[20] The rates of glycoside hydrolysis were dependent on the nature of the sugar moiety and also to some extent on the 5 substituent in the pyrimidine ring. In contrast, the ribosyl, arabinosyl, and lyxofuranosyl nucleosides, with and without 5-substituents in the pyrimidine ring, were generally completely stable to 1 *N* hydrochloric acid at 80°. Exceptions were OHUD, which underwent glycoside hydrolysis slowly and independently of acid concentration, and IUD in which the initial degradation was to UD.

Hydrolysis of N-glycosides has received the attentions of several previous workers. Kenner[21] and Dekker[22] suggested that acid solvolysis of purine nucleosides occurred as a result of initial protonation of the sugar ring oxygen (I → IIa). The sugar ring then opened to give the Schiff base intermediate IIIa. (Scheme I). Addition of water to the Schiff base leads to hydrolysis products. It was suggested that the alternative N-protonation (IIb) resulted in a much less reactive species which might undergo hydrolysis either by a slow dissociation of the glycoside link (IIb → IV), or by intramolecular proton transfer (IIb → IIa) and subsequent hydrolysis of the more reactive form. Differing rates of hydrolysis among various N-glycosides were considered to be explainable in terms of the ability of the glycosidic nitrogen to accept positive charge. Isbell and Frush[23] made similar proposals for the hydrolysis of glycosylamines. Initial O-protonation led to hydrolysis while the alternative N-protonation gave an unreactive ammonium salt (IIb) and thus inhibited hydrolysis. The latter effect predominated in strongly acidic solutions. A different viewpoint was adopted by Micheel and Heesing.[3] These workers postulated initial protonation of the glycosidic nitrogen in N-aryl glycosylamines to give a weak $N^+$–H bond which then decomposed after ring opening. A strongly bound proton in IIb prevented ring opening and thus explained the observed decreased rates of hydrolysis in strongly acidic solutions and the marked effect of benzene ring substituents.

More recent workers[24-26] have proposed that the rate-determining step involved the decomposition of the intermediate Schiff base (III) rather than the initial protonation. Capon and Connett[25] considered that N-aryl glycosylamines formed Schiff bases (IIIa) in acid and that these decomposed by the addition of water across the double bond followed by breakdown of the addition product, in a manner similar to the earlier proposals of Kenner.[21] Either step could be rate limiting depending on the conditions. Simon and Palm[26] proposed a similar scheme but considered that the final breakdown of the addition product was brought about by intramolecular attack of the C-5 hydroxyl of the hexose sugar. This had also been proposed by Isbel and Frush.[23]

It is apparent that the mechanism of acid hydrolysis of N-glycosides is still very much undecided. However, the process involves three key steps: (1) protonation with most workers[21-26] prefering O-protonation, (2) formation of the Schiff base intermediate (III), and (3) decomposition of the Schiff base intermediate.

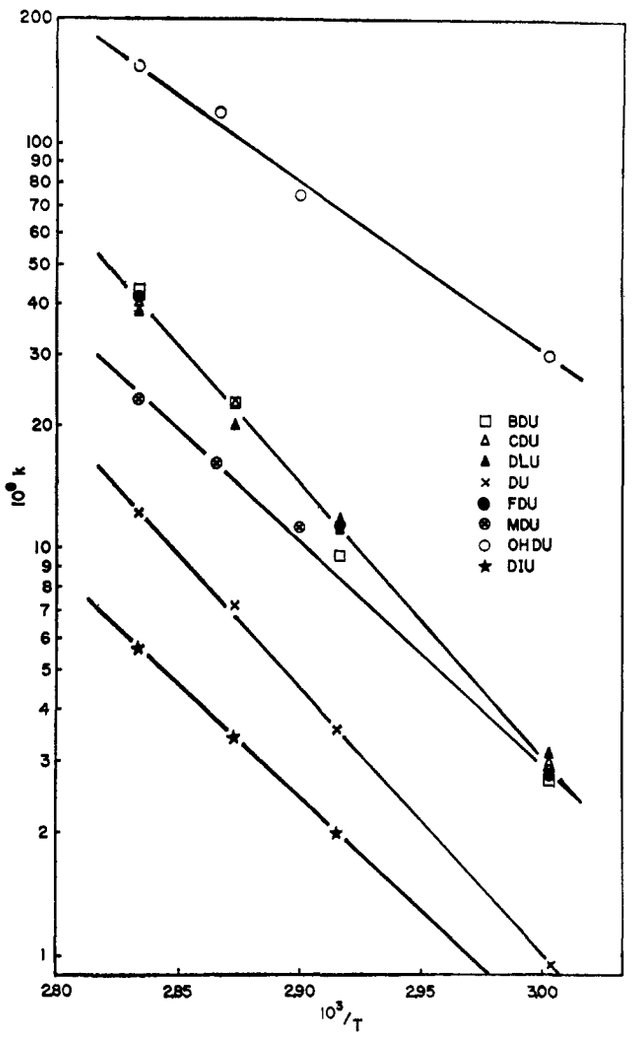


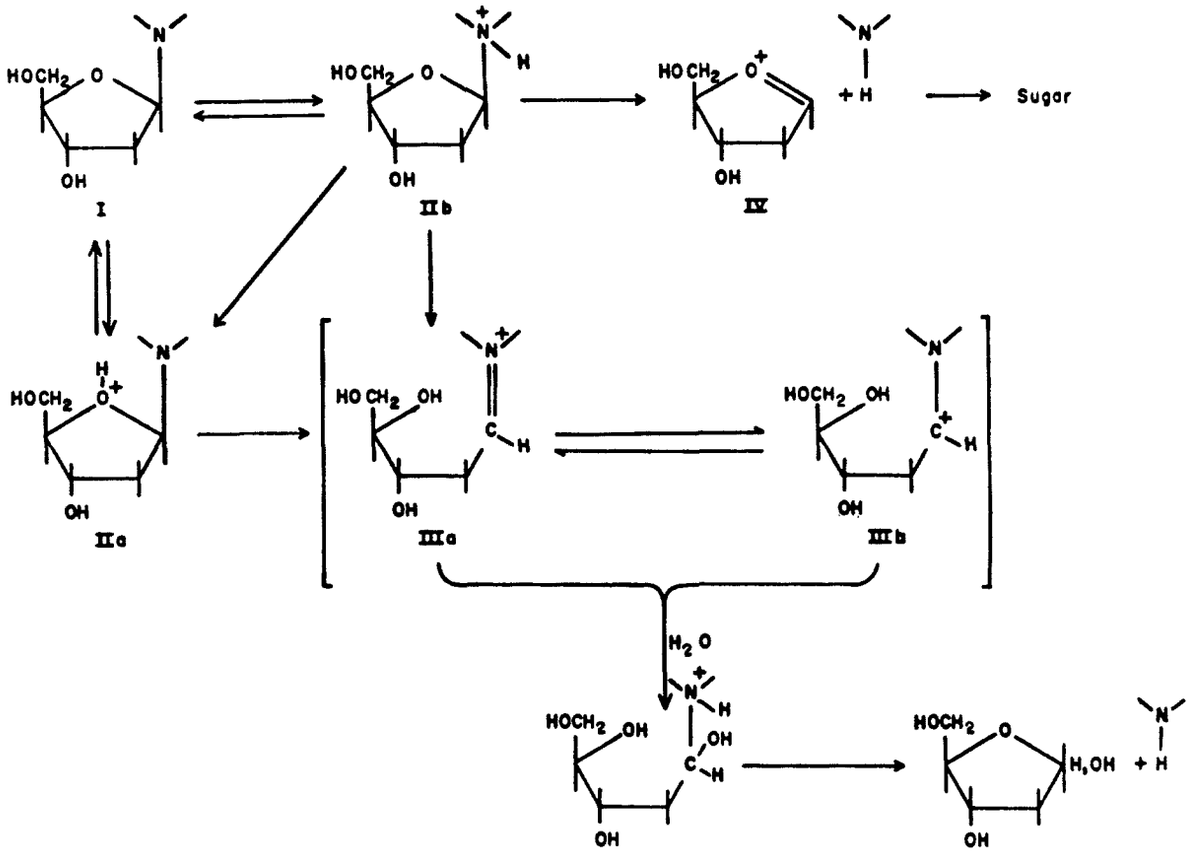

Figure 10.—Arrhenius plots for the apparent bimolecular rate constants for the acid-catalyzed solvolysis of various deoxynucleosides.

The high stability of the ribosides and other fully hydroxylated pentosides is best explained by effects occuring in step 2. The inductive effect of the 2′-hydroxyl could prevent formation of the carbonium ion (IIa → IIIb) and hence prevent hydrolysis. Alternatively the 2′-hydroxyl could decrease the rate of protonation of the sugar ring oxygen (step 1) either by acting as a competitive basic site or by long range inductive effects. This latter explanation is less satisfactory owing to the enormous influence on rate constant (*cf.* DU and UD) exerted by what must be relatively slight inductive and competitive effects. The increased stability resulting from substitution of iodine for the 3′-hydroxyl (DIU) in 2′-deoxyuridine and the decreased stability of 2′,3′-dideoxyuridine relative to DU[18] also can be explained by these hypotheses. If the 3′-deoxyribosides are as resistant to acid hydrolysis as the ribosides, the mechanism of carbonium ion inhibition would be favored. If their solvolytic rates are similar to those of the 2′-deoxyribosides, the greater significance of the protonation step would be more plausible.

Electron-donating 5-methyl and 5-hydroxy substituents in the pyrimidine ring increase the rate of

(21) G. W. Kenner, Ciba Foundation Symposium on the Chemistry and Biology of Purines, Little, Brown and Co., Boston, Mass., 1957, p 312.
(22) C. A. Dekker, *Ann. Rev. Biochem.*, **29**, 453 (1960).
(23) H. S. Isbell and H. I. Frush, *J. Org. Chem.*, **23**, 1309 (1958).
(24) B. Capon and B. E. Connett, *Tetrahedron Letters*, 1395 (1964).
(25) B. Capon and B. E. Connett, *J. Chem. Soc.*, 4497 (1965).
(26) H. Simon and D. Palm, *Chem. Ber.*, **98**, 433 (1965).

SCHEME I




solvolysis relative to DU. This increase is the expected result from previous work.[3,24-26] It also can be explained either on the basis of greater ease of formation of the Schiff base intermediate (step 2) or by the increased basicity of the pyrimidine ring resulting in preferential protonation (step 1) at the nitrogen and followed by breakdown according to the scheme of Micheel and Heesing.[3]

Electron-withdrawing pyrimidine ring substituents (halogen) increased the rate of solvolysis relative to DU by a factor of approximately 3.5. This is contrary to the effects expected from the above considerations. It can be argued that electron withdrawal decreases the stability of the Schiff base intermediate (step 3) once it has formed and thereby increases the rate of degradation. The same effect can also be considered to disfavor N-protonation (IIb) and thus increase the rate of O-protonation and hydrolysis (step 1).

Configuration of the 3′-hydroxyl influences the rate of hydrolysis and this effect can also be explained by the relative ease of breakdown of Schiff base intermediates. Examination of models shows that hydrogen bonding can occur between the 5′-hydroxyl of the sugar ring and the 2-carbonyl of the pyrimidine ring. When the 3′-hydroxyl is in the "up" position (DLU) it offers an additional possibility of hydrogen bonding to the pyrimidine carbonyl. Formation of the hydrogen bond will result in an electron drift out of the pyrimidine ring *via* the 2-carbonyl and hence an increased rate of hydrolysis as previously discussed for electronegative 5 substituents. The faster rate of hydrolysis of DLU relative to DU may thus be explained by the statistically greater degree of hydrogen bonding in the former case.

## Experimental Section

**Materials.**—The 5-fluoro-2′-deoxyuridine and 5-fluorouracil were kindly donated by Dr. W. E. Scott of Hoffmann-La Roche Inc., Nutley, N. J. Drs. J. R. Hoover and R. H. Blythe of Smith, Kline and French Laboratories, Philadelphia, kindly donated samples of 5-hydroxy-2′-deoxyuridine and 5-hydroxyuridine. Dr. J. J. Fox of the Sloan-Kettering Research Institute kindly gave samples of 5-fluoro-2′-deoxyuridine, 1-(β-D-lyxofuranosyl)-5-fluorouracil, and 1-(β-D-arabinofuranosyl)-5-fluorouracil. The remaining compounds were synthesized or obtained from commercial sources. The DU, UD, IUD, BDU, BUD, OHUD, and MDU were purchased from Nutritional Biochemical Corp.; CDU, CUD, IUD, BU, CU, and MU from Calbiochem; OHU from Mann Research Laboratories, New York, and Uracil from Eastman Organic Chemicals.

**Methods.**—An appropriate quantity of the nucleoside (mostly $10^{-2}$ mole/l.) was weighed into a 100-ml flask and made up to volume with nitrogen-purged distilled water. From this stock solution, an aliquot was diluted with an appropriate acid concentration (Table III) so that the concentration of the nucleoside was generally $2 \times 10^{-4}$ *M*. The solvent was previously equilibrated at the temperature of the kinetic study. No significant effect on rate constants was observed for studies where the substrate concentration was varied from $10^{-4}$ to $2 \times 10^{-4}$ *M*, for solutions wrapped and unwrapped in tinfoil in our temperature baths, for solutions purged and unpurged with nitrogen. Thus light and oxygen had negligible effects under the conditions of these studies. Iodouridine solutions became yellow on standing at room temperature when exposed to light but this did not occur under our kinetic conditions.

The solutions were maintained in constant-temperature baths at several temperatures between 60 and 80°. Spectrophotometric absorbances at pertinent wavelengths were obtained 24 hr after diluting 1:1 with 1.3 *N* NaOH. This removed any spectrophotometric interference from acid generated chromophores that might have been derived from sugars. Typical spectra as recorded using the Cary Model 15 spectrophotometer for the alkaline solutions are given in Figure 5. The acid absorbances at pertinent wavelengths were obtained on the samples acidified to *ca.* pH 2 with hydrochloric acid and corrected for the respective dilutions.

The apparent first-order rate constants for the increase of absorbance (alkaline readings) of the different uracil derivatives are given in Table II. The acid readings showing the decrease of intact nucleoside were in agreement. Typical first-order plots for alkali readings are given in Figure 6.

The stability of the nucleosides and uracils derived from them under the alkaline conditions at room temperature were checked. The compounds FUA, FUL, OHUD, OHDU, IUD, and OHU were not stable. Of these the first two did not degrade in acid. The remainder were assayed using different procedures.

A quantity of 5-iodouridine, sufficient to give a final concentration of $10^{-4}$ *M*, was weighed into a 100-ml flask and made up to volume with the appropriate concentration of hydrochloric acid, previously equilibrated at the desired temperature. The solutions were maintained at the temperature of study, samples were taken at suitable intervals, and the ultraviolet spectra of these solutions were determined using the Cary spectrophotometer. Apparent first-order rate constants (Tables II and III) were obtained for the decrease in absorbance at the 288 mμ maximum (IUD). Readings at 260 mμ showing the formation of uridine gave similar rates (Figure 1).

Acid solutions containg $1 \times 10^{-4}$ *M* 5-hydroxyuridine, prepared as above and degraded at the various temperatures (Tables II and III), showed a first-order loss of the 280-mμ maximum with time.

The appearance of a 261-mμ chromophore due to degraded deoxyribose did not interfere significantly with the measurement of absorbance at the 280-mμ maximum of 5-hydroxy-2′-deoxyuridine but interference was obtained from the degradation product OHU ($\lambda_{max}$ 285 mμ in acid). Two procedures were adopted for following the degradation of this compound. Solutions containing $10^{-4}$ *M* OHDU were prepared and degraded as described above for 5-iodouridine and the initial rate of decrease of the absorption maximum was followed. The samples were then made alkaline by the addition of a little more than the equivalent quantity of 10 *N* sodium hydroside solution, allowed to stand at room temperature for 6 hr, and the spectra of these alkaline solutions were also obtained. The alkali treatment was shown in a preliminary experiment to degrade all the interfering isobarbituric acid, OHU, and yet hardly affect the absorbance owing to residual OHDU. The rates of loss of OHDU due to acid hydrolysis were comparable when measured by either of these two procedures.

**Thin Layer Chromatography.**—The plates were prepared with a 0.4-mm layer of silica gel $GF_{254}$ according to Stahl (E. Merck, Darmstadt). A $10^{-2}$ *M* solution of the nucleoside in 1.0 *N* hydrochloric acid was reacted at 80° and at intervals 0.02 ml of this solution was spotted at the origin of the plate together with suitable standards. Chromatograms were developed for 12 cm generally using a chloroform–isopropyl alcohol (3:1) solvent system. After development, the spots were viewed under short-wave ultraviolet light, 2537 A. A typical thin layer chromatogram is given in Figure 4.

The silica gel corresponding to the $R_f$ value of each spot was scraped off, extracted with water, and the spectra of these extractions were run at acidic and alkaline pH values. Spectral shifts with pH were evident and confirmed the identities of the nucleoside, the pyrimidine, and the degraded sugar in the cases of acid-degraded BDU ($R_f$ 0.34) to give BU ($R_f$ 0.52); CDU ($R_f$ 0.32) to give CU ($R_f$ 0.55); FDU ($R_f$ 0.21) to give FU ($R_f$ 0.36); DLU ($R_f$ 0.24) to give uracil ($R_f$ 0.30); and DU ($R_f$ 0.17) to give uracil. The above $R_f$ values are for chloroform–isopropyl alcohol 3:1 developing solvent. 5-Iodouridine ($R_f$ 0.75 in *n*-butyl alcohol saturated with 0.2 *N* HCl) gave uridine ($R_f$ 0.50 in the same solvent) and OHUD ($R_f$ 0.60) gave OHU ($R_f$ 0.4 also in the *n*-butyl alcohol–HCl solvent system).

**Synthesis.**—1-(2′-Deoxy-β-D-lyxofuranosyl)uracil was prepared by a method based on that described for 1-(2-deoxy-β-D-lyxofuranosyl)thymine,[27] mp 167°, $R_f$ 0.35 (chloroform–isopropyl alcohol, 3:1). The method was essentially that subsequently described by Horwitz, *et al.*[28] who quote mp 163°.

2′,3′-Dideoxy-3′-iodouridine was prepared according to the method of Pfitzner and Moffatt:[18] mp 171–172° (from chloroform–ethyl acetate); $\lambda_{max}^{0.1\ N\ HCl}$ 263 mμ ($\epsilon_{max}$ 11,250); $\lambda_{max}^{0.1\ N\ NaOH}$ 263 mμ ($\epsilon_{max}$ 8700); $R_f$ 0.125 (chloroform–ethyl acetate, 1:2), 0.77 (chloroform–isopropyl alcohol, 3:1). Pfitzner and Moffatt[18] quote mp 162° dec (from acetone); $\lambda_{max}^{0.1\ N\ HCl}$ 262 mμ ($\epsilon_{max}$ 11,300); $\lambda_{max}^{0.1\ N\ NaOH}$ 263 mμ ($\epsilon_{max}$ 8720).

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# Amino Derivatives of Starches. Sulfonation Studies on Methyl 3,6-Anhydro-α-D-glucopyranoside and Related Derivatives[1]

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Selective *p*-toluenesulfonation of methyl 3,6-anhydro-α-D-glucopyranoside (VI) gives the 4-*p*-toluenesulfonate IX. The 2-*p*-toluenesulfonate (VII) and 2-methanesulfonate (V) of VI can be prepared by treatment of methyl α-D-glucopyranoside 2,6-di-*p*-toluenesulfonate (IV) and 2,6-dimethanesulfonate (I), respectively, with base. Reductive desulfonation of VII gives VI, and methanesulfonation of V gives II, proving that V and VII are pyranosides, and the structure of VII was further proved by methylation followed by desulfonation to give the known methyl 3,6-anhydro-4-*O*-methyl-α-D-glucopyranoside (XII). The *p*-tolylsulfonyloxy groups in VII and III were found to be remarkably resistant to displacement by hydrazine. A similar resistance to amination by hydrazine displacement was observed with methyl 3,6:3′,6′-dianhydro-2,2′,4′-tri-*O*-(*p*-tolylsulfonyl)-β-maltoside (XIII). *p*-Toluenesulfonated and methanesulfonated derivatives of 3,6-anhydroamylose were prepared, and were found to be likewise resistant to amination by hydrazine.

A number of reports[2-4] from this laboratory have been concerned with the preparation and characterization of an aminated derivative of amylose,[2] obtained from a slightly derivatized amylose by conversion into a di-*p*-toluenesulfonated derivative, followed by hydrazinolysis and reduction. Possible reaction pathways in the formation of this aminated derivative have been discussed,[3] leading to the production of residues in the polymer with the 3,6-diamino-3,6-dideoxy-D-altrose, 2,6-diamino-2,6-dideoxy-D-mannose, 3,6-anhydro-2-*O*-(*p*-tolylsulfonyl)-D-glucose, and 2-amino-3,6-anhydro-2-deoxy-D-mannose structures. Hydrazinolysis studies on the model compound methyl 2,6-di-*O*-(methylsulfonyl)-α-D-glucopyranoside have indicated[4] that 3,6-diamino-3,6-dideoxy-D-altrose resi-

(1) This work was supported by the Agricultural Research Service, U. S. Department of Agriculture, Contract No. 12-14-100-5760 (71) (The Ohio State University Research Foundation Project 1301), administered by the Northern Regional Research Laboratory, Peoria, Ill. The opinions expressed in this article are those of the authors and not necessarily those of the supporting agency.

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