THE MECHANISM OF BROMINATION OF URACIL, 5-BROMO-URACIL AND THEIR 1,3-DIMETHYL DERIVATIVES

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To my parents

Abstract

Charles Gary Berks

The Mechanism of Bromination of Uracil, 5-Bromouracil and Their 1,3-Dimethyl Derivatives

The kinetics of bromination of uracil, 1,3-dimethyluracil, 5-bromouracil and 5-bromo-1,3-dimethyluracil have been investigated in aqueous acid solution. Overall the reaction is second-order: first-order in substrate and first-order in bromine. Consequently all kinetic runs were performed under pseudo-first-order conditions with at least a ten fold excess of substrate. For both dimethyl derivatives the second-order rate constants are invariant with acidity whereas for uracil and 5-bromouracil they increase with decreasing acidity. These observations suggest that at lower acidities reaction with uracil and 5-bromouracil proceeds via electrophilic attack by bromine upon their anions resulting from deprotonation at N_1. An overall mechanism of bromination of uracil derivatives based on these and earlier results is presented.

Acknowledgements

I wish to express my sincere gratitude to Dr. O. S. Tee for his guidance and assistance throughout the course of this work. His dedication will always be appreciated. Thanks are also due to those faculty members who served on my advisory committee.

The cooperation of Mr. W. Fitch in the preparation of the drawings is greatly valued.

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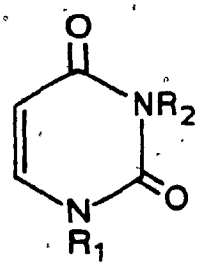
INTRODUCTION

General Introduction

The object of the work in this thesis was to study the rates of bromination of uracil, 5-bromouracil and their N,N-dimethyl derivatives in aqueous acid with a view to determining the mechanism(s) of these reactions.

Uracil (1a) and its 5-methyl derivative, thymine (21), are naturally occuring compounds of great importance owing to their presence in nucleic acids.[1] Thymine is the base component of the nucleoside thymidine found in DNA while uridine, the nucleoside containing uracil, is present in RNA. Both uracil and thymine are hydrogen-bonded with adenine (33) in nucleic acids.



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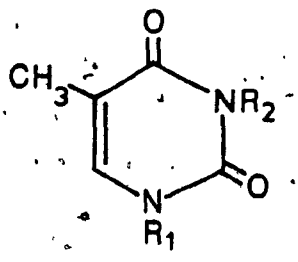
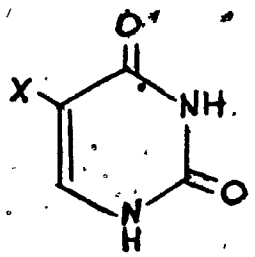


21

a) $R_1 = R_2 = H$
b) $R_1 = R_2 = CH_3$
c) $R_1 = CH_3$; $R_2 = H$
d) $R_1 = H$; $R_2 = CH_3$

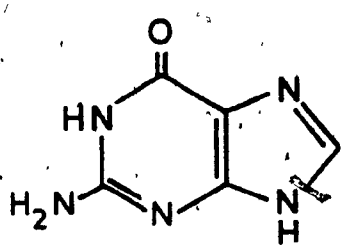
Much work has been devoted to studying the biological activity and clinical uses of 5-halouracils. 5-Bromo- (6a), 5-chloro- and 5-iodouracils (29) are usually considered as analogues of thymine since the van der Waals radii of the 5-halo substituents are about the same size as that of the 5-methyl group in thymine. For similar reasons, 5-fluorouracil (29, X=F) is regarded as an analogue of uracil since the flourine and hydrogen at the 5-position of these compounds are of roughly the same size.



29

X = F, Cl, or I

5-Bromouracil has been found to replace a considerable part of the thymine normally found in bacterial DNA. The incorporation of 6a in DNA increases the probability of incorrect base-pairing. That is, 5-bromo-



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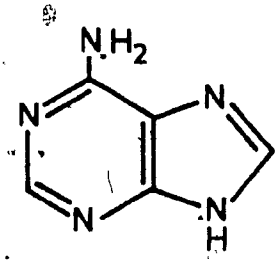

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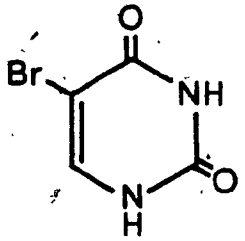
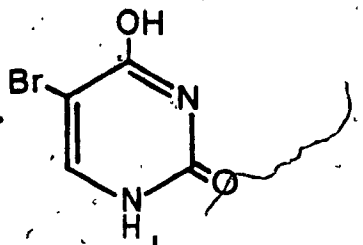


6a



6a'

uracil has a greater tendency to incorrectly pair with guanine (32) instead of adenine (33) than thymine has. This mutagenic activity of 6a could be a result of its hydroxy tautomeric form 6a' or perhaps due to the fact that 6a is appreciably ionized at neutral pH.[2]

5-Fluorouracil can be incorporated into RNA in place of uracil but can not be introduced into DNA. Since uracil can be used by mammalian tissues for nucleic acid synthesis, 5-fluorouracil is a very effective drug in mammals. Its main effect is inhibiting the methylation of uracil in thymine biosynthesis, resulting in a large reduction of DNA replication.[1]

In spite of the biological importance of uracil and

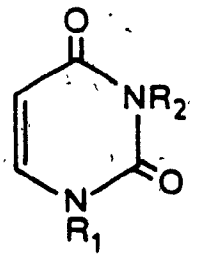
5-halouracils, it has only been recently that mechanistic studies of their reactions with electrophiles have been made. This is especially surprising since electrophilic substitution reactions at the 5-position of uracils have enzymic counterparts.[43] It is hoped that the results of the present work will be of aid in eventually determining the mechanisms of these complex reactions.

Structures of Uracils

Uracil (2,4(1H,3H)-pyrimidinedione) can, in principle, exist in a variety of tautomeric forms. However, various pieces of evidence suggest that it exists primarily in the diketo form 1a in solution, as well as the solid state.

The u.v. spectra of uracil, 1-methyluracil (1c), and 3-methyluracil (1d) in aqueous solution resemble that of 1,3-dimethyluracil (1b) which, necessarily, has a diketo structure.[3] Similar results have been obtained by the comparison of the double bond stretching region in the i.r. spectra (neat and in dioxane) for the compounds.[4] X-ray crystallographic studies, first performed by Parry and later repeated by Stuart and Jensen[6], support structure 1a. Less reliably, molecular orbital calculations also favour the diketo structure for uracil.[7]

Evidence for tautomerization of uracil comes from fluoresence emission spectroscopy where structure 1a' has been postulated as the fluorescing species. The above structures are presented on the next page.



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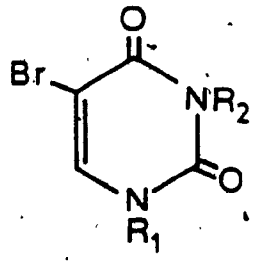

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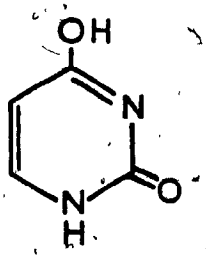
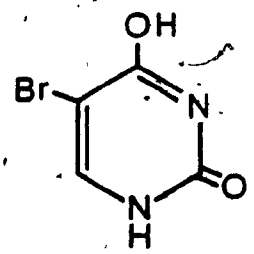


1a'

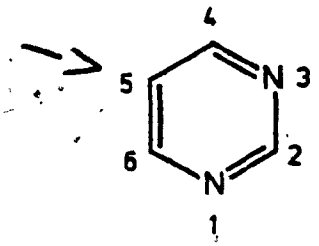


6a'

5-Bromouracil (6a) has a greater inclination towards tautomerization than does uracil.[9] N.m.r. studies indicate that the N_1 and N_3 protons of 5-bromouracil are in regions of lower electron density than they are in uracil and are therefore more acidic.[10] This increase in acidity is supported by pK_a measurements with the pK_a of 5-bromouracil being about 8.0 as compared to 9.5 for uracil.[11] As previously mentioned 6a can be incorporated into DNA in place of thymine. Due to the increased stability of its enol-form 6a' it is possible that it may pair with guanine instead of with adenine as thymine normally would.[12]

Electrophilic Substitution Reactions of Pyrimidines

The ring nitrogens of pyrimidine (30) can be considered as electron-withdrawing groups, each with an effect similar to that of a nitro substituent on a benzene ring,[13] which decrease the pi-electron density on the ring carbon atoms. Electrophilic substitution occurs mainly at the 5-position since it is the least electron deficient position[11a] in the ground state and in any transition state for electrophilic attack.



30

Bromination of pyrimidine hydrochloride is the only successful electrophilic substitution of pyrimidine reported[14] and requires relatively severe conditions. In all other cases at least one powerful electron-donating group such as hydroxyl or amino must be present

for the reaction to proceed.[11a]

Nitration of pyrimidines was erroneously believed to require at least two ring-activating substituents in order to occur. However, in the past decade or so, pyrimidines containing only one electron-donating group have been nitrated under vigourous conditions with varying degrees of success.[11b]

The nitration of uracil is usually performed in a nitric/sulphuric acid mixture but has been carried out as well in boiling fuming nitric acid.[15] N-alkyl derivatives of uracil such as 1- and 3-methyluracil may be nitrated in good yield in sulphuric acid at 40-50°C.[11a] 6-Methyluracil may be nitrated in a nitric/acetic acid mixture at 15-20°.[16]

Johnson et al[17] studied the rates of nitration of uracil and its 6-methyl- and 1,3-dimethyl derivatives in sulphuric acid and concluded that the uracil reacts as the free base diketo form (1a) rather than as some other tautomeric form or as the conjugate acid.

Nitrosation necessitates the presence of very strongly activating groups.[18] When three electron-releasing substituents are present reaction of the pyrimidine in aqueous acetic acid with sodium nitrite at room temperature results in a good yield of the 5-nitroso derivative.[11a] Some pyrimidines with only two electron-releasing groups can be nitrosated with mineral acid/sodium nitrite.[11a]

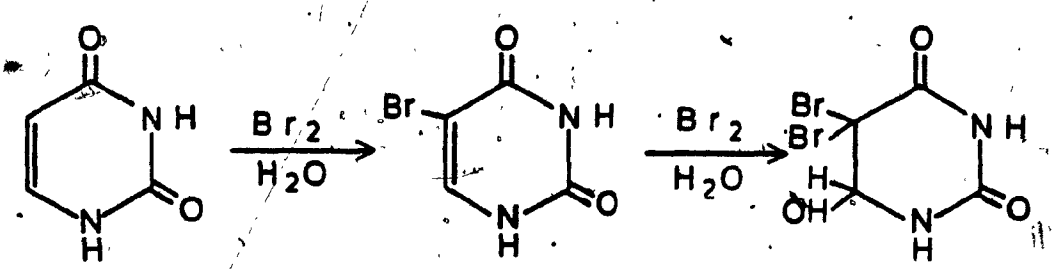
Deuterium exchange studies of uracils have been made in acidic and basic media.[19,20] The 5-deuteration of 1,3-dimethyluracil in acidic deuterium oxide probably involves direct electrophilic attack by D_3O^+ at C_5, followed by deprotonation.[19] Under basic conditions an addition-elimination scheme has been suggested[19] in which there is initial nucleophilic attack by OD^- at C_6 followed by capture of D^+, loss of H^+, and finally loss of OD^-.

The rates of loss of deuterium from 5-deuteriouracils in basic aqueous media have been measured.[20] For N_1 methylated uracils the addition-elimination mechanism was favoured, however for uracils lacking a methyl group at N_1 a scheme involving the electrophilic attack upon the anion resulting from deprotonation at N_1 was proposed.

The deuteration of pyrimidine nucleosides in basic media has been studied by Rabi and Fox, and by others.[21] These N_1 substituted derivatives all seem to undergo deuteration via an addition-elimination mechanism.

The bromination or chlorination of simple pyrimidines in aqueous solution gives the 5-halogeno compounds as the final product.[11a] The reaction proceeds easily if one or more electron-donating groups are present. Uracil and cytosine react with excess halogen in hydroxylic solvents to produce isolatable 5,5-dihalo

addition compounds.[22-25] For example

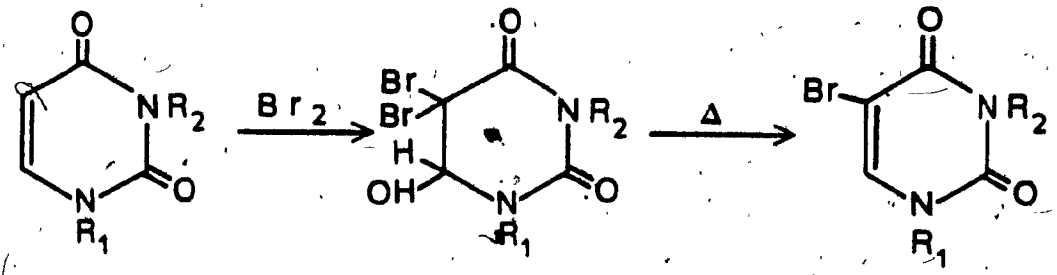


1a 6a 8a

Bromination of Uracils

Early studies concerning the bromination of uracils concluded that 8 was the final product formed.[25-29] In warm aqueous solution 8 decomposes into 6 and HOBr which behaves as an oxidizing agent.[25-28] Heating results in the destruction of HOBr and increases the rate of decomposition of 8. These structures are shown on the next page.

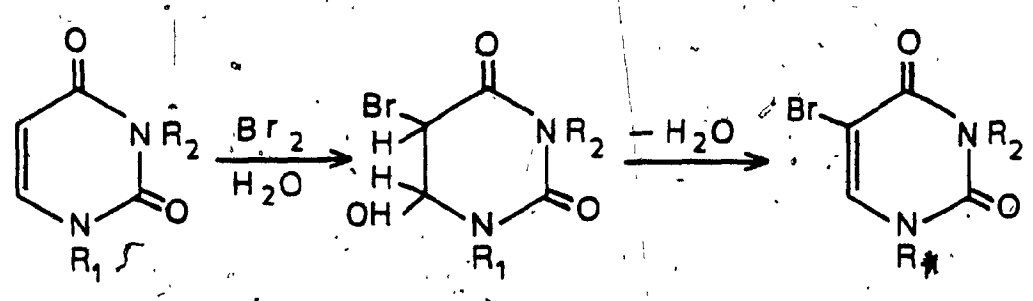
Wang postulated that the addition of 1 mole-equivalent of bromine to uracil (1a) or 1,3-dimethyl-uracil (1b) in aqueous solution results in the formation



1 8 6

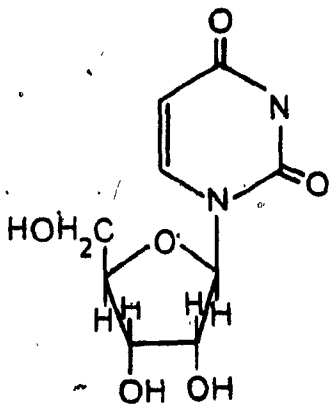
of 3 which undergoes spontaneous dehydration to 5-bromouracil (6). In the presence of excess bromine, 5-bromouracil may be further brominated to the 5,5-dibromo-6-hydroxy adduct 8. Wang concluded that the rate of dehydration of 3 was affected by the bulkiness of the substituent at the N_1 position.[22] These structures are presented overleaf.

Moore and Anderson[23] discounted the formation of 3 during the bromination of uracil. They performed spectroscopic and potentiometric titrations in acetate buffer of pH 4.7 and found that uridine (1e, see next page) and 1,3-dimethyluracil (1b) required 1 mole-equivalent of bromine for complete reaction while uracil



1 3 6

(1a) needed 2 mole-equivalents. They also noted what they believed to be the appearance of 5-bromouracil (6a) during the spectroscopic titration of uracil. They believed that the bromination of uracil initially produces 6a which then reacts with excess bromine to give 8a. They considered that the dehydration of adduct 3a at pH 4.7 would be too slow a step to account for the relatively quick appearance of 6a which was observed.

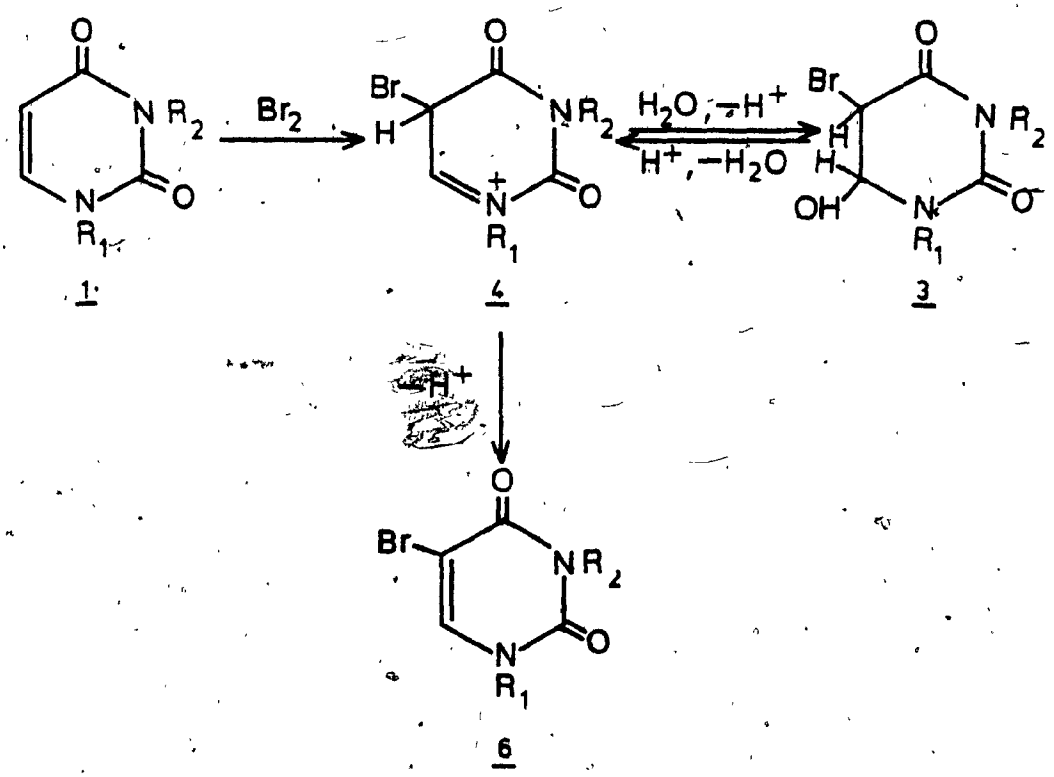


1e

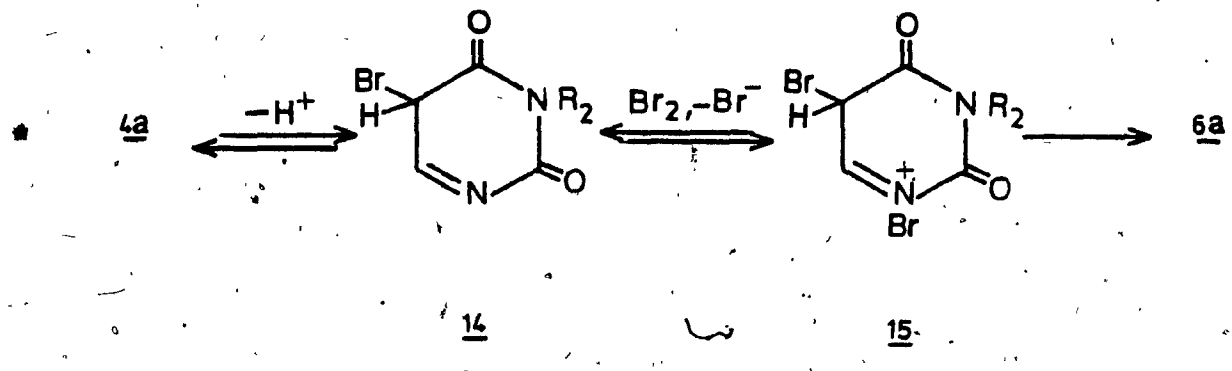
Banerjee and Tee[30-32] have studied the bromination of uracil and its N-methyl derivatives in aqueous sulphuric acid solutions. They performed titration experiments which confirmed that at low acidity N_1 unsubstituted uracils undergo a 1:2 reaction with bromine, but that N_1 substituted uracils undergo a 1:1 reaction. However at high acidity the reaction was found to be 1:1 for all uracils.

The hydrated adducts 3 proposed by Wang were characterized by p.m.r. spectroscopy, and showed two well resolved doublets in the region 4.30-5.40 for the 5- and 6-H. The formation of the adducts 3 was found to be too fast to be followed by the conventional techniques. However, it was possible to study the conversion of 3 to 6. This dehydration is acid catalyzed with the rate increasing with increasing acidity. The process is fairly slow having a $t_{1/2}$ of 1.96 hours for uracil in 4.0N sulphuric acid. The observed isotope effects for the 5-deuterio derivatives of uracil (1a) and 1,3-dimethyluracil (1b), k_H/k_D=4.3 and 3.4 for 1a and 1b respectively, indicated that the rupture of the C_5-H bond of the intermediate cation 4 with, presumably, water acting as the base is rate-determining (structures overleaf).[30,31]

To try and explain the rapid formation of 5-bromouracils from N_1 unsubstituted uracils in solutions of low acidity it was suggested that 4, for R_1=H, existed

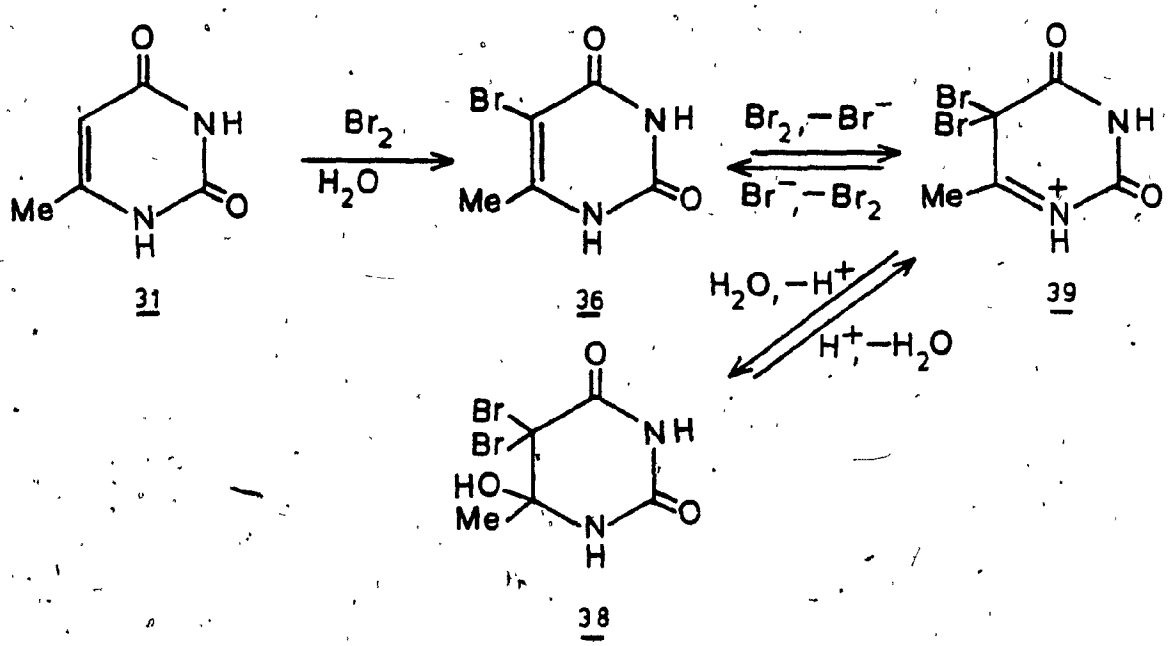


in equilibrium with 14. The conversion of 14 to 6 was postulated[30,31] to proceed via the formation of the N-bromo derivative 15, which should undergo rapid deprotonation at C_5, followed by aromatization to 6a.

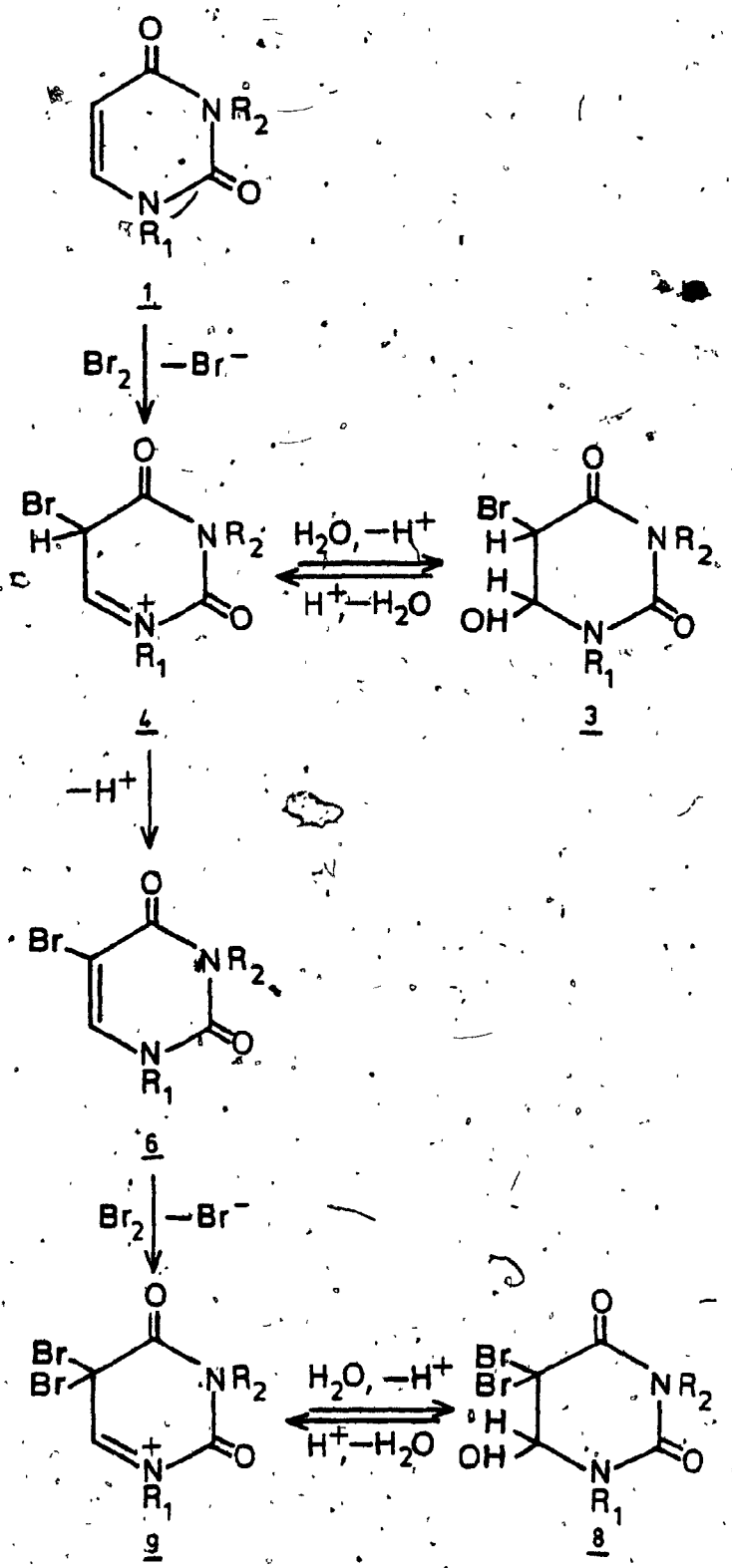


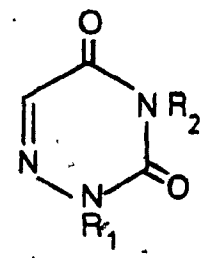
The overall mechanism proposed by Banerjee and Tee for the bromination of uracils is presented on the following page.

The kinetics of the debromination of the 5,5-dibromo-6-hydroxy adduct (38) formed by the reaction of 6-methyluracil (31) with two mole-equivalents of bromine in aqueous solution has also been studied by Banerjee and Tee.[33] In the presence of 6-methyluracil (31) the rate of reaction was found to be dependent upon the acid concentration and upon the concentration of bromide ion. It was postulated that the cation 39 in equilibrium with the substrate species 38, is transformed to 5-bromo-6-methyluracil (36) by the action of bromide ion. The debromination produces free bromine which then reacts with the 6-methyluracil to give a second equivalent of 5-bromo-6-methyluracil (36).



Scheme 1





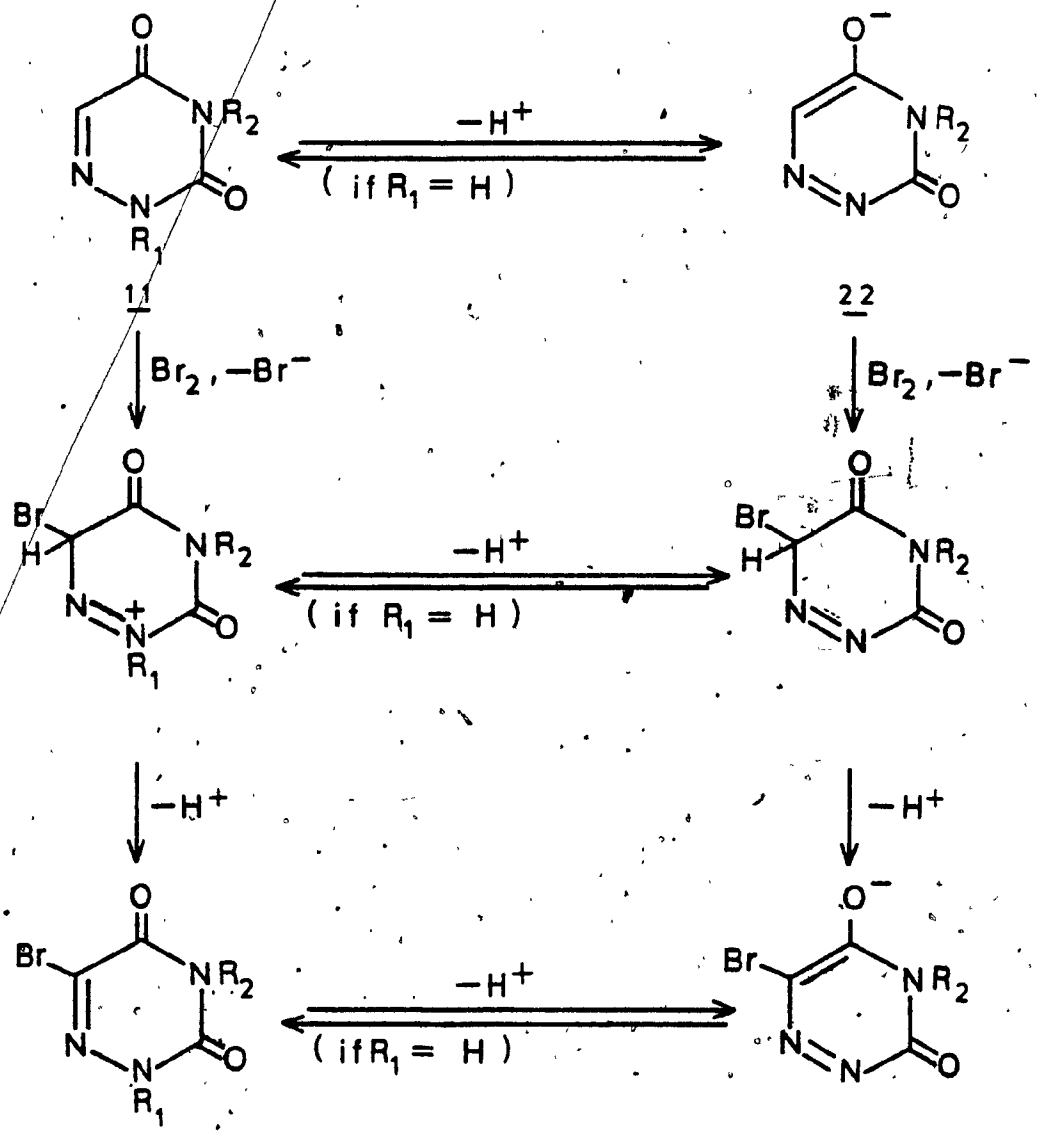
11

a) $R_1 = R_2 = H$
b) $R_1 = R_2 = CH_3$
c) $R_1 = H$; $R_2 = CH_3$

The same authors also made a study of the rate of bromination of 6-azauracils 11a, 11b, and 11c in dilute aqueous acid.[34] The rate of disappearance of bromine was found to vary inversely as the hydronium ion concentration when $R_1 = H$, but the reaction hardly proceeded at all for the dimethyl derivative, 11b, under the same conditions. The observations just noted were taken to indicate that 11a and 11c react with bromine via their anions (22) formed by deprotonation at N_1. The proposed mechanism is presented on the following page.

It was suggested[34] that uracil itself might react via a similar mechanism. The object of the work reported in this thesis was to apply stopped flow techniques to the bromination of uracils to determine whether anion formation is involved in the reaction scheme.

Scheme 2



Stopped Flow Method

This method can be used to study reactions with half-lives of a few milliseconds up to several minutes. The technique involves the rapid mixing of two reactant solutions which are forced through an observation cell. After a few milliseconds the flow of the mixed solution through the cell is suddenly stopped, and the progress of the reaction is followed by making observations of a suitable property of the solution by a sufficiently rapid method. The technique was first used for reaction times of about ten seconds[35] but was later improved for reactions of a few milliseconds duration.[36]

It is imperative that the reactant solutions be thoroughly mixed in a time which is much shorter than the $t_{1/2}$ of the reaction to be followed. The observation point is usually located some millimeters from the mixing chamber to ensure complete mixing. However, it must not be too far away since the time between mixing and observation must be minimized. The flow must be stopped very suddenly because a gradual decrease in the flow rate could result in incomplete mixing.[37] Also, the more rapidly the flow can be stopped, the faster the reactions that can be followed.

The progress of the reaction may be observed by a number of suitable methods. The Gibson apparatus,[38] the prototype of most of the contemporary stopped flow

machines, originally employed a spectrophotometric observation unit. Other modes of observation include e.m.f. measurements with a glass electrode[39] and conductivity measurements.[40,41]

The big advantage of the stopped flow technique over the continuous flow method is that very little solution is required which is most important when reactant solutions are difficult to make or very expensive.[42] The method allows one to obtain a permanent record of the progress of a reaction commencing a few milliseconds after mixing and extending as long as desired. Unlike the continuous flow method the results obtained are independent of the rate and character of the flow down the observation tube and are free from the distorting effect of mechanical disturbances.[42]

In summary, the stopped flow technique, with a suitable observation system, allows one to follow reactions with half-lives as short as a few milliseconds. For the purposes of this thesis the technique was used to study the fast initial stages involved in the bromination of some uracil derivatives.

EXPERIMENTAL

Materials

The uracil and 1,3-dimethyluracil used in this study were obtained from commercial sources (Aldrich and I.C.N. pharmaceuticals, respectively). The 5-bromo-uracil (previously prepared by Dr. S. Banerjee[30]) and 5-bromo-1,3-dimethyluracil were made by known literature methods.[22] All compounds except 1,3-dimethyluracil were recrystallized twice from water prior to use.

All inorganic reagents wre of analytical grade. Sulphuric acid solutions were prepared from commercial (Anachemia or Fisher) standard volumetric concentrates. Buffer solutions of 0.01M ionic strength were made after Perrin.[44]

Bromine solutions were prepared by either one of two methods depending upon the acidity of the reaction medium. Up to pH 2 bromine was generated by the reaction of bromate with bromide[45] (all reactant solutions were 0.1M in KBr for reasons dealt with elsewhere). The stoichiometric equation for the process is

$$BrO_3^- + 5Br^- + 6H^+ \rightarrow 3Br_2 + 3H_2O \qquad (1)$$

The advantage of this method is that one need not worry about the loss of bromine due to volatilization while transferring aliquots. Furthermore the salts used are very pure and can be easily handled and accurately weighed.[46]

At higher pH the bromate-bromide reaction proceeds too slowly to be useful for our purposes, and aqueous

bromine solutions were prepared by the dilution of appropriate amounts of a more concentrated solution. The latter was prepared by the addition of a drop of bromine to a weighed 25 mL volume of the reaction medium. Reweighing the solution and assuming the volume change to be negligible permitted the calculation of the bromine concentration.

Apparatus

All non-kinetic u.v. measurements were performed on an Aminco DW-2 U.V.-Visible Spectrophotometer operating in the split-beam mode. In this mode a single monochromator is utilized for the scanning and the monochromatic beam is chopped and alternately passed through the reference and sample cuvettes. The emergent beams are then compared by the detector system. For 5-bromo-1,3-dimethyluracil the rate of bromination is sufficiently slow that kinetic measurements were carried out using conventional u.v. techniques.

All other kinetic runs were made using the Aminco-Morrow Stopped Flow accessory[47] to the DW-2 with the optical unit functioning in the dual-wavelength mode. Under such conditions one monochromator was set at a reference wavelength where little or no change occurs during the reaction and the other monochromator at a convenient u.v. wavelength where there is a large change in absorbance. The reference and sample beams are alternately passed through the stopped flow

observation cell. The DW-2 was balanced with mixed reactants in the observation cell. Balancing equalizes the radiation intensities from both monochromators causing the two electrical signals from the photomultiplier to be equal even though two different wavelengths are being used. This procedure effectively removes the absorbance (at the monitoring wavelengths) due to the unreacted excess of substrate.

The major advantages of the dual-wavelength mode are: (a) light intensity fluctuations are minimized since both monochromators are illuminated from the same light source;[48] (b) the method minimizes artifacts due to scattering of light in turbid solutions because the reference and sample beams pass through the same observation cell;[48] (c) in double-beam design a difference signal is observed (rather than comparing the transmission ratio of two absorption cells seen at the same wavelength) compensating for the fluctuations in source intensity, the detector response and amplifier gain.[49,50]

When using the stopped flow attachment one drive syringe was filled with 2 mL of a solution of substrate in the desired reaction medium, and the other with 2 mL of bromine solution in the same medium. A driving pressure of 56 p.s.i. of nitrogen was used to drive both syringes simultaneously, and to force the reactant solutions into a dual path length flow

cell having a 10 mm light path along the line of flow. With this driving pressure the dead time (the time required for the reaction mixture to flow from the initial point of contact to the point of observation) is about 4 msec. The volume under observation is 0.04 mL.[47] The stopping block is a micrometer with an oscilloscope trigger switch mounted in its tip.

Acidity

The acidities of 2N and 1N sulphuric acid solutions were taken as H_0 values interpolated from the data of Johnson et al.[51] For all other solutions the acidity was determined using a Corning Digital 110 pH Meter.

Below pH 2 reactions were performed in aqueous sulphuric acid solutions. For higher pH's, buffers of constant ionic strength (0.01M) were made up following the directions of Perrin.[44] Since all solutions were 0.1M in KBr, the total ionic strength of the reactant solutions was 0.11M. Chloroacetate, acetate and succinate buffers were used in the pH intervals 2.20-3.40, 4.10-5.10, and 5.30-6.00, respectively.

Kinetic Procedure

All kinetic runs were performed at 30.00 ± 0.05°C. Temperature control was maintained by a Lauda (model no. RC-20B) constant-temperature circulating bath. The bath was allowed to stabilize and all reactant

solutions were equilibrated prior to the start of kinetic experiments.

Reactant solutions were 0.1M in KBr. The addition of this large concentration of bromide ion has several worthwhile benefits: (a) it swamps the effect Br^- produced by the bromination reaction; (b) it enhances the stability of the bromine solution since most of the bromine is present as Br_3^-; (c) it reduces the rate by reducing the free bromine concentration; (d) it facilitates measurements of rates since Br_3^- has a larger extinction coefficient than does Br_2; (e) it ensures that the ionic strength is high and constant (at 0.11M) for all the experiments in buffer solutions.

The rates of bromine disappearance were measured by monitoring the decrease in Br_3^- absorbance (λ_{max} = 266nm; log ϵ = 4.54[61]). The sample monochromator was set between 280 and 320 nm (depending upon where the best signal to noise ratio was obtained), with the reference monochromator at 350 nm.

In experiments with uracil and 1,3-dimethyluracil the DW-2 detector high voltage output (2 volts per absorbance unit) was connected to a Hewlett Packard Storage Oscilloscope (model no. 141A). The scope was triggered by the stop piston just prior (3 to 5 msec) to its stopping. If this stored trace appeared acceptable it was photographed with a Hewlett Packard Polaroid Camera (model no. 197A).

Before performing kinetic experiments the stopped flow system was flushed out 4 times with distilled water to ensure that no air bubbles were present in the flow cell or connecting tubes. Trapped air in the system may result in excessive noise in the DW-2 output signal. With the stopped flow accessory properly positioned in the DW-2, the total noise envelope of the output signal should be less than 1% transmittance.[52] One driving syringe was then filled with the bromine solution and the other with the desired substrate solution. The system was washed out three or four times to be sure that all the distilled water had been flushed out. The experiments were done using a response time of 5 msec[53] (denoted as FAST response). It is advised to choose the response time which produces the best signal to noise ratio. The optical chopper was operated at a rotating speed of 1000 Hz (one sample and one reference beam hit the mixing chamber every millisecond).[53]

The reactions of bromine with 5-bromouracil were sufficiently slow (except for the fastest run) to allow the detector output to be displayed on the DW-2 recorder. The recorder has a time constant of 300 msec,[53] too slow to successfully monitor fairly rapid reactions. A 150 msec[53] (MEDIUM) or FAST response was

used and the chopper was operated at a speed of 250 Hz (reference and sample beams are sampled every 4 msec[53]). For the experiments with 5-bromo-1,3-dimethyluracil the same response times and chopper speed were used. These reactions were slow enough to permit the mixing of the solutions in a 1 cm cuvette to be by hand, and the monitoring of the absorbance change using the split-beam mode.

Since all the reactions appeared to be, and were proven to be, second-order, all kinetic runs were carried out under pseudo-first-order conditions. Generally, substrate concentrations equalled 5×10^{-4}M, and the bromine concentration equalled 5×10^{-5}M after mixing (10^{-3}M and 10^{-4}M, respectively prior to 1:1 mixing in the stopped flow cell).

Normally 10 or more absorbance values were taken over at least 2 $t_{1/2}$'s from each decay curve recorded. Pseudo-first-order rate constants (k_1^{obsd}) were calculated using "normal", Swinbourne or Guggenheim treatments of the data.

"Normal" Treatment of Data[54]

For a first-order reaction where the Beer-Lambert Law is obeyed

$$(A - A_\infty) = (A_0 - A_\infty)e^{-k_1 t} \qquad (2)$$

and

$$\ln(A - A_\infty) = \ln(A_0 - A_\infty) - k_1 t \qquad (3)$$

where A_0 is the initial absorbance at t=0, A_∞ is the

final absorbance when the reaction is complete, A is the absorbance at time t and k_1 is the first-order rate constant.

A plot of $\ln(A - A_\infty)$ against time should give a straight line of slope $-k_1$. Only those data which in least squares analysis gave correlation coefficients ≥ 0.9995 were deemed acceptable. Low correlation coefficients are often obtained for data arising from excessively noisy decay curves. A small signal to noise ratio is an indication of either air being trapped in the stopped flow system, inefficient mixing, or the use of an improper response time.

The validity of such an analysis requires a correct assessment of A_∞. Small variations in A_∞ can cause relatively large differences in k_1 while hardly affecting the least squares correlation. For example, Collins[55] found that an error of one part in A_∞ can be enhanced up to fourteen times in the rate constant.

All runs done using the DW-2 recorder were observed for more than 10 $t_{1/2}$'s (>99.9% reaction) in order to accurately estimate A_∞. When the detector output was recorded on the storage oscilloscope a baseline was obtained by scanning a previously reacted solution in the observation cell immediately before initiating the reaction. Thus each picture contained a baseline and a decay curve. Errors in A_∞ can be attributed to drift in the DW-2, drift in the scope, or the onset of a

second step in the overall reaction. In such instances a Swinbourne treatment was performed to determine A_∞.

Swinbourne Treatment of Data[56]

For a first-order reaction if A_1, A_2, ... A_n are the absorbance readings at times t_1, t_2, ... t_n and A_1', A_2', ... A_n' are a second series of readings at times t_1+T, t_2+T, ... t_n+T, where T is a constant, then

$$(A' - A) = (A_0 - A_\infty)e^{-k_1(t+T)} \qquad (4)$$

Dividing equation (2) by equation (4) gives

$$\frac{(A - A_\infty)}{(A' - A_\infty)} = e^{k_1 T} \qquad (5)$$

Therefore $A = A_\infty(1 - e^{k_1 T}) + A'e^{k_1 T}$ (6)

Thus a plot of A versus A' should give a straight line of slope $e^{k_1 T}$ and intercept $A_\infty(1 - e^{k_1 T})$.

Therefore $A_\infty = (\text{intercept})/(1 - \text{slope})$ (7)

For best results the data should span a period of time greater than 1 $t_{1/2}$ and preferably greater than 2. The value of T should be between .5 and 1 $t_{1/2}$.

Guggenheim Treatment of Data[54]

This method may be used for a first-order reaction where A_∞ is not measurable. Subtracting equation (2) from equation (4) gives

$$(A' - A) = (A_0 - A_\infty)e^{-k_1 t}(e^{-k_1 T} - 1) \qquad (8)$$

and hence

$$\ln(A' - A) = \ln((A_0 - A_\infty)(e^{-k_1 T} - 1)) - k_1 t \qquad (9)$$

$$= \text{constant} - k_1 t$$

A plot of $\ln(A' - A)$ versus t should give a straight line of slope $-k_1$. For good results the data should be spread over 2 $t_{1/2}$'s and T should be between .5 and 1 $t_{1/2}$.

The values of k_1^{obsd} cited in the Results and Discussion sections of this thesis were all obtained using the "normal" method. Usually A_∞ was determined from the Swinbourne treatment rather than by direct measurement. The value of the rate constant thus obtained was then compared to that determined by the Guggenheim method for the same data. The rate constants were found to agree to within 5% in all cases.

Computer Programs

All computer calculations were performed on a CDC CYBER 172 Digital Computer using the following programs written in BASIC by Dr. O.S. Tee and by the author.

ALLKIN: This program is designed to calculate k_1 from absorbance data by the Guggenheim, Swinbourne and/or "normal" methods. It will carry out the appropriate least squares analysis utilizing equations (3), (6) or (8) given earlier. The program is set up to accept data in which there is a constant time interval between successive points. It should be noted that Swinbourne and usually Guggenheim treatments require

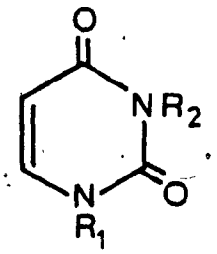
an even number of data points since these methods involve consideration of pairs of data points. Rate constants and intercepts with their respective standard deviations along with correlation coefficients are obtained for each type of treatment performed. The output of the Guggenheim and "normal" methods also contain A_0 determined from the intercept value. A_∞ is obtained by the Swinbourne analysis and may be automatically inserted into the "normal" treatment if desired. Otherwise, an observed value of A_∞ may be entered as input. The "normal" analysis permits the rejection of one poor middle point and as many poor end points as desired (that is, points whose values do not agree well (usually more than 5% different) with the calculated values obtained from the parameters of the linear regression analysis of the data). The program then redetermines the rate constant for this new set of data.

LSQ: This is a general least squares program used in substrate and acidity dependence studies to calculate second-order rate constants.

RESULTS

Introduction

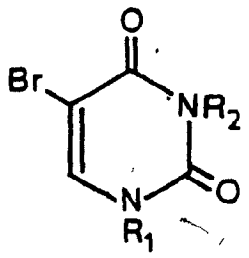
For this thesis the rates of reaction of bromine with uracil (1a), 1,3-dimethyluracil (1b), 5-bromouracil (6a), and with 5-bromo-1,3-dimethyluracil (6b) in aqueous media have been studied. Substrate dependence experiments were performed to determine the order of the reaction for each of the substrates concerned, and then the dependence of rates upon acidity was investigated to try to ascertain the charge type of the reactive species for each substrate.



1

a) $R_1 = R_2 = H$

b) $R_1 = R_2 = CH_3$



6

a) $R_1 = R_2 = H$

b) $R_1 = R_2 = CH_3$

Order of Reaction

In the presence of at least a tenfold excess of substrate (1 or 6) good first-order rate constants (k_1^{obsd}) were obtained for the rate of disappearance

of bromine. Hence

$$\text{rate} = -\frac{d[Br_2]_s}{dt} = k_1^{obsd}[Br_2]_s \qquad (10)$$

where $[Br_2]_s$ = stoichiometric bromine concentration

$= [Br_2] + [Br_3^-]$

Values of k_1^{obsd} were obtained for a range of substrate concentrations for each of the uracil substrates. The results, presented in Tables I and II, and plotted in Figures 1-4, show that the first-order rate constants for each substrate are a function of the substrate concentration. Thus

$$k_1^{obsd} = k_2^{app}[S] \qquad (11)$$

where k_2^{app} is the apparent-second-order rate constant and [S] is the substrate concentration. Therefore overall

$$\text{rate} = k_2^{app}[S][Br_2]_s \qquad (12)$$

That is, the reaction is second-order: first-order in substrate and first-order in bromine. The order for uracil was determined at two acidities to verify that it did not change with pH.

Since the excess of 1 or 6 over bromine was not particularly large, k_1^{obsd} should be directly proportional to $([S] - [Br_2]_s)$, the concentration of substrate which does not vary during the reaction, as proposed by Bell and Ramsden[57] and used by Tee et al.[34,58,59] Linear regression analysis of k_1^{obsd} did indeed give good correlations for all four substrates

Table I

Variation in the rates of bromination (k_1^{obsd}) of Uracils (1) with substrate concentration[a]

Uracil	pH	[S] x 10^4 (M)	k_1^{obsd} (S^{-1})	k_1^{calcd} (S^{-1})
1a	0.11[e]	5.0	8.41[b]	8.57
$R_1=R_2=H$		10.0	17.0	17.0
		15.0	25.8	25.4
		20.0	33.5	33.8
	4.64	5.0	17.5[c]	17.8
		7.5	28.2	27.6
		10.0	37.2	37.5
1b	1.26	5.0	16.3[d]	15.7
$R_1=R_2=Me$		7.5	24.0	25.0
		10.0	34.7	34.3
		15.0	53.0	52.9

[a] At 30°C, $[Br_2] = 5.0 \times 10^{-5}$M, [KBr] = 0.10M. Each k_1^{obsd} is the average of several determinations, at least 2, usually 4. The values of k_1^{calcd} were calculated from least squares parameters.

[b] Corr. coeff. = 0.9995

[c] Corr. coeff. = 0.9987

[d] Corr. coeff. = 0.9989

[e] This is a value of the acidity function H_o.[51]

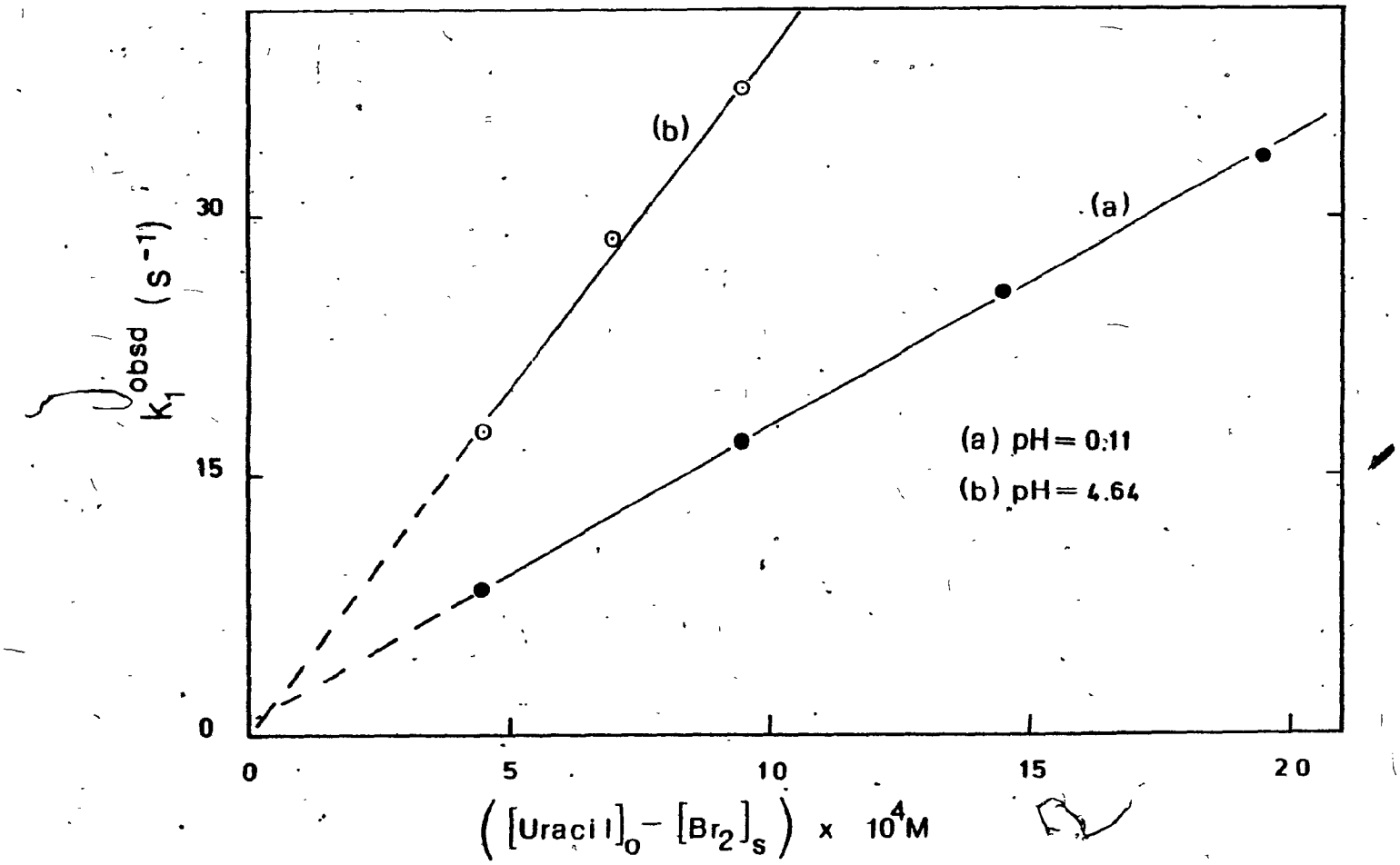


Figure 1. Variation in the Rate of Bromination of Uracil with Substrate Concentration.

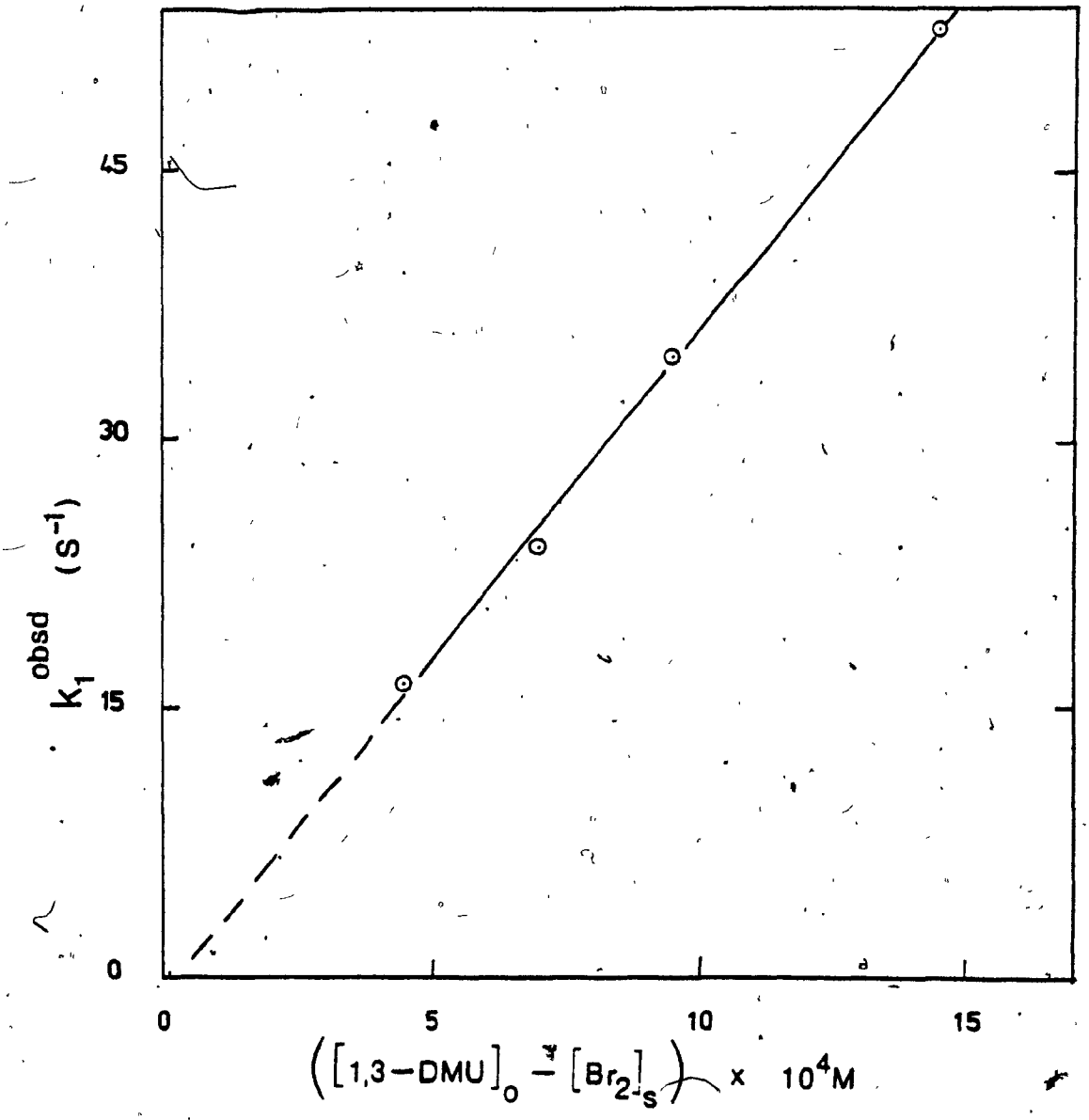


Figure 2. Variation in the Rate of Bromination of 1,3-Dimethyluracil at pH 1.26 with Substrate Concentration.

Table II

Variation in the rates of bromination (k_1^{obsd}) of 5-Bromouracils (6) with substrate concentration[a]

5-Bromouracil	pH	[S] x 10^4 (M)	k_1^{obsd} x10^3 (S^{-1})	k_1^{calcd} x10^3 (S^{-1})
6a	0.11[d]	5.0	10.4[b]	10.3
R_1=R_2=H		7.5	15.5	15.8
		10.0	21.4	21.3
6b	1.03	2.5[e]	1.80[c]	1.84
R_1=R_2=Me		5.0	3.19	3.25
		7.5	5.06	4.81
		10.0	6.22	6.37

[a] At 30°C, [Br_2] = 5.0x10^{-5}M, [KBr] = 0.10M. Each k_1^{obsd} is the average of several determinations, at least 2, usually 4. The values of k_1^{calcd} were calculated from least squares parameters.

[b] Corr. coeff. = 0.9991

[c] Corr. coeff. = 0.9960

[d] This is a value of the acidity function H_o.[51]

[e] [Br_2] = 2.5x10^{-5}M

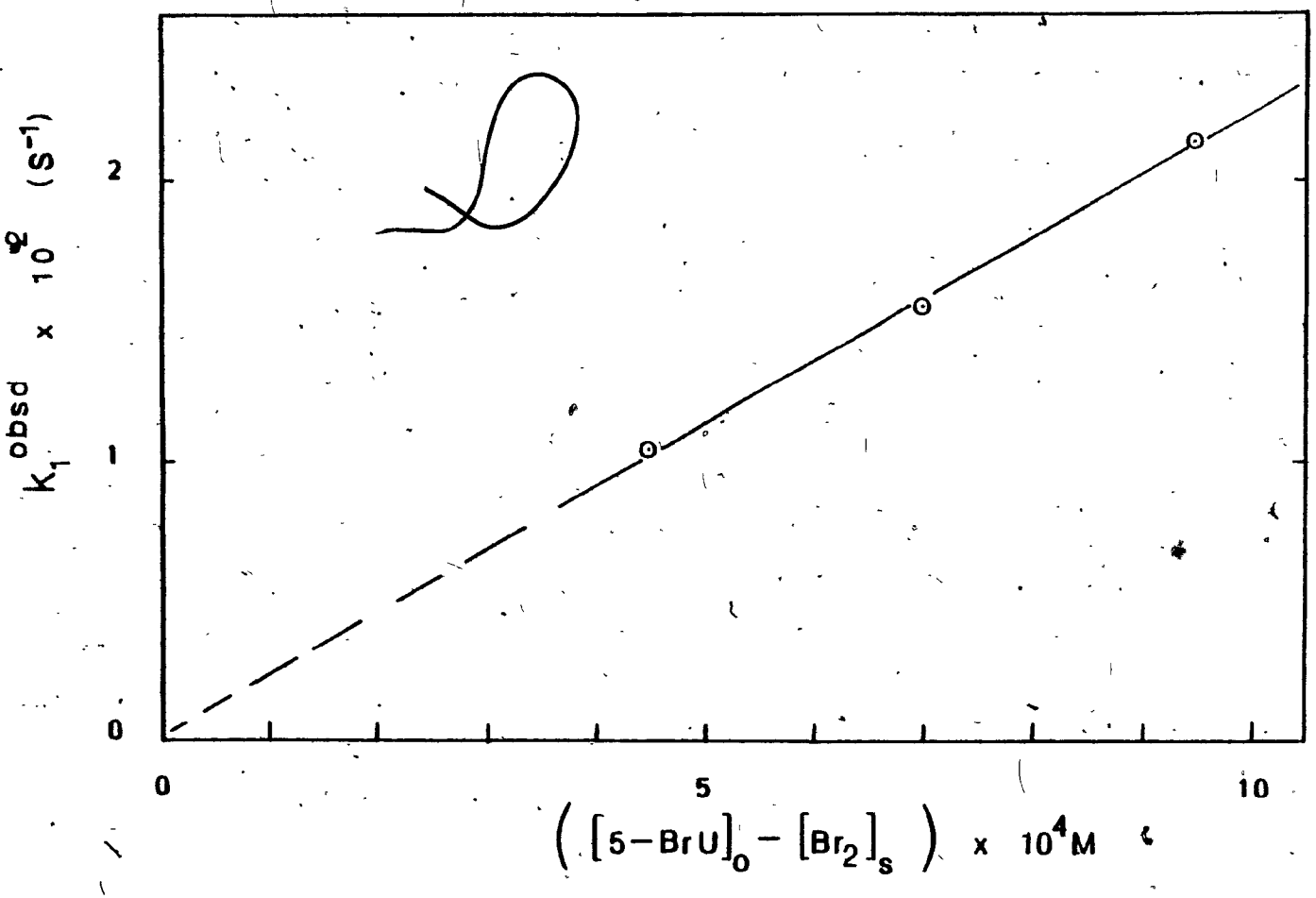


Figure 3. Variation in the Rate of Bromination of 5-Bromouracil with Substrate Concentration.

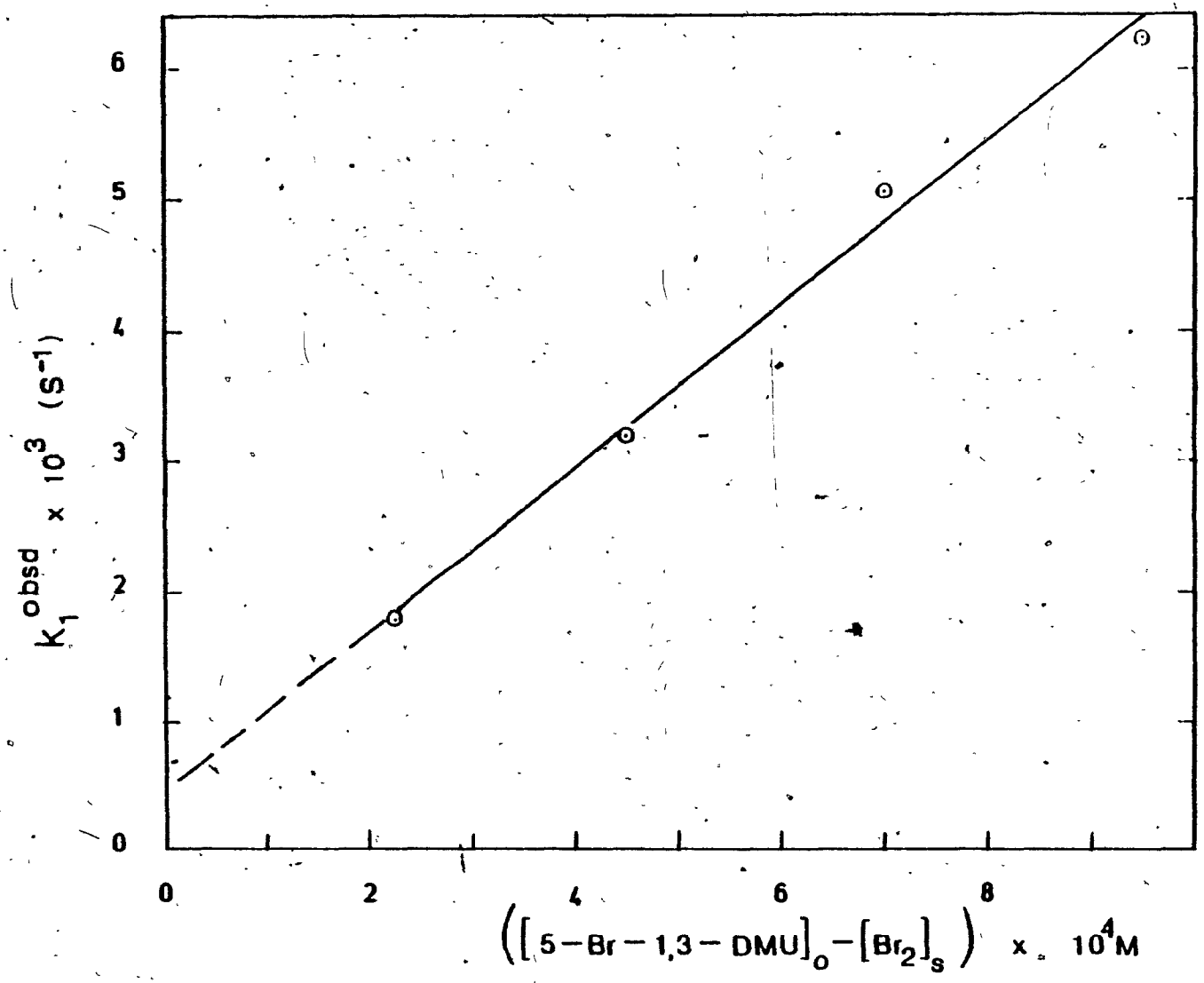


Figure 4. Variation in the Rate of Bromination of 5-Bromo-1,3-dimethyl-uracil at pH 1.03 with Substrate Concentration.

(1a (r = 0.9995 in aqueous sulphuric acid and 0.9987 in pH 4.64 acetate buffer), 1b (r = 0.9989), 6a (r = 0.9991) and 6b (r = .9960)). From the least squares parameters values for k_1^{calcd} were calculated and these are included in Tables I and II for comparative purposes. The differences between k_1^{obsd} and k_1^{calcd} are a few per cent or less, and are thus of the order of the experimental error.

In summary, for each uracil studied, substrate dependence experiments indicated that their reactions with bromine are second-order.

Effective Bromine Concentration

In the previous section it was concluded that the reaction of 1 or 6 with bromine is second-order, that is,

$$\text{rate} = k_1^{obsd}[Br_2]_s = k_2^{app}[S][Br_2]_s \qquad (13)$$

As mentioned earlier k_1^{obsd} is more accurately proportional to $([S] - [Br_2]_s)$. Therefore

$$k_1^{obsd} = k_2^{app}([S] - [Br_2]_s) \qquad (14)$$

For reasons explained in the Experimental section all solutions contained bromide ion, and so account must be taken of the depletion of free bromine due to formation of tribromide ion.[60]

$$Br_3^- \overset{K_1}{\rightleftharpoons} Br_2 + Br^- \qquad (15)$$

where

$$K_1 = \frac{[Br_2][Br^-]}{[Br_3^-]} = 0.0554 \text{ at } 30^0C^{59} \qquad (16)$$

As mentioned previously, the stoichiometric concentration of bromine is given by

$$[Br_2]_s = [Br_2] + [Br_3^-] \quad (17)$$

Accordingly, for the reaction of substrate S with free bromine

$$\text{rate} = k_1^{obsd}[Br_2]_s = k_2^{obsd}([S] - [Br_2]_s)[Br_2] \quad (18)$$

Hence one obtains the observed-second-order rate constants (k_2^{obsd}) using

$$k_2^{obsd} = \frac{k_1^{obsd}[Br_2]_s}{([S] - [Br_2]_s)[Br_2]} \quad (19)$$

Now

$$\frac{[Br_2]_s}{[Br_2]} = \frac{([Br_2] + [Br_3^-])}{[Br_2]}$$

$$= 1 + \frac{[Br_3^-]}{[Br_2]}$$

$$= \frac{(K_1 + [Br^-])}{K_1} \quad (20)$$

using the definition of K_1.

Thus

$$k_2^{obsd} = \frac{k_1^{obsd}}{([S] - [Br_2]_s)} \frac{(K_1 + [Br^-])}{K_1} \quad (21)$$

For all of the experiments bromide ion is in large excess (20,000 fold) relative to bromine and so its concentration is effectively constant at $[Br^-] = 0.1M$. Furthermore, for all the acidity dependence studies $[S] = 5\times10^{-4}M$ and $[Br_2]_s = 5\times10^{-5}M$. Making these substitutions in equation (21) gives

$$k_2^{obsd} = 6233k_1^{obsd} \quad (22)$$

The value of k_2^{obsd} was therefore calculated by multiplying k_1^{obsd} by 6233, a constant factor which takes into account the depletion of free bromine due to tribromide formation as well as the substrate concentration.

For pH > 5.10 the formation of hypobromous acid (HOBr) must be also considered. The appropriate equilibrium is

$$Br_2 + H_2O \overset{K_2}{\rightleftharpoons} H^+ + Br^- + HOBr \qquad (23)$$

where

$$K_2 = \frac{[H^+][Br^-][HOBr]}{[Br_2]}$$

$$= 9.6\times10^{-9} M^2 \text{ at } 25^0C^{61} \qquad (24)$$

and [HOBr] = actual hypobromous acid concentration

Under these conditions

$$\frac{[Br_2]_s}{[Br_2]} = \frac{([Br_2] + [Br_3^-] + [HOBr])}{[Br_2]}$$

$$= 1 + \frac{[Br_3^-]}{[Br_2]} + \frac{[HOBr]}{[Br_2]}$$

$$= 1 + \frac{[Br^-]}{K_1} + \frac{K_2}{[H^+][Br^-]} \qquad (25)$$

and so equation (21) becomes

$$k_2^{obsd} = \frac{k_1^{obsd}}{([S] - [Br_2]_s)} \left[\frac{K_1 + [Br^-]}{K_1} + \frac{K_2}{[H^+][Br^-]}\right] \qquad (26)$$

For the same concentrations of reactants and bromide

considered on the previous page

$$k_2^{obsd} = 6277 k_1^{obsd} \text{ for pH} = 5.31$$

and

$$k_2^{obsd} = 6368 k_1^{obsd} \text{ for pH} = 5.80 \qquad (27)$$

These values were employed in the studies described in the next section.

In the Experimental section it was stated that the progress of the brominations studied were monitored by following the decrease in absorbance due to Br_3^-. In the derivation of k_2^{obsd} just presented it has been assumed that the rate of Br_2 consumption is equal to that of Br_3^-. That is, Br_2 and Br_3^- are in fast equilibrium. If this were not the case the reactions would not show any acidity dependence (as was observed) since the ratio $[Br_3^-]/[Br_2]$ is acid independent (see equation (16)). Furthermore, if the dissociation of Br_3^- were rate-determining then one would not have observed a dependence upon substrate concentration as presented earlier.

Acidity Dependence

The acidity dependence of the second-order bromination rate constants of the four substrates were observed at various acidities in aqueous sulphuric acid solutions and in buffers. The results are shown in Tables III and IV for the uracils and 5-bromouracils respectively. The log k_2^{obsd} - pH rate profiles

appear in Figure 5.

The rate of reaction of bromine with 1,3-dimethyluracil (1b) was found to be invariant over the range of acidities considered, approximately pH 0 to 5 (see Figure 5). For uracil (1a), however, the rate was acid independent in strong acid but increased with increasing pH for pH >3. Similar results were obtained for the brominations of 5-bromouracils (6) except that the acidity dependence of 6a commenced at a lower pH.

It appears, therefore, that the observed rate constants for the bromination of uracil and 5-bromouracil are the sum of two terms, one acid invariant and one showing inverse dependence upon acidity. Accordingly, the data for these two substrates were analyzed in terms of an equation

$$k_2^{obsd} = k_2 + k_2'/[H_3O^+] \qquad (28)$$

Least squares analysis of k_2^{obsd} versus the inverse of the hydronium ion concentration for 1a and 6a gave straight lines with very acceptable correlation coefficients.

Uracil: $k_2^{obsd} = 5.14 \times 10^{4} + 1.31/[H_3O^+]$ (29)

5-Bromouracil: $k_2^{obsd} = 64.1 + 1.36/[H_3O^+]$ (30)

The values of k_2^{calcd} in Tables III and IV were obtained using the least squares parameters in equations (28) and (29) respectively. The calculated rate profiles are shown in Figure 5.

The value of k_2^{obsd} obtained for uracil at pH 5.8

Table III

Variation in the rates of bromination of uracils (1) with pH[a]

Uracil	pH	k_1^{obsd} (S^{-1})	$k_2^{obsd} \times 10^{-4}$ ($M^{-1}S^{-1}$)	$k_2^{calcd} \times 10^{-4}$ ($M^{-1}S^{-1}$)
1a	0.11[d]	8.41	5.24[b]	5.14
$R_1=R_2=H$	1.24	8.00	4.99	5.14
	1.84	8.21	5.12	5.15
	3.00	7.79	4.86	5.27
	4.20	11.5	7.17	7.22
	4.64	17.5	10.9	10.9
	4.91	26.6	16.6	15.8
	5.05	32.1	20.0	19.9
	5.31	50.3	31.6	32.0
	5.80	108	(68.8)[c]	----
1b	-0.30[d]	17.9	11.2	----
$R_1=R_2=Me$	0.11[d]	17.6	11.0	----
	1.26	16.3	10.2	----
	4.20	14.5	9.04	----
	5.00	14.3	8.91	----

[a] At 30°C, $[S] = 5.0 \times 10^{-4}M$, $[Br_2] = 5.0 \times 10^{-5}M$, [KBr] = 0.10M. Each k_1^{obsd} is the average of 3 or more determinations. The values of k_2^{calcd} were calculated from least squares parameters.

[b] Corr. coeff. = 0.9992

[c] Not included in the least squares analysis (see text).

[d] Values of H_o.[51]

Table IV

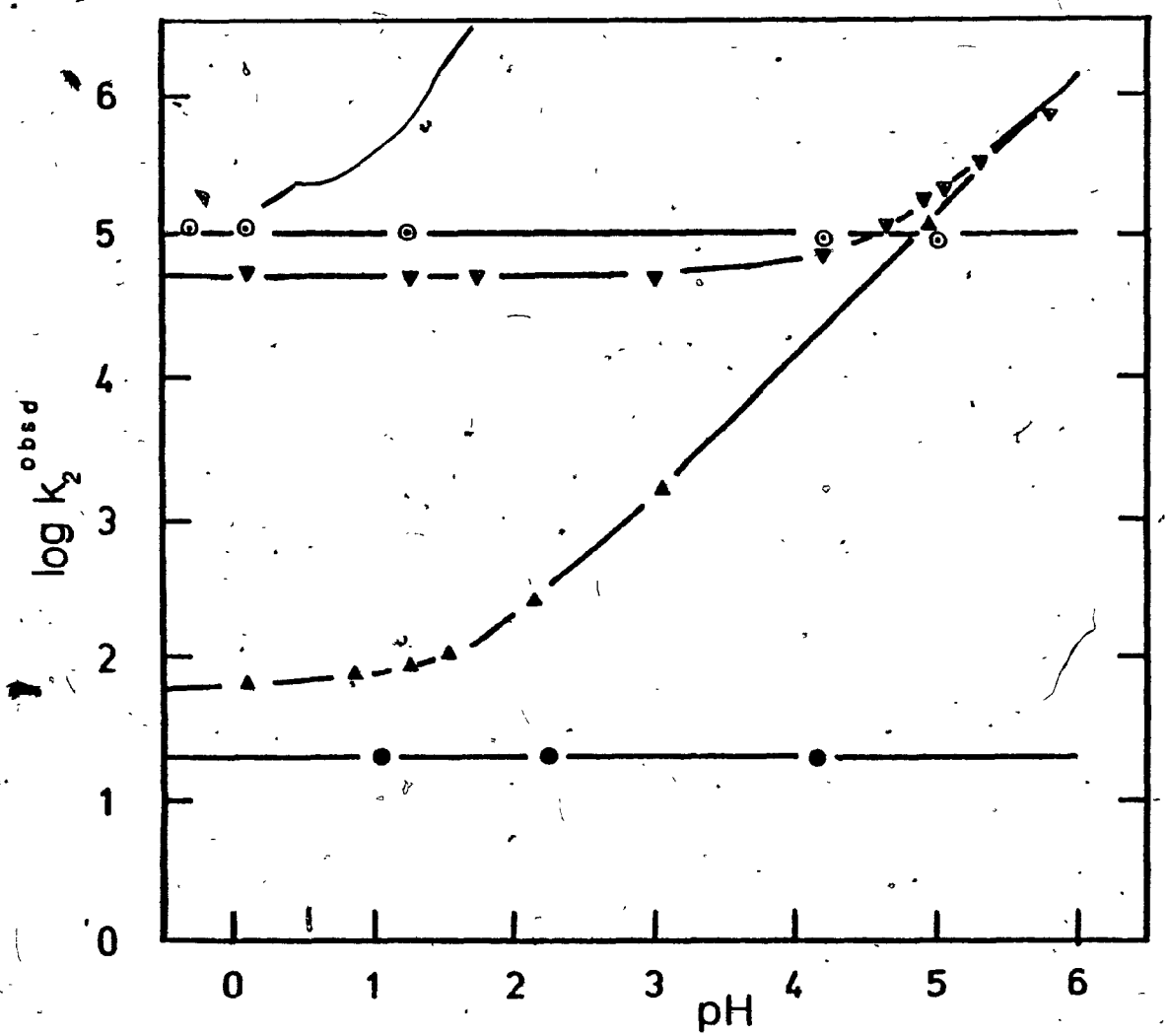
Variation in the rates of bromination of 5-bromouracils (6) with pH[a]

5-Bromouracil	pH	$k_1^{obsd} \times 10^3$ (S^{-1})	k_2^{obsd} ($M^{-1}S^{-1}$)	k_2^{calcd} ($M^{-1}S^{-1}$)
6a	0.11[c]	10.4	64.8[b]	65.9
$R_1=R_2=H$	0.89	12.6	78.5	74.7
	1.26	13.9	86.6	88.8
	1.52	16.9	105	109
	1.73	22.3	142	137
	2.13	40.1	249	248
	3.05	256	1580	1589
	3.30	432	2780	2777
	4.95	18400	115000	121190
6b	1.03	3.19	19.9	----
$R_1=R_2=Me$	2.25	3.22	20.1	----
	4.15	3.05	19.0	----

[a] At 30°C, [S] = 5.0×10^{-4}M, $[Br_2]$ = 5.0×10^{-5}M, [KBr] = 0.10M. Each k_1^{obsd} is the average of 3 or more determinations. The values of k_2^{calcd} were calculated from least squares parameters.

[b] Corr. coeff. = 0.9999

[c] Value of H_o.[51]



▽ Uracil

⊙ 1,3-Dimethyl-uracil

▲ 5-Bromouracil

● 5-Bromo-1,3-dimethyluracil

Figure 5. Acidity Dependence for the Rates of Bromination of Uracils and 5-Bromouracils.

was not included in the linear regression analysis since the value ($68.8 \times 10^4 M^{-1}S^{-1}$) is only 78.1% of k_2^{calcd} ($88.1 \times 10^4 M^{-1}S^{-1}$) for this pH, even after correction for HOBr formation. The most probable cause for the low value is the inability of the instrumental set-up to accurately measure a first-order rate as fast as that which is expected. Under the conditions of the experiment the expected $t_{1/2}$ should be 4.90 msec (i.e. $k_1^{calcd} = 141 S^{-1}$), approximately equal to the FAST instrument response (5 msec)[53] used for all the other experiments. Hence the reaction was 50% completed before the spectrophotometer was capable of monitoring it. A response time of less than or equal to 0.1 $t_{1/2}$ is recommended for optimum results.[62] The Aminco DW-2 Spectrophotometer can be operated with a still faster response time (22 microsec) designated as KINETIC response.[53] We found, however, that this response resulted in an output signal which was too noisy to be of any use. Since the completion of the work for this thesis the instrument set-up has been modified so that the DW-2 output signal (using KINETIC response) can be passed through a variable low-pass filter for the removal of unwanted high frequency noise which is mainly due to the chopper operating at the KINETIC speed of 1000 Hz.[53] In the future it is hoped to be able to follow reactions with $t_{1/2}$'s as small as 4msec, the dead-time of the stopped flow.[47]

DISCUSSION

Brominating Species

In this work the most likely brominating species are Br_2, Br_3^- and HOBr. Of these three the first is probably the only significant one, and so, in determining the second-order rate constants (k_2^{obsd}) it was assumed that all the substrates were being brominated by reaction with free Br_2.

Br_3^- is less reactive than Br_2 as a brominating species since the addition of Br^- to reactant solutions (resulting in Br_3^- formation) causes a decrease in the rates of bromination.[63-66] Br_3^- has been shown to be a significant brominating agent only for reactions with the most reactive organic substrates. Even with highly reactive phenoxide ions Br_3^- reactivity is only 1-3% of that of Br_2.[64] With phenol ($k_2^{obsd} = 1.8 \times 10^5$ $M^{-1}S^{-1}$[64], of the same magnitude as the largest k_2^{obsd} in this study) no evidence has been found for Br_3^- acting as a brominating species.[64]

For a halogenating agent X-Y, Norman and Taylor[67] generalize that the electrophilicity of the halogen atom X increases as the leaving group ability of the atom or group Y increases. In line with this generalization hypobromous acid (Br-OH) is a less effective brominating species than molecular bromine (Br-Br). For example, Derbyshire and Waters[68] studying the bromination of sodium-p-anisate in dilute solutions of phosphate buffer (pH 7-8) found HOBr to be about

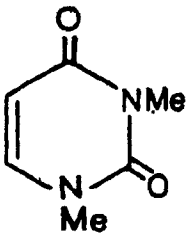
2000 times less reactive than Br_2. Many other examples may be found in the literature.[72] On the basis of these precedents it seems unlikely that HOBr is the brominating agent in the present study, particularly when one takes into account that at pH 6 only 9.6% of the Br_2 is present as HOBr, at pH 5 only .96% is present as HOBr, and so on (from equation (24)).

Positive bromination due to the formation of the highly reactive protonated hypobromous acid (H_2OBr^+) can also be discounted.[69] Reaction via this species usually proceeds only in strong mineral acid and should not be a factor in aqueous media. Positive bromination would require the reactions to be acid catalyzed which is contrary to the kinetic results (see Tables III and IV).

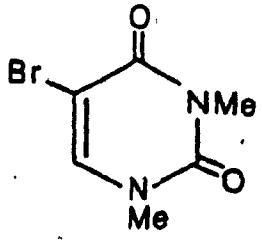
Therefore the assertion that Br_2 is the only brominating agent in the present brominations is a very reasonable one.

Acidity Dependence

1,3-Dimethyluracil (1b) and 5-bromo-1,3-dimethyluracil (6b) are necessarily fixed in the diketonic form and are not involved in any acid-base equilibria in the pH region. Shugar and Fox[3] studied the acid dependence of the u.v. spectrum of 1b and found no variation in its shape up to pH 14 indicating an absense of dissociable groups.



1b



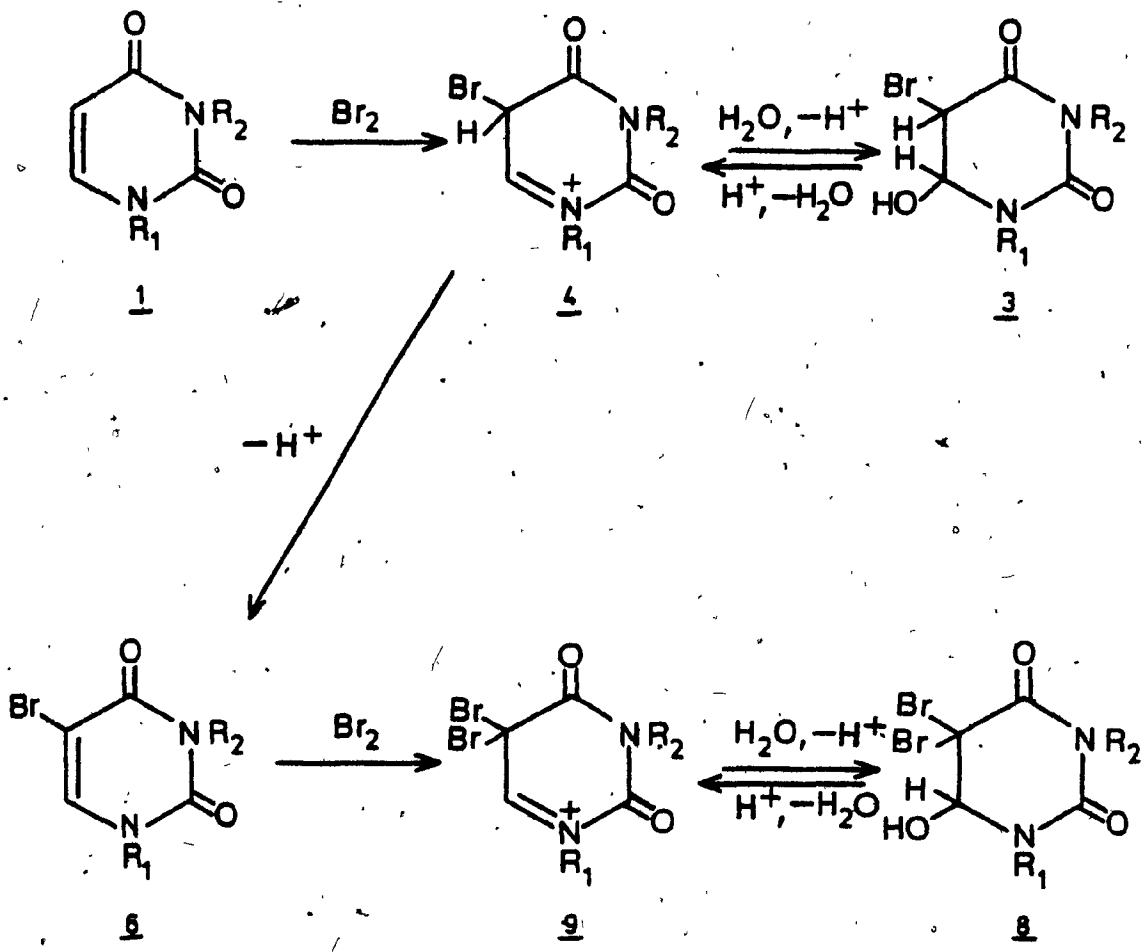
6b

It is most likely, therefore, that the bromination of these compounds involves direct attack by bromine on the neutral molecules which the results support. As shown in Tables III and IV the second-order rates of reaction are independent of acidity, as required for reactions involving neutral species. The kinetic data for 1b thus support direct attack by Br_2 leading to the formation of the cationic intermediate 4b, and hence 3b (see Scheme 3, next page).

$$\underline{1b} + Br_2 \longrightarrow Br^- + \underline{4b} \overset{H_2O,-H^+}{\rightleftharpoons} \underline{3b}$$

Adduct 3b is a long-lived observable intermediate, whose dehydration kinetics (to 6b) have been studied by Banerjee and Tee.[30,31] For 6b the results may be

Scheme 3

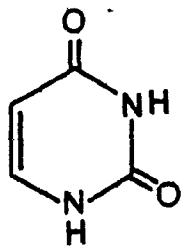


explained by an analogous mechanism.

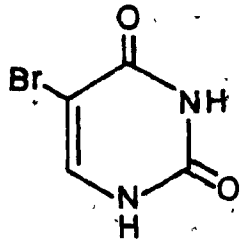
$$\underline{6b} + Br_2 \longrightarrow Br^- + \underline{9b} \overset{H_2O,\,-H^+}{\rightleftharpoons} \underline{8b}$$

Combining the two processes just described for 1b and 6b together with the dehydration step (3b ⟶ 6b) the overall bromination mechanism proposed for uracil derivatives 1 (R_1 = Me; R_2 = H or Me) is that shown in Scheme 3. This mechanism is in accord with the titration results of Moore and Anderson that the reaction be 1:1, since the dehydrations 3 ⇌ 4 ⟶ 6 are quite slow in weak acid.[23]

The rates of bromination of uracil (1a) and of 5-bromouracil (6a) are constant in strong acid, while they vary inversely as the acidity in weak acid. Thus



1a



6a

the observed second-order rate constants (k_2^{obsd}) are composed of two terms: one acid independent, one acid dependent. This was expressed earlier in equation (28)

$$k_2^{obsd} = k_2 + k_2'/[H_3O^+] \qquad (28)$$

which gives an excellent description of the observed data (see equations (29) and (30) and Figure 5). The acid independent term, k_2, is presumably due to reaction upon the neutral molecule, whereas the acid dependent term, $(k'/[H_3O^+])$, may be ascribed to reaction via an anion. The overall situation may be presented as

$$\begin{array}{ccc} SH & \overset{K_a}{\rightleftharpoons} & S^- + H_3O^+ \\ \downarrow Br_2,\ k_2^1 & & \downarrow Br_2,\ k_2^2 \\ \text{pdts} & & \text{pdts} \end{array} \qquad (31)$$

where

SH = neutral 1a or 6a

S^- = anion of 1a or 6a

k_2^1 = second-order rate constant for the bromination of 1a or 6a

k_2^2 = second-order rate constant for the bromination of the anions of 1a or 6a

$$K_a = \frac{[S^-][H_3O^+]}{[SH]} \qquad (32)$$

$= 10^{-9.5}$ for 1a

$= 10^{-8.0}$ for 6a

Now
$$[SH]_s = [SH] + [S^-]$$
$$= \frac{[SH]([H_3O^+] + K_a)}{[H_3O^+]} \qquad (33)$$

All experiments were done at pH < 6 therefore $[H_3O^+] \gg K_a$ and $[SH]_s = [SH]$. Considering that two reaction pathways are possible

$$-\frac{d[Br_2]}{dt} = k_2^{obsd}[SH]_s[Br_2]$$

$$= k_2^1[SH][Br_2] + k_2^2[S^-][Br_2]$$

$$= \left[k_2^1 + \frac{k_2^2 K_a}{[H_3O^+]}\right][SH]_s[Br_2] \qquad (34)$$

Therefore $$k_2^{obsd} = k_2^1 + \frac{k_2^2 K_a}{[H_3O^+]} \qquad (35)$$

Equation (35) is of the same form as equation (28), and the correlations expressed by equations (29) and (30) for 1a and 6a respectively. Thus the observed data for 1a and 6a are consistent with reaction taking place upon their neutral species at higher acidity, and upon their anions at lower acidity.

In Table V are presented the second-order rate constants (k_2^1 and k_2^2) for 1a and 6a obtained by equating the correlation equations (29) and (30) with the theoretical equation (35). Also included are the corresponding values for the 1,3-dimethyluracils (1b and 6b) and for the analogous 6-azauracil system examined by Banerjee and Tee.[34]

In strong acid it is the acid independent term which makes the largest contribution to the value of k_2^{obsd} for 1a and 6a. It should be noted that the values of k_2^1 for 1a and 1b and for 6a and 6b are of

Table V

Comparison of the kinetic parameters for the brominations of uracils (1), 5-bromouracils (6), 6-azauracils (11) and 1,2-dihydro-1,3-dimethyl-2-oxopyrimidinium cation (52)

Compound	K_a (M)	k_2^1 ($M^{-1}S^{-1}$)	$k_2^2K_a$ (S^{-1})	k_2^2 ($M^{-1}S^{-1}$)
1a	$10^{-9.5}$ [a]	5.14×10^{4}	1.31	4.15×10^{9}
1b	------	1.00×10^{5}	------	--------
6a	$10^{-8.0}$ [a]	64.1	1.36	1.36×10^{8}
6b	------	20.0	------	--------
11a [b] $R_1=R_2=H$	$\sim10^{-9.5}$	1.2×10^{-3}	2.2×10^{-4}	$\sim7\times10^{5}$
11c [b] $R_1=H;R_2=Me$	$10^{-9.52}$	5.6×10^{-4}	3.5×10^{-4}	1.0×10^{6}
11b [b] $R_1=R_2=H$	-------	$\sim1\times10^{-4}$	------	--------
52 [c] X=H	-------	2×10^{9}	------	--------
52 [c] X=Br	-------	4×10^{6}	------	--------

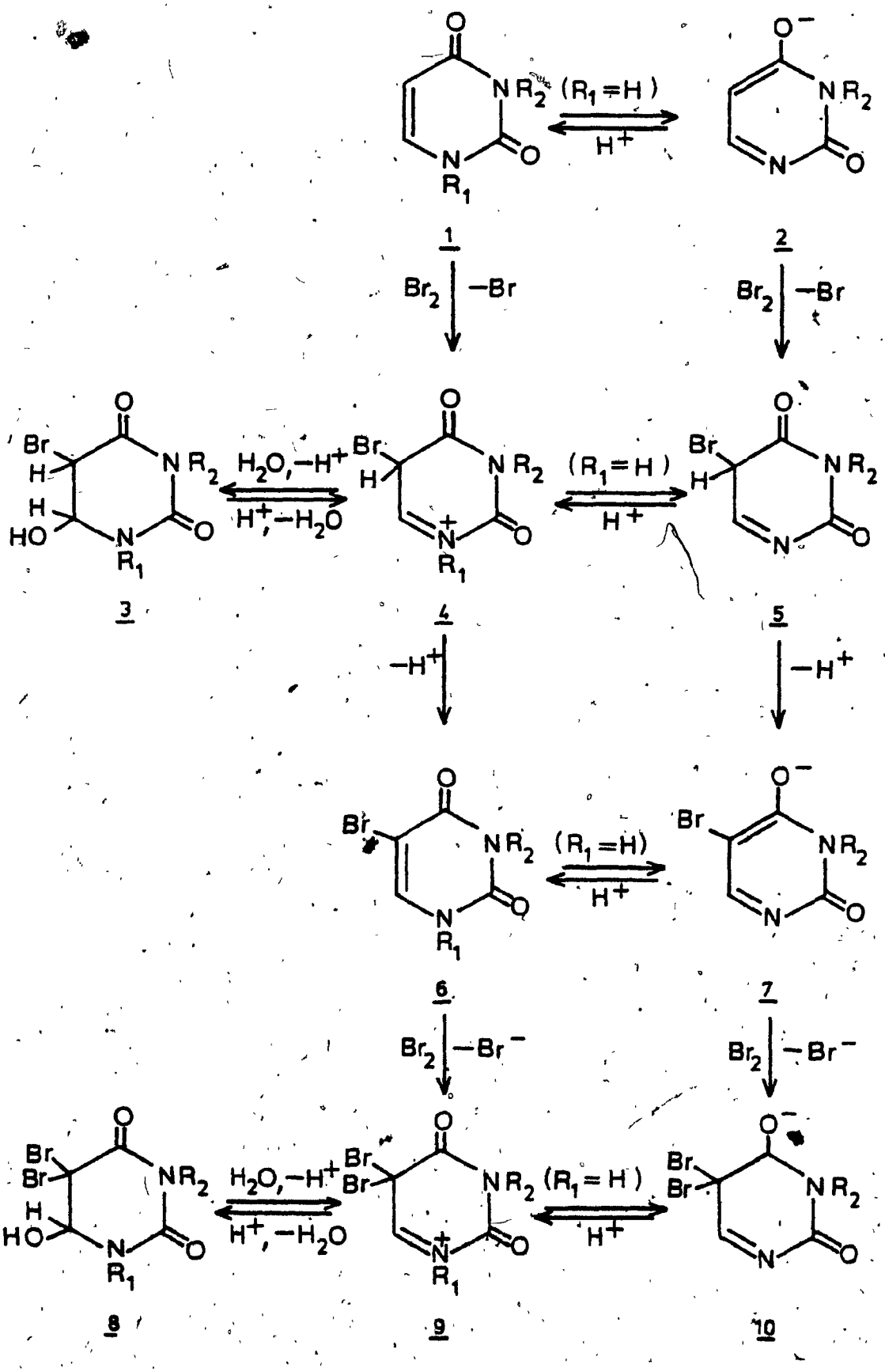
[a] Ref. 11 [b] Ref. 34 [c] Ref. 74

the same order of magnitude. Furthermore, titration results for the bromination of uracils 1 (R_1 = H, R_2 = H or Me) have shown the reaction to be 1:1 in strong acid.[31] These observations suggest that the reaction under these conditions can also be expressed by the mechanism previously described in Scheme 3 for the dimethyl derivatives.

At higher pH 1a and 6a appear to react via the anions 2 and 7 respectively (see Scheme 4). It is proposed that bromine reacts rapidly with 2 to give a neutral species 5 which may be in equilibrium with the hydrated adduct 3a (analogous to 3b described in Scheme 3). Loss of a proton from the C-5 position of 5 results in the anion of 5-bromouracil, 7, in equilibrium with 5-bromouracil, 6a, itself. Likewise, the bromination of 5-bromouracil may proceed via the anion 7 which may react with bromine to give the neutral species 10 which is in equilibrium with the dibromo product 8. These mechanisms are presented in Scheme 4.

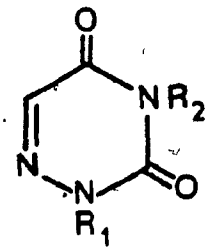
Titration studies of N_1 unsubstituted uracils with bromine have previously shown the reaction to be 1:2 in weakly acidic solutions.[23] In terms of the mechanism in Scheme 4 this implies that the deprotonation 5 $\longrightarrow$ 7 occurs quite rapidly. Kinetic studies performed in strong acid (> 0.25N H_2SO_4) have shown, however, that the rate of this proton loss is slow ($k_1 = 10^{-5}s^{-1}$, $t_{1/2}$= 20 hr)[30,31] with water acting

Scheme 4



as the base. At higher pH (> 1) it is possible that hydroxide ion is the base involved and one should expect the rate of deprotonation of 5 to increase greatly as the pH increases. In the future it is hoped to obtain evidence to support this proposal.

Other systems besides uracil have been shown to react with bromine via their anions. Phenols are known to react as phenoxide anions except in strongly acidic media.[63,64,70] Work done in this laboratory has suggested that N_1 unsubstituted 6-azauracils (11 R_1 = H, or Me) react with bromine as anions even

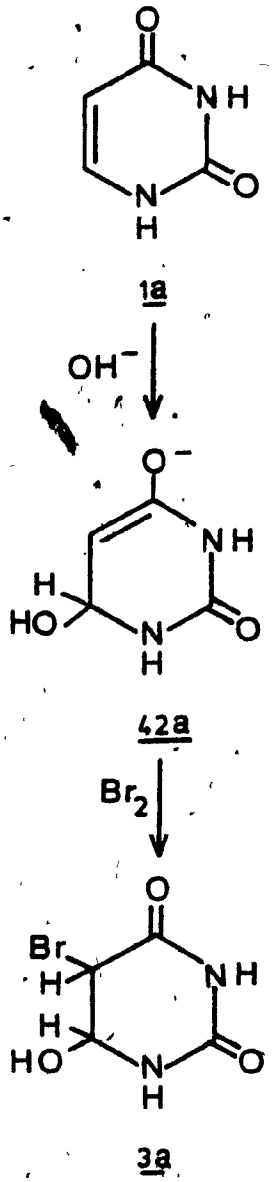


11

in 0.05-0.50N sulphuric acid solutions.[34] The mechanism is presented in the Introduction section (Scheme 2, page 17).

Besides the anion mechanism (Scheme 4) presented above, other possibilities were considered. The inverse acidity dependence could also be explained by an

Scheme 5



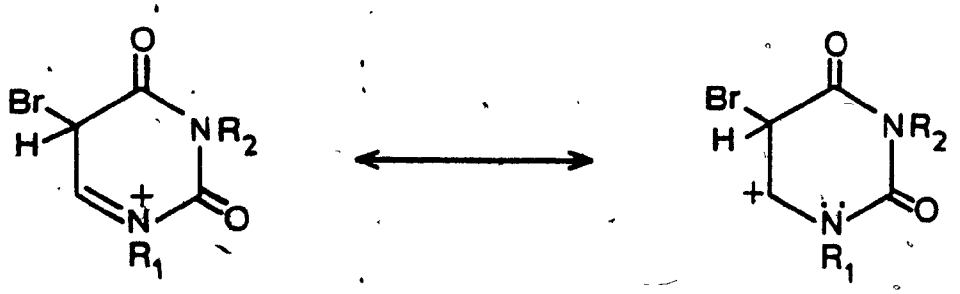
addition-elimination mechanism (Scheme 5) in which there is firstly nucleophilic attack by OH^- to form an enolate 42a which then reacts with bromine to give the intermediate 3a. A similar mechanism has been proposed for the base catalyzed H-D exchange of N_1 substituted uracils.[19,20]

The mechanism in Scheme 5 appears improbable for several reasons. Firstly, if uracil 1a reacts this way, one would expect 1,3-dimethyluracil 1b to react likewise at a similar rate but it does not. Secondly, the enolate (42b) would react with bromine at a diffusion controlled rate which would result in zero-order (in bromine) kinetics (cf. acetone halogenation[71]). However, this also was not observed. Thirdly, the sequence 1a ⟶ 42a ⟶ 3a in no way contributes to an explanation of the 1:2 titrations since the 3a converts to 5-bromouracil quite slowly.[30,31]

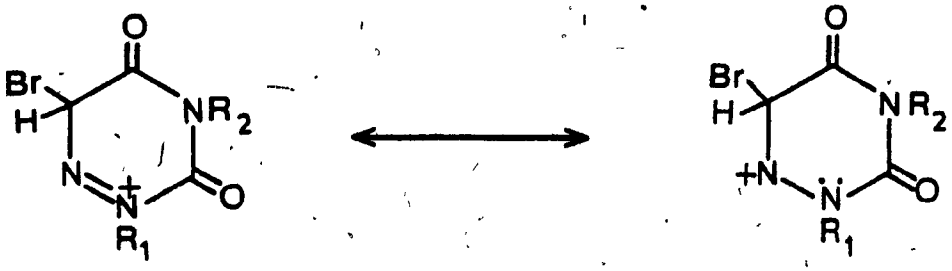
Comparison of Reactivities

Uracil is much more reactive than 6-azauracil with respect to electrophilic attack (see Table V). The reactivity of uracil towards bromine is 4×10^7 more than that of 6-azauracil and 1,3-dimethyluracil is 10^9 more reactive than 1,3-dimethyl-6-azauracil. The reason for this increased reactivity may be understood by considering the relative stabilities of the intermediates formed by the attack of bromine

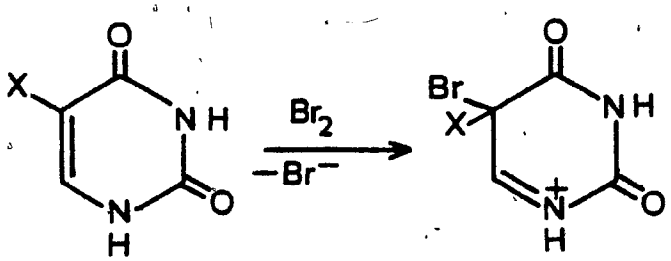
in each case. For uracils, 4 has carbonium ion character and is therefore relatively stable. With 6-azauracils, the corresponding intermediate has its positive charge residing solely on nitrogen atoms and should be less stable resulting in decreased reactivity.



4

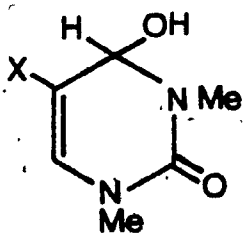


Uracil was found to be 800 times as reactive towards bromine as 5-bromouracil is. This not surprising since the presence of a bromine at the 5-position in 25 should retard the rate of bromine attack (25 ⟶ 26) for steric as well as electronic reasons (see next page). By way of comparison, the pseudobase formed from 1,2-dihydro-1,3-dimethyl-2-oxo-



25 26

pyrimidinium cation (52, X = H) reacts with bromine 500 times as fast as its 5-bromo derivative (52, X = Br).[74]



52

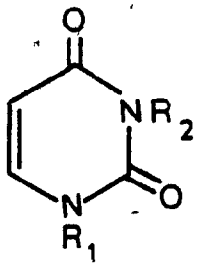
The value of k_2^1 for uracil (5.14×10^4 $M^{-1}S^{-1}$) is of the same order as that for phenol (1.8×10^5 $M^{-1}S^{-1}$ at 25^0C[63]). Similarly, k_2^2 for the uracil anion (4×10^9 $M^{-1}S^{-1}$)

is close to that for simple phenoxides (10^9-10^{10} $M^{-1}S^{-1}$), and approaches the diffusion controlled limit.

From Figure 5 it can be seen that the reactivity of 5-bromouracil approaches that of uracil in very weak acid (pH > 4). In this pH range the value of k_2^{obsd} is predominantly determined by the product $k_2^2K_a$ (see equation (35)). From Table V it can be seen that this value is virtually the same for uracil and 5-bromouracil. Hence, at acidities where the two compounds react principally as anions, but below their pK_a's, their rates of bromination are nearly the same. Moore and Anderson[23] found that during the 1:2 titration of uracil with bromine at pH 4.7 there was not a large build-up of 5-bromouracil. These observations are in agreement with the present kinetic results which show that 5-bromouracil can successfully compete with uracil for free bromine at higher pH's.

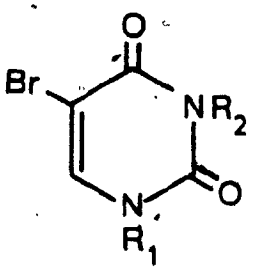
Conclusion

The brominations of the uracils 1a, 1b, 6a, and 6b in aqueous acid solutions are second-order: first-order in bromine and first-order in uracil. The mechanism for the reaction involves two discrete schemes, Schemes 3 and 4. For uracils bearing a substituent at N_1 there is a rapid attack by bromine leading to an observable 'HOBr' adduct which then undergoes a slow acid-catalyzed dehydration to the



1

a) $R_1 = R_2 = H$
b) $R_1 = R_2 = CH_3$



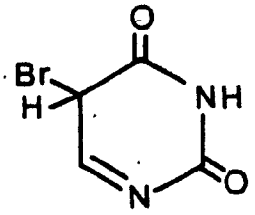
6

a) $R_1 = R_2 = H$
b) $R_1 = R_2 = CH_3$

5-bromo derivative (see Scheme 3, page 51). The kinetics of this dehydration have been studied elsewhere.[30,31] A similar scheme applies to N_1 unsubstituted uracils at higher acidities.

For uracil (1a), the rate of bromination increases with increasing pH for pH > 3. Similar reactivity is shown by 5-bromouracil (6a), except that the acidity dependence of 6a commences earlier. It has been concluded (see Scheme 4, page 57) that the bromination occurs upon the anion resulting from deprotonation at the N_1 position. Of the intermediates found in Scheme 4 only the existence of 5 and 10 (see next page) have not been proven directly or indirectly.

Further work in this area could include a study of the behaviour of 5-bromo-6-methoxy-5,6-dihydrouracil (13a) a compound most likely stable at neutral



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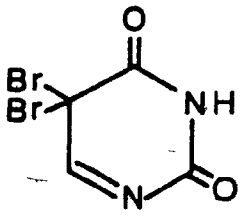

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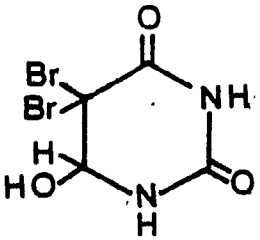
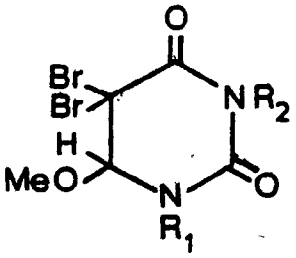


3a



13

pH. This would serve as a model for intermediate 3a. Compound 13b has been successfully prepared and isolated by Szabo et al.[73] If 13 should react with Br_2 an understanding of the mechanism would enhance our knowledge of uracil bromination.

This study could also be extended to other related pyrimidines such as thymine, 5-fluorouracil, 5-chlorouracil, etc.. This would provide comparative data concerning the effect of the substituent at the C_5 position. Cytosine, which reacts with Br_2 in a manner similar to uracil, should also be included in the investigation.

Therefore it can be seen that work has still to be done before the reaction of uracil with Br_2 is completely understood. It is hoped that the results and conclusions reported in this thesis will shed valuable light towards rationalizing much of the observed phenomena of the reaction as well as indicating areas where future investigations must be undertaken.

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