Studies on the Preparation and Exchange Reactions of 5-Deuterated Uracils (I)

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The mechanism of base catalyzed proton exchange at the 5-position of uracil and its N-methylated derivatives has been studied. These reactions proceed by addition - elimination across the 5,6-double bond when the 1-nitrogen is substituted with a methyl group, or with anchimeric assistance of the N-1 anion if the 1-position is unsubstituted. The base catalyzed hydrolyses of 1,3-dimethyluracil and 3-methyluracil also appear to proceed through hydrated intermediates. A facile method for an acid catalyzed preparation of 5-deuterated uracils is described as well as a simple and accurate method for analysis of deuterium content.

Electrophilic substitution reactions at the 5-position of uracil and some of its derivatives are of importance because of their many enzymic counterparts (3-7). In particular, we have been interested (8,9) in the mechanistic features of 5-H exchange (7b) and reductive methylation (7a) of 2'-deoxyuridylic acid as catalyzed by thymidylate synthetase. Recently it has been demonstrated (8,10) that the base catalyzed 5-H exchange of certain 1-substituted uracils and uracil nucleosides is facilitated by the intramolecular addition of a neighboring nucleophile of the 1-substituent (viz. oxyanion) to the 6-position of the heterocycle to form cyclic carbanionic intermediates. For example, exchange of the 5-H of 2',3'-O-isopropylideneuridine for deuterium in sodium methoxide-deuteriomethanol is some 500 times faster than 5-H exchange of derivatives which do not possess a sterically disposed ionizable 5'-hydroxyl group, such as 5'-deoxy-2',3'-O-isopropylideneuridine or 1-methyluracil. It has also been demonstrated (8) that the relative rates of the base catalyzed hydroxymethylation of a number of uracil furanosides closely parallel those of 5-H exchange. In view of the apparent mechanistic analogies of these reactions, the observation that uracil (11) but not 1-methyluracil (8) can be readily hydroxymethylated at the 5-position in basic solutions of formaldehyde suggested to us that uracil should also undergo base catalyzed 5-H exchange. The results of our investigations of the base catalyzed exchange of uracil-5-d and its N-methylated derivatives are reported herein as well as a facile, efficient method of preparation of uracil derivatives isotopically labeled at the 5-position.

Prior to investigating isotope exchange reactions it was necessary to ascertain the stability of the uracil derivatives under the experimental extremes to be used. 1,3-Dimethyluracil (DMU) has been reported to lose uv absorption when treated with sodium hydroxide at ambient temperature overnight (12). In response to our need for a semi-quantitative description of the stability of DMU, a cursory

study of its degradation in highly alkaline media (> pH 10.4) at 90.0° was performed. Typical spectra of a degrading solution of DMU at pH 12.0 are shown in Figure 1a; they indicate a complex sequence of reactions involving uv absorbing intermediates which are not readily amenable to analysis. However, upon adjusting aliquots removed from reaction mixtures to pH 7.0 the interfering uv absorption disappeared, leaving only that of the DMU remaining in solution (Figure 1b). The pseudo first-order rate constants obtained by monitoring the amount of DMU present in solution after quenching to pH 7.0 can be expressed by equation 1

$$k_{obs} = k_{OH}(OH^-)^2 \qquad (1)$$

where $k_{OH} = 12.9 \ M^{-2} \ hr^{-1}$ at 90°. It has been well established that the 5,6-double bond of uracils is susceptible to addition reactions (8,10,13), and that the resultant saturated derivatives are susceptible to base catalyzed cleavage of the C-4 amide bond. Pertinent examples are the hydroxide catalyzed cleavages of the C-4 amide bonds of a) 5-fluoro-1-β-D-arabinofuranosyluracil (14) and 1-β-D-arabinofuranosyluracil [$t_{1/2}$ in N sodium hydroxide at 60° is 2.5 hours (15)], which proceed by initial attack of the ionized 2'-hydroxyl of the sugar at the 6-position of the heterocycle, and b) 5,6-dihydrouracil derivatives to give the corresponding β-ureidopropionic acids (16). In consideration of these facts, and the mechanism recently proposed by Sander (16c) for the base catalyzed hydrolysis of 5,6-dihydrouracils, we believe the pathway for the degradation of DMU in strongly basic media proceeds as depicted in Scheme I. It is suggested that at high pH the 5,6-double bond of DMU is in effect saturated by formation of the hydrated intermediate **a**. Hydroxide attack at the 4-carbonyl of **a** would give the tetrahedral intermediate **b** which could undergo further ionization to the dianionic species **c**. In accord with hydrolytic data it would appear that breakdown of **c** to yield products is

rate determining and that cleavage of the C-4 amide bond of intermediate **b** is sufficiently slow that it is not relevant to hydrolysis in the pH range examined. Using the value of $K \simeq 10^{-5}$ (17) and the expression $k_{OH} = k_{OH} K(OH^-)^2$, the rate constant ($k_{OH}$) for the hydrolysis of the hydrated intermediate (**a**) may be calculated to be $1.29 \times 10^5 \ M^{-2} \ hr^{-1}$.

SCHEME I
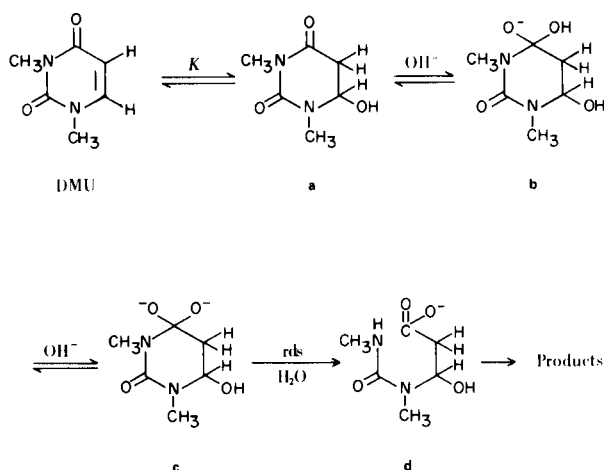
3-Methyluracil (3-MU) was also observed to be unstable towards aqueous base, the decomposition being commensurate with a loss of the pyrimidine chromophore and the stoichiometric generation of acidic products. The apparent first-order rate constants can be expressed by equation 2

$$k_{obs} = k_{OH}(OH^-)^2 \ \frac{a_H}{K_a + a_H} \tag{2}$$

where k_{OH} is the specific rate constant for the hydrolysis of the neutral species of 3-MU (second-order in hydroxide ion, $k_{OH} = 2.1 \times 10^2 \ M^{-2} \ hr^{-1}$), K_a is the dissociation constant for 3-MU at 90° (2.0×10^{-9}), and $a_H/K_a + a_H$ the mole fraction of the unionized form of 3-MU. Although a kinetically equivalent expression can be written for the reaction of the monoanion with hydroxide, it has tentatively been eliminated on the basis of the expected similarities of the hydrolysis of the neutral species to that of DMU. Although the ultimate identity of the products of this reaction are not known at this time, it is likely that the reaction also involves cleavage of the C-4 amide linkage. Further studies on the hydrolytic reactions of DMU and 3-MU are in progress.

The stability of uracil (U) under conditions of high pH and temperature has been verified by a number of workers (18), and under conditions used for the exchange experiments degradation of uracil was not noticeable. Similarly, 1-methyluracil (1-MU) was shown to be completely stable towards the most extreme conditions used in the exchange

studies. In accord with the mechanisms proposed for the hydrolysis of dihydrouracil derivatives (16c) and DMU (Scheme I), it would appear that the high base stability of U and 1-MU is a result of ionization of the 3-NH subsequent to hydration which would preclude hydroxide attack at the 4-carbonyl.

Because of the greater experimental convenience of performing exchange studies in water, it was considered expedient to investigate the loss of deuterium from the substrates rather than its incorporation. It was therefore necessary to obtain uracil derivatives specifically labeled with deuterium in the 5-position. The preparation of uracil-5-d has been reported by Parkanyi and Sorm (19). Their procedure involves treatment of 5-bromouracil with deuterium gas on palladium-barium sulfate and yields 63-69% isotopically pure uracil-5-d. In studies directed at ascertaining the position of the isotopic label of commercially tritiated pyrimidines, Fink (20) observed that tritium is released from uracil-5-³H in hot N sulfuric acid. Based on this result, an investigation of the feasibility of introducing deuterium into the 5-position of uracil under strong acid catalysis was undertaken. When uracil was treated with 6 N deuterium chloride at reflux, the nmr spectra showed an appearance of a singlet (δ 7.48) midway
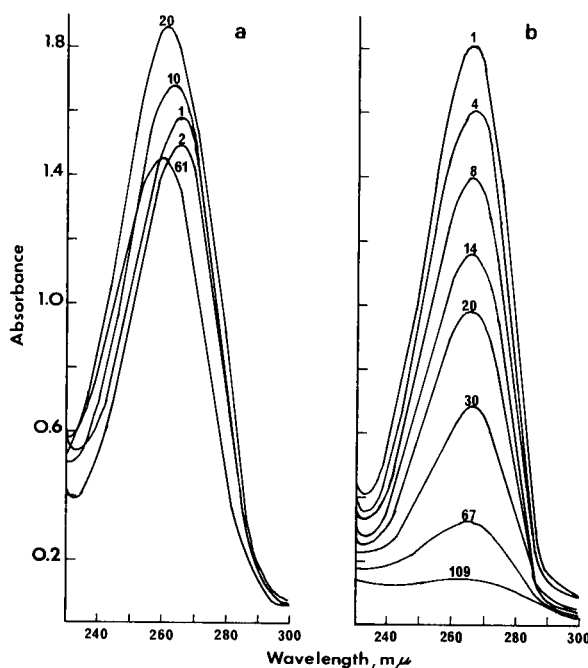

Figure 1. Typical spectral changes of degrading solutions of 1,3-DMU at pH 12.0 and 90°: (a) $2.2 \times 10^{-4} \ M$ solution at pH 12.0, (b) $2.2 \times 10^{-3} \ M$ solution in which removed aliquots were diluted 10-fold with 0.5 M phosphate (pH 7.0). Each curve is labeled as to the number of minutes after initiation of the reaction.
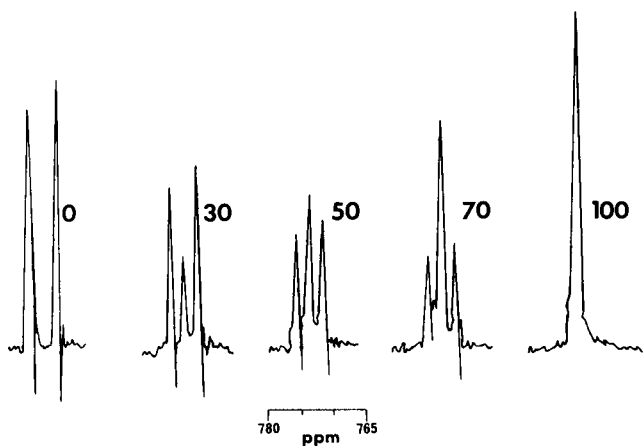
Figure 2. Typical 100 MHz nmr spectra of the 6-proton signal of known mixture of the hydrochloride salts of 1-(4-aminobutyl)uracil-5-*d* and 1-(4-aminobutyl)uracil. The percent deuterated compound in the mixture is indicated to the right of each signal.
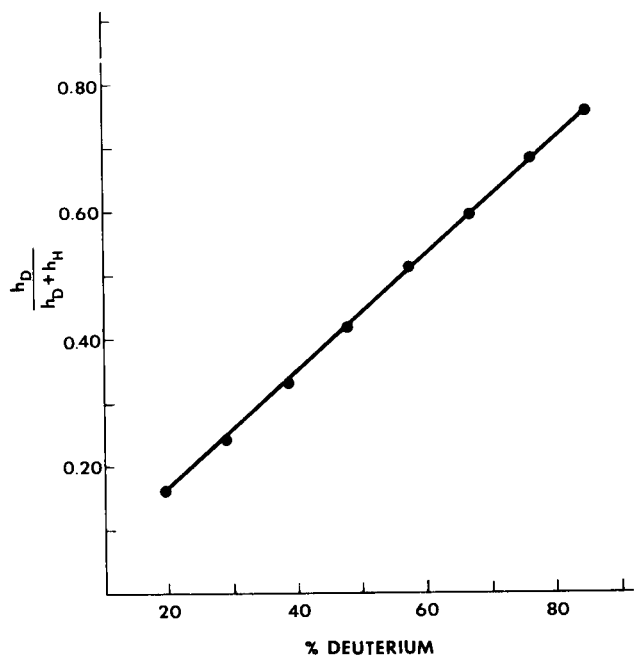

Figure 4. A typical pseudo first-order plot for the dedeuteration of 1-methyluracil-5-*d* at pH 11.1 at 90°.

Figure 3. A typical calibration curve recorded at 100 MHz for a plot of h_D/h_D+h_H versus percent deuterium in the mixture.
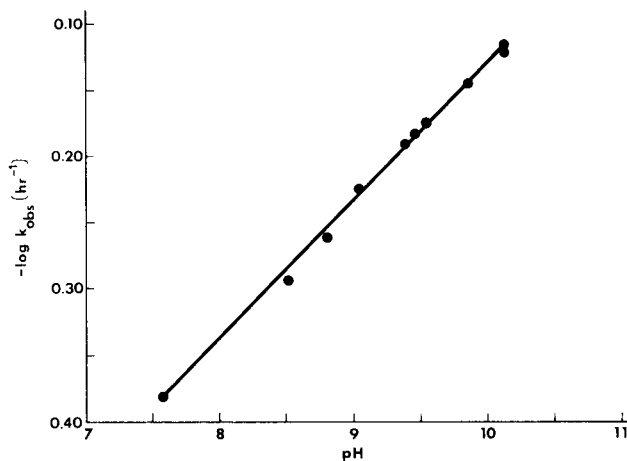
Figure 5. The pH-log k_{obs} profile for the dedeuteration of 1,3-dimethyluracil-5-*d* at 90°.

between the signals of the C-6 proton doublet ($J_{5,6}$ = 6.3 cps), with concomitant decrease of the intensities of the C-5 (δ 5.61) and C-6 doublets. After three hours, only the singlet expected of uracil-5-*d* could be detected. The product was isolated in over 90% yield and shown to be
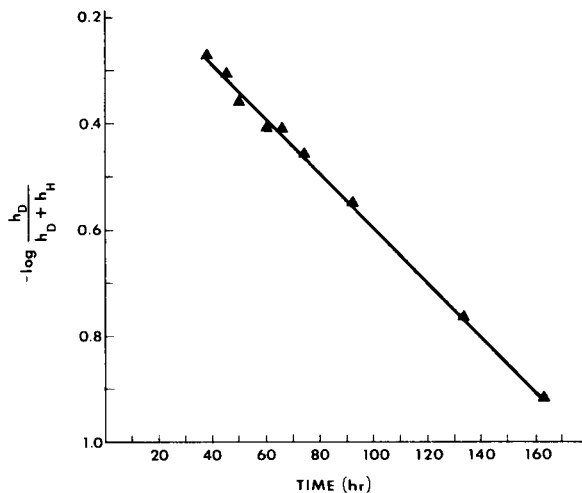
identical to uracil in its chromatographic properties, uv spectra and melting point. Mass spectral analysis showed a parent peak at m/e 113 and isotopic purity of 96.4%. Analogous procedures were utilized for the preparation of 1-methyluracil-5-*d*, 3-methyluracil-5-*d*, 1,3-dimethyluracil-5-*d*, and 1-(4-aminobutyl)uracil-5-*d*. In no instance could we detect exchange of the 6-*H* under the conditions used.

Since we desired to perform multiple nmr analyses of fairly dilute solutions without resorting to tedious isolation of sample or time averaging, it was considered expedient to devise an alternative method for ascertaining the deuterium content of the uracil derivatives under investigation. A number of solutions with varying known concentrations
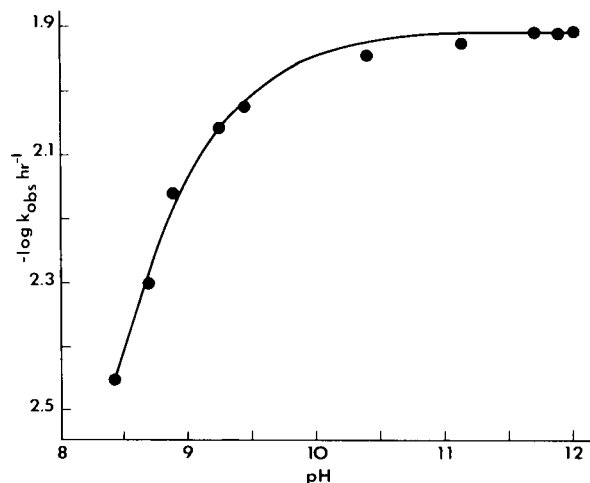
Figure 6. The pH-log k_{obs} profile for the dedeuteration of 1-methyluracil-5-d at 90°. Points are experimental and the curve calculated from equation 5.

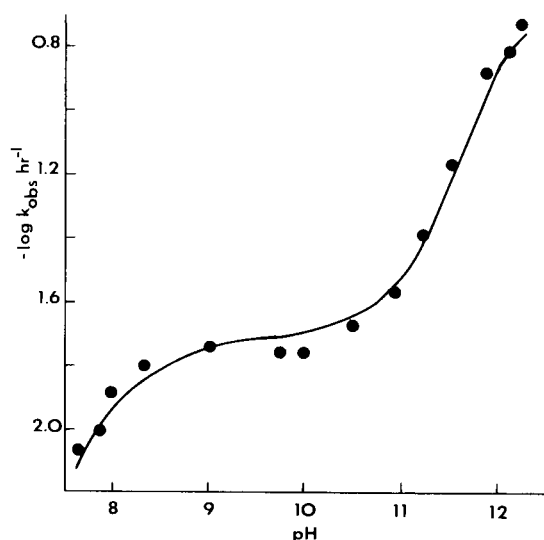Figure 7. The pH-log k_{obs} profile for the dedeuteration of uracil-5-d at 90°. Points are experimental and the curve calculated from equation 7.

of the hydrochloride salts of 1-(4-aminobutyl)uracil and 1-(4-aminobutyl)uracil-5-d were prepared and the nmr spectra of their 6-H determined (Figure 2). A function was sought which would allow us to relate the relative peak heights of the C-6 hydrogens of the deuterated and undeuterated species to the fractional deuterium content at the C-5 position. It was observed that the ratio of the

peak height of the H-6 singlet (h_D) of the deuterated pyrimidine to the sum of the peak heights of the C-6 signals of both species ($h_H + h_D$) was a linear function of the fraction of the deuterated uracil present in the mixture (Figure 3); similarly, the mole-fraction of undeuterated uracil was a linear function of the ratio of the combined heights of the H-6 doublet (h_H) of the undeuterated uracil to combined peak heights of both species. By use of a calibrated plot of this type it was possible to directly determine by nmr as little as 15% (0.015 M) of a uracil-5-d in a 0.1 M solution of substrate without resorting to time averaging or tedious isolation of reaction components. This relationship was also demonstrated to be valid for uracil, 1-methyluracil, and 2',3'-O-isopropylideneuridine under a variety of conditions. The linearity, but not necessarily the slope, of these plots was found to be independent of concentration of the pyrimidine in the range of 0.1 to 0.5 M, the solvent composition (methanol, water, DMSO-water mixtures) and pH. Thus, rate constants for the dedeuteration could be simply determined in situ by plots of log ($h_D/h_H + h_D$) versus time; a typical plot is shown in Figure 4. The error of this method is approximately 5% and inaccuracies encountered are more likely the result of experimental variances.

Below pH 10 at 90°, the degradation of DMU-5-d was sufficiently slow to allow the determination of rates of 5-D exchange. The pH-log k_{obs} profile for the dedeuteration of DMU-5-d at 90° is given in Figure 5. The apparent first-order rate constants for exchange can be described by equation 3

$$k_{obs} = k_{OH}(OH^-) \tag{3}$$

where k_{OH} = 13.4 M^{-1} hr^{-1} at 90°. The absence of general catalysis was demonstrated over a ten-fold range in concentration of carbonate buffer at pH 9.6. A mechanism for 5-D exchange of DMU-5-d which is in accord with this data (Scheme II) involves rate determining nucleophilic attack on the 6-position of the pyrimidine by hydroxide, leading to an intermediate in which water has added across the 5,6-double bond of DMU, as in the initial step of the pathway proposed for the base catalyzed hydrolysis of DMU. Rapid reversal of this process would account for the observed isotope exchange at the 5-position. Ongoing studies (17) on the mechanism of dehydration of 1-methyl-5-hydro-6-hydroxyuracil suggests that the equilibrium depicted in Scheme II lies far in

SCHEME II
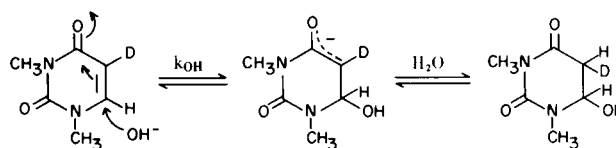
favor of DMU, the rate constant for base catalyzed dehydration of the unsaturated intermediate being some 10^5 times greater than that of its formation.

The pH-log k_{obs} profile for the dedeuteration of 1-methyluracil-5-d at 90° is given in Figure 6. The apparent first-order rate constants (k_{obs}) for 5-D exchange at pH values above 8.4 can be expressed by equation 4

$$k_{obs} = k\, f_{MU^-} = k\, \frac{K_a}{K_a + a_H} \qquad (4)$$

where k is the specific rate constant for the reaction of the monoanion (MU^-) with water, f_{MU^-} is the fraction of anion (MU^-) present, K_a the acid-dissociation constant of 1-methyluracil (1-MU), and a_H the hydrogen ion activity as measured by the glass electrode. At high pH ($a_H \ll K_a$) no significant change in rate is observed and k_{obs} becomes equal to k (0.012 hr^{-1}). By solving for K_a in equation 4, the apparent pK_a of 1-MU is 8.82, in excellent agreement with the measured value at 90° of 8.89. Alternatively, the rate data may be described by the kinetically equivalent hydroxide attack on the neutral species of 1-MU (equation 5)

$$k_{obs} = k_{OH}(OH^-)f_{MU} = \frac{k_{OH}K_w}{K_a + a_H} \qquad (5)$$

where k_{OH} is the second-order rate constant associated with specific base catalyzed exchange, and f_{MU} the fraction of 1-methyluracil-5-d present. An interpretation of these data is given in Scheme III.

SCHEME III

1-Methyluracil-5-H
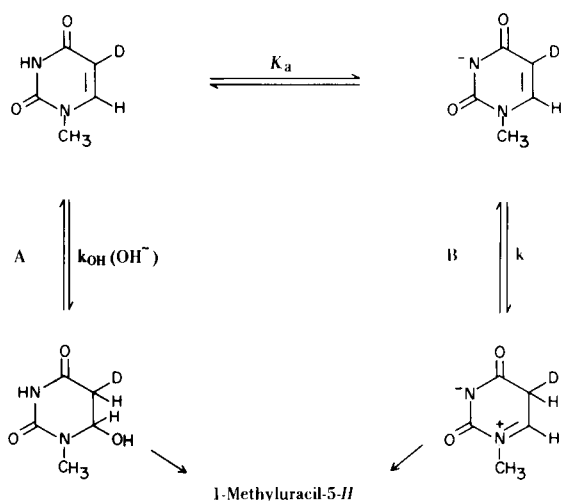
In pathway B, the monoanion (1-MU$^-$) reacts spontaneously with water via anchimeric assistance by the lone pair of electrons of the N-1-nitrogen; the N-3 anion can be envisioned as enhancing the electron density at N-1 and

thus facilitating this reaction. The alternative pathway A involves hydroxide catalyzed addition of water to the 5,6-double bond of the neutral species to form a saturated intermediate via an incipient carbanion at the 5-position of the heterocycle. Although these kinetically equivalent mechanisms cannot be unequivocally distinguished, we tend to favor pathway A at this time. Precedent for this mechanism can be found in the addition of neighboring alkoxide to the 6-carbon in 5-H exchange of certain 1-(β-D-furanosyl)uracils (8,10), the apparent intermolecular nucleophilic catalysis of 5-H exchange of uridine by 2-mercaptoethylamine (21), and the addition of bisulfite to 1-(β-D-furanosyl)uracils (13). Furthermore, the calculated second-order rate constant for pathway A (k_{OH} = 48.6 M^{-1} hr^{-1}) is only about four-fold greater than that obtained in the exchange of DMU-5-d (k_{OH} = 13.4 M^{-1} hr^{-1}), where the mechanism analogous to that depicted in pathway B is of course precluded. This small rate difference might be attributed to electronic and solvation effects resulting from the nature of the N-3 substituent but does not warrant proposal of an alternative mechanism.

Complete studies of 5-D exchange of 3-MU-5-d were prohibited by its instability in strongly basic media, the generation of acidic products resulting in variation of hydroxide ion concentration throughout the course of the reaction. However, within the limited pH range where 3-MU-5-d is sufficiently stable to permit exchange studies, the base catalyzed dedeuteration can be expressed by equation 4 (substituting 3-MU for 1-MU). Utilizing the measured dissociation constant at 90° (K_a = 2.0 x 10^{-9}), the specific rate constant (k) for the reaction of the nomoanion of 3-MU-5-d with water may be calculated to be 5.95 x 10^{-2} hr^{-1}. Alternatively, solution of the kinetically equivalent equation 5, which would represent hydroxide attack on the neutral species of 3-MU in an expectedly similar manner as depicted for DMU (Scheme II) gives k_{OH} = 330 M^{-1} hr^{-1}. Although it is difficult to unequivocally distinguish between these kinetically equivalent mechanisms, the 25-fold rate difference between the calculated second-order rate constants associated with specific base catalysis for dedeuteration of DMU-5-d and 3-MU-5-d suggests that exchange of 3-MU-5-d involves participation of its N-1 monoanion (Scheme IV). Furthermore, precedent has been established for analogous anchimeric assistance of the N-1 anion of pyrimidines in hydrolytic reactions of 5-acetoxymethyluracils (9,22) and 5-trifluoromethyluracils (23).

SCHEME IV
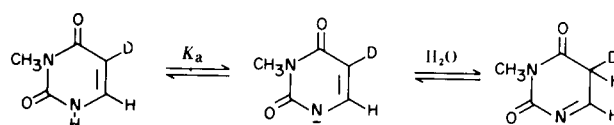
The pH-log k_{obs} profile for 5-D exchange of uracil-5-d at 90° (Figure 7) indicates the involvement of two acidic protons with apparent pK_a values of 7.8 and ca. 12.5. The first pK_a corresponds to formation of the 1- and 3-monoanion, which are present in approximately equal proportions (24), and the latter to the formation of the 1,3-dianion from either of the monoanionic species. The apparent first-order rate constants (k_{obs}) for the exchange of uracil-5-d above pH 7 may be expressed by equation 6

$$k_{obs} = k_{OH}(OH^-) f_{UH_2} + k_1 f_{UH-1}^- + k_2 f_U^= \quad (6)$$

where k_{OH} is the second-order rate constant associated with reaction of hydroxide ion with the mole-fraction of unionized uracil (f_{UH_2}), and k_1 and k_2 are the specific catalytic rate constants for the spontaneous reaction of the mole-fractions of uracil 1-anion (f_{UH-1}^-) and uracil dianion ($f_U^=$), respectively. The fractions of ionic species present in solution can be expressed through material balance, a_H, and the dissociation constants of each species. The resulting expression, which is depicted in Scheme V, is given in equation 7 which can be fitted to the pH-log k_{obs} profile (Figure 6) to give the values: $K_{a_2} = 7.0 \times 10^{-9}$, $K_{a_2} = 8.8 \times 10^{-9}$, $K_{a_3} = 3.0 \times 10^{-13}$, $k_{OH} = 48.3$ M^{-1} hr^{-1}, $k_1 = 0.04$ hr^{-1} and $k_2 = 0.91$ hr^{-1}.

$$k_{obs} = \frac{k_{OH} K_w a_H + k_1 K_{a_1} a_H + k_2 K_{a_1} K_{a_3}}{a_H^2 + a_H(K_{a_1} + K_{a_2}) + K_{a_1} K_{a_3}} \quad (7)$$

It is apparent that each term of equation 6 has one or more kinetic equivalents. However, if the assumption is made that the major effect of the N-methyl groups of DMU-5-d, 3-MU-5-d and 1-MU-5-d upon 5-D exchange is that of precluding ionization, the ionic state of the reacting species of uracil-5-d may be deduced. Thus, if the mechanism of 5-D exchange of 3-MU-5-d involves anchimeric assistance of the 1-anion, it is anticipated that the

N-1 anion of uracil-5-d undergoes exchange by an analogous mechanism. Likewise, since 5-D exchange of DMU-5-d and 1-MU-5-d proceed via hydroxide attack on the 6-position of the neutral heterocycle, a similar pathway for exchange is expected for 5-D exchange of the unionized form of uracil-5-d. On the basis that the monoanion of 1-MU does not appear to be an intermediate in 5-D exchange, it may be concluded that the 3-monoanion of uracil is also unreactive.

At pH over 10.5 the pH-log k_{obs} profile for the dedeuteration of uracil-5-d describes the reaction of the dianion, U$^=$, with water (Scheme V) ($k_{obs} = k_2 f_U^= = 0.91$ hr^{-1}) which is the kinetic equivalent to the reaction of one of the monoanions with hydroxide [$k_{obs} = k(OH^-)f_U^-$]. That the former is likely to be the case is indicated by comparison to the 1- and 3-monomethyluracils. If either of the uracil monoanions could react with hydroxide, then 1-CH$_3$U$^-$ or 3-CH$_3$U$^-$ would also be expected to react by a similar mechanism, albeit at slightly different rates. It is apparent from the rate of saturation effect observed for the dedeuteration of 1-methyluracil-5-d (Figure 6) that there is no reaction of 1-CH$_3$U$^-$ with hydroxide. Although the exchange of 3-methyluracil-5-d is complicated by its instability, even after maximal adjustments for errors, the rates do not approach those observed for U$^=$. In addition, from a chemical standpoint one would not anticipate the 6-position of the N-1 anion of 3-MU to be susceptible to hydroxide attack, because of the adjacent negative charge. The rate enhancement of the dianion is also observed in other models we have examined (22,23) and can be rationalized in terms of the increased electron density of the N-1 anion induced by ionization of N-3, and anchimeric assistance by the former.

EXPERIMENTAL

All nmr spectra were recorded with either Varian HA-100 or Jeolco C-60H spectrometers; chemical shifts are referred to

SCHEME V
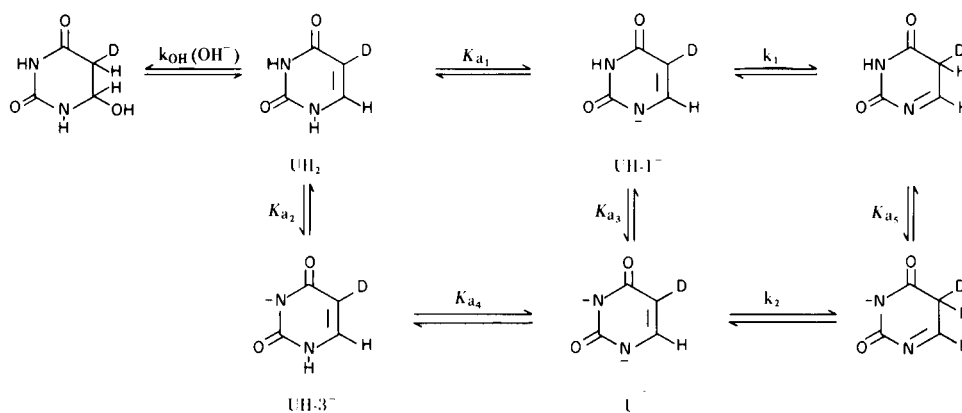
sodium 3-(trimethylsilyl)-1-propane sulfonate. Mass spectra were obtained on a AEI-MS902 spectrometer which was equipped with a Mosley 7101 B linear streak recorder for analysis of deuterium content. All pH measurements for kinetic runs were taken at 90 ± 0.2° with a Radiometer Model 26 pH meter and Metrohm 121H or 125U combined electrodes. Ultraviolet spectra were recorded on a Cary 15 spectrophotometer at ambient temperature. Deuterium chloride (6 N) was prepared by the method of Blomquist (25). All materials used were reagent grade unless otherwise specified. Dissociation constants were determined by pH measurements of half-neutralized solutions of the uracil derivative at $\mu = 1$ (potassium chloride). All melting points were corrected.

Kinetics.

Reactions were conducted at 90 ± 0.2° in boron-free glass (Corning No. 7280) containers or teflon vessels tightly capped with rubber septums. Solutions contained 0.20 M of the deuterated uracil, the required amount of potassium hydroxide, and potassium chloride to give a calculated ionic strength of 1.0. Normally aliquots were removed at appropriate time intervals by means of a syringe, quenched by rapid cooling, and their nmr spectra recorded shortly thereafter. For kinetic solutions containing uracil at pH values lower than 11.0, aliquots were neutralized with glacial acetic acid and cooled to 0°. The precipitated uracil was filtered and redissolved in 0.5 N potassium hydroxide-0.5 N potassium chloride and the nmr spectrum recorded. For exchange studies of DMU-5-d, appropriate buffers were used throughout the pH range studied. In all cases titration and pH measurements demonstrated that base consumption did not occur throughout the period of the run, and thus pseudo first-order kinetics were obtained. For certain preliminary runs, experiments were performed and monitored in teflon nmr tubes (Nmr Specialties, New Kensington, Pa.); appropriate corrections were made for the time taken for recording of spectra. Pseudo first-order rate constants for dedeuteration were determined from plots of log $h_D/h_H + h_D$ versus time (or log $h_H/h_H + h_D$ versus time for deuteration) where h_D is the peak height of the C-6 singlet of the uracil-5-d present in a mixture, and h_H the combined peak heights of the C-6 doublet of the unlabeled uracil.

Stability experiments of the pyrimidines used in this study were conducted at 90° ± 0.2 in boron-free glass ampoules. Solutions of DMU were made to 1.17×10^{-3} M, and contained appropriate amounts of potassium hydroxide and potassium chloride to give the desired base concentration and ionic strength ($\mu = 1$). At appropriate intervals aliquots were removed by syringe, accurately diluted to 1.17×10^{-4} M with phosphate buffer (pH 7.0), and their uv spectra recorded. Solutions of 3-MU (1.00×10^{-4} M) were prepared in a similar manner. Aliquots were removed at appropriate time intervals, quenched by rapid cooling, and their uv spectra recorded. Pseudo first-order rate constants were calculated from plots of log ($OD_\infty - OD_t$) versus time at 267 mμ for DMU and 284 mμ for 3-MU.

1-(4-Phthalimidobutyl)uracil.

A suspension of 4.84 g. (43.2 mmoles) of uracil, 5.97 g. (43.2 mmoles) of finely powdered potassium carbonate, 4.03 g. (14.4 mmoles) of 4-bromobutylphthalimide, and 0.23 g. (1.5 mmoles) of sodium iodide in 60 ml. of dimethylsulfoxide was stirred at 90° for 7 hours protected from moisture. After allowing to cool, the mixture was neutralized with glacial acetic acid and partitioned between 250 ml. of chloroform and 250 ml. of 10% aqueous acetic acid. The chloroform extract was washed with five 100 ml. portions of water, dried over magnesium sulfate and spin-

evaporated to a white solid. Recrystallization from chloroform-ethyl acetate first gave 0.37 g. (0.72 mmoles) of N^1,N^3-bis-(4-phthalimidobutyl)uracil followed by 1.19 g. (26.4%) white crystals, m.p. 184-187°. A small portion was recrystallized from chloroform-ethyl acetate to give the analytical sample, m.p. 187-188°; λ max (pH 2), 266 mμ (ϵ, 1.11 x 10^4), λ min (pH 2), 247 mμ; λ max (pH 7), 266 mμ (ϵ, 1.08 x 10^4), λ min (pH 7), 241 mμ; λ max (pH 12), 264 (ϵ, 8.43 x 10^3), λ min (pH 12), 246 mμ. The compound moved as a single spot in ethyl acetate-ethanol (8:2) on tlc.

Anal. Calcd. for $C_{16}H_{15}N_3O_4$: C, 61.34; H, 4.83; N, 13.41. Found: C, 61.56; H, 4.94; N, 13.26.

1-(4-Aminobutyl)uracil Hydrochloride.

A solution of 1.0 g. (0.32 mmole) of 1-(4-phthalimidobutyl)-uracil in 10 ml. of concentrated hydrochloric acid was refluxed for 12 hours and poured into 20 ml. of ice water. After allowing to stand for 30 minutes the mixture was filtered and concentrated to ca. 5 ml. After filtration of the insoluble phthalic acid, the solution was spin-evaporated to dryness and triturated with ethanol to give 0.53 g. (75.9%) of a white solid, m.p. 216-219°. Recrystallization from ethanol-water gave the analytical sample, m.p. 230-231°; λ max (pH 2), 266 mμ (ϵ, 9.8 x 10^3), λ min (pH 2), 231 mμ; λ max (pH 7), 266 mμ (ϵ, 9.7 x 10^3), λ min (pH 7), 231 mμ; λ max (pH 12), 265 mμ (ϵ, 6.8 x 10^3), λ min (pH 12), 241 mμ.

Anal. Calcd. for $C_8H_{14}ClN_3O_2$: C, 43.74; H, 6.42; N, 19.13. Found: C, 43.54; H, 6.36; N, 18.94.

Uracil-5-d.

A suspension of 1.12 g. (10 mmoles) of uracil (twice lyophilized from deuterium oxide) in 25 ml. of 6 N deuterochloric acid was refluxed for 3.5 hours. After cooling, the product was filtered and washed with water. Recrystallization from water gave 1.05 g. (93%) of white crystals, m.p. 333-335° dec. Mass spectra showed a parent peak at m/e 113 and an isotopic purity of 96.4%. Nmr showed a singlet at δ 7.54, and the absence of doublets centered at δ 5.71 and δ 7.54.

1-Methyluracil-5-d.

This was prepared as described for uracil-5-d. After spin-evaporation of the reaction mixture, the residue was recrystallized from water to give 88.1% of white solid, m.p. 233-234°. Mass spectra showed a parent peak at m/e 127 and isotopic purity of 97.8%.

3-Methyluracil-5-d.

This was prepared as described for uracil-5-d. After spin-evaporation of the reaction mixture the residue was evaporated from ethanol several times, and recrystallized from benzene-dichloromethane to give 90% of a white solid, m.p. 179-180°. Mass spectra showed a parent peak at m/e 127 and isotopic purity of 93.5%.

1,3-Dimethyluracil-5-d.

This was prepared as described for uracil-5-d. After spin-evaporation of the reaction mixture the residue was recrystallized from ethanol-ether to give 92% yield of a white crystalline material, m.p. 123-124°. Mass spectra showed a parent peak at m/e 141 and isotopic purity of 93.6%.

1-(4-Aminobutyl)uracil-5-d Hydrochloride.

This was prepared as described for uracil-5-d. After spin-evaporation of the reaction mixture the residue was recrystallized from ethanol-water to give 83.3% yield of white crystals, m.p.

230-231°. Mass spectra showed a parent peak at m/e 184 and isotopic purity of 95.4%.

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