The Mechanisms of the Brominations of Cytosines and Uracils

Charles Gary Berks

A Thesis

in

the Department

of

Chemistry

Presented in Partial Fulfillment of the Requirements
for the degree of Doctor of Philosophy at
Concordia University
Montréal, Québec, Canada.

August 1983.

Abstract

The Mechanisms of the Brominations of Cytosines and Uracils

Charles Gary Berks

Concordia University, 1983

The work reported in this thesis falls into three areas:

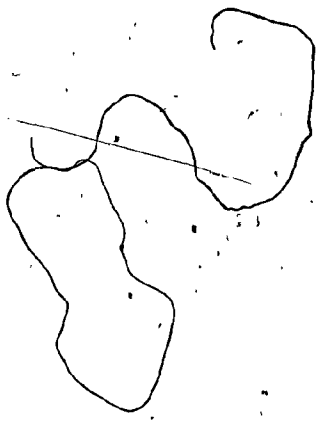
1) The primary project was a mechanistic study of the bromination of cytosine, 1-methylcytosine, 3-methylcytosine and cytidine in aqueous acid. The reaction follows an addition-elimination pathway with the initial rapid formation of long-lived intermediates observable by pmr. These adducts undergo relatively slow elimination of water to yield the appropriate 5-bromocytosine product. The acidity dependence of the initial reaction rate plus the fact that cytosine iodination is about 1000 times slower than its bromination suggest that the attack of bromine occurs on the free base form of the cytosine substrate followed by capture of the cation so produced by water.

2) The kinetics of bromination of 1-methyl- and 3-methyluracil has been examined in the pH range 0 - 5. For

1-methyluracil the reaction rate is invariant with acidity whereas for the 3-methyl substrate the rate increases markedly with decreasing acidity above pH 3. These observations are in agreement with our earlier findings that N_1 unsubstituted uracils may undergo bromination via their anions formed by deprotonation at N_1.

3) A kinetic study of the equilibrium between the 1,2-dihydro-1,3-dimethyl-2-oxopyrimidinium cation ($\underline{Q}^+$) and its pseudobase (QOH) has been undertaken in aqueous media (pH 5.6 - 9.5). Using the spectrophotometric method of Albert and Serjeant[108] pK_R^+ for the equilibration has been found to be 7.16 at 30 °C. The rate constants for the formation and decomposition of QOH compare well to those obtained from an earlier study of the bromination of $\underline{Q}^+$ carried out by Tee and Thackray[21] for which reaction was postulated to proceed via bromine attack upon QOH. These results, therefore, furnish additional support for the involvement of QOH in the bromination reaction.

To Elena



Acknowledgements

I wish to thank Dr. O. S. Tee for his guidance and assistance throughout the course of this work. His interest and patience will always be appreciated. Thanks are also due to those faculty members both external and internal to the Department who have served on my examining committee.

I am also grateful to the Department of Chemistry for financial support and to the Graduate Studies Office for the awarding of a Concordia University Graduate Teaching Fellowship.

Lastly, I wish to thank my fellow graduate students whose friendship has helped to make my stay at Concordia the rewarding experience it was.

Table of Contents

Page

Part III - RESULTS AND DISCUSSION (Cont'd)

Index to Tables

Index to Tables (cont'd)

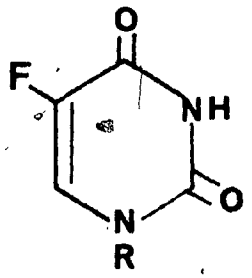
Index to Figures

INTRODUCTION

General Introduction

Pyrimidines are compounds of great importance owing, to a large extent, to their presence in nucleosides and nucleotides.[1,14] Any carbohydrate derivative of a N-heterocyclic compound, whether the heterocycle is naturally occurring or not, is termed a nucleoside. Nucleotides, the phosphate esters of nucleosides, are the structural components of nucleic acids just as amino acids are the structural components of proteins.[1]

A number of nucleosides have been found to have useful antibiotic and antitumor properties. 5-Fluorouracil (5-FU) and its N_1 ribosyl (5-FUR) and N_1 deoxyribosyl (5-FUDR) derivatives are known to have considerable anticancer activity.[2] 5-FU and 5-FUDR are converted, in vivo, to



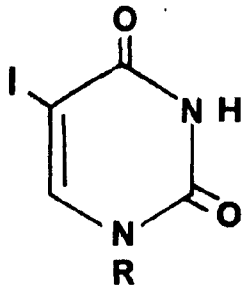
5-FU : R = H

5-FUR : R = ribosyl

5-FUDR : R = deoxyribosyl

5-FUDRP : R = deoxyribosyl monophosphate

the nucleotide 5-fluorouracil deoxyribose monophosphate (5-FUDRP) which is a strong inhibitor of thymidylate synthetase. The size of the fluorine atom is similar to that of hydrogen and 5-FUDRP competes with deoxyuridine monophosphate at the enzyme site, but its 5-position can not be methylated inhibiting DNA synthesis.[1] (One of the main distinguishing features between DNA and RNA is that whereas thymidine is present in DNA uridine takes its place in RNA.) 5-FUDR has also been shown to inhibit orotic acid metabolism and the incorporation of phosphate into DNA.[3]

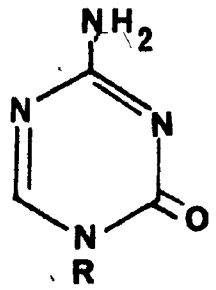


5-Iodouracil : R = H

5-IUDR : R = deoxyribosyl

5-Iodouracil also exhibits some antitumor activity.[1] It is converted in vivo to 5-iododeoxyuridine (5-IUDR) which can be incorporated into DNA. 5-IUDR thus differs in its mechanism of action from 5-FUDR in that it

acts in place of thymidine whereas 5-FUDR inhibits the biosynthesis of thymidine. 5-FUDR can however be incorporated into RNA thus causing it to be highly toxic if not used properly.[5]

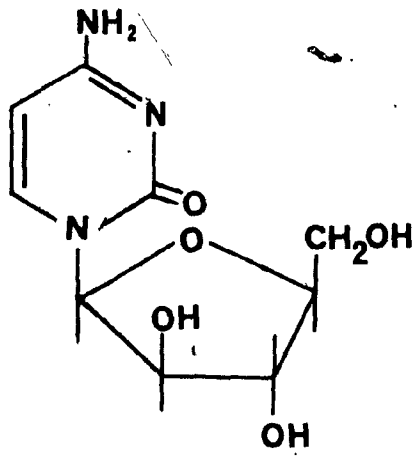


5-Azacytidine
(R = ribosyl)

5-Azacytidine is a very potent antileukaemic agent and clinical tests have shown potential as a treatment for children with acute leukaemia.[6] It is also known to be bacteriostatic and causes chromasomal and genetic mutations in bacteria and plants.[6] 5-Aza-2'-deoxycytidine also exhibits potent antibacterial activity although it may be more toxic than 5-azacytidine.[6,7]

Cytosine arabinoside (ARA-C), a synthetic

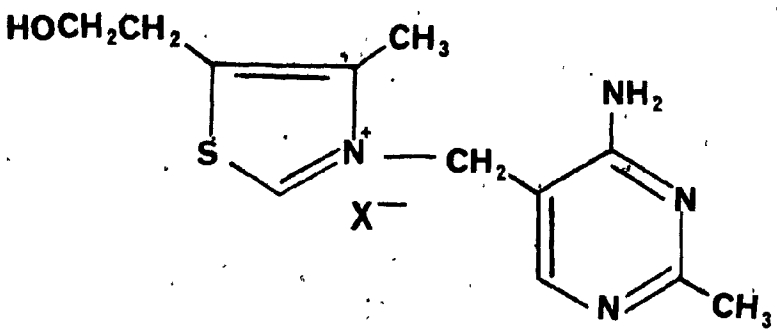
nucleoside, has also been used as an anticancer agent.[6] It inhibits the reduction of cytidine monophosphate to deoxycytidine monophosphate in DNA synthesis. ARA-C also exhibits antibiotic properties. It has been shown to inhibit certain neoplasms in mammals and also to be active against herpes simplex virus.[8,9]



Cytosine arabinoside
(ARA-C)

Nucleotide coenzymes have been studied due to their importance as vitamins and due to their involvement as the active sites for several important enzyme-mediated biochemical reactions.[1] Thiamine (vitamin B_1) is a pyrimidine derivative which also contains a thiazole ring.[1] A deficiency of thiamine results in beri-beri in man whilst animals suffer the condition of polyneuritis. Dietary sources of thiamine include green plants, yeast, whole meal flour and the husks

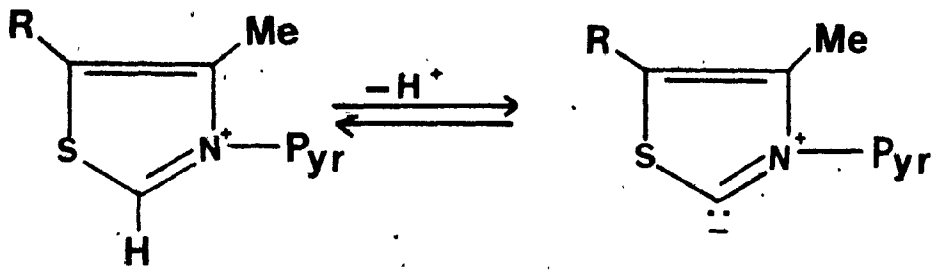
of rice and other grains.[1]



Thiamine

Prior to its use as a coenzyme thiamine must undergo pyrophosphorylation (on the OH group) to its active form thiamine pyrophosphate (TPP).[1] Thiamine can readily pass through cell membranes whereas TPP can not, so TPP is synthesized within the cell which requires it, the liver and kidney being particularly active sites of synthesis.

TPP serves as the coenzyme in three types of reactions: oxidative and non-oxidative decarboxylation of α-keto acids and transketolizations of α-hydroxyketones.[1] The mechanism of action of TPP is believed to involve the formation of the zwitter ion generated by the loss of the proton from the 2-position of the thiazole ring.[10,11,12]

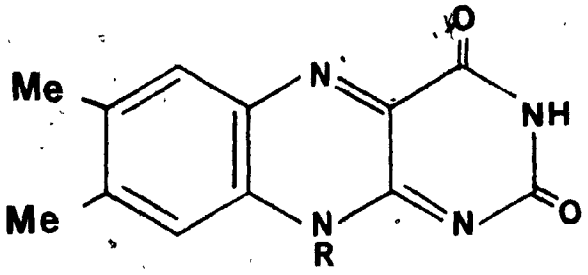


TPP

An important example of the role of TPP in oxidative decarboxylation is the pyruvate dehydrogenase enzyme complex.[1] The initial reaction is attack of the carbanoid thiazole ring position at the carbonyl carbon atom. The function of this enzyme complex is to convert pyruvic acid and coenzyme A to acetyl coenzyme A. The deficiency symptoms for a lack of thiamine are principally due to an accumulation of excess pyruvic acid in the system since the pyruvate decarboxylase process can not then be carried out properly.[1]

Riboflavin (vitamin B_2) is one of a group of compounds known collectively as the flavins, the common

feature of which is a substituted benzopteridine.[1] Riboflavin



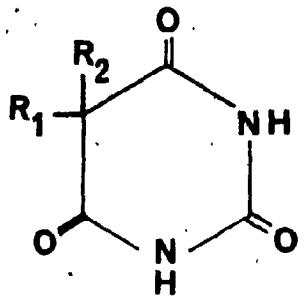
Riboflavin

R = $CH_2(CHOH)_3CH_2OH$

is a combination of the flavin ring system with the carbohydrate ribitol. Like thiamine, riboflavin is only active in a phosphorylated form (phosphorylated at the ribitol primary OH) and in this form it is commonly known as flavin mononucleotide. A deficiency of riboflavin does not lead to any specific disease but it does lead to inflammation of the tongue, lesions at the mucotaneous junctions of the eyes and lips and several other problems of this kind.[1]

Barbiturates are perhaps the best known class of pyrimidines of pharmaceutical importance. 5,5-Diethylbarbituric acid, the first known hypnotic barbiturate, was introduced into medicine by Fischer and von Mering in 1903.[13]

Barbiturates are normally prescribed in combination with other drugs for use as sedatives, tranquillizers, hypnotics, anti-convulsants and antiepileptics.[1]



Barbituric acid

$R_1 = R_2 = H$

The principal requirement for barbiturates to show activity is that there be 5,5-disubstitution by two lipophilic groups, both of which have at least two carbon atoms.[1] Hypnotic properties tend to increase with the length of the 5-substituents and commercial derivatives usually have up to 7 carbon atoms. Above 7 or 8 carbon atoms drug activity decreases, convulsant properties do not appear and the toxicity becomes a critical factor.[1]

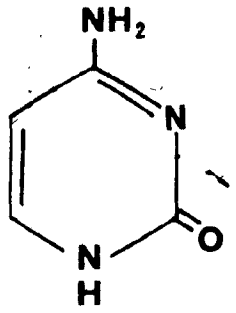
Barbiturates are known to have profound effects

upon the central nervous system but for the most part studies have only led to descriptions of their actions.[1] They are known to elevate the threshold of neurons by stabilization of the cell membrane and prolongation of the time required for recovery from excitation. Barbiturates are also known to cause increased activity in the hepatic microsomal poly-functional oxidases.[1]

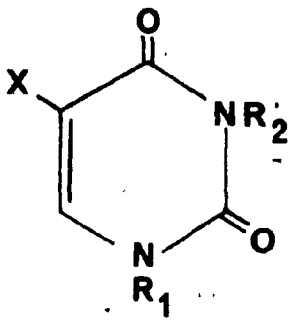
From this brief review it can be seen that pyrimidines have great biological, medicinal and pharmacological roles. A knowledge of the chemistry of these compounds is crucial for a complete understanding of the chemistry of nucleic acids and the biochemical pathways by which pyrimidine drugs, such as barbiturates, act. Surprisingly, however, relatively few mechanistic studies concerning the reactions of pyrimidines with electrophiles and nucleophiles have been reported.

This work includes the results of a kinetic study of the bromination of cytosine (1, structure next page) and some N-methylcytosine derivatives in aqueous acid. The reaction has been postulated to proceed via an addition-elimination process[15,16] and is now recognized to involve the formation of an observable long-lived intermediate.[17,18] The object of our study has been to elucidate the reaction mechanism in detail especially with regards to the identification of the species undergoing bromine attack and the

characterization of the long-lived intermediate.



1



21; X = H

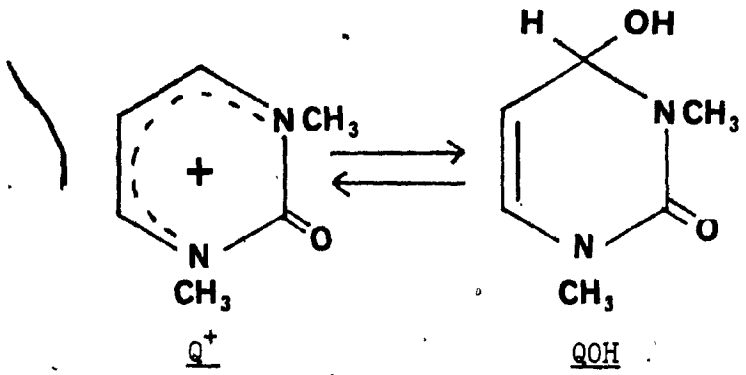
26; X = Br

Earlier[19] we presented evidence that the rates of bromination of 1,3-dimethyluracil (21, R_1=R_2=methyl) and 5-bromo-1,3-dimethyluracil (26, R_1=R_2=methyl) are invariant with pH in aqueous solution whereas for the parent molecules, lacking methyl groups, the rates increase with pH. We suggested that uracil (21, R_1=R_2=H) and 5-bromouracil (26, R_1=R_2=H) most likely can react as their anions formed by deprotonation at their N_1 positions.

We have now extended this study to include 1-methyluracil (21, R_1=methyl, R_2=H) and 3-methyluracil (21, R_1=H, R_2=methyl).[20] Should the mechanism proposed previously[19]

be correct, then the reactivity of 3-methyluracil with bromine should show definite acid dependency while the reactivity of 1-methyluracil should be acid independent.

We will also present a kinetic investigation of the equilibration of the 1,2-dihydro-1,3-dimethyl-2-oxo-pyrimidinium cation, $\underline{Q}^+$, and its pseudobase, $\underline{QOH}$, in aqueous solution. Our interest in this process has been prompted by



$\underline{Q}^+$ $\underline{QOH}$

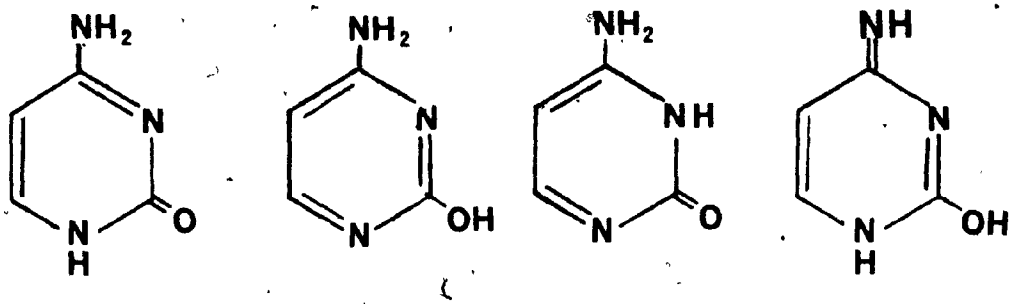
a study of the aqueous bromination of $\underline{Q}^+$ which postulated that bromine attack occurred on the pseudobase, $\underline{QOH}$.[21] A comparison of the rate constants describing the equilibration as obtained by the present work to those derived from the bromination study allow for an independent verification of the bromination results.[21]

Structure of Cytosine and Uracil

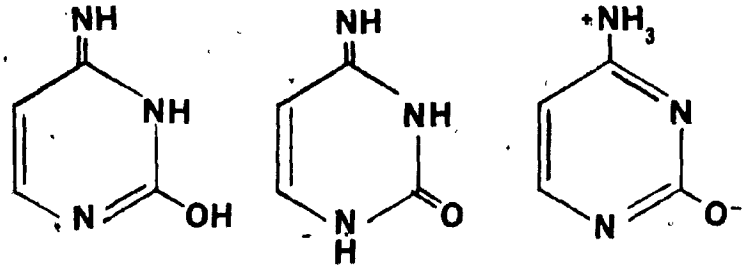
An understanding of the tautomeric properties of the fundamental biological pyrimidines, cytosine, uracil and thymine, is of extreme importance in molecular biology, especially with respect to the theory of mutations.[22] These properties are ascribed to the presence of the electron-releasing substituents NH_2 or OH. The labile hydrogen of these substituents may migrate to one of the ring nitrogens resulting in tautomer formation. Some important analogs of the above compounds may also contain the electron-donating group SH.[22]

Cytosine is usually represented as the 1(H)-amino-oxo form 1 (structure next page) both for the free molecule and in its nucleosides and nucleotides. X-ray crystal studies on cytosine [23-25] and its 1-ribosyl derivative, cytidine[26], support their existence in the amino-oxo form. However as many as six other tautomeric structures (structures next page) are theoretically possible[27,28], so that small amounts of rare tautomeric forms may exist along with 1 and may be important in the chemistry of cytosine. Nucleosides and nucleotides contain ribose or ribose phosphate substituents at the N_1 position and therefore may exist only in the forms 1, 15 or 17.

Until recently, evidence for the occurrence of cytosine tautomers has been based on indirect arguments.

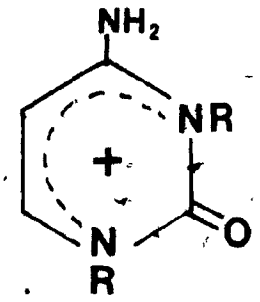


1 14 5 15



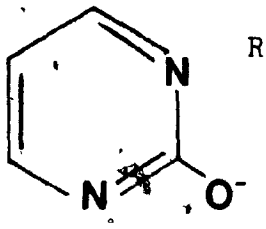
16 17 18

Katritzky and Waring[28] found that the uv spectrum of cytosine in both aqueous and dimethylsulphoxide solutions resembles that of of 1-methylcytosine but differs from that of 3-methylcytosine and of the anion of 2-pyrimidone, 100 (structure next page). These results support cytosine existing mainly as 1. They also showed that the spectra of the cations of cytosine and its 1- and 3-methyl derivatives are similar, and different from that of 2-pyrimidone, 101 (structure next page), indicating that cytosine undergoes protonation at the N_3 position (see structure 6, next page).

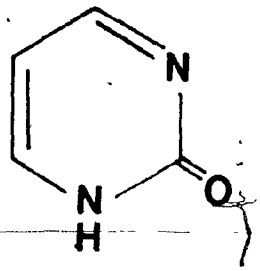


6

R = H or Me



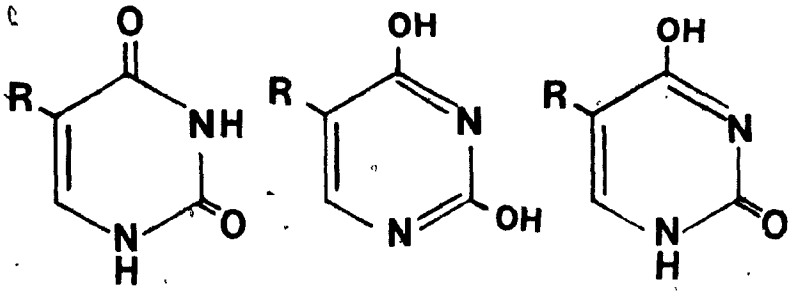
100



101

More recently, Dubois and co-workers[29] undertook a thermodynamic and kinetic study of tautomerization in cytosine and 3-methylcytosine using the temperature-jump relaxation technique together with uv and ir spectroscopy. They found that in aqueous solution cytosine exists primarily as the 1(H)-amino-oxo form 1 but tautomerizes slightly (0.25%) to the 3(H)-amino-oxo form 5 as well.

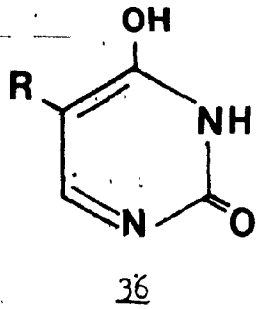
Uracil (or thymine, its 5-methyl derivative) like cytosine, can, in principle, exist in various tautomeric forms, 21 and 34-38 (structures next page).[27] However, a variety of experimental evidence points to uracil and thymine having the diketo structure 21. This form is suggested by



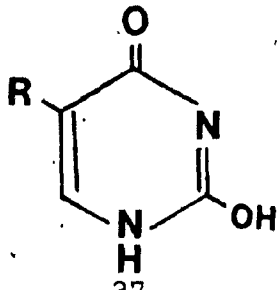
21

34

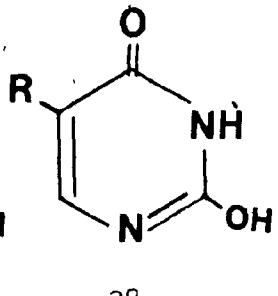
35



36



37



38

R = H or Me

X-ray crystallographic studies of uracil[30,31], thymine[32,33] and of derivatives of these molecules.[34-37] This structure has also been confirmed for uridines and deoxyuridines.[38-41] For all these uracils the lengths of the ring C-O bonds are 120-125 pm, corresponding to a very high double bond character.[27]

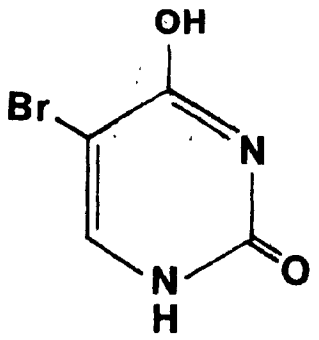
Analysis of the ir spectra of uracil, thymine and their nucleosides and nucleotides support these results. Miles[42,43], for example, has found that the spectra of uracil and thymine carrying N_1 and/or N_3 positions substituted by a methyl group or a sugar moiety are very similar to each

other in the carbonyl region but quite different from the spectrum of a corresponding enol ether. Strong additional evidence has been obtained by the comparison of the double bond stretching regions of uracil, 1-methyluracil and of 3-methyluracil with that of 1,3-dimethyluracil, which must have the diketo structure.[44] These results have also been confirmed by Raman[45,46], pmr[47-49] and uv[44,50-52] spectroscopy studies.

Direct evidence for the existence of uracil and thymine tautomeric forms comes from fluorescence emission spectroscopy.[53,54] Daniels[54] has shown that the fluorescing tautomer contains an enol group and based on uv data he assigned it to the form 35 (see page 15 for structure). The second tautomer, in equilibrium with 35, he assumed to be the predominant diketo form 21.

5-Bromouracil is known to have a greater tendency towards tautomerization than does uracil.[49] The bromine substituent at the 5-position helps to stabilize the enol form 39 (structure next page) by decreasing electron density about the ring nitrogens. This is borne out by pK_a measurements, with the pK_a of 5-bromouracil being about 8.0 as compared to 9.5 for uracil.[55] 5-Bromouracil can replace a considerable amount of the thymine normally found in bacterial DNA. This incorporation has been shown to increase the probability of incorrect base-

pairing. That is, 5-bromouracil has a greater inclination to incorrectly pair with guanine instead of with adenine than does thymine. This mutagenic activity may due to the increased stability of the enol tautomer[56] of 5-bromouracil, 39.

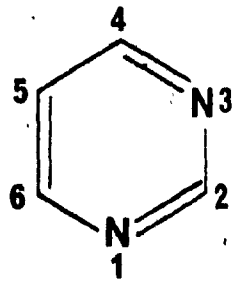


39

The Chemistry of Pyrimidines

The pyrimidine ring exhibits definite aromatic properties including resistance to oxidation.[55a] The electron-withdrawing capability of the two aza nitrogens results in the 2,4, and 6-positions being markedly electron deficient[42] (structure next page). In this, the positions resemble, in a qualitative fashion, the 2, 4, and 6-positions

of nitrobenzene and 1,3-dinitrobenzene. As a result pyrimidines readily undergo a wide variety of nucleophilic substitution reactions, but electrophilic substitution is confined to the 5-position and is very difficult in the absence of electron-releasing substituents.[55]



Pyrimidine

Thus, for example, a halogen at one of the three electron deficient positions (2, 4, or 6) is easily replaced by an amino group by reaction with ammonia or amines.[55] The 5-position, on the other hand, is the least electron deficient position in pyrimidine. Therefore a 5-halogen atom does not very readily react with ammonia or amines.[55]

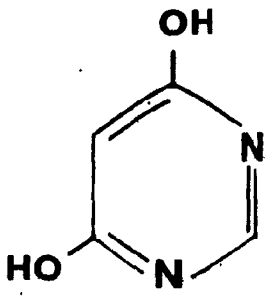
The presence of electron-releasing groups

such as hydroxy or amino substituents, particularly at positions 2, 4, and 6, counters the inductive effects of the ring nitrogens and helps to strengthen the pi electron system.[55] This increases the aromatic character of the ring. Hence the 5-position in cytosine (1, structure page 13), for example, readily undergoes bromination in aqueous media.[57] Conversely, potential leaving groups in the 2, 4, and 6-positions show reduced reactivity towards nucleophilic reagents.[55] Thus halogens at these positions are less easily replaced by amines (vide supra).

Electron-withdrawing groups with large resonance or inductive effects have only been successfully introduced at the 5-position of pyrimidine since they are labile at positions 2, 4, and 6.[55] A 5-nitro substituent, for instance, strongly accentuates the electron deficiency of the other positions, and thereby increases the rates of nucleophilic substitution reactions at these sites. As a result 2,4-dichloro-5-nitropyrimidine reacts quickly with ammonia at 0 °C to produce 4-amino-2-chloro-5-nitropyrimidine. The new amino group, which is of course electron-releasing, now acts in opposition to the electron-withdrawing effect of the nitro group causing substitution of the second chlorine to occur only at temperatures above 100 °.[55a]

For many years it was believed that at least two electron-supplying substituents must be present for

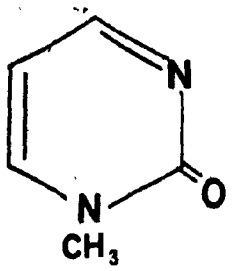
pyrimidines to successfully undergo nitration or nitrosation.[55] The positions of these substituents is also of critical importance. That is, whereas, 4,6-dihydroxypyrimidine, 40, can be nitrosated uracil, 21, does not undergo nitrosation at all.[55a]



40

In the past fifteen years, however, it has been shown that 5-nitration can occur under very rigorous conditions for pyrimidines containing only one electron-donating group. Thus 1,2-dihydro-1-methyl-2-oxopyrimidine (102, structure next page) can be nitrated to yield its 5-nitro derivative.[55b]

The nitration of uracil is usually performed in boiling fuming nitric acid.[58,59] Cytosine[60] and N-alkyl



102

derivatives of uracil[61] require less harsh conditions. The best procedure is to carry out the reaction in sulfuric acid with the addition of the theoretical amount of nitric acid at 40-50 °.[55a]

5-Nitrosopyrimidines are most commonly prepared by the addition of sodium nitrite to an acidic solution of the appropriate pyrimidine. When three elecron-donating groups are present the reaction may take place in aqueous acetic acid with sodium nitrite at ambient temperature.[55a]

Pyrimidines with at least one electron-releasing substituent can couple with diazotized aryl amines to yield 5-arylazo derivatives.[55] Subsequent reduction under very mild conditions provides a convenient method for producing 5-aminopyrimidines. This route is especially useful when sugar moieties or other potentially labile groups are present.

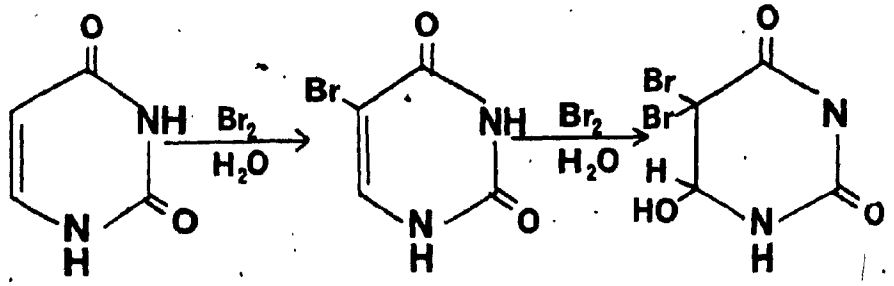
Sulphonation at position 5 takes place normally only under very rigorous conditions and requires the presence of at least one amino or hydroxy group. Chlorosulphonation of uracil at 110 ^{o} forms 5-chlorosulphonyluracil in reasonable yields.[62-64] Similar treatment of cytosine and 6-methylcytosine, however, produces the corresponding 5-sulphonic acid rather than the desired 5-sulphonyl chloride.[64,65]

Direct halogenation always occurs at the 5-position. These reactions proceed very easily if more than one electron-releasing substituent is present.[55a] Pyrimidine hydrochloride under specific conditions, however, can be brominated to yield 5-bromopyrimidine.[66,67]

Chlorination by gaseous elemental chlorine can be difficult to perform and often leads to poor yields. Nevertheless, 2,4-diamino-6-chloropyrimidine can be converted with this reagent to its 5-chloro derivative.[68] Often

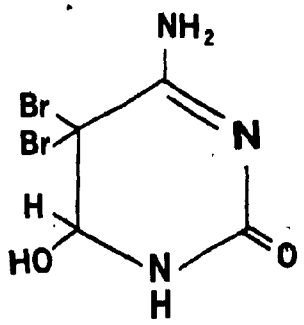
sulphuryl chloride or N-chlorosuccinimide (NCS) are used as convenient chlorinating agents[55b], for instance, NCS in acetic acid has been used to make 5-chlorocytosine.[69]

Direct bromination using bromine, often in acetic acid, usually gives yields greater than 82%.[55b] Uracil (21) reacts with excess bromine in aqueous solution to produce an isolatable 5,5-dibromo addition product, namely 5,5-dibromo-6-hydroxy-5,6-dihydrouracil (28).[16,57,60-62]



21 26 28

Although 28 is quite stable, it can be converted into 5-bromouracil (26) by boiling in dilute mineral acid.[15] Cytosine also reacts with excess bromine, but produces the uracil derivative 28 as the major product[18], rather than the expected cytosine derivative, 4 (structure next page).



4

This is due to the ease with which dihydrocytosines undergo deamination in aqueous media.[18]

Brominations are sometimes performed with N-bromosuccinimide, especially where the pyrimidine bears acid-sensitive groups. In this way, for example, the 5-bromo derivative of 4,6-dimethoxypyrimidine has been made.[73]

Iodinations are best carried out using N-iodosuccinimide.[55b] Iodine monochloride, however, can be used to prepare 5-iodouracil in good yield.[74] It has also been used to convert 1-benzyloxy and 3-benzyloxyuracil to their corresponding 5-iodo derivatives.[75]

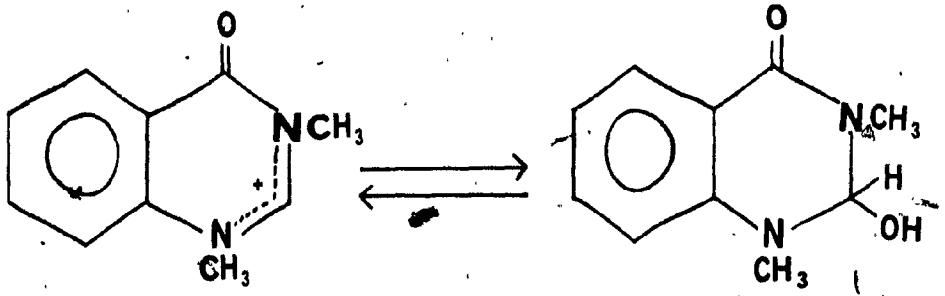
Pseudobase Formation

A pseudobase is the covalent adduct formed by the formal addition of hydroxide ion to an unsaturated heterocyclic cation in aqueous solution. Even though such adducts were first observed by Decker[76] and Hantzsch[77,78] near the close of the 19th century it has only been recently that the kinetics of pseudobase-cation equilibria have been studied.[89] One such investigation is presented further on in this thesis (see Table of Contents for page number).

The concept of pseudobase formation is very similar to that of covalent hydration[79-82] and to Meisenheimer complex formation.[83-88] These reactions involve sigma bond formation by nucleophilic addition to an electron deficient aromatic species.

Spectroscopic studies have been extensively used in pseudobase investigations since pseudobase formation results in pH dependent electronic and nmr spectra.[89] Therefore should the cation contain no easily ionizable protons, such spectral changes accompanying the addition of hydroxide ion can usually be attributed to pseudobase formation, particularly when very similar changes are seen upon the addition of methoxide ion.[89]

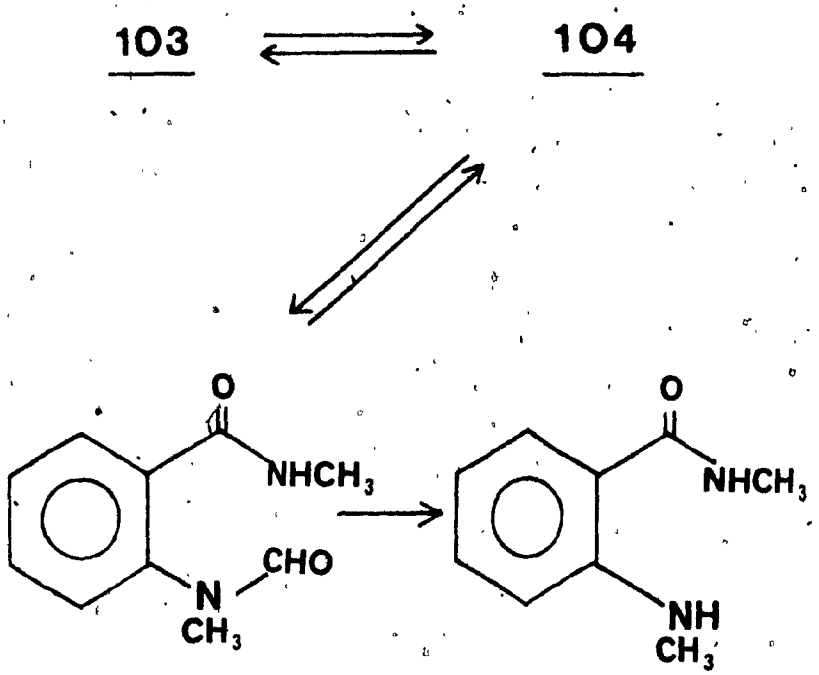
Relatively large spectral changes accompany pseudobase formation especially if it disrupts the aromatic character of a ring. For example, the 3,4-dihydro-1,3-dimethyl-4-oxoquinazolium cation (103) recently investigated by Tee, McClelland and co-workers[90] has λ_{max} ~295 nm (ϵ~6000) whereas its pseudobase (104) has λ_{max} ~ 340 nm. (ϵ~2500).



103 104

Normally these spectral changes are reversible. Appropriate acidification of a solution of the pseudobase results in the reformation of the cation, a test which may be used to rule out irreversible chemical reactions. Sometimes, however, the pseudobase may undergo ring-opening to produce its amino-carbonyl tautomer. For example 104 with

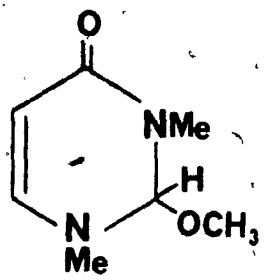
increasing pH undergoes reversible ring-opening via its anion to a formanilide derivative, 105.[90] The formanilide in aqueous base can undergo irreversible hydrolysis to ortho-aminobenzamide, 106.



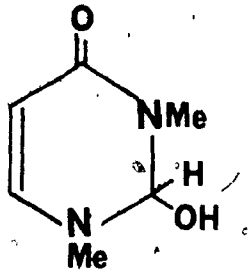
105 106

The methoxide adduct of a heterocyclic cation in basic methanol gives rise to a uv spectrum very similar to that of the pseudobase. For instance, the methoxide adduct 107 and the pseudobase 108 of the 1,4-dihydro-1,3-dimethyl-4-oxopyrimidinium cation 109 have almost identical spectral properties (see next page). Any differences between the spectra can be attributed to solvent effects. Since methoxide adducts can not undergo ring-opening any marked difference between the spectrum of a cation dissolved in

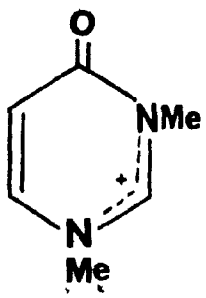
basic methanol and the spectrum in aqueous base suggests that the pseudobase is undergoing ring-opening to some extent in the aqueous solution.



107



108

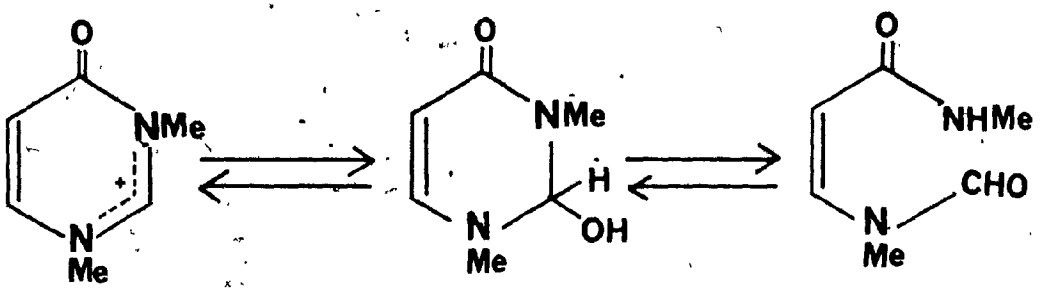


109

	107	108	109
λ_{max}	286	289	231, 262
log ϵ	4.00	3.96	4.06, 3.47
ref.	91	92	92

The pseudobase 108, for example, undergoes reversible ring-opening via its anion to the β-formamido acrylamide 110 (see next page). The uv spectrum of this solution shows maxima at 250 and 289 nm a noticeable change from that of the methoxide adduct.[92]

Another, though often less satisfactory, test for ring-chain tautomerization is a comparison of the spectrum of the dihydro derivative of the cation with that of the pseudobase. Even though the different electronic

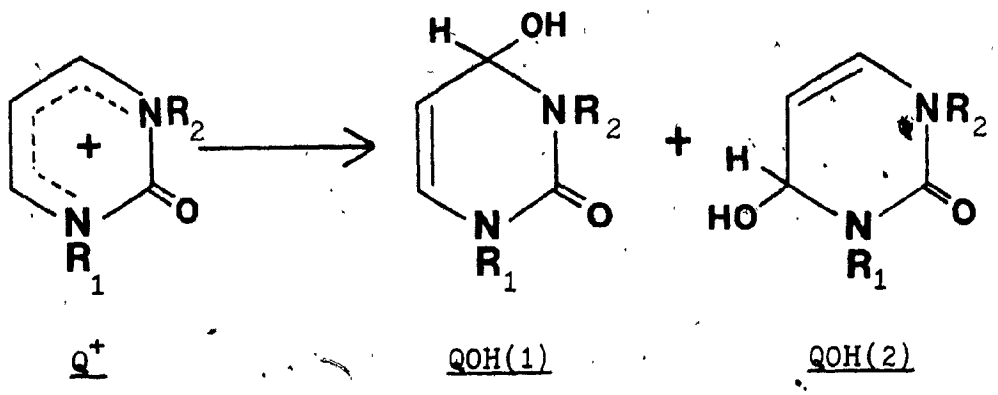


109 108 110

effects of OH and H can sometimes lead to relatively large differences in absorption maxima, Bunting and Meathrel[93] have successfully employed this approach to show that the N-methyl cations of diazanaphthalenes exist predominantly as their pseudobases in aqueous base.

Pseudobase formation can also be identified by pmr spectroscopy. The saturation of a formerly unsaturated carbon, which occurs in pseudobase production, results in a characteristic upfield chemical shift of approximately 4 ppm for the signal from a hydrogen on that carbon.[89] The chemical shifts of other hydrogens on nearby carbons may

also be affected, especially if disruption of an aromatic or conjugated pi system has taken place. For example, Tee and Endo[94] have used pmr to characterize the pseudobases of various 1,3-disubstituted-1,2-dihydro-2-oxopyridinium cations, $\underline{Q}^+$, for $R_1 \neq R_2$. Additionally, pmr allowed for the estimation of the relative amounts of the two pseudobases QOH(1) and QOH(2) formed.



$\underline{Q}^+$ QOH(1) QOH(2)

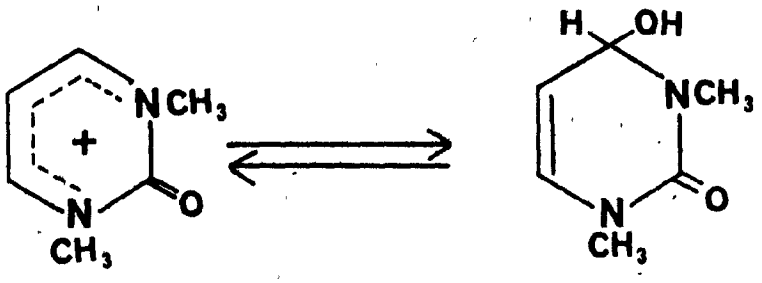
There are examples known of reactions of heterocyclic compounds which are believed to proceed via pseudobase or via covalent hydrate intermediates. In very few cases have detailed mechanistic studies of these reactions been carried out. However, work originating from our laboratory in recent years has definitely shown the

involvement of pseudobases and covalent hydrate intermediates in the bromination of various pyrimidine derivatives. It has been established from the pH dependence for the rates of bromination of derivatives of 4(3H)-quinazolinone[95], 2(1H)-pyrimidone[128], 5-bromo-2(1H)-pyrimidone[96] and 4(3H)-pyrimidinone[129] cations that bromine attack occurs upon the pseudobases or covalent hydrates of the starting substrates.

This thesis includes a report on the formation and decomposition of the pseudobase of the 1,2-dihydro-1,3-dimethyl-2-oxopyrimidinium cation, $\underline{Q^+}$ (structure next page), in aqueous media. The bromination of $\underline{Q^+}$ was postulated to proceed via its pseudobase, $\underline{QOH}$.[148] To provide support for this hypothesis and to complement the bromination studies of Thackray[21], we have observed the uv spectrum of the $\underline{QOH}$ and measured the equilibrium and rate constants associated with its formation and decomposition.

Stopped-flow Methods

For the most part the kinetics presented in this thesis required the stopped-flow technique. The origins and characteristics of this method were extensively reviewed in our earlier thesis[19], and therefore they are not discussed here.



Q^+ QOH

Objectives

The work carried out for this thesis falls into three areas:

1) The primary project was to study the bromination of cytosine and related derivatives in aqueous solution; to observe, if possible, the postulated intermediates, and to determine the mechanism of their formation. In this we were successful, and the bulk of the work has been published recently.[149,152]

2) Another objective was to provide additional support for the conclusions of our earlier thesis[19] on the bromination of uracils. For the most part this was also successful and a paper on these studies has appeared.[20,150] However, there are still some anomalies of the uracil system that we have not solved.

3) It seemed profitable to study the equilibration of the cation $\underline{Q^+}$ and its pseudobase $\underline{QOH}$ in aqueous solution (vide supra). The object here was to provide an independent determination of the rate constants associated with this equilibration, and to provide complementary evidence for the bromination study of $\underline{Q^+}$ carried out by Thackray.[21] These results were quite concordant and were published sometime ago.[21,151]

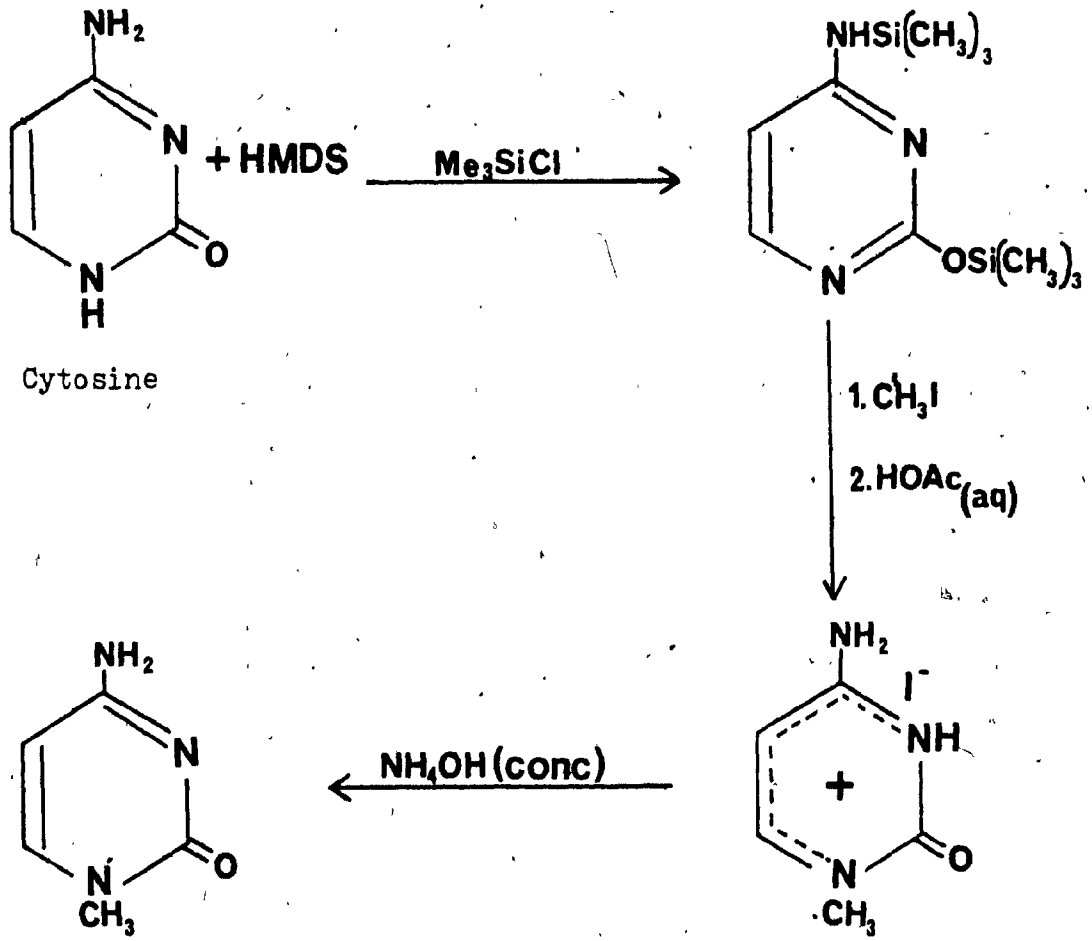
EXPERIMENTAL

Materials

Cytosine, cytidine and 3-methylcytosine were obtained from commercial sources and recrystallized before being used. 1-Methyluracil (prepared by C. Solomon) and 1-methylcytosine were prepared by procedures based on the method of Santi[97], which was developed for the preparation of micro-quantities of these compounds. 5-Bromocytosine was made by the direct reaction of bromine with cytosine.[18,57] N-acetylcytosine[98] and N-acetyl-1-methylcytosine[99] were obtained by reaction of the parent compounds with acetic anhydride. 3-Methyluracil[61] was prepared by the methylation of 2-thiouracil followed by hydrolysis in aqueous acid. All melting points were measured with a Gallenkamp Melting Point Apparatus and are uncorrected.

1-Methyluracil[97] (Prepared by C. Solomon): Uracil was converted to its bis(trimethylsilyl) derivative using the method of Nishiumura and Iwai (see outline of procedure, next page).[100] This was followed by "in situ" reaction with an excess of methyl iodide in benzene to give 1-methyluracil, 28% yield from uracil. The trimethylsilyl groups were lost during the work-up of product.

1-Methylcytosine[97]: This procedure involves the preparation of 1-methylcytosine hydroiodide which is then converted to the free base by treatment with base (see outline, next page).



Cytosine

1-Methylcytosine

Synthesis of 1-Methylcytosine

Cytosine (4.8 g, 43 mmol) and chlorotrimethylsilane (4 mL, 32 mmol) were mixed together in 200 mL 1,1,1,3,3,3-hexamethyldisilazane (HMDS, 943 mmol) and refluxed for 1 hour. After cooling to room temperature, iodomethane (40 mL, 643 mmol) was added and the resulting mixture was refluxed for 2 hours. The yellowish mixture was stirred at ambient temperature for 12 hours and then rotary evaporated leaving yellow crystals. Dissolution of the crystals in 110 mL of 10% aqueous acetic acid produced a two phase mixture. The mixture was then rotary evaporated until only one phase remained and a precipitate was beginning to form. The yellowish-white crystals were collected at the pump and the filtrate was reduced to half its volume which, after cooling, allowed more 1-methylcytosine hydroiodide to be obtained: 6.5 g (60%, later preparations gave yields as high as 77%); mp 260-265 °C (dec).

Conversion to the free base was achieved by stirring a suspension of pulverized 1-methylcytosine hydroiodide (14.9 g, 59 mmol) in 47 mL of concentrated aqueous ammonia for 2 hours at ambient temperature followed by refrigeration at 0 °C for 1 hour. The white crystals were collected and recrystallized from absolute ethanol: 6.1 g (82%); mp 290 °C (dec) (lit mp 285 ° (dec), 300 °)[97,99].

5-Bromocytosine[18,57]: This compound was first obtained as its hydrobromide. Bromine (1.16 mL, 23 mmol) was added

dropwise to a warm solution of cytosine (2.5 g, 23 mmol) in 70 mL water. The yellowish crystals of the hydrobromide salt which separated from solution were collected and recrystallized from ammoniacal H_2O: 2.2 g (35%); mp 245-248 °(dec) (lit mp 255-256 ° (dec), 244-255 ° (dec)).[18,101]

The hydrobromide was converted to its free base by stirring in base. A suspension of pulverized 5-bromocytosine hydrobromide (0.38 g, 1.4 mmol) in 7 mL of water was treated with concentrated aqueous ammonia (1.1 mL). After stirring at ambient temperature for 2 hours the mixture was kept at 0 ° for 12 hours. The white crystals of 5-bromocytosine were collected, washed with cold water and then recrystallized from water: 0.24 g (90%); mp 235-240 ° (dec) (lit mp 240 ° (dec)).[102,103]

N-acetylcytosine[98]: Cytosine (4.9 g, 44 mmol) was added to a mixture of acetic anhydride (50 mL) and acetic acid (10 mL). After refluxing the resulting suspension for 24 hours, the white crystals of product were collected and washed with cold ethanol followed by ether: 6.3 g (93%); mp 316-318 ° (reddened) (lit mp 326-328 °).[104] N-acetyl cytosine was successfully recrystallized from dimethylsulphoxide.

N-acetyl-1-methylcytosine[99]: 1-Methylcytosine (0.5 g, 4 mmol) was added to a solution of acetic anhydride (1.2 mL) in pyridine (11 mL). After refluxing the mixture for 1 hour white crystals of N-acetyl-1-methylcytosine were collected:

0.64 g (96%); mp 269 ^{o} (lit mp 268 ^{o}).[99]

3-Methyluracil[61]: 2-Thiouracil (10 g, 78 mmol) in 5N NaOH (45 mL) was reacted with dimethyl sulphate (211 mmol). After standing, yellow crystals of 1,6-dihydro-1-methyl-2-methylthio-4-oxopyrimidine (I, 2.4 g, 12%) were collected. Refluxing I (0.97 g) for 1 hour in aqueous HCl (20% w/w, 10 mL) afforded, after the removal of solvent, 3-methyluracil: 0.55 g (70% from I); mp 176-177 ^{o} (lit mp 179 ^{o}).[61]

Bromine solutions were prepared by the dilution of appropriate amounts of a more concentrated solution. The latter was usually made 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.

All inorganic reagents used for kinetic experiments were of analytical grade. Buffer solutions of 0.01 M or 0.02 M ionic strength were prepared according to Perrin.[105] Perchloric acid solutions were made from commercial 60-62% solutions (Anachemia or Fisher) by weight.

Procedures

Kinetic experiments were performed using

an Aminco-Morrow Stopped-flow Accessory [19,106] attached to an Aminco DW-2 Uv-Visible Spectrophotometer operating in the dual wavelength mode. In this mode one monochromator is set at a reference wavelength where little or no absorbance change occurs during the reaction under observation and the other monochromator is placed at a sampling wavelength where there is a relatively large change in absorbance. The reference and sample beams are then alternately passed through the stopped-flow observation cell at 250 or 1000 Hz. The procedure for setting up the stopped-flow apparatus has been described in our earlier thesis.[19]

Temperature control was maintained by a Lauda 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.

Absorbance changes from stopped-flow experiments were recorded with a Biomation 805 Waveform Recorder, and the stored traces were displayed on an auxiliary oscilloscope. Acceptable traces were plotted onto large graph paper by connecting the Biomation 805 to the X-Y recorder of the Aminco DW-2. For a few cases the stored digitized traces were outputted from the Biomation 805 via a Datos 305 interface to a Teletype Model 43 printer. However, obtaining hard copy of the stored traces in their

analog forms proved to be the method of choice especially when dealing with extremely fast reactions or with traces of small absorbance changes which contain considerable noise.

Reactant solutions for the bromination studies were 0.1 M in KBr. The use of this relatively large concentration of bromide ion (substrate concentrations $\leq 5 \times 10^{-4}$ M) has several advantages:

1) It swamps the effect of Br^- produced by the bromination reaction.

2) It increases the stability of the bromine solution since most of the bromine is present as Br_3^-.

3) It decreases the rate of reaction by reducing the free bromine concentration.

4) It facilitates measurements of rates since Br_3^- has a much larger extinction coefficient than Br_2.

5) It ensures that the ionic strength is high and constant (0.11 M) for all the experiments in buffer solutions.

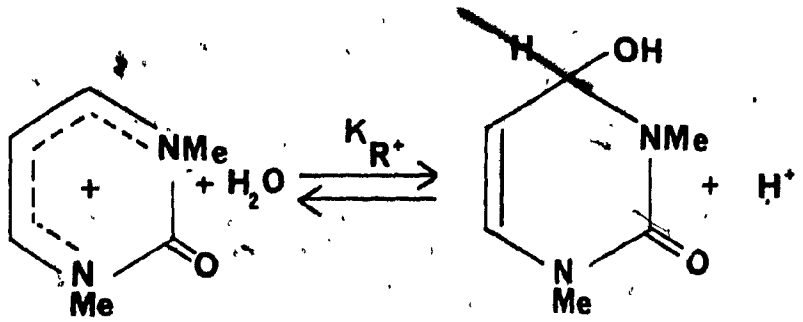
The rates of bromine disappearance were measured by monitoring the decrease in Br_3^- absorbance (λ_{max}=266 nm; logϵ=4.54)[107] with time. The sample

monochromator was set as close to 266 nm as possible, depending upon which wavelength gave the largest signal to noise ratio, and the reference monochromator was placed between 300 and 350 nm depending on the substrate used.

Solutions used in the cytosine iodination experiments were 0.1 M in KI. The presence of I^- provides benefits analogous to those ascribed to Br^- in the bromination experiments (vide supra). The rates of iodine consumption were monitored at 350 nm, where I_3^- strongly absorbs, relative to 400 nm.

The value of pK_{R^+} for the equilibrium between 1,2-dihydro-1,3-dimethyl-2-oxopyridinium cation, $\underline{Q^+}$, and its pseudobase, $\underline{QOH}$, was measured spectrophotometrically[108] at 30 °C, [KBr]=0.1 M and a total ionic strength of 0.11 M. The reason for choosing these experimental conditions will be discussed in the Results and Discussion section. $\underline{Q^+}$ and $\underline{QOH}$ have uv absorption maxima well separated from one another, and since the absorbance differences between $\underline{Q^+}$ and $\underline{QOH}$ are very great at these wavelengths, both wavelengths were suitable as analytical wavelengths.

The equilibrium under consideration may be represented as (see next page)



(1)

Q^+ QOH

where[109]

$$K_{R^+} = \frac{[QOH]\,[H^+]}{[Q^+]} \qquad (2)$$

The value of pK_{R^+} may be obtained using the following equation:[108]

$$pK_{R^+} = pH + \log\left[\frac{d_M - d}{d - d_I}\right] \qquad (3)$$

where

d_M = OD of QOH at the analytical wavelength,

d_I = OD of Q^+ at the analytical wavelength,

and d = OD of a buffer solution containing a mixture of Q^+ and QOH.

The analytical wavelengths were 239 nm, λ_{max} of QOH, and 314 nm, near the λ_{max} of Q^+. The value for d_M was obtained from the spectrum of QOH in a NaOH solution of pH 9.10. The value for d_I was measured from the spectrum of Q^+ in a buffer solution of pH 2.67. Using λ = 239 nm as the analytical wavelength gave 7.15 ± 0.04 for pK_R^+, whereas with λ = 314 nm as the analytical wavelength a value of 7.17 ± 0.03 was obtained. Accordingly in the text we have adopted the value of pK_R^+ = 7.16.

Kinetic studies of the formation and decomposition of QOH were performed in the manner of Bunting and Meathrel.[110] One drive syringe of the stopped-flow was filled with a solution 5×10^{-4} M in Q^+ in pH 2.00 sulphuric acid solution (or QOH in pH 9.10 sodium hydroxide solution) and 0.1 M in KBr. The other drive syringe contained a buffer solution, prepared after Perrin[105], but of double the usual ionic strength of 0.01 M with $pH > pK_R^+$ (or $pH < pK_R^+$) and 0.1 M in KBr. Hence after mixing the ionic strength of the solution under observation was 0.11 M.

The disappearance of $\underline{Q^+}$ (or $\underline{QOH}$) at 316 nm (or 239 nm) relative to 270 nm (spectrum minimum) was then monitored.

Proton nmr spectra were obtained on Varian A-60 and T-60 spectrometers. Chemical shifts are reported relative to internal DSS. Bromination reactions were initiated by the addition of one or two drops of bromine to relatively concentrated solutions of substrates (0.5 to 1 M) in acid, usually 1 N DCl. This acid masks the effect of HBr produced and facilitates solubilization.

Treatment of Data

For reactions exhibiting first-order behavior rate constants, k_1^{obsd}, were obtained using "normal", Swinbourne or Guggenheim analyses of the data. For certain bromination reactions the substrate concentration was not present in any large excess and therefore the data for these runs were analyzed directly for second-order behavior.

"Normal" Treatment[111]: For a reaction which follows first-order kinetics the rate may be represented as

$$\frac{dx}{dt} = k_1(b - x) \qquad (4)$$

where b is the initial concentration of the chemical species B at t = 0, x is the amount of B which has reacted

by time t and k_1 is the first-order rate constant.

Integration of equation 4 gives

$$k_1 t = \ln \frac{b}{(b - x)} \qquad (5)$$

Consider the following reaction which goes to completion in which a, the initial concentration of A, is in a large excess with respect to b, the initial concentration of B. If the progress of the reaction (represented by equation 6) is monitored spectrophotometrically,

$$A + B \longrightarrow P \qquad (6)$$

assuming the Beer-Lambert Law[112] is obeyed and unit sample thickness, then the following relations will hold.

$$A_0 = \varepsilon_A a + \varepsilon_B b \qquad (7)$$

$$A = \varepsilon_A(a - x) + \varepsilon_B(b - x) + \varepsilon_P x \qquad (8)$$

and

$$A_\infty = \varepsilon_A(a - b) + \varepsilon_P b \qquad (9)$$

where A_0 is the initial absorbance at t = 0, A_∞ is the final absorbance when the reaction is complete and A is the absorbance at a time t during the course of the reaction.

The extinction coefficients ε_A, ε_B, and ε_P refer to those associated with A, B, and P at the analytical wavelengths used.

For $a \gg b$, equations 7 to 9 may be manipulated to give

$$b = \frac{A_0 - A_\infty}{\varepsilon_B - \varepsilon_P} \qquad (10)$$

and

$$b - x = \frac{A - A_\infty}{\varepsilon_B - \varepsilon_P} \qquad (11)$$

Equation 5 may then be rewritten as

$$k_1 t = \ln \frac{b}{(b - x)} = \ln \frac{(A_0 - A_\infty)}{(A - A_\infty)} \qquad (12)$$

or

$$\ln (A - A_\infty) = \ln (A_0 - A_\infty) - k_1 t \qquad (13)$$

The value of k_1 can then be obtained from the slope given by the least-squares analysis of $\ln (A - A_\infty)$ against time. Only those data which covered 3 to 4 half-lives (about 90% reaction) and which in least-squares analysis gave correlation coefficients ≥ 0.9995 were deemed acceptable.

The validity of the above analysis requires

a correct assessment of A_∞. Small variations in A_∞ while hardly affecting the least-squares analysis can cause relatively large differences in k_1.[153] Therefore it is important to monitor the reaction for as long a period of time as possible.

Hence all first-order reactions were followed for greater than 10 half-lives in order to obtain the best possible assessment for the value of k_1. However, since absorbances become small relative to noise towards the end of a reaction the Swinbourne treatment was performed and the value of A_∞ determined by this approach was compared to that obtained by direct measurement.

Swinbourne Treatment of Data[113,161]: For a reaction exhibiting first-order kinetics 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 recorded at times $t_1 + T$, $t_2 + T$, ... $t_n + T$, where T is a constant, then equation 13 may be rewritten as

$$(A - A_\infty) = (A_0 - A_\infty)e^{-k_1 t} \qquad (14)$$

for the first set of readings and as

$$(A' - A_\infty) = (A_0 - A_\infty)e^{-k_1(t + T)} \qquad (15)$$

for the second set of data.

Dividing equation 14 by equation 15 gives

$$\frac{(A - A_\infty)}{(A' - A_\infty)} = e^{k_1 T} \qquad (16)$$

Therefore $$A = A_\infty(1 - e^{k_1 T}) + A' e^{k_1 T} \qquad (17)$$

Least-squares analysis of A versus A' allows k_1 to be obtained from the slope (slope = $e^{k_1 T}$) and A_∞ from the intercept and slope since

$$A_\infty = \frac{\text{intercept}}{(1 - \text{slope})} \qquad (18)$$

For best results the data should span a period of time greater than one half-life and preferably greater than two. Normally our data spanned three. The value of T should be between one half and one half-life.

The value of A_∞ obtained in this manner can be inserted into the "normal" method. The values for k_1^{obsd} referred to in this thesis were all determined by the "normal" treatment utilizing the Swinbourne value for A_∞.

Guggenheim Treatment of Data[111,161]: This method can also be used for first-order reactions where A_∞ is not known. Subtracting equation 14 from equation 15 gives (see next page)

$$(A' - A) = (A_0 - A_\infty)e^{-k_1 t}(e^{-k_1 T} - 1) \qquad (19)$$

and hence

$$\ln (A' - A) = \ln ((A_0 - A_\infty)(e^{-k_1 T} - 1)) - k_1 t \qquad (20)$$

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

Linear regression analysis of ln (A' - A) against t yields a straight line of slope $-k_1$. For optimum results the data should cover at least two half-lives and T should be between one half and one half-life, as for the Swinbourne treatment.

The rate constants determined by the "normal" method were always compared to those obtained by the Guggenheim treatment. The rate constants for acceptable runs were found to agree to within 5% for all cases.

Second-Order Treatment[111]: Consider the reaction between chemical species A and B to produce P. If the reaction follows second-order kinetics then the rate equation may be written as

$$\frac{dx}{dt} = k_2(a - x)(b - x) \qquad (21)$$

where x is the concentration of A or B that has reacted by

time t. If a > b then integration of equation 21 gives

$$\frac{1}{a - b} \ln \frac{a(b - x)}{b(a - x)} = k_2 t \qquad (22a)$$

or $$\ln \frac{(b - x)}{(a - x)} = \ln \frac{b}{a} + (a - b)k_2 t \qquad (22b)$$

Should the reactions be monitored spectrophotometrically (assuming unit sample width and that the Beer Lambert Law[112] is obeyed) then equations 7 - 9 would be applicable. Using these equations it can be shown that since

$$A_0 - A = x(\varepsilon_A + \varepsilon_B - \varepsilon_P) \qquad (23)$$

then $$x = \frac{(A_0 - A)}{\varepsilon_A + \varepsilon_B - \varepsilon_P} \qquad (24)$$

Subtracting equation 9 from equation 7 leads to

$$A_0 - A_\infty = (\varepsilon_A + \varepsilon_B - \varepsilon_P)b \qquad (25)$$

and therefore $$(\varepsilon_A + \varepsilon_B - \varepsilon_P) = \frac{A_0 - A_\infty}{b} \qquad (26)$$

Equation 24 may then be rewritten as

$$x = \frac{(A_0 - A)}{(A_0 - A_\infty)} b \qquad (27)$$

Thus x at various times t during the life of a reaction can be obtained from absorbance measurements. Linear regression analysis of equation 22b may now be carried out allowing k_2 to be obtained from the slope.

For the special case where a = b equation 21 reduces to

$$\frac{dx}{dt} = k_2(b - x)^2 \qquad (28)$$

which upon integration becomes

$$\frac{1}{(b - x)} - \frac{1}{b} = k_2 t \qquad (29a)$$

or

$$\frac{x}{b(b - x)} = k_2 t \qquad (29b)$$

Again if the reaction is monitored spectrophotometrically and the Beer Lambert Law[112] applies then for a = b equations 7 to 9 may be manipulated to give

$$A_0 - A = x(\varepsilon_A + \varepsilon_B - \varepsilon_P) \qquad (30)$$

and

$$A - A_\infty = (b - x)(\varepsilon_A + \varepsilon_B - \varepsilon_P) \qquad (31)$$

Therefore $$\frac{x}{(b - x)} = \frac{A_0 - A}{A - A_\infty} \qquad (32)$$

and equation 29b may be written as

$$\frac{A_0 - A}{b(A - A_\infty)} = k_2 t \qquad (33a)$$

or $$\frac{A_0 - A}{A - A_\infty} = b(k_2 t) \qquad (33b)$$

Hence least-squares analysis of equation 33b allows k_2 to be determined from the slope. For the proper analysis of the data it is necessary to know A_0 and A_∞ (see equations 27 and 33b). All reactions were followed by monitoring an absorbance decrease with time. Reasonable estimates for A_0 were obtained by extrapolating the recorded absorbance curves back to t = 0. The value for A_∞ was estimated by observing the reaction for greater than 20 half-lives.

Computer Programs

Most computer calculations were performed on a CDC CYBER 172 Computer using programs written in BASIC by Dr. O. S. Tee or the author. Programs were written which permitted linear regression analysis of kinetic data by

either first- or second-order treatments detailed above. An important feature of these programs is that they were designed to read data stored in permanent ASCII files so that analysis of the data by various methods could be easily carried out if necessary. First-order programs calculated k_1 from absorbance data by the Guggenheim, Swinbourne or "normal" methods. Rate constants from the "normal" treatment were obtained using both the observed and Swinbourne values for A_∞.

Recently our data acquisition and analysis have been improved by the purchase of an Apple II microcomputer system. It is now possible to analyze absorbance data "on site" while carrying out the kinetic experiments. The absorbance data, stored in the Biomation 805 in digital form, can be transferred through the Datos 305 directly into the memory of the Apple II.[90] The data can then be analyzed by the methods outlined above.

RESULTS AND DISCUSSION

Effective Halogen Concentration

The reactions of bromine with uracils and cytosines were found to be overall second-order (vide infra): first-order in bromine concentration and first-order in substrate concentration. Hence

$$\text{rate} = k_2^{app}[S][Br_2]_s \qquad (34)$$

where $[S]$ is the substrate concentration, $[Br_2]_s$ is the stoichiometric bromine concentration and k_2^{app} is the apparent-second-order rate constant.

Kinetic experiments were normally carried out with at least a tenfold excess of substrate. Under such conditions, where the excess of substrate over bromine is not particularly large, the observed first-order rate constant, k_1^{obsd}, is directly proportional to $([S] - [Br_2]_s)$, the concentration of substrate that remains constant during the reaction, as first proposed by Bell and Ramsden[114] and later used by Tee and co-workers.[20,96] That is,

$$k_1^{obsd} = k_2^{app}([S] - [Br_2]_s) \qquad (35)$$

For reasons explained in the Experimental section (see page 40) all solutions contained a large

excess of bromide ion, and so account must be taken of the depletion of free bromine due to the formation of tribromide ion.[95] At higher pH hypobromous acid formation becomes significant which also leads to a reduction in the free or effective bromine concentration. Therefore

$$\begin{aligned} \text{rate} &= k_2^{obsd}[S][Br_2] \\ &= k_2^{app}[S][Br_2]_s \end{aligned} \qquad (36)$$

where $[Br_2]$ is the free bromine concentration and k_2^{obsd} is the second-order rate constant as reported in the text. Obviously

$$k_2^{obsd} = k_2^{app}\frac{[Br_2]_s}{[Br_2]} \qquad (37a)$$

which, with the use of equation 35, may be rewritten as

$$k_2^{obsd} = \frac{k_1^{obsd}}{([S'] - [Br_2]_s)}\frac{[Br_2]_s}{[Br_2]} \qquad (37b)$$

The important equilibria involving Br_2 under our conditions are

$$Br_3^- \underset{}{\overset{K_1}{\rightleftharpoons}} Br_2 + Br^- \qquad (38)$$

and

$$Br_2 + H_2O \overset{K_2}{\rightleftharpoons} H^+ + Br^- + HOBr \qquad (39)$$

where $$K_1 = \frac{[Br_2][Br^-]}{[Br_3^-]} \qquad (40)$$

$$= 0.0554 \text{ M at } 30\ ^{\circ}C^{95}$$

$$= 0.0562 \text{ M at } 25\ ^{\circ}C^{114}$$

and $$K_2 = \frac{[H^+][Br^-][HOBr]}{[Br_2]} \qquad (41)$$

$$= 9.6 \times 10^{-9} \text{ M}^2 \text{ at } 25\ ^{\circ}C^{107}$$

Hence the stoichiometric bromine concentration is given by

$$[Br_2]_s = [Br_2] + [Br_3^-] + [HOBr] \qquad (42)$$

Therefore one can derive

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

$$= \frac{K_1 + [Br^-]}{K_1} + \frac{K_2}{[H^+][Br^-]} \qquad (43)$$

using equations 40 to 42 since $[Br^-]$ and $[H^+]$ remain constant during each reaction. Equation 37b may then be rewritten as equation 44 (next page)

$$k_2^{obsd} = \frac{k_1^{obsd}}{([S] - [Br_2]_s)} \left[\frac{K_1 + [Br^-]}{K_1} + \frac{K_2}{[H^+][Br^-]} \right] \quad (44)$$

The last term of equation 43 is only significant (> 0.01) for pH $>$ 5 under our reaction conditions ($[Br^-] = 0.1$ M). For pH $<$ 5 equation 44 therefore reduces to

$$k_2^{obsd} = \frac{k_1^{obsd}}{([S] - [Br_2]_s)} \left[\frac{K_1 + [Br^-]}{K_1} \right] \quad (45)$$

For brominations carried out under second-order conditions k_2^{obsd} was obtained directly from k_2^{app} using equation 37a. That is,

$$k_2^{obsd} = k_2^{app} \frac{[Br_2]_s}{[Br_2]} \quad (37a)$$

$$= k_2^{app} \left[\frac{K_1 + [Br^-]}{K_1} + \frac{K_2}{[H^+][Br^-]} \right] \quad (46)$$

For iodination experiments all solutions were 0.1 M in KI. Second-order rate constants need correcting for the fraction of iodine existing as tri-iodide ion. That is (see equation 47, next page),

$$k_2^{obsd} = k_2^{app}\frac{[I_2]_s}{I_2} \qquad (47)$$

and

$$\frac{[I_2]_s}{[I_2]} = \frac{K_3 + [I^-]}{K_3} \qquad (48)$$

where

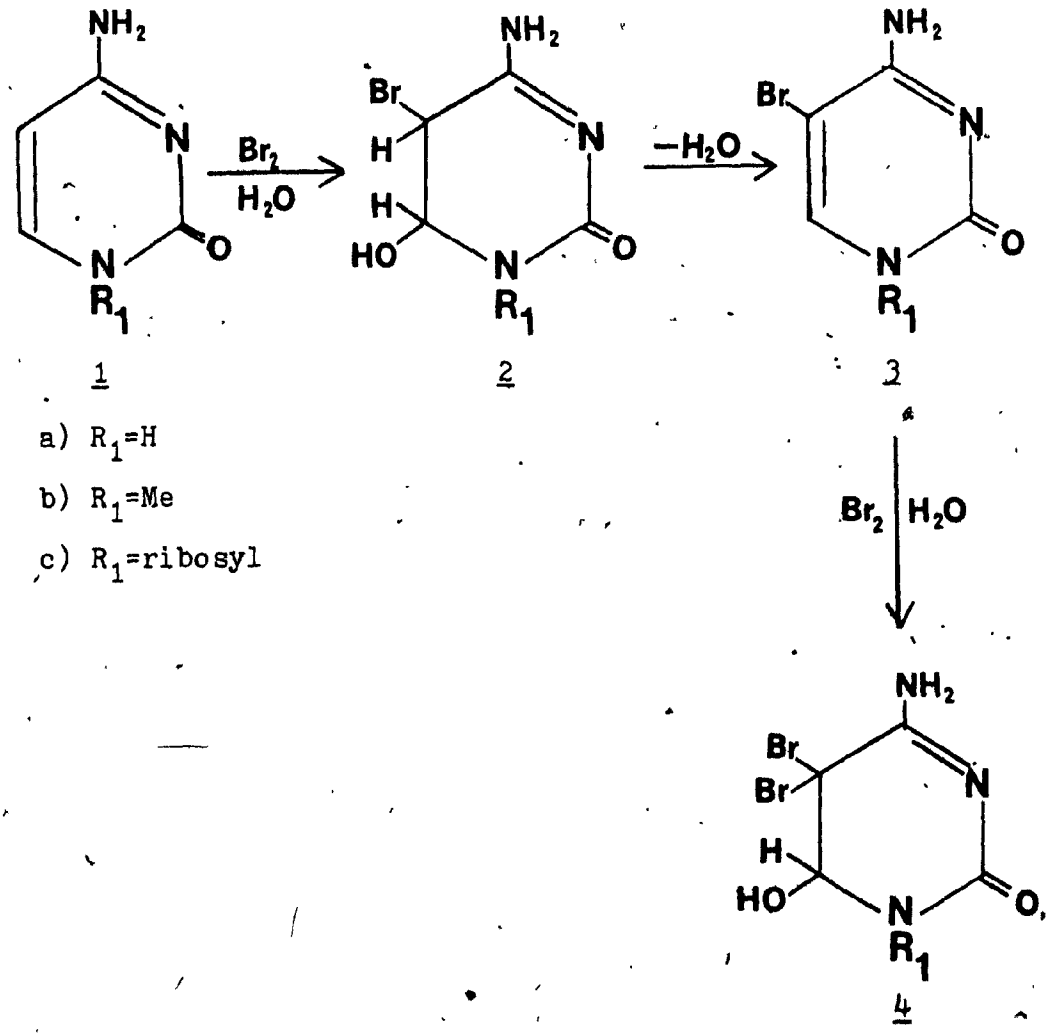
$$K_3 = \frac{[I_2][I^-]}{[I_3^-]} \qquad (49)$$

$$= 0.00130 \text{ M at } 25\ ^oC^{115}$$

The Bromination of Cytosine and Its N-substituted Derivatives

It has been known for some time[57] that cytosine (1a) reacts with excess bromine in aqueous solution to form 5,5-dibromo-5,6-dihydro-6-hydroxycytosine (4a) as the final product (see Scheme I).

Scheme I



1

a) R_1=H

b) R_1=Me

c) R_1=ribosyl

2

3

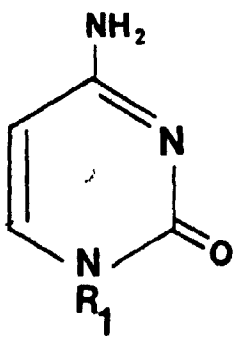
4

During the course of his study of uracil bromination Banerjee observed by pmr the formation of a long-lived intermediate 2a resulting from the attack of aqueous bromine upon cytosine.[17] It seemed probable, therefore, that the reaction proceeded via an addition-elimination mechanism such as presented in Scheme I. To verify Scheme I we have undertaken a mechanistic study of cytosine bromination employing both stopped-flow and pmr techniques. We particularly wished to investigate the manner by which bromine attack occurs and to characterize the adducts 2 formed during the course of the reaction.

After our studies had been initiated Taguchi and Wang[18] reported uv spectral evidence which supports Scheme I. For a number of cytosine derivatives they were able to observe the involvement of adducts 2 (or their 6-methoxy analogs). The final products of bromination using excess bromine were 5,5-dibromo-6-hydroxy-5,6-dihydrouracils, an indication of the ease with which dihydrocytosines such as 2 or 4 can undergo deamination in aqueous media. This observation is consistent with the work of Shapiro[116,125,154,156] and others[157] on the hydrolysis of dihydrocytosines.

The substrates we chose for our study were cytosine (1a), 1-methylcytosine (1b), cytidine (1c) and

3-methylcytosine (5b). Dubois and co-workers[29] have found

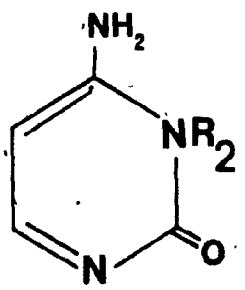


1

a) R_1 = H

b) R_1 = Me

c) R_1 = ribosyl

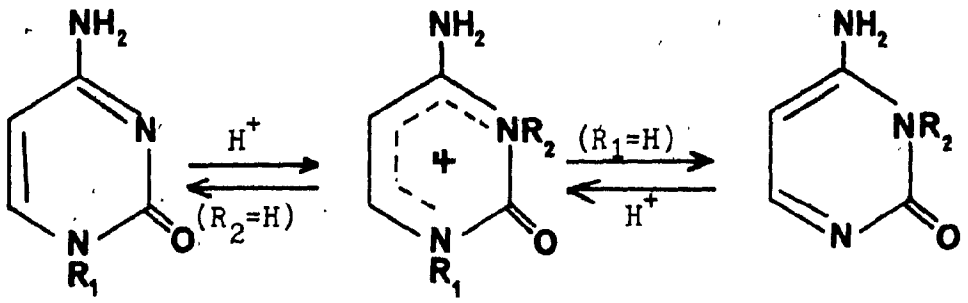


5

a) R_2 = H

b) R_2 = Me

that in aqueous solution cytosine exists to a small degree, ~0.25%, as its 3-H tautomer 5a. These two tautomers most likely interconvert through the common cation 6a in acidic media (structures next page). The two methyl derivatives 1b and 5b were chosen, therefore, to serve as models for the principle tautomers of cytosine. Moreover, their protonated forms, 6b and 6d respectively, can be used as models for the cytosine cation 6a.



1 6 5

a) $R_1=R_2=H$

b) $R_1=Me$, $R_2=H$

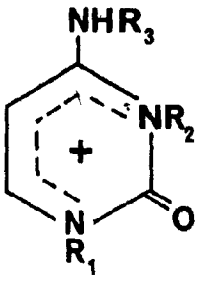
c) $R_1=$ribosyl, $R_2=H$

d) $R_1=H$, $R_2=Me$

Proton Nmr Studies

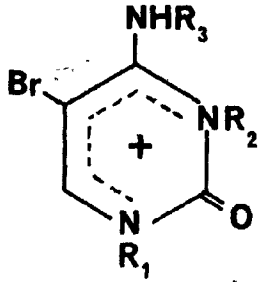
We carried out a pmr study of the reaction in order to observe the formation and decomposition of the bromohydrin intermediates 2. To ensure the complete solubility of the cytosines and also to mask the effects of increasing acidity (HBr is produced by the reaction) 1 N DCl/D_2O was usually used as the solvent. Besides using the substrates chosen for the kinetic experiments (1 and 5b) pmr studies were performed with 5-bromocytosine 3,

N-acetylcytosine and N-acetyl-1-methylcytosine. N^4-acetylcytosines undergo acid-catalyzed hydrolysis rather easily.[98] For the 1-methyl compound 6f our pmr study was carried out in D_2O as the starting solvent to minimize the effects of such hydrolysis. The data obtained is presented in Table I (next page). At the acidities used the substrates exist as their protonated forms 6 or 9a.[22,28,124,125]



6

a) $R_1=R_2=R_3=H$
b) $R_1=Me$, $R_2=R_3=H$
c) $R_1=ribosyl$, $R_2=R_3=H$
d) $R_1=R_3=H$, $R_2=Me$
e) $R_1=R_2=H$, $R_3=acetyl$
f) $R_1=Me$, $R_2=H$, $R_3=acetyl$



9

a) $R_1=R_2=R_3=H$
b) $R_1=Me$, $R_2=R_3=H$
c) $R_1=ribosyl$, $R_2=R_3=H$
d) $R_1=R_3=H$, $R_2=Me$
e) $R_1=R_2=H$, $R_3=acetyl$
f) $R_1=Me$, $R_2=H$, $R_3=acetyl$

The spectra of the starting materials are quite simple. The 5- and 6-protons appear as doublets

Table I

Pmr spectral data for (i) cytosines, (ii) bromination adducts, (iii) 5-bromocytosines and (iv) 5,5-dibromo derivatives in aqueous acid solution.[a]

Structure		Chemical Shifts (δ)				$J_{5,6}$ (Hz)
		5H	6H	N_1-Me	N_3-Me	
(i)	6a	6.20	7.85			8.0
	6b	6.40	8.12	3.59		7.9
	6c	6.39	8.31			8.0
	6d	6.49	7.99		3.62	7.6
	6e	6.59	8.39			7.2
	6f	7.56	8.41	3.71		7.6
(ii)	7a	5.23	5.52			2.1
	7b	5.22	5.53	3.26		2.4
	7c	5.18	5.71			2.1
	7d[b]	5.21	5.32		3.45	2.2
	7e	4.51	5.27			2.5
	7f	4.70	5.46	3.25		2.3
(iii)	9a[c]		8.37			
	9a		8.40			
	9b		8.55	3.61		
	9c		8.83			
	9d		8.24		3.71	
	9e		8.21			
	9f		8.41	3.56		
(iv)	10a[d]		5.61			
	10a		5.58			
	10b		5.61	3.22		
	10d		5.52		3.27	
	10e		5.45			
	10f		5.59	3.26		

Table I (cont'd)

[a] Initially in 1 N DCl/D_2O except for 6f which was initially in D_2O. After addition of bromine acidity increases due to the formation of HBr and so for 7, 9 and 10 the acidity is greater.

[b] 1:1 concentrated DCl/D_2O. In 1 N DCl/D_2O the solvent peak (HOD) obscured the 6-proton peak.

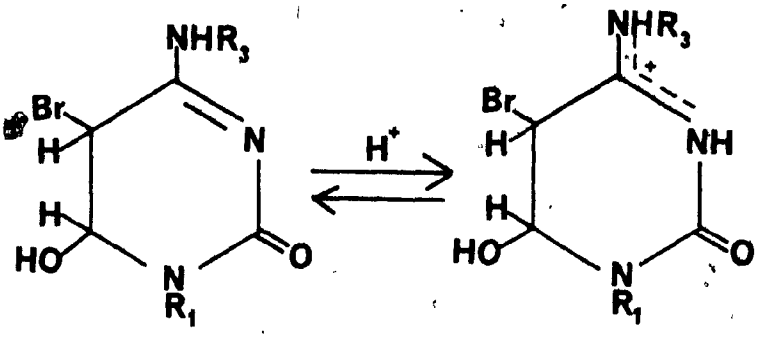
[c] Authentic sample of 9a in 1 N DCl/D_2O.

[d] Formed by the addition of bromine to authentic 9a in initially 1 N DCl/D_2O.

having chemical shifts at approximately 6.5 and 8 ppm respectively and coupling constants of about 8 Hz. The presence of an electron-withdrawing acetyl group at the N^4-position results in a slight downfield shift in the position of the 5- and 6-hydrogen peaks (see 6e and 6f in Table I).

The addition of Br_2 results in the reduction of the spectral lines due to the starting materials 6 and the appearance of peaks at higher field attributable to intermediates such as 2. For 3-methylcytosine the observable adduct would be 8 (structure next page). In all cases the intermediates are most likely in their protonated forms 7 since the protonation pK_a's of simple 5,6-dihydrocytosines are around 6.5.[116] The 5- and 6-hydrogens appear as a simple AB quartet ($J_{5,6} \sim 2.2$ Hz, see Table I) at $\delta \sim 5.2$ and 5.5 ppm, respectively. These chemical shifts are definitely further downfield than those observed by Banerjee[17,117] for the corresponding uracil adducts 22 (structure next page), consistent with the cationic structures 7.

The values for the coupling constants $J_{5,6}$ of adduct 7 are very similar to those found by Banerjee for the uracil intermediates 22 ($J_{5,6} \sim 2.3$ Hz).[17,117] This suggests that the stereochemistry of 7 and 22 should be similar with bromine trans to the hydroxyl group, as was



2 7

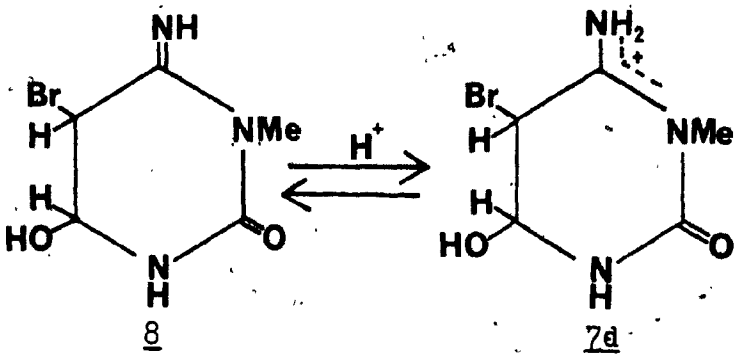
a) $R_1=R_3=H$

b) R_1=Me, $R_3=H$

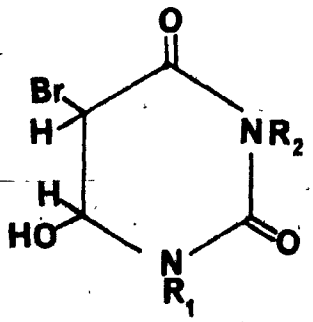
c) R_1=ribosyl, $R_3=H$

e) $R_1=H$, R_3=acetyl

f) R_1=Me, R_3=acetyl



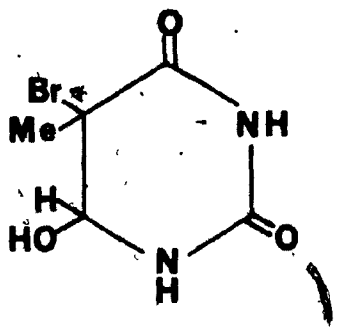
8 7d



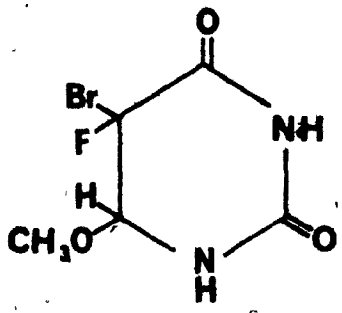
22

R_1, R_2=H or Me

shown to be the case for 22.[117] Nofre and co-workers[118-121] have also concluded that the thymine adduct 41 and the dihydrouracil 22 (R_1=R_2=Me) have this same trans stereochemistry. Likewise, Robins and co-workers[122] have prepared the adduct 42 from 5-fluorouracil and found the bromine to trans to the methoxyl substituent.



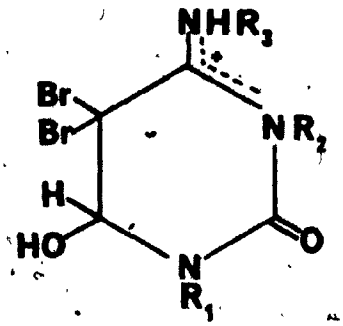
41



42

For the intermediate 7c, derived from cytidine, the spectral lines for the 5- and 6-protons appear somewhat broadened. This may be the result of the chirality of the ribose moiety giving rise to two diastereomeric forms of 7c, and/or rotation about the N_1 ribosyl bond.

The absorption peaks due to the intermediates 7 gradually decrease with time and are replaced by those characteristic of 5-bromocytosines in their protonated forms 9 (structure page 63). Table I also includes the spectral properties of an authentic sample of 9a recorded in 1 N DCl/D_2O. This spectrum compares well to that of 9a produced in the pmr tube by the addition of Br_2 to cytosine in 1 N DCl/D_2O. The addition of further bromine leads to the formation of the 5,5-dibromo derivatives 10 which are also most likely protonated.



10

a) $R_1=R_2=R_3=H$

b) $R_1=Me$, $R_2=R_3=H$

d) $R_1=R_3=H$, $R_2=Me$

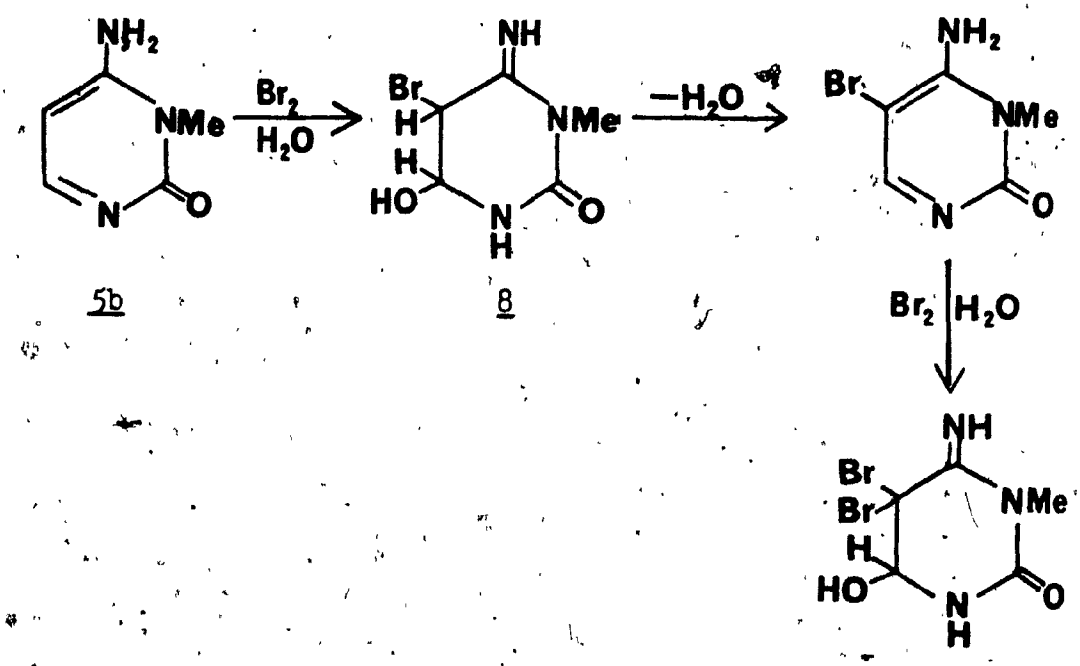
e) $R_1=R_2=H$, $R_3=$acetyl

f) $R_1=Me$, $R_2=R_3=H$

Some final spectra contained a singlet ~8.15 ppm due to the 6-H of the appropriate 5-bromouracil formed via the hydrolysis of the dihydrocytosine adducts 7 and 10. As mentioned earlier Taguchi and Wang[18] have reported that the bromination of 5-bromocytosine leads to 5,5-dibromo-6-hydroxy-5,6-dihydrouracil, 22 (structure page 67), as the isolatable product. This adduct is known to undergo acid-catalyzed decomposition to 5-bromouracil.[16]

These pmr spectral study results lend support to the validity of the addition-elimination mechanism outlined in Scheme I (page 59) for cytosine and 1-substituted cytosines. An analogous mechanism applicable to 3-methylcytosine is shown in Scheme II.

Scheme II



5b 8

Kinetic Studies

We have studied the kinetics of the bromination reaction with the cytosine substrates 1a-c and 5b in aqueous acid. At these acidities (pH 0-5) the reactions are fast and so stopped-flow methods were employed to monitor their progress.

In the presence of at least a tenfold excess of substrate good first-order rate constants, k_1^{obsd}, are obtained for the rates of bromine disappearance. Values of k_1^{obsd} were determined for a range of concentrations for each substrate. The results, presented in Table II (next page), show k_1^{obsd} to be a direct function of the cytosine concentration. Thus

$$k_1^{obsd} = k_2^{app}([S] - [Br_2]_s) \qquad (50)$$

where k_2^{app} is the apparent second-order rate constant and $([S] - [Br_2]_s)$ is the concentration of substrate which remains constant during the reaction. For further discussion concerning the form of equation 50 please refer to page 54.

Linear regression analysis of k_1^{obsd} versus $([S] - [Br_2]_s)$ gave good correlations for all four substrates (1a, r = 0.9999; 1b, r = 0.9997; 1c, r = 0.9996; 5b, r = 0.9999). From the least-squares parameters values for

Table II

Variation in the rates of bromination (k_1^{obsd}) of cytosines 1a-c and 5b with substrate concentration.[a]

Substrate	pH	[S]x10^4 (M)	k_1^{obsd}x10^2 (S^{-1})	k_1^{calcd}x10^2 (S^{-1})
1a[b]	0.98[f]	5.0	5.74	5.72
(R_1=H)		7.5	9.36	9.40
		10.0	13.1	13.1
1b[c]	0.98	1.0	23.2	23.5
(R_1=Me)		2.0	46.5	45.9
		3.0	68.0	68.3
1c[d]	2.30	5.0	5.94	6.00
(R_1=Ribosyl)		7.5	10.1	9.98
		10.0	13.9	14.0
5b[e]	0.98[f]	2.0	1.64	1.64
(R_2=Me)		3.75	2.42	2.42
		5.0	3.21	3.21

Table II (cont'd)

[a] At 25 °C with [KBr] = 0.1 M. The values for k_1^{calcd} were calculated from the least-squares parameters from analysis of k_1^{obsd} versus ([S] - $[Br_2]_s$).

[b] $[Br_2]_s = 5.0 \times 10^{-5}$ M, r = 0.9999.

[c] $[Br_2]_s = 1.0 \times 10^{-5}$ M, r = 0.9997.

[d] $[Br_2]_s = 5.0 \times 10^{-5}$ M, r = 0.9996.

[e] $[Br_2]_s = 2.5 \times 10^{-5}$ M, r = 0.9999.

[f] Value of H_0.[123]

k_1^{calcd} were determined and are included in Table II for comparative purposes. As expected from the values for the correlation coefficients agreement between k_1^{obsd} and k_1^{calcd} is very good, differing by only a few percent or less in all cases.

Therefore overall the reaction must be second-order: first-order in substrate and first-order in bromine, at fixed pH. Accordingly, observed first-order rate constants were converted to second-order constants (k_2^{obsd}) as explained earlier (see page 54).

The acidity dependence of the value of k_2^{obsd} for the four substrates was observed at various acidities in aqueous perchloric acid and in buffer solutions. The results are shown in Table III (next page).

Most of the experiments were carried out under pseudo first-order conditions with the value of k_1^{obsd} obtained converted to k_2^{obsd} by taking into account the substrate concentration and correcting for the reduction in free molecular bromine due to the formation of tribromide ion and at higher pH's hypobromous acid (see equation 44 and discussion on pages 56 and 57). In two cases the substrate was only in a 2:1 excess. Here the absorbance data were directly analyzed as second-order with k_2^{obsd} obtained from the measured apparent second-order

Table III

Variation in the rates of bromination of cytosines 1a-c and 5b with pH.[a]

Substrate	pH	k_1^{obsd} (S^{-1})	$k_2^{obsd} \times 10^{-3}$ ($M^{-1}S^{-1}$)
1a	0.98[b]	0.0574	0.358
(R_1=H)	1.30	0.116	0.723
	2.00	0.564	3.52
	2.45	1.41	8.79
	3.01	4.88	30.4
	3.41	12.6	78.5
	4.19	52.1	325
	4.65	98.4	613
1b	0.98[b]	0.0158	0.488
(R_1=Me)	2.30	0.232	7.16
	2.93	1.12	34.6
	3.21	2.42	74.7
	3.59	6.86	212
	3.95	18.1	559
	4.25	31.7	978
	4.53	38.0	1.17×10^3
	4.83	52.1	1.61×10^3
	5.37	66.3	2.06×10^3

Table III (cont'd)

Substrate	pH	k_1^{obsd} (S^{-1})	$k_2^{obsd} \times 10^{-3}$ ($M^{-1}S^{-1}$)
1c	0.98[b]	0.0594	0.367
(R_1=Ribosyl)	1.30	0.141	0.871
	2.00	0.708	4.37
	2.43	1.96	12.1
	2.94	6.44	39.8
	3.24	18.4	114
	3.39	28.4	173
	4.14	90.1	557
5b	0.98[b]	0.0164[c]	0.203
(R_2=Me)	2.00	---	1.47[e]
	2.42	0.608	3.76
	2.94	0.367[d]	11.3
	3.24	0.709[d]	21.9
	3.84	---	69.8[e]
	4.03	3.99[d]	123
	4.37	8.90[d]	275
	4.85	26.7[d]	825

Table III (cont'd)

[a] At 25 °C with $[KBr]$ = 0.1 M. $[S] = 5.0 \times 10^{-4}$ M and $[Br_2]_s = 5.0 \times 10^{-5}$ M, except where noted otherwise.

[b] Value of H_0.[123]

[c] $[S] = 2.5 \times 10^{-4}$ M and $[Br_2]_s = 2.5 \times 10^{-5}$ M.

[d] $[S] = 1.0 \times 10^{-4}$ M and $[Br_2]_s = 1.0 \times 10^{-5}$ M.

[e] $[S] = 1.0 \times 10^{-4}$ M and $[Br_2]_s = 5.0 \times 10^{-5}$ M. Absorbance data analyzed directly for second-order behavior.

rate constant, k_2^{app}, by correcting for tribromide ion production using equation 37a (page 55).

The log k_2^{obsd} - pH rate profiles appear in Figures 1 - 4 (see pages 79-82). The profiles for 1a, 1b and 1c are very similar being virtually superimposable one upon the other. That for 5b shows an inverse dependence of reactivity with respect to acidity throughout the pH range studied. For all cases the rate profiles have slopes of +1 for their linear portions. These profiles are consistent with bromine attack occurring upon the free base forms of the cytosines. Since compounds 1a-c have protonation pK_a's ~ 4.5[22,124,125] a levelling off in reactivity is expected $\sim$ pH > 4. 3-Methylcytosine, 5b, however, has its $pK_a = 7.49$[28] considerably higher than those of the other substrates studied. Therefore a levelling off in the rate profile of 5b should not be observable until pH $\sim$ 7, at which acidity the rate of reaction is much too fast to be monitored by our equipment.

For cytosines 1 a possible mechanism for the reaction is presented in Scheme III (page 83). Bromination of the free base 1, which is in equilibrium with its protonated form 6, leads to the formation of the cationic intermediate 11 which undergoes rapid attack by water to form the adducts which are observable by pmr (2 $\rightleftharpoons$ 7). These long-lived adducts can undergo slow

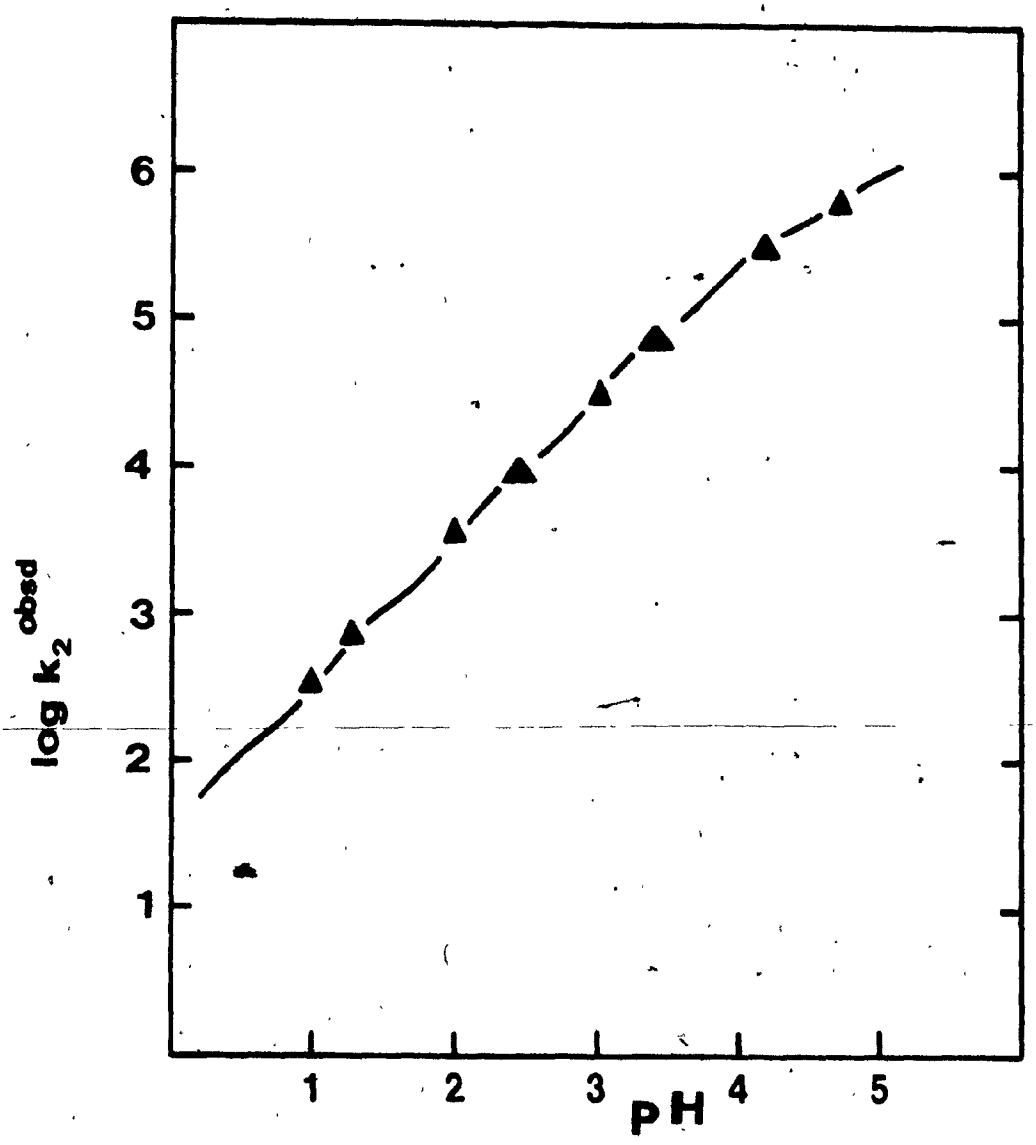


Figure 1. Acidity dependence for the rate of bromination of cytosine.

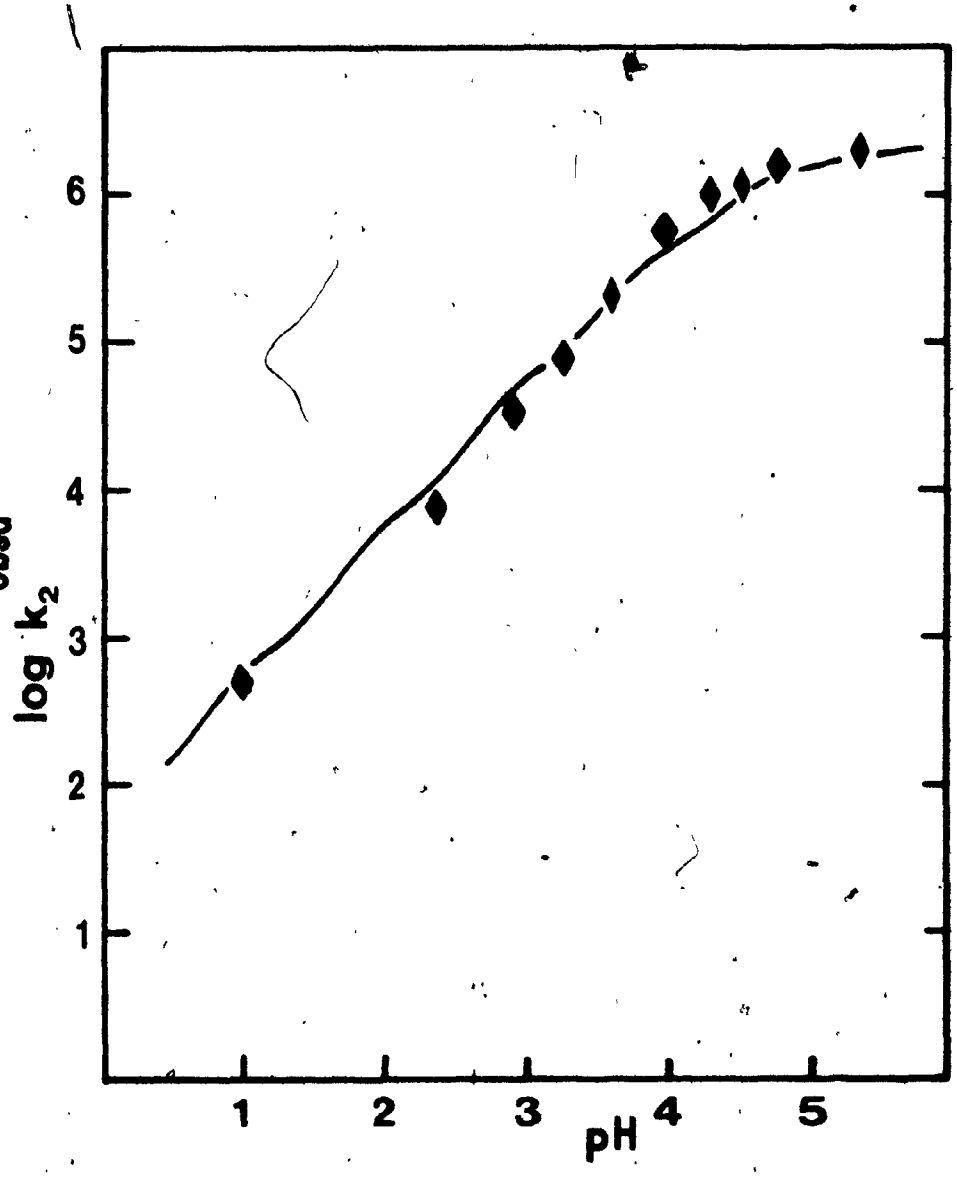


Figure 2. Acidity dependence for the rate of bromination of 1-methylcytosine.

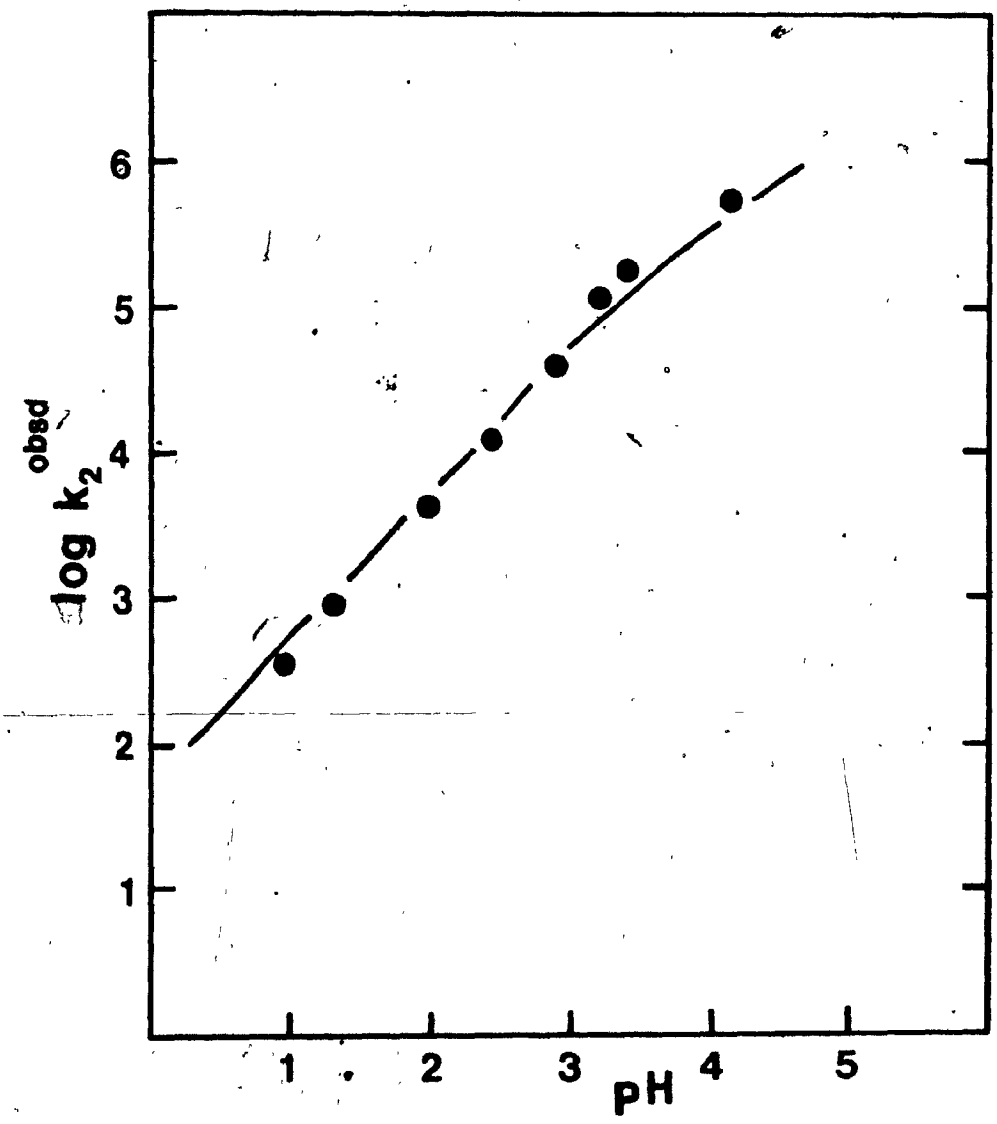


Figure 3. Acidity dependence for the rate of bromination of cytidine.

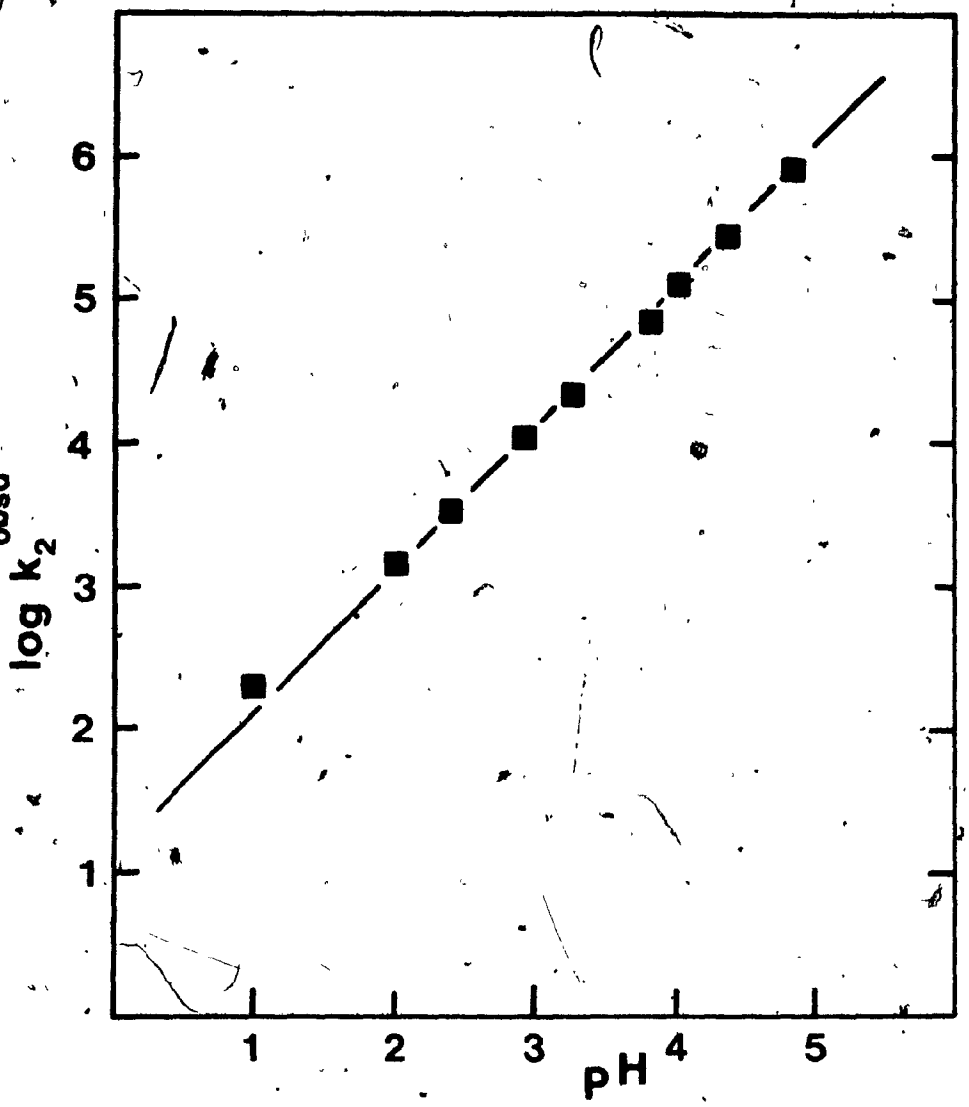
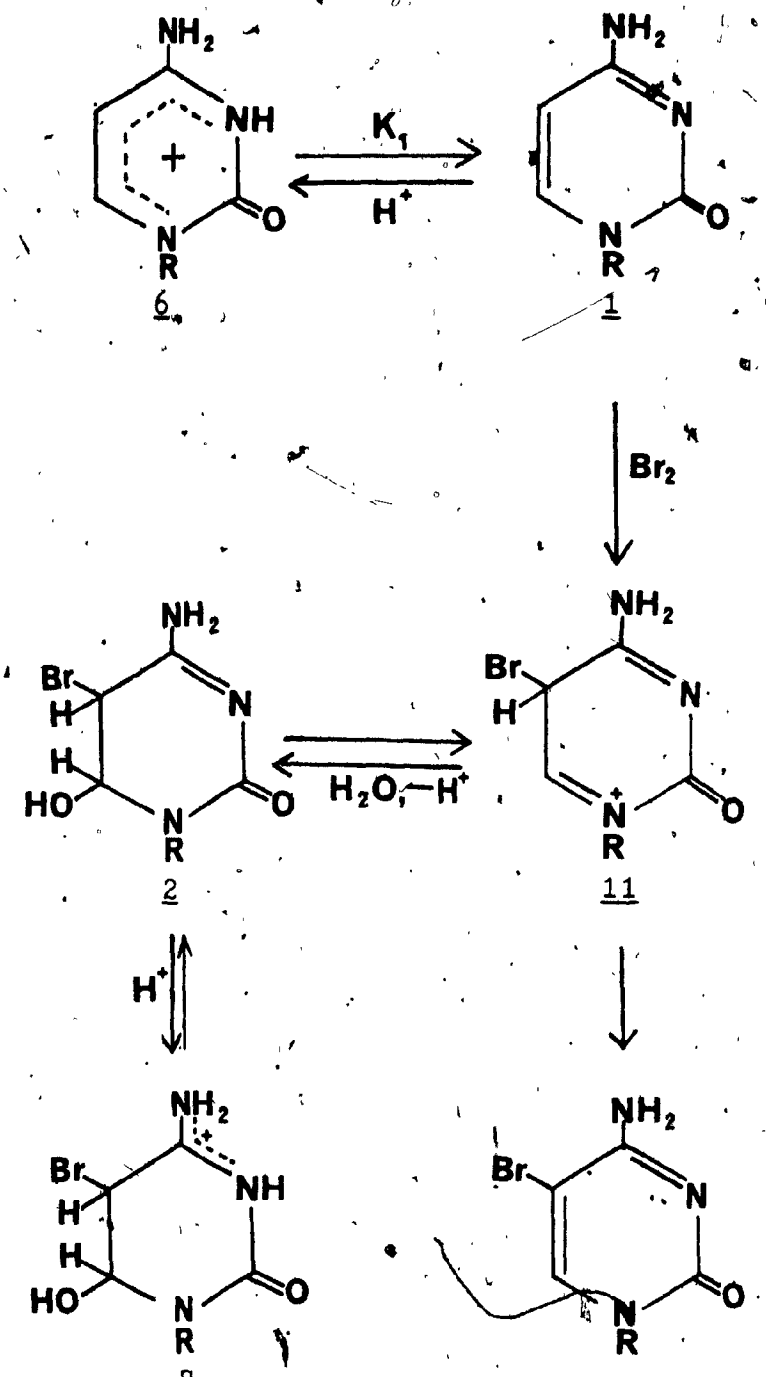


Figure 4. Acidity dependence for the rate of bromination of 3-methylcytosine.

Scheme III

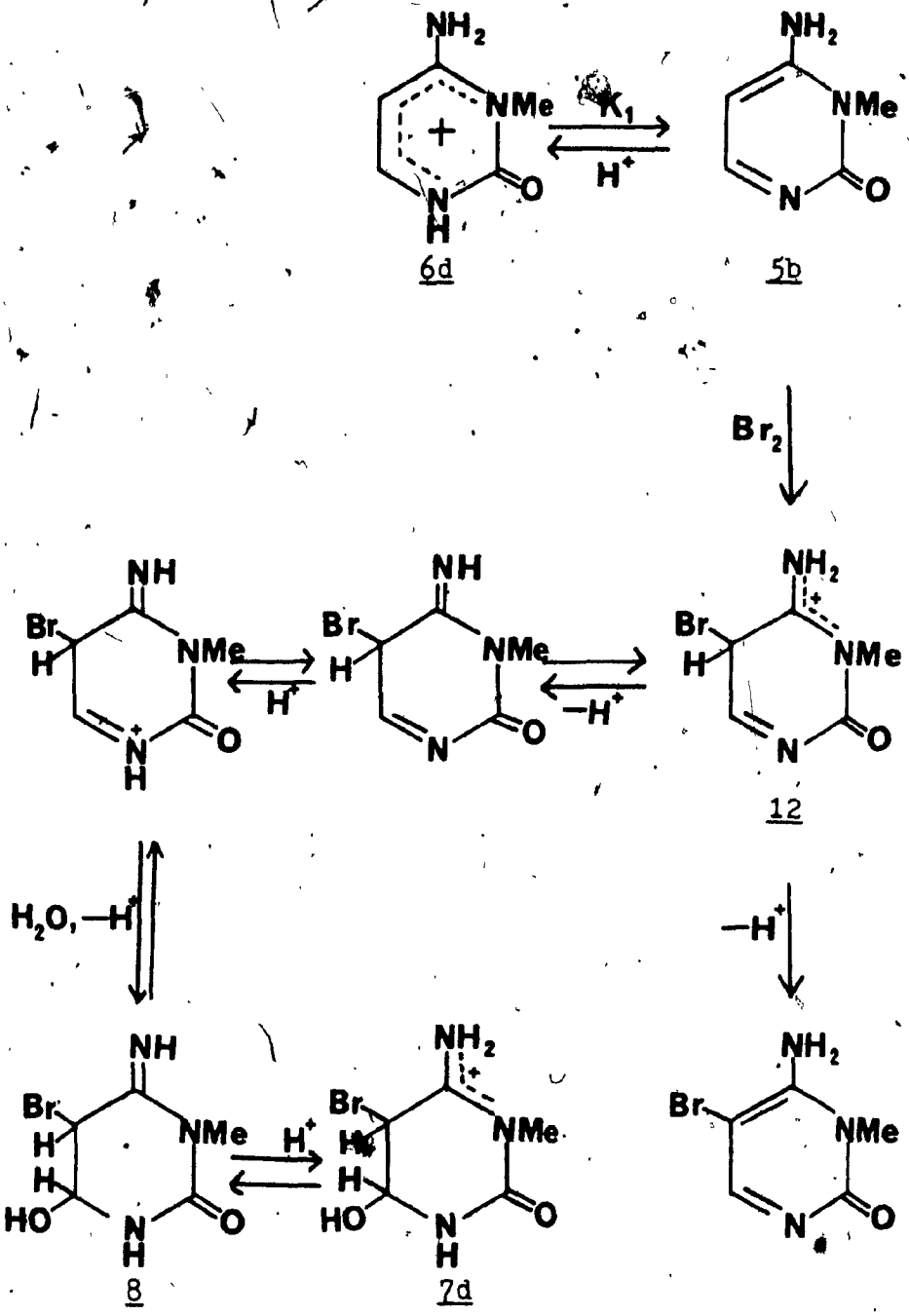


dehydration to the appropriate 5-bromocytosine.

Banerjee and Tee[17,117,126] have studied the kinetics of the dehydration of the analogous uracil intermediates 22 (structure page 67) formed during uracil brominations. They found the dehydration to be acid-catalyzed with the rupture of the C_5-H bond being rate determining. We were able to follow the decomposition of these bromohydrin adducts (2 $\rightleftharpoons$ 7) to 5-bromocytosines during our pmr study of the reaction (vide supra).

For 3-methylcytosine, 5b, a similar mechanism must be involved (see Scheme IV, next page). Here too, the acidity dependence data point to reaction involving the free base form of the substrates. Scheme IV is somewhat different from Scheme III in that the cationic intermediate 12 formed by the attack by bromine on the free base 5b must pass through two proton-transfer equilibria before capturing water to yield the observable adducts 8 $\rightleftharpoons$ 7d.

Scheme IV



The bromination reaction presented in Scheme III may be expressed as

$$\underline{6} \underset{}{\overset{K_1}{\rightleftharpoons}} \underline{1} + H_3O^+ \qquad (51)$$

$$\underline{1} \xrightarrow[Br_2]{k_2} \text{products}$$

where k_2 is the second-order rate constant for the bromination of $\underline{1}$

and $$K_1 = \frac{[\underline{1}][H_3O^+]}{[\underline{6}]} = 10^{-4.47} \text{ for } \underline{1a}^{50} \qquad (52)$$

$$= 10^{-4.55} \text{ for } \underline{1b}^{50}$$

$$= 10^{-4.22} \text{ for } \underline{1c}^{127}$$

Now $$[\underline{1}]_s = [\underline{1}] + [\underline{6}] \qquad (53)$$

$$= [\underline{1}]\left[\frac{K_1 + [H^+]}{K_1}\right]$$

where $[\underline{1}]_s$ is the stoichiometric concentration of $\underline{1}$.

Hence,

$$\text{rate} = k_2[\underline{1}][Br_2] \qquad (54)$$

$$= k_2\left[\frac{K_1}{K_1 + [H^+]}\right][\underline{1}]_s\,[Br_2]$$

Therefore $$k_2^{obsd} = \frac{k_2K_1}{(K_1 + [H^+])} \qquad (55)$$

The calculated curves shown in Figures 1 - 3 were constructed using equation 55 and the appropriate values found in Table IV (next page).

From Table IV it can be seen that cytosines 1 undergo direct bromine attack with $k_2 \sim 10^6\ M^{-1}S^{-1}$. The close similarity in the behavior of cytosine, 1a, to that of its N_1 substituted derivatives, 1b and 1c, strongly suggests that it reacts via its 1H tautomer. However the 3H tautomer 5a (structure page 61) may also undergo reaction with bromine (vide infra).

For 3-methylcytosine (5b) $pK_1 = 7.49$[28] (see Table IV) and therefore $[H^+] >> K_1$ in the pH range studied. Therefore equation 55 reduces to equation 56 (see page 89).

Table IV

Kinetic parameters for the reaction of bromine with cytosines.

Cytosine	pK_1	k_2K_1 (S^{-1})	k_2 ($M^{-1}S^{-1}$)
1a	4.45[a]	34	9.5×10^5
1b	4.55[a]	60	2.1×10^6
1c	4.22[b]	56	9.3×10^5
5b	7.49[c]	13	4.0×10^8

[a] Reference 50.

[b] Reference 127.

[c] Reference 28.

Equation 56 was used to generate the calculated curve in Figure 4 with $k_2K_1 = 13\ S^{-1}$ and $k_2 = 4.0 \times 10^8\ M^{-1}S^{-1}$.

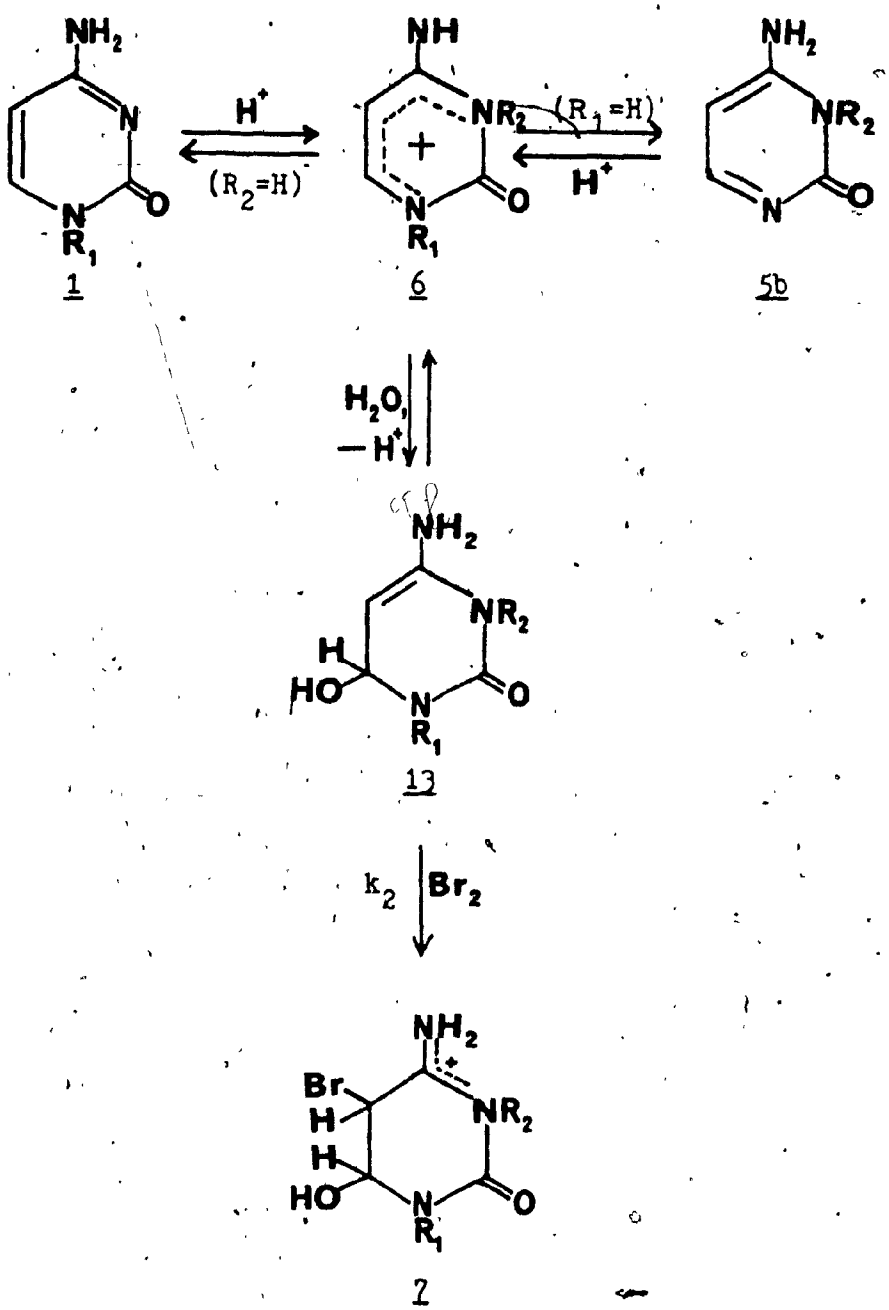
$$k_2^{obsd} = \frac{k_2K_1}{[H^+]} \qquad (56)$$

The kinetic data then can be successfully interpreted in terms of Schemes III and IV. Reaction of 5b with bromine results initially in the formation of the relatively stable amidinium cation, 12; whereas bromination of the cytosines 1 leads to a less stable iminium ion, 11. This would account for the reactivity of 3-methylcytosine (5b) being 200 times greater than that of 1-methylcytosine (1b) towards bromine (see Table IV).

In summary, the kinetic results are consistent with the adducts 7 arising by way of bromine attack, followed by attack of water. We now consider the possibility that the timing of these events is reversed.

Should the formation of the adducts 7 come about due to bromine attack occurring upon the covalent hydrate (13) of the free base then a mechanism such as detailed in Scheme V would apply (see next page). This mechanism seems a plausible alternative since Tee and

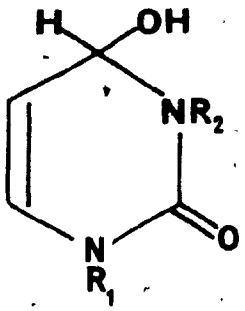
Scheme V



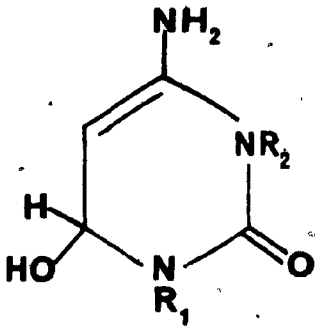
1 6 5b 13 7

Paventi have shown that the bromination of 2-pyrimidinones involves a similar mechanism.[128] Moreover, mechanisms involving covalent hydrates have been found by Tee and co-workers to apply for 4-pyrimidinones[129] and for 4-quinazolinones.[95]

The covalent hydrate 13 should be extremely reactive towards bromine. The rate constant k_2 for bromine



QOH



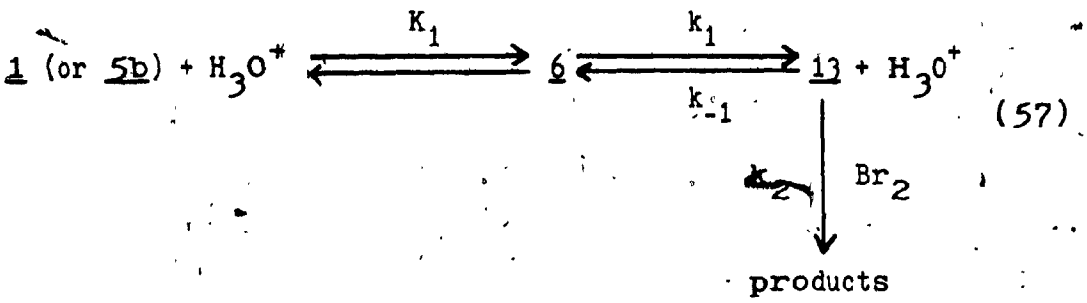
13

attack upon the related species QOH (R_1=R_2=H), the covalent hydrate of 2-pyrimidone, is $\sim 10^9$ $M^{-1}S^{-1}$, approaching the diffusion-controlled limit.[128]

Additionally, 13 may be regarded as being

an enamine twice over and should react with bromine as quickly as the solvent allows ($k_2 \sim 10^{10}$ $M^{-1}S^{-1}$ [158]). It is known, for instance, that simple enamines react with iodine, which is normally less reactive than bromine, at the diffusion-controlled limit.[130]

Scheme V can be represented as



(57)

Assuming a steady-state concentration for the highly reactive covalent hydrate[128,129] gives

$$-\frac{d[Br_2]}{dt} = k_2[13]_{ss}[Br_2] \qquad (58)$$

where $[13]_{ss}$ is the steady-state concentration of 13.

Now,

$$-\frac{d[13]_{ss}}{dt} = k_2[13]_{ss}[Br_2] + k_{-1}[H^+][13]_{ss} - k_1[6] \qquad (59)$$

$$= 0$$

therefore $$[\underline{13}]_{ss} = \frac{k_1[\underline{6}]}{k_2[Br_2] + k_{-1}[H^+]} \qquad (60)$$

Hence equation 58 becomes

$$\frac{-d[Br_2]}{dt} = \frac{k_2 k_1[\underline{6}][Br_2]}{k_2[Br_2] + k_{-1}[H^+]} \qquad (61)$$

Additionally, equation 53 (page 86) may be rewritten as

$$[\underline{1}]_s = [\underline{6}]\frac{(K_1 + [H^+])}{[H^+]} \qquad (62)$$

thus $$\frac{-d[Br_2]}{dt} = \frac{k_2 k_1[H^+][\underline{1}]_s[Br_2]}{(k_2[Br_2] + k_{-1}[H^+])(K_1 + [H^+])} \qquad (63)$$

To obtain a second-order rate law which is compatible with the observed kinetics the condition $k_{-1}[H^+] \gg k_2[Br_2]$ must apply. Equation 63 thereby reduces to

$$\frac{-d[Br_2]}{dt} = \frac{k_2 K_R^+[\underline{1}]_s[Br_2]}{(K_1 + [H^+])} \qquad (64)$$

where $$K_R^+ = \frac{k_1}{k_{-1}} \qquad (65).$$

Therefore $$k_2^{obsd} = \frac{k_2 K_R^+}{(K_1 + [H^+])} \qquad (66)$$

Equation 66 is of the same form as equation 55 and would therefore provide a good description of the kinetic data. However, the values which must be ascribed to k_1, k_{-1}, and K_R^+ in order to use equation 66 do not seem reasonable.

As mentioned above, k_2, the second-order rate constant for bromine attack upon the covalent hydrate should be $\sim 10^{10}\ M^{-1}S^{-1}$ and $[Br_2] = 5\times10^{-5}$ M initially (see Table III). Therefore

$$k_2[Br_2] \sim 10^{10}\ M^{-1}S^{-1} \times 5\times10^{-5}\ M$$
$$\sim 5\times10^{5}\ S^{-1} \qquad (67)$$

At the lowest acidity under consideration, pH = 5,

$$[H^+] = 10^{-5}\ M \qquad (68)$$

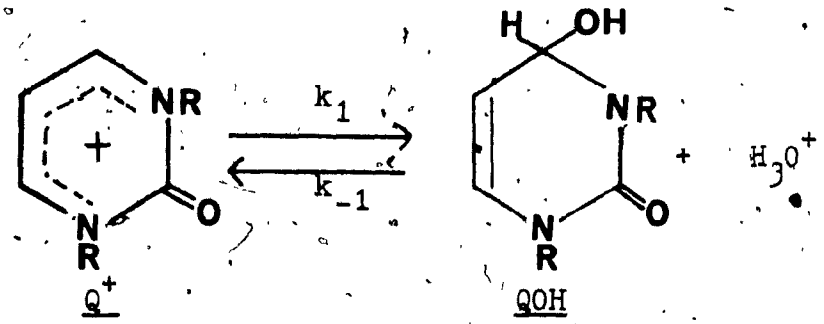
For equation 64 to be applicable the condition is $k_{-1}[H^+] \gg k_2[Br_2]$ (vide supra), hence (see next page)

$$k_{-1} >> \frac{k_2[Br_2]}{H^+}$$

$$>> \frac{5\times10^5\ S^{-1}}{10^{-5}\ M}$$

$$>> 5\times10^{10}\ M^{-1}S^{-1} \qquad (69)$$

Known values of k_{-1} for various heterocyclic cations are $\leq 6\times10^8\ M^{-1}\ S^{-1}$ [89] and for the closely related QOH,



(70)

R = Me or H

studied by Tee and co-workers[128,131], are $\sim 10^7\ M^{-1}S^{-1}$. Furthermore, only the proton transfer between H_3O^+ and OH^- has a rate constant appreciably greater than

$5x10^{10}$ $M^{-1}S^{-1}$ in aqueous solution.[132] In our case k_{-1} represents the proton transfer to the pseudobase 13 accompanied by a concerted C-O bond breakage[89] and therefore should be significantly smaller.

Additionally, applying equation 66 to the kinetic data in Table III yields values of $k_2K_R^+$ = 13 - 60 S^{-1} (i.e. corresponding to k_2K_1 in Table IV). Assuming $k_2 \sim 10^{10}$ $M^{-1}S^{-1}$, as discussed above, leads to K_R^+ = $1.3x10^{-9}$ - $6.0x10^{-9}$ M (pK_R^+ = 8.2 - 8.9).

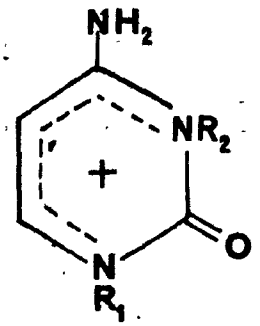
Now,
$$K_R^+ = \frac{k_1}{k_{-1}} \qquad (65)$$

$$= 1.3x10^{-9} - 6.0x10^{-9} \text{ M}$$

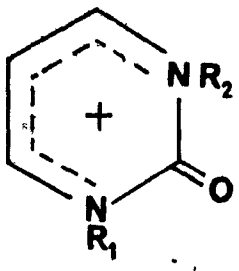
and
$$k_{-1} >> 5x10^{10} \text{ } M^{-1}S^{-1} \qquad (69)$$

hence
$$k_1 >> 65 - 300 \text{ } S^{-1} \qquad (71)$$

These values for K_R^+ and k_1 also do not seem acceptable. For the 2-pyrimidinone cations, Q^+ (equation 70), pK_R^+ = 5.7 - 7.2 and k_1 = 1.5 - 10 S^{-1}.[128,131] Since the cytosine cations 6 are more stable than the pyrimidinone cations Q^+, due the presence of an electron-supplying amino group at the 4-position, the attack by water on 6 should be



6



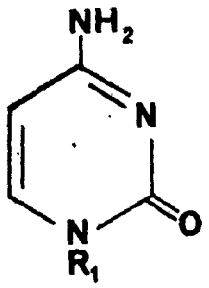
Q^+

slower than upon Q^+. Hence the value of k_1 for the cytosine equilibria should be smaller rather than larger than those obtained for the 2-pyrimidinone systems ($Q^+ \rightleftharpoons QOH$).

Even more convincing evidence against the covalent hydrate mechanism, but supporting bromine attack occurring upon the cytosine free bases (Schemes III or IV) comes from our iodination study. The highly reactive covalent hydrate 13 (Scheme V) should react with iodine and bromine at the same rate[130], the diffusion-controlled limit. In fact, cytosine iodination is about 1000 times slower than its bromination, much more in keeping with

direct halogen attack as presented in Schemes III or IV.

It was mentioned earlier (page 87) that the very similar reactivities of cytosine (1a), 1-methylcytosine (1b) and cytidine (1c) suggest that the 1H tautomer of cytosine (1a) is the reactive form. However, 3-methylcytosine (5b) is about 200 times more reactive than is 1b. If the reactivities of 5a and 1a exhibit a similar relationship then it is possible that these two

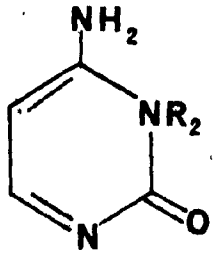


1

a) R_1=H

b) R_1=Me

c) R_1=ribosyl



5

a) R_2=H

b) R_2=Me

tautomers of cytosine may compete to some extent for bromine since the tautomeric ratio

$$K_T = \frac{[1a]}{[5a]} = 400^{29} \qquad (72)$$

To summarize, we have completed a study of the reactions of bromine with cytosine, 1-methylcytosine, cytidine and 3-methylcytosine in aqueous media. Initially adducts (5-bromo-5,6-dihydro-6-hydroxycytosines) are produced which we were able to observe by proton nmr as their protonated forms in acidic media. With time these adducts undergo elimination of water to yield 5-bromocytosines, the substitution products of the reaction. Kinetic measurements of the initial reaction in the pH range 0 - 5 are consistent with the adducts resulting from rate-determining attack of bromine on the free base form of the cytosine substrates followed by capture of the cation so produced by water.

The Bromination of 1- and 3-Methyluracil

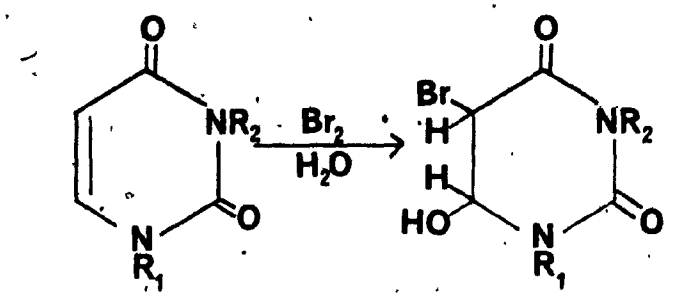
Much has already been presented from this laboratory concerning the bromination of uracil.[155] Prior to these studies there were no kinetic investigations of the mechanism of uracil halogenation.

Wang[16] proposed that uracil (21a) and 1,3-dimethyluracil (21d) react rapidly with bromine in aqueous solution via an addition-elimination process as presented in Scheme VI (next page). Banerjee and Tee[117,126,133,134] have provided evidence which supports this overall mechanism. They were able to characerize by pmr the hydrated adducts 22 and studied the kinetics of the relatively slow acid-catalyzed dehydration of 22 ⟶ 26 in acidic media. We have shown that cytosine bromination follows a similar reaction pathway (see page 59ff).

5-Bromouracils (26) can react rapidly with bromine to form the 5,5-dibromo adducts 28.[16] These adducts are also the major products formed by the reaction of 5-bromocytosines with bromine (see ref. 18 and page 70). Decomposition of 28 to 26 is known to take place in strong acid.[16] The kinetics of this type of debromination were investigated by Banerjee and Tee[133] for 6-methyluracil.

Moore and Anderson[135] have made observations

Scheme VI



21 22

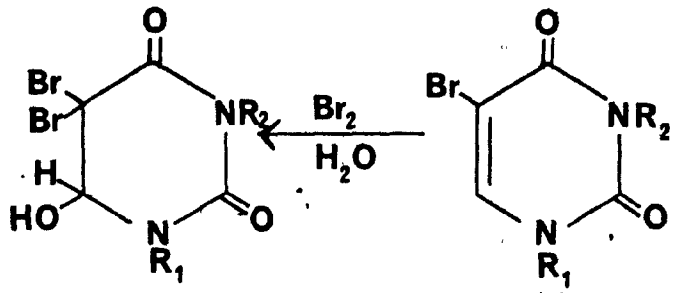
a) $R_1=R_2=H$

b) $R_1=Me$, $R_2=H$

c) $R_1=H$, $R_2=Me$

d) $R_1=R_2=Me$

$-H_2O$

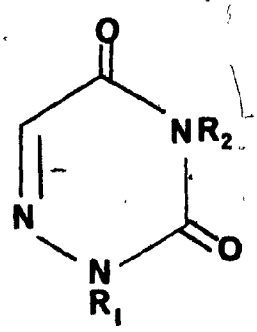


28 26

which they felt conflicted with Scheme VI. They carried out spectroscopic and potentiometric titrations of uracils with bromine in acetate buffer of pH 4.7. It was found that whereas 1,3-dimethyluracil (21d) and uridine (21; R_1=ribosyl, R_2=H) react with 1 equivalent of bromine to form, presumably, 22, uracil (21a) itself appears to consume 2 mole equivalents to quickly form initially 5-bromouracil (26a) and then 5,5-dibromo-6-hydroxy-5,6-dihydouracil (28a). However, the dehydration of the adduct 22a at pH 4.7 is too slow to account for the rapid appearance of 26a observed.[117]

For an earlier thesis[19] we carried out stopped-flow kinetic studies of the reactions of bromine with uracil (21a), 5-bromouracil (26a) and their 1,3-dimethyl derivatives, 21d and 26d, respectively.[19,20] For all four substrates the reaction is second-order, being first-order in uracil or 5-bromouracil and first-order in bromine. The rates of bromination for the dimethyl derivatives (21d and 26d) are invariant with acidity throughout the entire pH range 0 - 5. This is consistent with the reaction steps 21 ⟶ 22 and 26 ⟶ 28 shown in Scheme VI. For the parent compounds (21a and 26a) the kinetic data demonstrated definite acidity dependence, With uracilthe rate of reaction varies little between pH 0 and 3, but increases significantly with decreasing acidity above pH 3. 5-Bromouracil exhibits

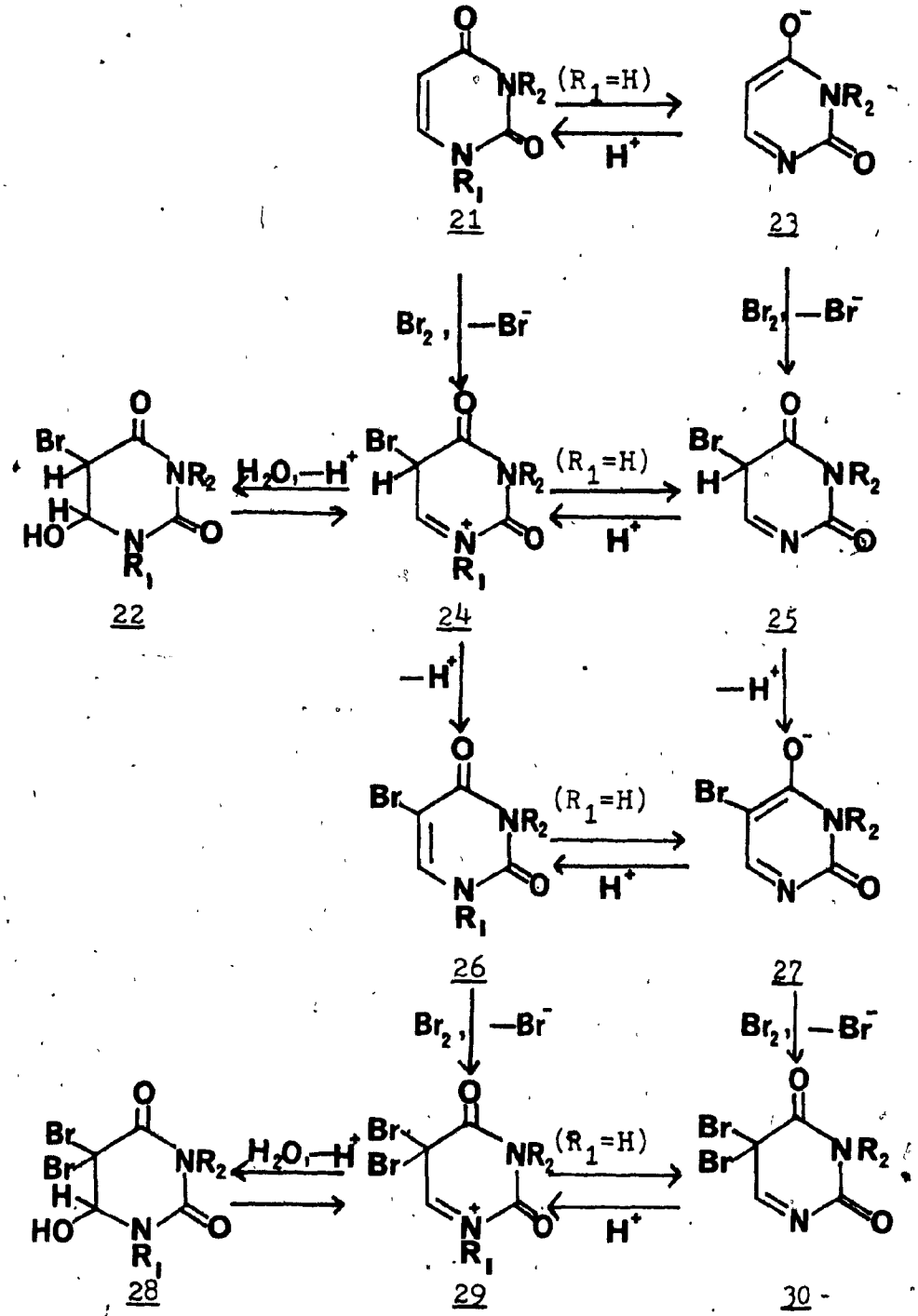
similar behavior except that the onset of observable acidity dependence occurs above pH 1.5. These results suggested that at lower acidities (eg pH > 3 for 21a) the brominations may proceed via bromine attack upon the anions formed by deprotonation at the N_1 positions of the free bases. This overall mechanism is presented in Scheme VII (next page). The brominations of 6-azauracils (43) also proceed in a similar fashion.[134]



43

We now report that we have extended this study to 1-methyluracil (21b) and 3-methyluracil (21c) to obtain further support for Scheme VII. In the presence of at least a tenfold excess of substrate the disappearance of bromine exhibits first-order behavior (>90% reaction),

Scheme VII



and the derived rate constants, k_1^{obsd}, were converted to second-order rate constants, k_2^{obsd}, taking into account the depletion of free bromine due to the formation of tribromide ion and hypobromous acid (see page 54ff).

The values of k_2^{obsd} for the uracil substrates 21b and 21c at various pH's are listed in Table V. Over the whole pH range studied the rate constants for the reaction of bromine with 21b remain virtually constant. For 21c, however, the values of k_2^{obsd} show marked acidity dependence above pH 3. Thus 1-methyluracil (21b) behaves like 1,3 dimethyluracil (21d), whereas 3-methyluracil (21c) behaves like the parent uracil (21a)[19,20] (see Table V, next page and Figure 5, page 107). It appears, therefore, that the observed rate constants for the bromination of 3-methyluracil (21c) are the sum of two terms, one acid invariant and one showing inverse dependence upon acidity. The data for this substrate are well reproduced by equation 73 (compare k_2^{obsd} and k_2^{calcd} in Table V).

$$k_2^{calcd} = 6.47 \times 10^4 + \frac{0.89}{[H^+]} \qquad (73)$$

These results support the validity of Scheme VII. The kinetic data for 1-methyluracil (21b) are consistent with the simple pathway 21 + Br_2 ⟶ 24 ⟷ 22 (where 22 is the long-lived intermediate

Table V

Variation in the rates of bromination of 1-methyluracil (21b) and 3-methyluracil (21c) with pH.[a]

Uracil	pH	k_1^{obsd} (S^{-1})	$k_2^{obsd}{}_{x10^{-4}}$ ($M^{-1}S^{-1}$)	$k_2^{calcd}{}_{x10^{-4}}$ ($M^{-1}S^{-1}$)
21b	1.26	12.0	7.48	----
	2.94	11.6	7.23	----
	4.90	11.6	7.23	----
21c	1.26	10.4	6.48[b]	6.47
	2.94	10.5	6.54	6.55
	4.34	13.5	8.41	8.42
	4.90	21.3	13.3	13.5
	5.01	25.0	15.6	15.6

a At 30 °C, $[S] = 5.0 \times 10^{-4}$ M, $[Br_2] = 5.0 \times 10^{-5}$ M and [KBr] = 0.1 M. The values of k_2^{calcd} were calculated from least-squares parameters.

b r = 0.9997

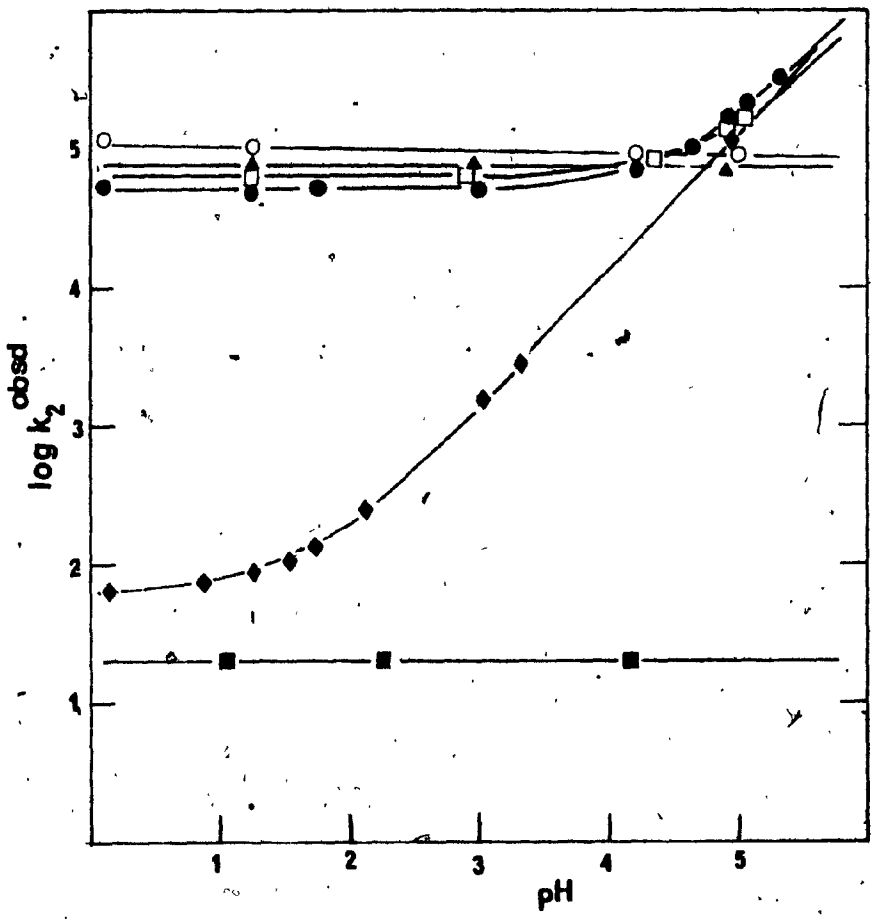


Figure 5. Acidity dependence for the rates of bromination of uracils and 5-bromouracils.

● Uracil ▲ 1-Methyluracil □ 3-Methyluracil

○ 1,3-Dimethyluracil

◆ 5-Bromouracil ■ 5-Bromo-1,3-dimethyluracil

observable by pmr), the mechanism predicted for N_1 substituted uracils.[19,20]

The pH dependency of k_2^{obsd} for 21c has a form which indicates that at high acidity it also reacts by the pathway $21 + Br_2 \longrightarrow 24 \rightleftharpoons 22$ but that at low acidity it reacts via its anion according to the route $21 \rightleftharpoons 23 + Br_2 \longrightarrow 25 \rightleftharpoons 24 \rightleftharpoons 22$. The entire situation may be represented as

$$\begin{array}{ccc} & \overset{K_a}{\rightleftharpoons} & \\ 21 & & 23 + H_3O^+ \\ Br_2 \downarrow k_2^{HA} & & Br_2 \downarrow k_2^{A^-} \\ \text{pdts} & & \text{pdts} \end{array} \qquad (74)$$

where

k_2^{HA} = second-order rate constant for the bromination of the free base, 21, protonated at N_1.

$k_2^{A^-}$ = second-order rate constant for the bromination of the N_1 deprotonated anion 23.

and $$\hat{K}_a = \frac{[23][H_3O^+]}{[21]} \qquad (75)$$

Now $$[\underline{21}]_s = [\underline{21}] + [\underline{23}]$$

$$= [\underline{21}]\frac{([H_3O^+] + K_a)}{[H_3O^+]} \qquad (76)$$

where $[21]_s$ = stoichiometric concentration of 21.

All experiments were performed under conditions where $[H_3O^+] \gg K_a$ and therefore $[\underline{21}] \sim [\underline{21}]_s$. Since two reaction pathways are possible

$$-\frac{d[Br_2]}{dt} = k_2^{obsd}[\underline{21}]_s [Br_2]$$

$$= k_2^{HA}[\underline{21}][Br_2] + k_2^{A^-}[\underline{23}][Br_2]$$

$$= k_2^{HA} + \frac{k_2^{A^-}K_a}{[H_3O^+]}[21]_s [Br_2] \qquad (77)$$

Therefore $$k_2^{obsd} = k_2^{HA} + \frac{k_2^{A^-}K_a}{[H_3O^+]} \qquad (78)$$

Equation 78 is of the same form as equation 73, the correlation equation containing the least-squares parameters used to calculate the values of k_2^{calcd} listed in Table V. Equating the correlation equation 73 to the theoretical equation 78 gives for 21c (next page).

$$k_2^{HA} = 6.47 \times 10^4\ M^{-1}S^{-1} \quad (79)$$

and $$k_2^{A^-} K_a = 0.89\ S^{-1} \quad (80)$$

Similar theoretical equations have been obtained for the other N_1 unsubstituted uracils, 21a (R_1=R_2=H) and 26a (R_1=R_2=H), that we studied earlier.[19,20] A complete tabulation of all the kinetic parameters ascribed to the reaction of bromine with the uracils that we have studied appears in Table VI (next page). For comparative purposes Table VI also lists the rate constants for the bromination of 6-azauracils (43, structure on page 103) investigated by Banerjee and Tee.[134]

In strong acid it is the acid independent term k_2^{HA} which makes the largest contribution to the value of k_2^{obsd} (see equation 78 and Table VI) for the N_1 unsubstituted uracils 21a, 21c and 26a. Also the values for k_2^{HA} for 21 (R_1=H, R_2=H or Me) and 26a are very similar to those obtained for their analogs containing a methyl substituent at N_1. This would seem to indicate that all the substrates are reacting as their free bases at these acidities, a covalent hydrate mechanism having been ruled out.[20]

At higher pH(> 3 for uracils 21 (R_1=H, R_2= H or Me) and > 1.5 for 5-bromouracil (26a)) the term $k_2^{A^-} K_a$ makes the largest contribution to the value of

Table VI

Kinetic parameters for the reaction of bromine with uracils (21), 5-bromouracils (26) and 6-azauracils (43).

Compound	pK_a [a]	k_2^{HA} ($M^{-1}S^{-1}$)	$k_2^{A^-}K_a$ (S^{-1})	$k_2^{A^-}$ ($M^{-1}S^{-1}$)
21a [b] ($R_1=R_2=H$)	~9.65 [d]	5.00×10^{4}	1.33	$\sim6\times10^{9}$ [d]
21b (R_1=Me, R_2=H)	9.75	7.2×10^{4}	----	----
21c (R_1=H, R_2=Me)	9.95	6.47×10^{4}	0.89	7.9×10^{9}
21d [b] ($R_1=R_2$=Me)	----	10×10^{4}	----	----
26a [b] ($R_1=R_2=H$)	8.0	64	1.36	1.4×10^{8}
26d [b] ($R_1=R_2$=Me)	----	20	----	----
43a [c] ($R_1=R_2=H$)	9.5	12×10^{-4}	2.2×10^{-4}	7×10^{5}
43c [c] (R_1=H, R_2=Me)	9.52	5.6×10^{-4}	3.5×10^{-4}	12×10^{5}
43d [c] ($R_1=R_2$=Me)	----	$\sim1\times10^{-4}$	----	----

[a] Values taken from references 55b and 124 except where otherwise noted.

[b] Reference 19. [c] Reference 134. [d] Reference 136

k_2^{obsd} (see equation 78 and Table VI), corresponding to reaction via the anions 23. Similar behavior has been observed for various phenols[138,139,159,160] and for 6-azauracils (43) unsubstituted at the N_1 position (Ref. 134 and Table VI). In fact, 43 (R_1=H, R_2=H or Me) can react with bromine as an anion even in 0.05 - 0.50 N sulphuric acid solutions.[134] Furthermore, Santi and co-workers[140] have proposed that hydrogen-deuterium exchange of uracil and 3-methyluracil proceeds by way of their anions in basic media (pH 7 - 10) at 90 °C. Since bromine can be as much as 10^5 - 10^{14} times as reactive an electrophile as hydronium ion[141], it is quite reasonable that bromination via the same anions is operative at pH > 3.

From Table VI and Figure 5 it can be seen that the reactivity of 5-bromouracil (26a) approaches that of uracil (21a) (and even surpasses that of 3-methyluracil (21c)) for pH > 3 since the product $k_2^{A^-}K_a'$, the term making the largest contribution to k_2^{obsd} in weak acid, is virtually the same for 21a and 26a. This would seem to explain Moore and Anderson's[135] observation that during the 1 : 2 titration of uracil with bromine at pH 4.7 there did not appear to be any large buildup of 5-bromouracil (see page 100). This implies that at high pH the deprotonation 25a $\longrightarrow$ 27 can happen fast with, perhaps, hydroxide ion acting as the base.

We have found that even in 0.1 N sulphuric

acid the titration of uracil with bromine is 1 : 2. This is most surprising since at this acidity (pH 1.26) reaction must occur via the free bases 21a or 26a. Our kinetic results, however, show 21a to be ~800 times as reactive towards bromine as 26a is (see Table VI and Figure 5). Furthermore, the deprotonation of 24a to 26a (see Scheme VII) proceeds too slowly at this acidity to account for any rapid formation of 5-bromouracil.[126] A 1 : 1 mixing of bromine with uracil produces an absorption spectrum with a λ_{max} of 264 nm. This spectrum can not be associated with the long-lived intermediate 22a, which does not absorb in the uv, or 5-bromouracil, 26a, which has an absorption maximum at 276 nm. Addition of a second equivalent of bromine results in the disappearance of the absorption spectrum of the intermediate indicating that it is capable of reaction with bromine. This would seem to account for the observed results for the titration of uracil with bromine in 0.1 N sulphuric acid.

We have attempted to study the kinetics of the reaction between the intermediate and bromine. The intermediate, after being generated by a 1 : 1 mixing of uracil with bromine (mixed together in the stopped-flow apparatus), was reacted 1 : 1 with bromine and monitored using the stopped-flow apparatus. The results of these experiments, however, do not analyze well by traditional methods for the determination of reaction order.[162]

Likewise, the 1 : 2 reaction between uracil and bromine gives kinetic results which do not analyze well by conventional means of first- or second-order analysis.

We have also carried out kinetic experiments under conditions where the uracil bromine ratio was 1 : 1 and 3 : 1. The absorbance/time data do not give satisfactory second-order analysis using the observed A_∞ values. However, they do analyze well for first-order behavior. Use of the van't Hoff method of determining order[163] also supports the order being one, and not two. These results differ from those previously obtained using a sizeable excess of uracil over bromine which appeared consistent with second-order behavior (see page 102).

Table VII (next page) shows values of k_1^{obsd} obtained at two pH's (4.7 and 5.15) by conventional analysis using the observed values of A_∞. At pH 4.7 the rate constant increases monotonically with the uracil concentration. This is best seen by the values in the column headed $k_1^{obsd}/[uracil]$ which remain essentially constant. Moreover, there is agreement with the analogous value obtained when uracil was in ten-fold excess. Unfortunately, there is no such consistency amongst the results obtained at pH 5.15 (see Table VII).

The exact significance of these results

Table VII

Observed first-order rate constants (k_1^{obsd}) for the reaction of uracil with bromine at pH's 4.7 and 5.15.

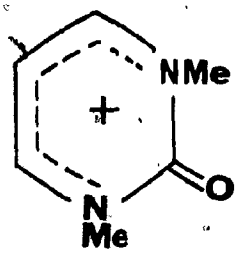
pH	[Uracil] (M)	[Bromine] (M)	k_1^{obsd} (S^{-1})	$\frac{k_1^{obsd}}{[Uracil]}$ ($M^{-1}S^{-1}$)	$\frac{[Uracil]}{[Bromine]}$
4.70	2×10^{-5}	2×10^{-5}	0.710	3.55×10^{4}	1:1
4.71	5×10^{-5}	5×10^{-5}	1.73	3.46×10^{4}	1:1
4.75	1.5×10^{-4}	5×10^{-5}	5.32	3.55×10^{4}	3:1
4.70[a]	5×10^{-4}	5×10^{-5}	18.8	3.76×10^{4}	10:1
5.17	5×10^{-5}	5×10^{-5}	3.31	6.62×10^{4}	1:1
5.15	1.5×10^{-4}	5×10^{-5}	5.34	3.56×10^{4}	3:1
5.15[a]	5×10^{-4}	5×10^{-5}	37.9	7.58×10^{4}	10:1

[a] Rate constant obtained from the rate profile for the bromination of uracil presented in reference 19.

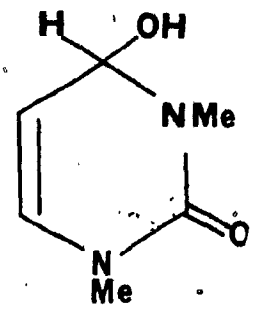
remains unclear at this time. It does appear, however, that the mechanistic picture is more complex than we thought earlier[19,20], at least for the uracils having an ionizable hydrogen at N_1. It may be the case that the principal pathway followed depends not only on pH, but also on the concentrations of the uracil and the bromine and the way in which they are mixed (stopped-flow versus titration). The possible incursion of N-bromo species needs to be seriously considered.

Pseudobase Formation and Decomposition of the 1,2-Dihydro-1,3-dimethyl-2-oxopyrimidinium Cation

An investigation of the bromination of 1,2-dihydro-1,3-dimethyl-2-oxopyrimidinium cation ($\underline{Q^+}$) by Tee and Thackray[21] had found that the reaction involves bromine attack upon the pseudobase, QOH. The proposed mechanism is presented in Scheme VIII. In relatively strong



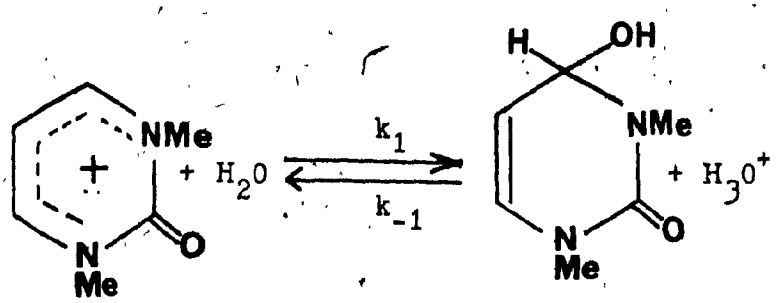
$\underline{Q^+}$



QOH

acid (pH 0 - 2) bromine attack at the 5-position of QOH is rate-determining, whereas at lower acidities (pH 4 - 5) the formation of QOH is the slow step. This change occurs because at high acidities the rate of acid-catalyzed

Scheme VIII



Q^+ QOH

$K_{R^+} = k_1/k_{-1}$

k_2 Br_2

product

decomposition of QOH is faster than the rate of bromine attack. At lower acidities, however, the reverse is true, and so formation of QOH becomes rate-limiting.

These results prompted us to undertake a kinetic study of the equilibrium between Q^+ and QOH. This would provide values of k_1 and k_{-1} which could be compared to the values obtained from the bromination investigation (see Scheme VIII). Good agreement would furnish additional support for the validity of Scheme VIII.

The experimental approach followed (see also page 41ff) was that which Bunting and co-workers have used in their studies of quaternary heterocyclic cations (Refs. 99,100 and references therein). The studies were performed by monitoring the disappearance of Q^+ (or QOH) spectrophotometrically. The reaction was initiated by mixing in a stopped-flow apparatus a solution of Q^+ (or QOH) with a buffer (ionic strength of 0.02 M) of $pH > pK_R^+$ (or $pH < pK_R^+$). Since both solutions were 0.1 M in KBr the total ionic strength of the mixed solution was 0.11 M. Experimental conditions (30 °C, ionic strength = 0.11 M) were chosen to be the same as those used in the bromination studies of Tee and Thackray.[21]

The approach of the system to equilibrium

followed first-order kinetics with good first-order behavior observed for >90% reaction. The measured first-order rate constant for opposing first-order reactions approaching equilibrium is the sum of the first-order rate constants for the forward-and reverse reactions.[146] Therefore the observed pseudo first-order rate constants, k_1^{obsd}, for the equilibration of $\underline{Q^+}$ with $\underline{QOH}$ is the sum of the pseudo first-order rate constants k_f and k_d for the formation and decomposition of $\underline{QOH}$. The situation may be presented as

$$\underline{Q^+} \underset{k_d}{\overset{k_f}{\rightleftharpoons}} \underline{QOH} \qquad (81)$$

for which $$k_1^{obsd} = k_f + k_d \qquad (82)$$

Now at equilibrium

$$k_f[\underline{Q^+}]_{eq} = k_d[\underline{QOH}]_{eq} \qquad (83)$$

where $[\underline{Q^+}]_{eq}$ and $[\underline{QOH}]_{eq}$ represent the equilibrium concentrations of $\underline{Q^+}$ and $\underline{QOH}$ respectively.

Additionally, $$K_{R^+} = \frac{[\underline{QOH}]_{eq}[H^+]}{[\underline{Q^+}]_{eq}} \qquad (84)$$

And so $$K_{R^+} = \frac{k_f[H^+]}{k_d}$$

The symbol K_{R^+} is used to point out the similarity of cation-pseudobase equilibria such as $\underline{Q^+} \rightleftharpoons \underline{QOH}$ to carbonium ion-carbinol equilibria for which K_{R^+} was originally intended.[147] Equation 82 may then be rewritten as

$$k_1^{obsd} = k_f + \frac{k_f[H^+]}{K_{R^+}} \qquad (85)$$

or

$$k_1^{obsd} = k_d + \frac{k_d K_{R^+}}{[H^+]} \qquad (86)$$

Therefore rearranging equation 85 gives

$$k_f = \frac{k_1^{obsd} K_{R^+}}{(K_{R^+} + [H^+])} \qquad (87)$$

and rearranging equation 86 gives

$$k_d = \frac{k_1^{obsd} [H^+]}{(K_{R^+} + [H^+])} \qquad (88)$$

Thus the values of k_f and k_d at each acidity studied can be calculated from the appropriate k_1^{obsd} provided pK_{R^+} is known.

Using the spectrophotometric method of Albert and Serjeant[108] pK_R^+ was found to be 7.16 at 30 °C. This agrees well with the potentiometrically determined value of 7.12 reported by Tee and Endo[94] at 23-24 °C.

In Table VIII (next page) are listed the values of k_f and k_d calculated from the measured first-order rate constants, k_1^{obsd}, by using equations 87 and 88. Outside of the pH range shown the equilibrations are too rapid to follow with our instrumentation.

The pH profiles for k_f and k_d are given in Figure 6 (page 124). It can be seen that k_f and k_d are composed of acid independent and acid dependent terms. The curve for k_f remains fairly level up to about pH 7 after which the formation of QOH is a base-catalyzed process. The decomposition of QOH is acid dependent throughout the pH range studied. However, a levelling off in k_d is definitely apparent above pH 9. The rate profiles for k_f and k_d intersect at pH = pK_R^+, where the equilibrium mixture contains equal concentrations of cation and pseudo-base.[89] The deviations of the data from the calculated curves is attributable to buffer catalysis (vide infra).

The pH profiles displayed in Figure 6 may be described by equations 89 and 90 (page 125).

Table VIII

Rate constants for the formation and the decomposition of pseudobase QOH.[a]

pH	k_1^{obsd} (S^{-1})	k_f (S^{-1})	k_d (S^{-1})
5.64	70.6	1.38	69.2
6.05	22.8[b]	1.64	21.2
6.20	16.8	1.66	15.1
6.50	10.6	1.90	8.70
6.96	5.60	2.16	3.44
7.42	4.30	2.78	1.52
8.00	4.07	3.56	0.514
8.55	8.35	8.02	0.327
8.96	31.5	31.0	0.491
9.45	61.3	61.0	0.313

[a] At 30 °C, KBr = 0.1 M, ionic strength = 0.11 M. Each k_1^{obsd} is the average of 3 or more runs.

[b] Average value (± 1.0) obtained in a phosphate buffer. Average value obtained in a succinate buffer was 28.8 ± 2.0 S^{-1}.

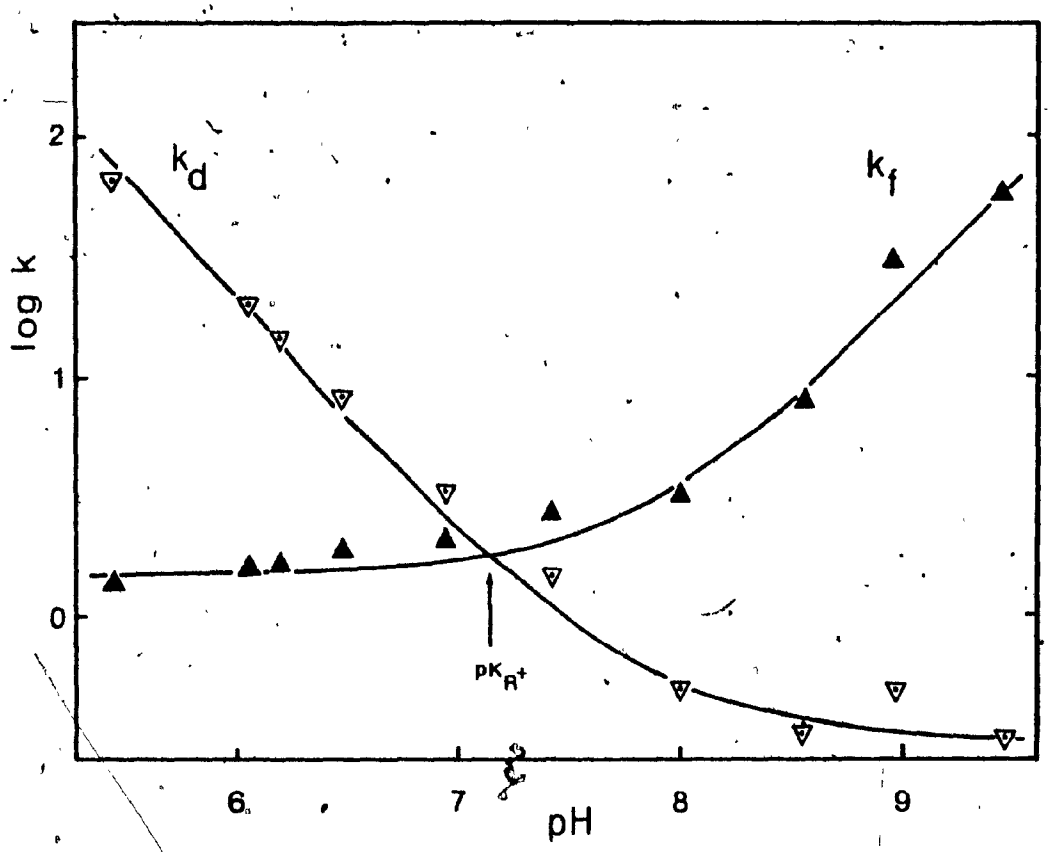


Figure 6. Acidity dependence of rate constants for formation (k_f) and decomposition (k_d) of pseudobase QOH.

$$k_f = k_1 + k_{OH}[OH^-] \qquad (89)$$

and

$$k_d = k + k_{-1}[H^+] \qquad (90)$$

The rate constants k_1, k_{OH}, k and k_{-1} are defined by the following equations.

$$\underline{Q^+} + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underline{QOH} + H^+ \qquad (91)$$

$$\underline{Q^+} + OH^- \underset{k}{\overset{k_{OH}}{\rightleftharpoons}} \underline{QOH} \qquad (92)$$

The calculated curves in Figure 6 were constructed from equations 89 and 90 with $k_1 = 1.5\ S^{-1}$, $k_{OH} = 2.1 \times 10^6\ M^{-1}S^{-1}$, $k = 0.31\ S^{-1}$ and $k_{-1} = 2.2 \times 10^7\ M^{-1}S^{-1}$.

The acidity dependence of k_1^{obsd} is shown in Figure 7 (next page). The calculated curve is the sum of the calculated values for k_f and k_d at each pH.

In the region pH < 6.5 k_1^{obsd} is primarily a measure of the rate constant for the decomposition of $\underline{QOH}$, k_d, whereas for pH > 7.5 it is roughly equal to the rate constant for the formation of $\underline{QOH}$, k_f. In the pH region between 6.5 and 7.5 k_f and k_d are of the same order of magnitude. Therefore the formation and decomposition

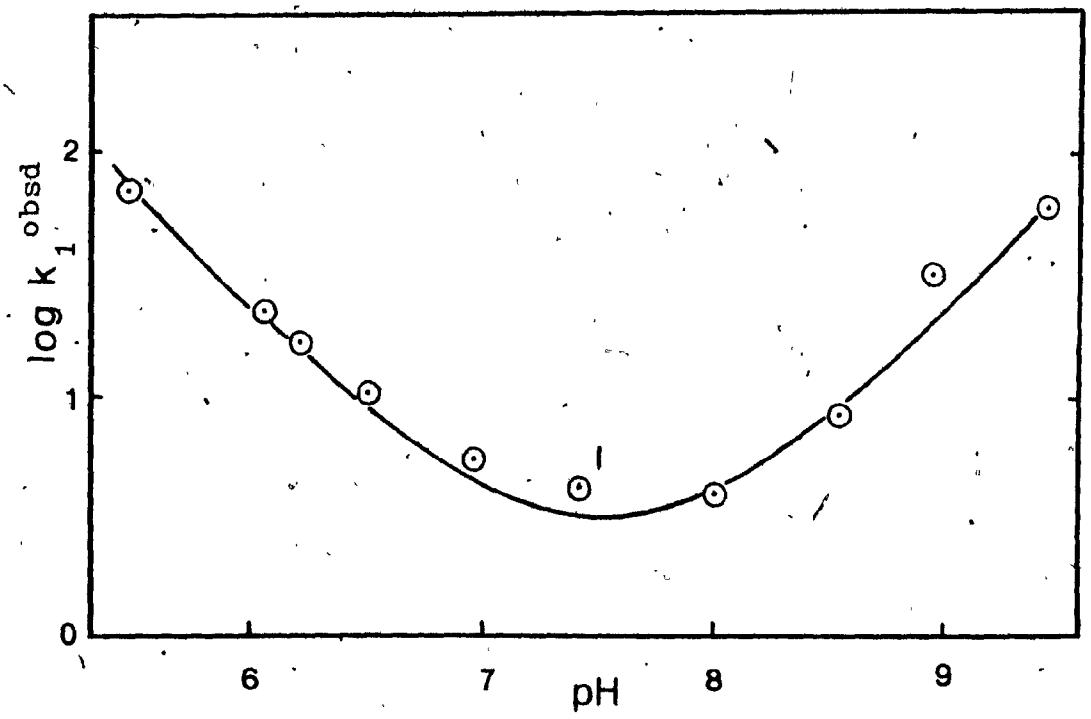


Figure 7. Acidity dependence of k_1^{obsd} for formation and decomposition of pseudobase QOH.

of QOH occur simultaneously at these acidities.

The deviations of the data points from the calculated curves in Figures 6 and 7, as mentioned previously, are most likely due to buffer catalysis. That is, the mechanism of QOH formation may involve general base assisted nucleophilic attack by water. Therefore the reverse reaction, pseudobase decomposition, would be general acid-catalyzed. The situation can be represented as

$$Q^+ + H_2O + B^- \underset{k_A}{\overset{k_B}{\rightleftharpoons}} QOH + HB \qquad (93)$$

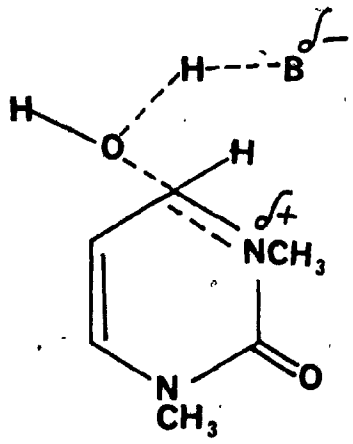
where $$k_f = \sum_B k_B[B^-] \qquad (94)$$

and $$k_d = \sum_A k_A[HB] \qquad (95)$$

Hence, k_f is the sum of terms resulting from general base-catalyzed attack by water on Q^+ (equation 94) and k_d is the sum of terms due to general acid assisted loss of hydroxyl from QOH (equation 95). These processes are illustrated on the next page.

Bunting, based on the evidence obtained from solvent isotope effects, entropies of activation and buffer catalysis experiments, has proposed a similar mechanism for the cation-pseudobase systems he has investigated.[89,109,110]

A possible effect of buffer catalysis is that at pH 6.05 it was found that $k_1^{obsd} = 28.8 \pm 2.0\ S^{-1}$ in a succinate buffer and that $k_1^{obsd} = 22.8 \pm 1.0\ S^{-1}$ in a phosphate buffer.



It is interesting to note that Bunting and Norris[109] have reported that the ratio k_{OH}/k_1 varied very little ($\sim 10^7\ M^{-1}$) for a variety of cations whose K_{R^+} values covered a 10^6 range. With repect to a mechanism such as equation 93, k_{OH} and k_1 are ascribed to hydroxide ion and a water molecule, respectively, acting as general bases assisting in the nucleophilic attack of water upon

QOH. The ratio k_{OH}/k_1 is therefore a measure of the relative basicities of hydroxide ion and water. For our system the ratio is 0.14×10^7 M^{-1} which is in good agreement to those values obtained by Bunting and Norris.[109]

The values of k_1 and k_{-1} from our study (1.5 S^{-1} and 2.2×10^7 $M^{-1}S^{-1}$ respectively), although a little high, are in good agreement with those derived from the bromination studies of Tee and Thackray[21] (k_1 = 1.3 S^{-1} and k_{-1} = 1.9×10^7 $M^{-1}S^{-1}$). Thus the present study of the equilibration of Q^+ and QOH provides strong independent support for the mechanism shown in Scheme VIII (page 118), which was proposed to explain the bromination kinetics.[21]

REFERENCES

REFERENCES

1. D. T. Hurst, "An Intro. to the Chemistry and Biochemistry of Pyrimidines, Purines, and Pteridines", John Wiley and Sons, New York, 1980.

2. R. Duschinsky, E. Plever and C. Heidelberger, J. Am. Chem. Soc., 1957, 79, 4559.

   C. Heidelberger and F. J. Ansfield, Cancer Res., 1963, 23, 1226.

3. C. C. Cheng, "Progress in Medicinal Chemistry", (Edited by G. P. Ellis and G. B. West), Vol. 6, Butterworths, London, 1969.

4. H. J. Schaeffer, L. Beauchamp, P. de Miranda, G. B. Elion, D. J. Bauer and P. Collins, Nature, 1978, 272, 583.

5. T. C. Hall, J. Griffith, G. Watters, R. Baringer and S. Katz, Pharmacologist, 1968, 10, 171.

6. R. J. Suhadolnik, "Nucleoside Antibiotics", Wiley-Interscience, New York, 1970.

7. F. Sorm and J. Vesely, Neoplasma, 1968, 15, 339.

8. G. E. Underwood, Proc. Soc. Exp. Biol. Med., 1962, 111, 660.

9. H. E. Kaufman, Ann. N.Y. Acad. Sci., 1965, 130, 168.

10. R. Breslow, J. Am. Chem. Soc., 1957, 79, 1762.

11. R. Breslow, J. Am. Chem. Soc., 1958, 80, 3719.

12. J. Ullrich and A. Mannschreck, Biochim. Biophys. Acta., 1966, 115, 46.

13. W. J. Doran, "Medicinal Chemistry", (Edited by F. F. Blicke and H. Cox), Vol. 4, Wiley, New York and London, 1959.

14. A. Albert, "Heterocyclic Chemistry", 2nd Edition, The Athlone Press, London, 1968.

15. S. Y. Wang, Nature (London), 1957, 180, 91.

16. S. Y. Wang, J. Org. Chem., 1959, 24, 11.

17. S. Banerjee, Ph.D. Thesis, Concordia University, 1974.

18. H. Taguchi and S. Y. Wang, J. Org. Chem., 1979, 44, 4385.

19. C. G. Berks, M.Sc. Thesis, Concordia University, 1978.

20. O. S. Tee and C. G. Berks, J. Org. Chem., 1980, 45, 830.

21. O. S. Tee, D. C. Thackray and C. G. Berks, Can. J. Chem., 1978, 56, 2970.

22. A. Pullman, "Electronic Aspects of Biochemistry", (Edited by B. Pullman), Academic Press, New York, 1964.

23. D. L. Barker and R.E. Marsh, Acta Crystallogr., 1964, 17, 1581.

24. G. A. Jeffrey and Y. Kinoshita, Acta Crystallogr., 1963, 16, 20.

25. R. J. McClure and B. M. Craven, Acta Crystallogr., Section B, 1973, 29, 1234.

26. S. Furberg, C. S. Peterson and C. Romming, Acta Crystallogr., 1965, 18, 313.

27. J. S. Kwiatkowski and B. Pullman, Adv. Heterocycl. Chem., 1975, 18, 200.

28. A. R. Katritzky and A. J. Waring, J. Chem. Soc., 1963, 3046.

29. M. Dreyfus, O. Bensaude, G. Dodin and J. E. Dubois, J. Am. Chem. Soc., 1976, 98, 6338.

30. G. S. Parry, Acta Crystallogr., 1954, 7, 313.

31. R. F. Stewart and L. H. Jansen, Acta Crystallogr., 1967, 23, 1102.

32. K. Ozeki, N. Sakabe and J. Tanake, Acta Crystallogr., Section B, 1969, 25, 1038.

33. R. Gerdil, Acta Crystallogr., 1961, 14, 333.

34. D. W. Green, F. S. Mathews and A. Rich, J. Biol. Chem., 1962, 237, 3573.

35. B. M. Craven, Acta Crystallogr., 1967, 23, 376.

36. E. Shefter, J. Chem Soc. B, 1970, 903.

37. P. Singh and D. J. Hodgson, J. Chem. Soc. Chem. Commun., 1962, 439.

38. D. R. Harris and W. M. MacIntyre, Biophys. J., 1964, 4, 203.

39. J. Iball, C. H. Morgan and H. R. Wilson, Proc. Roy. Soc. Ser. A, 1967, 295, 320.

40. D. Suck and W. Saenger, J. Am. Chem. Soc., 1972, 94, 6520.

41. C. H. Schwalbe and W. Saenger, J. Mol. Biol., 1973, 75, 129.

42. H. T. Miles, Biochim. Biophys. Acta, 1956, 22, 247.

43. H. T. Miles, Biochim. Biophys. Acta, 1958, 27, 46.

44. K. Nakanishi, N. Suzuki and P. Yamazaki, Bull. Chem. Soc. Jap., 1961, 34, 53.

45. R. C. Lord and G. J. Thomas, Spectrochim. Acta, Part A, 1967, 23, 2551.

46. R. C. Lord and G. J. Thomas, Develop. Appl. Spectrosc., 1968, 6, 179.

47. J. P. Kokko, J. H. Goldstein and L. Mandell, J. Am. Chem. Soc., 1962, 84, 1042.

48. L. Gatlin and J. C. Davis, J. Am. Chem. Soc., 1962, 84, 4464.

49. J.P. Kokko, L. Mandell and J. H. Goldstein, J. Am. Chem. Soc., 1962, 84, 1042.

50. J. J. Fox and D. Shugar, Biochim. Biophys. Acta, 1952, 9, 199.

51. J. J. Fox and D. Shugar, Biochim. Biophys. Acta, 1952, 9, 369.

52. A. R. Katritzky and A. J. Waring, J. Chem. Soc., 1962, 1540.

53. W. Hauswirth and M. Daniels, Photochem. Photobiol., 1971, 13, 157.

54. M. Daniels, Proc. Nat. Acad. Sci. U.S., 1972, 69, 2488.

55. (a) D. J. Brown, "The Pyrimidines", Vol. 16, "Chem. of Heterocycl. Compounds", (Edited by A. Weissberger), Interscience, New York, 1962.

(b) D. J. Brown, "The Pyrimidines. Supplement I", Wiley-Interscince, New York, 1970.

56. J. N. Davidson, "The Biochemistry of the Nucleic Acids", 7th Edition, Academic Press, New York, 1972.

57. G. E. Hilbert and E. F. Jansen, J. Am. Chem. Soc., 1934, 56, 134.

58. D. J. Brown, J. Appl. Chem. (London), 1952, 2, 239.

59. T. B. Johnson and I. Matsuo, J. Am. Chem. Soc., 1919, 41, 782.

60. C. O. Johns, Amer. Chem. J., 1911, 45, 79.

61. D. J. Brown, E. Hoerger and S. F. Mason, J. Chem. Soc., 1955, 211.

62. G. R. Barber, N. G. Luthy and M. M. Dhar, J. Chem. Soc., 1954, 4206.

63. R. R. Herr, T. Enkoji and T. J. Bardos, J. Am. Chem. Soc., 1956, 78, 401.

64. N. V. Khromov-Borisov and R. S. Karlinskaya, J. Gen. Chem. (USSR), 1957, 27, 2518.

65. N. V. Khromov-Borisov and R. S. Karlinskaya, J. Gen. Chem. (USSR), 1954, 24, 2212.

66. H. Bredereck, R. Gompper and H. Herlinger, Angew. Chem., 1958, 70, 571.

67. H. Bredereck, R. Gompper and H. Herlinger, Chem. Ber., 1958, 91, 2832.

68. A. P. Phillips, N. B. Mehta and J. Z. Strelitz, J. Org. Chem., 1963, 28, 1488.

69. I. Wempen and J. J. Fox, J. Med. Chem., 1963, 6, 688.

70. H. L. Wheeler and T. B. Johnson, J. Biol. Chem., 1907, 3, 183.

71. M. Soodak, A. Pircio and L. R. Cercedo, J. Biol. Chem., 1949, 181, 712.

72. T. B. Johnson, J. Am. Chem. Soc., 1940, 62, 2269.

73. M. P. L. Caton, M. S. Grant, D. L. Pain and R. Slack, J. Chem. Soc., 1965, 5467.

74. N. M. Bui, R. Gillet and P. Dumont, Internat. J. Appl. Radiation Isotopes, 1965, 16, 337.

75. W. Klotzer and M. Herberg, Monatsh., 1968, 99, 847.

76. H. Decker, J. Prakt. Chem. (N. S.), 1893, 47, 28.

77. A. Hantzsch, Chem. Ber., 1899, 32, 575.

78. A. Hantzsch and M. Kalb, Chem. Ber., 1899, 32, 3109.

79. A. Albert and W. L. F. Armarego, Adv. Heterocycl. Chem., 1965, 4, 1.

80. D. D. Perrin, Adv. Heterocycl. Chem., 1965, 4, 43.

81. A. Albert, Angew Chem., Internat. Ed. Engl., 1967, 6, 919.

82. A. Albert, Adv. Heterocycl. Chem., 1976, 20, 117.

83. E. Buncel, A. R. Norris and K. E. Russel, Q. Rev., Chem. Soc., 1968, 22, 123.

84. P. Buck, Angew. Chem., Internat. Ed. Engl., 1969, 8, 120.

85. M. R. Crampton, Adv. Phys. Org. Chem., 1969, 7, 211.

86. M. J. Strauss, Chem. Rev., 1970, 70, 667.

87. R. Foster and C. A. Fyfe, Rev. Pure Appl. Chem., 1966, 16, 61.

88. C. F. Bernasconi, Acc. Chem. Res., 1978, 11, 147.

89. J. W. Bunting, Adv. Heterocycl. Chem., 1979, 25, 1.

90. O. S. Tee, M. Trani, R. A. McClelland and N. E. Seaman, J. Am. Chem. Soc., 1982, 104, 7219.

91. M. Trani, Undergraduate Research Project, Concordia University, 1982.

92. O. S. Tee and S. Banerjee, J. Org. Chem., 1979, 44, 3256.

93. J. W. Bunting and W. G. Meathrel, Can. J. Chem., 1972, 50, 917.

94. O. S. Tee and M. Endo, Can. J. Chem., 1976, 54, 2681.

95. O. S. Tee and G. V. Patil, J. Org. Chem., 1976, 41, 838.

96. S. Banerjee, O. S. Tee and K. D. Wood, J. Org. Chem., 1977, 42, 3670.

97. T. T. Sakai, A. L. Pagolotti Jr. and D. V. Santi, J. Heterocycl. Chem., 1968, 5, 849.

98. I. Wempen, G. B. Brown, T. Ueda and J. J. Fox, Biochem., 1965, 4, 54.

99. G. W. Kenner, C. B. Reese and A. R. Todd, J. Chem. Soc., 1955, 855.

100. T. Nishimura and I. Iwai, Chem. Pharm. Bull., 1964, 12, 352.

101. H. L. Wheeler and T. B. Johnson, Amer. Chem. J., 1904, 31, 591.

102. "Aldrich Catalog Handbook of Fine Chemicals", Aldrich Chemical Company, Milwaukee, 1979.

103. Taguchi and Wang, reference 18, report a melting point of 170-171 °C. This most likely is a misprint.

104. J. F. Codington, R. Fecher, M. H. Maguire, R. Y. Thompson and G. B. Brown, J. Am. Chem. Soc., 1958, 80, 5164.

105. D. D. Perrin, Aust. J. Chem., 1963, 16, 572.

106. J. J. Morrow, Chem. Instrum., 1970, 2, 375.

107. J. M. Pink, Can. J. Chem., 1970, 48, 1169.

108. A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases", Methuen, London, 1962.

109. J. W. Bunting and D. J. Norris, Can. J. Chem., 1977, 99, 1189.

110. J. W. Bunting and W. G. Meathrel, Can. J. Chem., 1973, 51, 1965.

111. A. A. Frost and R. G. Pearson, "Kinetics and Mechanisms", John Wiley and Sons, New York, 1953.

112. R. L. Pecsok and L. D. Shields, "Modern Methods of Chemical Analysis", Wiley, New York, 1968.

113. E. S. Swinbourne, J. Chem. Soc., 1960, 2371.

114. R. P. Bell and E. N. Ramsden, J. Chem. Soc., 1958, 161.

115. M. Davies and E. Gwynne, J. Am. Chem. Soc., 1952, 74, 2748.

116. S. Slae and R. Shapiro, J. Org. Chem., 1978, 43, 1721.

117. O. S. Tee and S. Banerjee, Can. J. Chem., 1979, 57, 626.

118. C. Nofre, M. Murat and A. Cier, Bull. Soc. Chim. Fr., 1965, 1749.

119. M. Chabre, D. Gagnier and C. Nofre, Bull. Soc. Chim Fr., 1966, 108.

120. P. Rouillier, J. Delman and C. Nofre, Bull. Soc. Chim. Fr., 1966, 3515.

121. P. Rouillier, J. Delman, J. Duplan and C. Nofre, Tetrahedron Lett., 1966, 4189.

122. M. J. Robins, M. MacCoss, S. R. Naik and G. Ramani, J. Am. Chem. Soc., 1976, 98, 7381.

123. C. D. Johnson, A. R. Katritzky and S. A. Shapiro, J. Am. Chem. Soc., 1969, 91, 6654.

124. J. J. Christensen, J. H. Rytting and R. M. Izatt, J. Phys. Chem., 1967, 71, 2700.

125. R. Shapiro, V. Difate and M. Welcher, J. Am. Chem. Soc., 1974, 96, 906.

126. S. Banerjee and O. S. Tee, J. Chem. Soc., Chem. Commun., 1974, 535.

127. Reference 124 gives pK_1=4.08 (ionic strength=0 at 25 °C).

128. O. S. Tee and M. Paventi, J. Org. Chem., 1980, 45, 2072.

129. O. S. Tee and M. Paventi, J. Org. Chem., 1981, 46, 4172.

130. C. Verny-Doussin, Ph.D. Thesis, University of Paris, 1979.

131. See pages 114ff.

132. M. Eigen, Angew. Chem., Internat. Ed. Engl., 1964, 3, 1.

133. S. Banerjee and O. S. Tee, J. Org. Chem., 1974, 39, 3120.

134. S. Banerjee and O. S. Tee, J. Org. Chem., 1976, 41, 4004.

135. A. M. Moore and S. M. Anderson, Can. J. Chem., 1959, 37, 590.

136. The literature pK_a for uracil (=9.46) applies to ionization of the N_3-H bond.[124, 137] Since the pK_a's for 1-methyluracil and 3-methyluracil are 9.74 and 9.95 respectively[55b], it is likely that the pK_a for ionization of the N_1-H bond is ~9.65.

137. J. Elguero, C. Marzin, A. R. Katritzky and P. Linda, "The Tautomerism of Heterocycles", Academic Press, New York, 1976.

138. R. P. Bell and D. J. Rawlinson, J. Chem. Soc., 1961, 63.

139. J. Kulic and M. Vecera, Collect. Czech. Chem. Commun., 1974, 39, 171.

140. D. V. Santi, C. F. Brewer and D. Farber, J. Heterocycl. Chem., 1970, 7, 903.

141. Bromine is 10^5 times as reactive as H_3O^+ toward the very reactive enol of acetone[142,143], toward much less reactive styrene it is 10^{14} times as reactive[144,145] (all reactions in water at 25 °C).

142. J. E. Dubois and J. Toullec, J. Chim. Phys. Phys.-Chim. Biol., 1968, 65, 2166.

143. J. E. Dubois and J. Toullec, J. Chem. Soc., Chem. Commun., 1969, 478.

144. R. Bolton, "Comprehensive Chemical Kinetics", (Edited by C. H. Bamford and C. F. H. Tipper), Vol. IX, Elsevier, Amsterdam, 1973.

145. M. F. Ruasse and J. E. Dubois, J. Am. Chem. Soc., 1975, 97, 1977.

146. K. J. Laidler, "Chemical Kinetics", McGraw-Hill, New York, 1965.

147. N. C. Deno, J. J. Jaruzelski and A. Schriesheim, J. Org. Chem., 1954, 19, 155.

148. O. S. Tee and S. Banerjee, Can. J. Chem., 1974, 52, 451.

149. O. S. Tee, M. J. Kornblatt and C. G. Berks, J. Org. Chem., 1982, 47, 1018.

150. S. Banerjee, C. G. Berks and O. S. Tee, "Abstracts of Papers - 2nd Joint Conference Chemical Institute of Canada / American Chemical Society", Montreal, May 29 - June 2, 1977.

151. C. G. Berks and O. S. Tee, "Abstacts of Papers - 61st Canadian Chemical Conference", Winnipeg, June 4 - 7, 1978.

152. C. G. Berks and O. S. Tee, "Abstracts - 63rd Canadian Chemical Conference", Ottawa, June 8 - 11, 1980.

153. C. J. Collins, Adv. Phys. Org. Chem., 1964, 2, 1.

154. S. Slae and R. Shapiro, J. Org. Chem., 1978, 43, 4197.

155. See references 19, 20, 117, 126, 133 and 134.

156. R. Shapiro, R. E. Servis and M. Welcher, J. Am. Chem. Soc., 1970, 92, 1422.

157. M. Shiragani, I. Kudo, S. Iida and H. Hayatsu, Chem. Pharm. Bull., 1975, 23, 3027.

158. J. H. Ridd, Adv. Phys. Org. Chem., 1978, 16, 1.

159. O. S. Tee, N. Rani Iyengar and M. Paventi, J. Org. Chem., 1983, 48, 759.

160. O. S. Tee and M. Paventi - Unpublished results.

161. E. S. Swinbourne, "Analysis of Kinetic Data", Thomas Nelson and Sons Ltd, London, 1971, section 4.1.

162. Reference 161, sections 3.2, 4.1 and 4.2.

163. Reference 161, section 3.2.