temperature and then acidified with dilute HCl while cooling. The initial red color turned to yellow and then white. After the mixture was cooled in ice, a solid precipitate was collected, washed with H_2O, and dried under vacuum (0.1 mm). Taken up in 2 L of boiling CH_3CN containing Norit, filtered, concentrated to 1 L, and cooled, it gave a first crop, and after concentration to 250 mL, a second was obtained for a total yield of tetranitrile **2** amounting to 3.15 g (81%): mp 325–330 °C dec (lit.[2] mp >360 °C); ^1H NMR (Me_2SO-d_6) δ 8.38, 8.28 (s, 7.75 H, aromatic), 6.68 (s, 2.26 H, $C(CN)_2\mathbf{H}$); IR (KBr) 3025 (w), 2944 (s), 2255 (w, saturated CN), 1608 (m), 1448 (w), 1408 (w), 1327 (m), 1002 (m), 900 (s), 875 (s), 712 (s), 694 (m), 382 cm^{-1} (m). UV (CH_3CN) λ_{max} 273 nm (log ϵ 4.53), 246.5 (4.73), 264 (4.33), 275 (4.39), 309 (4.26), 323 (4.65), 337 (4.60), 372 (3.83). Anal. Calcd for $C_{22}H_{10}N_4$: C, 79.99; H, 3.05; N, 16.96. Found: C, 80.22; H, 3.21; N, 16.75.

Bis(tetra-*n*-butylammonium) Salt of 2 (15). The tetranitrile **2** (0.203 g, 0.62 mmol), tetra-*n*-butylammonium hydroxide (0.352 g, 1.36 mmol, 40% solution/H_2O), and 50 mL of CH_3CN were added to a nitrogen-flushed 100-mL round-bottomed flask containing a stirring bar. The reaction mixture was stirred until all the solid had dissolved (ca. 45 min), and H_2O (125 mL) was added to precipitate the product, which was collected and dried under vacuum (0.1 mm), giving an orange solid, 0.41 g (82%). An analytical sample was prepared by recrystallization from 2:3 EtOH/H_2O: mp 209–210.5 °C (red liquid); ^1H NMR (CD_3CN) δ 7.69, 7.50 (s, 7.94 H, aromatic), 3.02 (t, asymmetric, skewed, 16.05 H, J = 8.64 Hz, $N^+\mathbf{CH_2}$), 1.72–1.08 (m, br, 32.10 H), 0.94 (t, asymmetric, skewed, 23.91 H, J = 6.40 Hz, $CH_2\mathbf{CH_3}$); IR (KBr) 2957 (s), 2870 (m), 2157 (s, $^-C(CN)_2$), 2120 (s, $^-C(CN)_2$), 1600 (s), 1578 (m), 1478 (m), 1450 (s), 1377 (m), 1318 (s), 1208 (m), 1150 (m), 764 (m); UV (CH_3CN) λ_{max} 234 nm (log ϵ 4.43), 295 (4.38), 310 (4.75), 323 (5.18), 337 (4.83), 365 (4.83). Anal. Calcd for $C_{54}H_{80}N_6$: C, 79.75; H, 9.92; N, 10.33. Found: C, 79.82; H, 9.73; N, 10.28.

Purple Solid. Tetranitrile **2** (0.119 g, 0.36 mmol) and 140 mL of dry CH_3CN were added to a flame-dried, N_2-flushed, 250-mL round-bottomed flask equipped with a magnetic stirring bar and a reflux condenser with a N_2 gas inlet. The suspension was heated to reflux until **2** dissolved, and DDQ (0.40 g, 1.76 mmol) in 1 mL of dry CH_3CN was then syringed in over a 1-min period. A purple precipitate formed, and the reaction mixture was refluxed further for 15 min. After the mixture cooled, the solid was collected, washed with CH_3CN, and dried under vacuum (0.15 torr), yielding a purple solid: 0.101 g (85%, assuming mol wt 328); mp >330 °C. The product was too insoluble for the ^1H NMR spectrum (Fourier transform, 80 MHz) to be measured: IR (KBr) 3055 (w), 3025 (w), 2924 (w), 2255 (w), 1605 (m), 1598 (m, sh), 1470 (w), 1450 (m), 1402 (m), 1385 (w), 1317 (m), 1212 (m), 1162 (m), 1137 (w), 1008 (m, br), 910 (m), 874 (vs), 808 (m), 738 (w), 724 (m), 704 (s), 570 (w), 532 (vw), 497 cm^{-1} (w).

The purple solid was also prepared by chemical oxidation with *N*-chloro-, *N*-bromo-, or *N*-iodosuccinimide/TEA/CH_3CN or DMF, with pyridinium hydrobromide perbromide/CH_3CN, with Br_2 or I_2/NaH/CH_3CN or DMF, and with DDQ/dioxane under N_2. In each case it was identified by the IR spectrum which was identical with that obtained in the DDQ/CH_3CN reaction. Elemental analysis of four different samples are shown in Table III.

Acknowledgment. We thank G. Kolks for the magnetic susceptibility measurements, D. O. Cowan for a preprint of his paper on TCNP and for helpful discussions, W. Reinmuth and R. Bersohn for helpful discussions, and R. Breslow for the use of his electrochemical laboratory. We thank the National Science Foundation (Grant CHE75-20621) and the National Institutes of Health (Grant GM19173) for their support.

Registry No. 2, 76357-78-3; **3**, 81-30-1; **4**, 31996-10-8; **5**, 80293-90-9; **6**, 80293-91-0; **7**, 80293-92-1; **7** free acid, 80293-93-2; *cis*-**8**, 80293-94-3; *trans*-**8**, 80293-95-4; *cis*-**9**, 80293-96-5; *trans*-**9**, 80293-97-6; *cis*-**10**, 80293-98-7; *trans*-**10**, 80293-99-8; *cis*-**11**, 80294-00-4; *trans*-**11**, 80294-01-5; *cis*-**12**, 80294-02-6; *trans*-**12**, 80294-03-7; **13**, 80294-04-8; **14**, 80294-05-9; **15**, 80294-06-0; **17**, 80301-14-0; diethyl malonate, 105-53-3; diethyl carbonate, 105-58-8.

Mechanisms of Bromination of Uracil Derivatives. 6.[1] Cytosine and N-Substituted Derivatives

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The reactions of bromine with cytosine, 1-methylcytosine, cytidine, and 3-methylcytosine in acidic aqueous solutions have been studied. Initially adducts (5-bromo-5,6-dihydro-6-hydroxycytosines) are produced which are clearly observable by proton NMR, albeit in their protonated forms in the acidic media used. In time the adducts undergo elimination of water to give substitution products, 5-bromocytosines. 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. An alternative mechanism involving first hydration and then bromine attack can be ruled out.

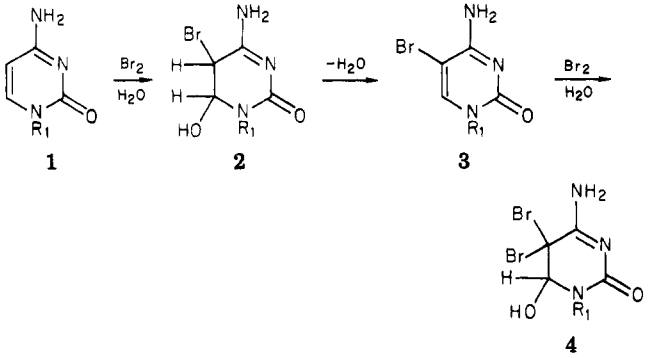
Simple uracils undergo electrophilic bromination in aqueous solution by an addition–elimination mechanism, as first proposed by Wang.[2] The validity of his proposal was shown by studies carried out in this laboratory.[1,3] Kinetic studies provided details of both the addition step[1] and the elimination step,[3] and the postulated adducts were clearly observed by proton NMR.[3] The product of the addition–elimination sequence is a 5-bromouracil which can also react with aqueous bromine to undergo the formal addition of HOBr and give a 5,5-dibromo derivative.[1-3]

During the course of our earlier studies[3] Banerjee also observed by NMR the formation of an adduct, **2a**, resulting from the reaction of aqueous bromine with cytosine (**1a**).[4] It seemed probable, therefore, that bromination of cytosines also proceeded by an addition–elimination mechanism such as shown in Scheme I, and so we undertook a study of the reaction by methods similar to those used in our earlier work on uracils.[1,3] While this study was in

(1) Part 5: O. S. Tee and C. G. Berks, *J. Org. Chem.*, **45**, 830 (1980).
(2) S. Y. Wang, *Nature (London)*, **180**, 91 (1957); *J. Org. Chem.*, **24**, 11 (1959).
(3) O. S. Tee and S. Banerjee, *Can. J. Chem.*, **57**, 626 (1979).
(4) S. Banerjee, Ph.D. Thesis, Sir George Williams University, 1974.

0022-3263/82/1947-1018$01.25/0

Scheme I[a]



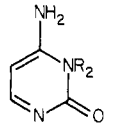
[a] a, R_1 = H; b, R_1 = Me; c, R_1 = ribosyl.

progress Taguchi and Wang[5] reported UV spectral evidence which supports the involvement of such a mechanism. For some cytosine derivatives they were able to isolate the unstable adducts **2** (or their methoxy analogues) and also 5,5-dibromo derivatives **4** resulting from the addition of HOBr to 5-bromo cytosines **3**.[5]

In this paper we present NMR spectral evidence that further supports the intermediacy of the adducts **2** and the sequence of the events shown in Scheme I. We also report kinetic results for the reaction of bromine with cytosines which provide insight into the mechanism of formation of the intermediates **2**.

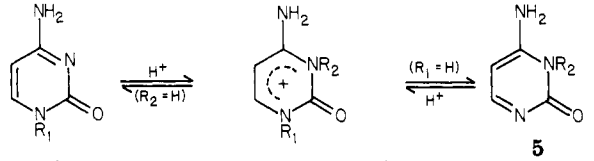
Results

As substrates for study we chose cytosine (**1a** ⇄ **5a**), 1-methylcytosine (**1b**), cytidine (**1c**), and 3-methylcytosine (**5b**).



5a, R_2 = H
b, R_2 = Me

In aqueous solution cytosine exists mainly as the 1*H* tautomer **1a** and to a lesser extent (~0.25%) as the 3*H* tautomer **5a**.[6] Almost certainly these two tautomers interconvert through the common protonated form **6a**[6] in



6a, 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

acidic media such as were used in the present study. The two methyl derivatives **1b** and **5b** serve, therefore, as models for the principal tautomers of cytosine, just as their protonated forms **6b** and **6d** can act as models for the cytosine cation **6a**.[7]

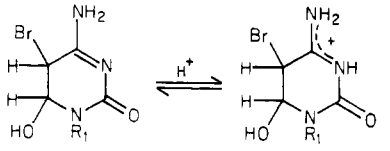
(5) H. Taguchi and S. Y. Wang, *J. Org. Chem.*, **44**, 4385 (1979).
(6) (a) D. J. Brown and J. M. Lyall, *Aust. J. Chem.*, **15**, 851 (1962); (b) A. R. Katritzky and A. J. Waring, *J. Chem. Soc.*, 3046 (1962); (c) M. Dreyfus, O. Bensaude, G. Dodin, and J. E. Dubois, *J. Am. Chem. Soc.*, **98**, 6338 (1976).

Table I. ^{1}H NMR Spectral Data for Cytosines, Bromination Adducts, 5-Bromocytosines, and 5,5-Dibromo Derivatives in Acid Solution[a]

structure	shifts, δ 5H	6H	N_1 Me	N_3 Me	$J_{5,6}$, Hz
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
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
9a		8.40			
9b		8.55	3.61		
9c		8.83			
9d		8.24		3.71	
10a		5.58			
10b		5.61	3.22		
10d		5.52		3.27	

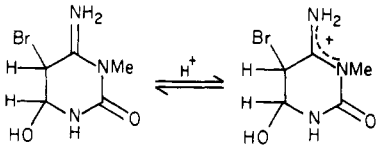
[a] Initially in 1 N DCl/D_2O. After addition of bromine, DBr is produced, and so for **7**, **9**, and **10** the acidity is higher. Appropriate absorptions are observed for the ribose moiety of cytidine derivatives. [b] 1:1 concentrated DCl/D_2O. In 1 N DCl/D_2O the solvent (HDO) peak obscured the 5- and 6-proton peaks.

Proton NMR Studies. For reasons of solubility and to mask the effects of HBr produced during bromination, we used 1 N DCl/D_2O as the solvent for these spectral studies. In this medium the substrates exist as their protonated forms **6** (but with deuterium replacing protium at exchangeable positions) and show very simple spectra (Table I). The 5- and 6-protons appear as doublets ($J_{5,6}$ ≈ 8 Hz) at δ ~6.5 and ~8, respectively. Upon addition of bromine these peaks diminish, and new peaks attributable to adducts such as **2** appear at higher field. In fact,



a, R_1 = H; b, R_1 = Me; c, R_1 = ribosyl

these adducts are observed also in their protonated forms **7**, since the protonation p*K*s of simple 5,6-dihydrocytosines are ~6.5.[8] Likewise, the adduct **8**, derived from 3-methylcytosine **5b**, is in the cationic form **7d**.



For these protonated adducts **7** the 5- and 6-protons appear as doublets at δ ~5.2 and ~5.5, respectively (Table I). These shifts are lower than those of the corresponding uracil adducts,[3] consistent with the cationic structures **7**. Moreover, the coupling constants $J_{5,6}$ ≈ 2.2 Hz suggest that the stereochemistry of the adducts **7** has bromine trans to

(7) We also intended to study the 1,3-dimethyl cation **6** (R_1 = R_2 = Me) to serve as a further model of the cytosine cation **6a**. However, various literature methods for the preparation of this cation led to materials contaminated with monomethyl derivatives which were difficult to remove completely. Such adulterated material would be useless for comparitive rate studies and so was not pursued.
(8) S. Slae and R. Shapiro, *J. Org. Chem.*, **43**, 1721 (1978).

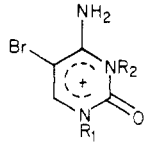
Table II. Substrate Dependence of First-Order Rate Constants for Reaction of Bromine with Cytosines[a]

substrate (S)	10^4[S], M	k_1^{obsd}, s^{-1}	k_1^{calcd}, s^{-1}
1a[b]	5.0	0.0574	0.0572
	7.5	0.0936	0.0940
	10.0	0.131	0.131
1b[c]	1.0	0.232	0.235
	2.0	0.465	0.459
	3.0	0.680	0.683
1c[b]	5.0	0.0594	0.0600
	7.5	0.101	0.0998
	10.0	0.139	0.140
5b[d]	2.5	0.0164	0.0164
	3.75	0.0242	0.0242
	5.0	0.0321	0.0321

[a] At 25 °C with [KBr] = 0.1 M and pH 0.98. Values of k_1^{calcd} are from least-squares analysis of k_1^{obsd} vs. [S] − $[Br_2]_0$ (see ref 10). [b] $[Br_2]_0 = 5 \times 10^{-5}$ M. [c] $[Br_2]_0 = 1 \times 10^{-5}$ M, pH 2.30. [d] $[Br_2]_0 = 2.5 \times 10^{-5}$ M.

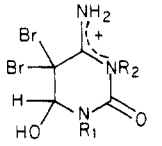
hydroxyl.[3] The peaks for the 5- and 6-protons of the adduct **7c**, derived from cytidine, appear slightly broadened. This apparent broadening is probably due to the existence of two diastereomeric forms of **7c** arising from the chirality of the ribose moiety.

In time the absorptions due to the adducts **7**[9] give way to those due to the substitution products, the 5-bromocytosines, in their protonated forms **9** (see Table I). The



9a, $R_1 = R_2 = H$
b, R_1 = Me; $R_2 = H$
c, R_1 = ribosyl; $R_2 = H$
d, $R_1 = H$; R_2 = Me

addition of further bromine brings about formation of 5,5-dibromo derivatives which are also probably protonated (**10**; Table I).



10a, $R_1 = R_2 = H$
b, R_1 = Me; $R_2 = H$
c, R_1 = ribosyl; $R_2 = H$
d, $R_1 = H$; R_2 = Me

Our NMR studies, then, provide added support for following the sequence of events: addition, elimination, addition, as depicted in Scheme I. However, in the acidic medium used the derivatives **1–4** were observed in their protonated forms **6**, **7**, **9**, and **10**. An analogous scheme for the bromination of 3-methylcytosine (**5b**) is also supported.

Kinetics. We have measured the rates of reaction of bromine with the four cytosine substrates **1a–c** and **5b** in aqueous media. In the pH range used (0–5) the reactions are fast, necessitating use of the stopped-flow method.

In the presence of an excess of the cytosine substrate (tenfold or greater) the disappearance of bromine follows a first-order rate law. The corresponding rate constants

Table III. Rate Constants for Reaction of Bromine with Cytosines[a]

substrate	pH	k_1^{obsd}, s^{-1}	k_2^{obsd}, M^{-1} s^{-1}
1a	0.98[b]	0.0574	358
	1.30	0.116	723
	2.00	0.564	3 520
	2.45	1.41	8 790
	3.01	4.88	30 400
	3.41	12.6	78 500
	4.19	52.1	325 000
	4.65	98.4	613 000
1b[c]	0.98[b]	0.0158	488
	2.30	0.232	7 160
	2.93	1.12	34 600
	3.21	2.42	74 700
	3.59	6.86	212 000
	3.95	18.1	559 000
	4.25	31.7	978 000
	4.53	38.0	1 170 000
	4.83	52.1	1 610 000
	5.37	66.3	2 060 000
1c	0.98[b]	0.0594	367
	1.30	0.141	871
	2.00	0.708	4 370
	2.43	1.96	12 100
	2.94	6.44	39 800
	3.24	18.4	114 000
	3.39	28.4	173 000
	4.14	90.1	557 000
5b	0.98[b]	0.0164[d]	203
	2.00		1 470[e]
	2.42	0.608	3 760
	2.94	0.367[c]	11 300
	3.24	0.709[c]	21 900
	3.84		69 800[e]
	4.03	3.99[c]	123 000
	4.37	8.90[c]	275 000
	4.85	26.7[c]	825 000

[a] At 25 °C with [KBr] = 0.1 M. [Substrate] = 5×10^{-4} and $[Br_2]_0 = 5 \times 10^{-5}$ M, except where noted otherwise. [b] Value of H_0. [c] [Substrate] = 1×10^{-4} M; $[Br_2]_0 = 1 \times 10^{-5}$ M. [d] [Substrate] = 2.5×10^{-4} M; $[Br_2]_0 = 2.5 \times 10^{-5}$ M. [e] [Substrate] = 1×10^{-4} M. Absorbance data analyzed directly for second-order behavior.

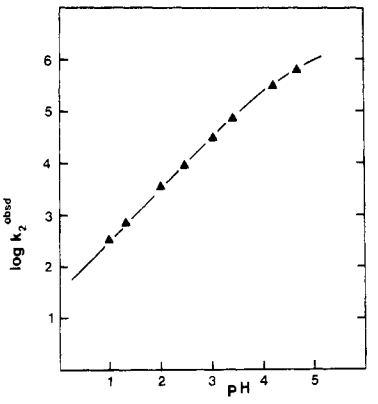


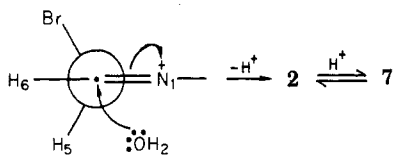
Figure 1. pH dependence of log k_2^{obsd} for cytosine (**1a**). Rate profiles for **1b** and **1c** are virtually superimposable. That for 3-methylcytosine (**5b**) is a straight line of slope 1.

(k_1^{obsd}) vary linearly with substrate concentration (Table II), indicating overall second-order behavior at fixed pH. Accordingly, values of k_1^{obsd} were converted to second-order rate constants (k_2^{obsd}), taking into account the substrate concentration[10] and correcting for the reduction in free molecular bromine due to the formation of tribromide ion and, where necessary, hypobromous acid.[1] In two instances

(9) Cytosine and 1-methylcytosine bearing an acetyl group on N_4 also give adducts similar to **7** upon reaction with bromine. C. G. Berks, unpublished results.

(10) S. Banerjee, O. S. Tee, and K. D. Wood, *J. Org. Chem.*, **42**, 3670 (1977).

Scheme II



11

where the [substrate]:$[Br_2]_0$ ratio was only 2:1 the absorbance data was directly analyzed for second-order behavior,[11] and the apparent second-order rate constant so obtained was corrected for tribromide ion formation[1] (see entries for **5b** in Table III).

The values of k_2^{obsd} obtained at various pHs are given in Table III, and the pH–rate profile for **1a** is shown in Figure 1. The corresponding profiles for **1b** and **1c** are very similar and show similar curvature around pH 4.[12] That for **5b** simply shows a linear dependence of log k_2^{obsd} on pH with a slope of 1. These profiles are all consistent with attack of bromine upon the free base forms of the substrates since compounds **1** have protonation pKs ≈ 4.5,[13] and **5b** has a value of 7.49.[6b]

We have also carried out a few kinetic experiments measuring the rate of iodination of cytosine **1a** under similar reaction conditions. At pH 3 the iodination proceeds relatively slowly with $k_2^{obsd} \approx 30\ M^{-1}\ s^{-1}$.

Discussion

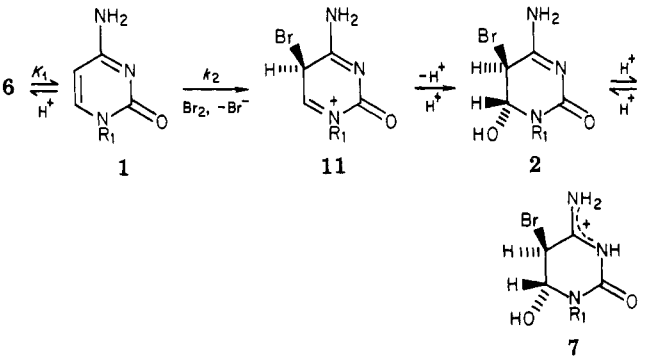
Electrophile. Under the reaction conditions used in the present study (pH 0–5, $[Br^-] = 0.1$ M) the predominant electrophile is almost certainly molecular bromine (Br_2). The reasons for this assertion are exactly the same as those given earlier for the bromination of uracils[1] and need not be repeated here. Thus all the observed second-order rate constants (k_2^{obsd}) reported in the present paper have been corrected for the fraction of free bromine existing in solution at the appropriate pHs.[1]

Intermediates. The proton NMR studies reported above fully support the intermediacy of the adducts **2** for which Taguchi and Wang[5] gave UV spectral evidence. However, under the acidic conditions used in our spectral studies these adducts exist as their conjugate acids, **7**. Likewise the ultimate products of bromination, the 5,5-dibromo-6-hydroxy derivatives **4**, are probably protonated (as **10**) under these same conditions.

The cationic adducts **7** show spin–spin coupling constants for the 5- and 6-protons of about 2.2 Hz. This is the same as has been found for various uracil adducts where the stereochemistry was shown to be bromine at C_5 trans to hydroxyl at C_6 (see ref 3 and references therein). In terms of the most probable mechanism of formation of the adducts **2** and **7** (discussed below), this stereochemistry results from the nucleophilic attack of water at C_6 of the transient cation **11** occurring anti to the bulky bromine at C_5 (Scheme II). The same must hold for the formation of uracil adducts.[1,3]

During their studies Taguchi and Wang[5] observed several instances in which to some extent the cytosine adducts **2** underwent hydrolysis to yield uracil adducts. This observation is not unexpected in that 5,6-dihydrocytosines are fairly susceptible to hydrolysis.[8] In the strongly acid media used in our ^1H NMR studies hydrolysis of the adducts was not a complicating feature. This may be a reflection of the mechanism of hydrolysis of 5,6-dihydrocytosines which involves general base-catalyzed attack of water upon the protonated substrate.[8]

Scheme III[a]



[a] a–c compounds are as indicated previously.

Scheme IV

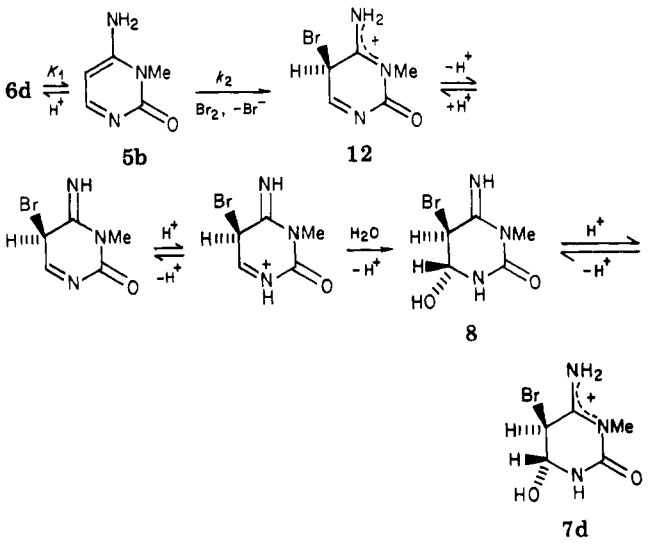


Table IV. Kinetic Parameters for the Reaction of Bromine with Cytosines

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] As in ref 13a. [b] Reference 13b gives 4.08 ($\mu = 0$ at 25 °C); ref 13c gives 4.24 ($\mu = 2.25$ M at 25 °C). [c] Reference 6b.

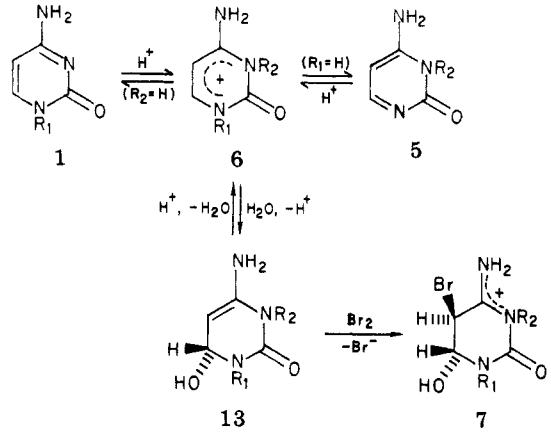
Mechanisms. The simplest interpretation of the pH–rate data in Table III is that the reaction involves rate-limiting electrophilic attack of bromine upon the free base form of the substrate (**1** or **5**). For the substrates **1** a reasonable proposal is that the adducts **7** are formed as shown in Scheme III. Attack of bromine leads to the formation of a cation **11** which is captured by water (as in Scheme II) to lead to the observable adducts. For the substrate **5b** a slightly different mechanism is necessary (Scheme IV).

At the acidities used the substrates **1** exist to a greater or lesser extent in their protonated forms **6**.[13] Thus the expected form of the acidity dependence of k_2^{obsd} is that given by eq 1, where K_1 is the acid-dissociation constant

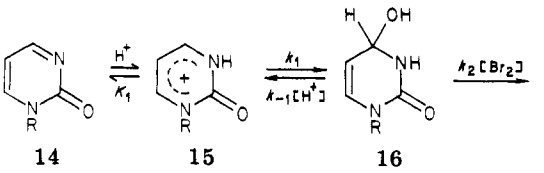
$$k_2^{obsd} = k_2K_1/(K_1 + [H^+]) \quad (1)$$

(11) O. S. Tee and M. Paventi, *J. Org. Chem.*, **46**, 4172 (1981).
(12) The profiles for **1b,c** and **5b** are not shown in Figure 1 as they are almost superimposable on that for **1a**.
(13) (a) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1962); (b) J. J. Christensen, J. H. Rytting and R. M. Izatt, *J. Phys. Chem.*, **71**, 2700 (1967); (c) R. Shapiro, V. Difate, and M. Welcher, *J. Am. Chem. Soc.*, **96**, 906 (1974).

Scheme V



Scheme VI[a]



[a] R = H, Me.

of the conjugate acid **6** and k_2 is the second-order rate constant for the attack of bromine upon the substrate **1** (i.e., **1** → **11**). The calculated curve shown in Figure 1 was obtained by using this equation and the values of k_2 and K_1 in Table IV. All of the data in Table III are equally well represented by eq 1 with the appropriate parameters from Table IV.

On this basis the substrates cytosine, 1-methylcytosine (**1b**), and cytidine (**1c**) all react directly with bromine with $k_2 \approx 10^6$ M^{-1} s^{-1}. The close similarity of the behavior of cytosine to that of the N_1-substituted derivatives **1b** and **1c** is consistent with it reacting via its predominant 1*H* tautomer **1a**, but the 3*H* tautomer **5a** may also contribute (vide infra). Moreover, the close similarity of the reactivities of **1b** and **1c** militates against the involvement of any hydroxyl of the ribose moiety of cytidine **1c** in the rate-determining step.[14]

For 3-methylcytosine (**5b**) the value of $pK_1 = 7.49$.[6b] Therefore, at the pHs used in the present study $[H^+] \gg K_1$, and so a simplified form of eq 1 can be used, namely, $k_2^{obsd} = k_2K_1/[H^+]$. This relationship fits the observed data well with $k_2K_1 = 13$ s^{-1} and $k_2 = 4.0 \times 10^8$ M^{-1} s^{-1}.

If the formation of the adduct **8** (and its protonated form **7d**) results from the direct attack of bromine upon 3-methylcytosine (**5b**), then the mechanism of adduct formation is likely that shown in Scheme IV. This mechanism is slightly more complex than that in Scheme III since deprotonation of the initially formed cation **12** is probably necessary before hydration of the $C_6{=}N_1$ double bond takes place.

The rate data, then, can be rationalized in terms of direct attack of bromine upon the substrate (**1** or **5**). One question which this interpretation raises is why 3-methylcytosine (**5b**) should be 420 times more reactive than 1-methylcytosine (**1b**) toward bromine (see Table IV). The probable answer is that electrophilic attack upon **5b** leads to a relatively stable amidinium cation, **12**, whereas attack on **1b** leads to a less stable iminium ion, **11**.

From the outset we also considered that formation of the adducts **2** and **7** might result from bromine attack upon a covalent hydrate of the cytosine substrate as depicted in Scheme V. Earlier work has shown that such a mechanism is involved in the bromination of 2-pyrimidinones[15] **14** (Scheme VI) and that similar mechanisms operate for 4-pyrimidinones,[11] and for 4-quinazolinones.[16]

Moreover, the covalent hydrate **13** should be extremely reactive toward bromine with a second-order rate constant k_2 at the diffusion-controlled limit ($\sim 10^{10}$ M^{-1} s^{-1}).[17] The covalent hydrate of 2-pyrimidinone, **16** (R = H), reacts with bromine with $k_2 \approx 10^9$ M^{-1} s^{-1},[15] and the presence of the strongly activating 4-amino group in **13** should easily increase this value to the diffusion-controlled limit. From another viewpoint **13** is an enamine twice over and as such is expected to react with bromine as fast as the solvent allows since the normally less reactive iodine reacts with simple enamines at the limit.[18] The mechanism shown in Scheme V is also attractive since it would readily explain why 1-methylcytosine (**1b**) and 3-methylcytosine (**5b**) show similar reactivities at pHs where both exist as their respective cations **6b** and **6d**.

For the covalent hydrate mechanism, with constants according to eq 2, one can derive the rate law shown in eq

$$1 \text{ (or } 5) \underset{K_1}{\overset{H^+}{\rightleftharpoons}} 6 \underset{k_{-1}[H^+]}{\overset{k_1}{\rightleftharpoons}} 13 \xrightarrow{k_2[Br_2]} 7 \qquad (2)$$

$$\text{rate} = \frac{k_1k_2[1]_{st}[H^+][Br_2]}{(K_1 + [H^+])(k_{-1}[H^+] + k_2[Br_2])} \qquad (3)$$

3 by assuming a steady-state concentration of the highly reactive enamine **13**.[1,11,15,19] In this equation the total concentration of substrate **1** remaining at any given time is $[1]_{st} = [1] + [6]$.

For this rate law to be compatible with observed second-order kinetics (rate = $k_2^{obsd}[1]_{st}[Br_2]$), the condition $k_{-1}[H^+] \gg k_2[Br_2]$ must apply. In this case eq 4 can be

$$k_2^{obsd} = k_2K_{R^+}/(K_1 + [H^+]) \qquad (4)$$

written, where $K_{R^+} = [13][H^+]/[6] = k_1/k_{-1}$ is the equilibrium constant[11,15,20] relating the protonated substrate **6** and the covalent hydrate **13**. Clearly eq 4 is compatible with the observed acidity dependences since it has the same form as eq 1. However, the values of k_1, k_{-1}, and K_{R^+} that are required to explain the observed data do not seem reasonable.

As pointed out above we can safely assume that $k_2 \sim 10^{10}$ M^{-1} s^{-1}, and initially we have $[Br_2] = 5 \times 10^{-5}$ M. Thus for the inequality $k_{-1}[H^+] \gg k_2[Br_2]$ to hold even at the highest pHs studied (~ 5) values of $k_{-1} \gg 5 \times 10^{10}$ $M^{-1}s^{-1}$ are required. Values of k_{-1} known for various heterocyclic cations are $\leq 6 \times 10^8$ M^{-1} s^{-1},[20] and values for the closely related **16** are $\sim 10^7$ M^{-1} s^{-1}.[15] Moreover, in aqueous solution only the proton transfer between H_3O^+ and OH^- has a rate constant significantly above 5×10^{10} M^{-1} s^{-1},[21] whereas here k_{-1} represents a proton transfer concerted with C–O bond rupture.[20] Thus from several viewpoints the required values of k_{-1} seem implausible.

(14) Participation of ribosyl and arabinosyl hydroxyl groups has been implicated in some reactions of pyrimidine nucleosides even in aqueous solution. (a) J. A. Rabi and J. J. Fox, *J. Am. Chem. Soc.*, **95**, 1628 (1973), and references therein; (b) R. E. Notari, M. L. Chin, and A. Cardoni, *J. Pharm. Sci.*, **59**, 28 (1970).

(15) O. S. Tee and M. Paventi, *J. Org. Chem.*, **45**, 2072 (1980).

(16) O. S. Tee and G. V. Patil, *J. Org. Chem.*, **41**, 828 (1976).

(17) J. H. Ridd, *Adv. Phys. Org. Chem.*, **16**, 1 (1978).

(18) (a) J. Toullec and C. Verny-Doussin, Abstracts of 5th IUPAC Conference on Physical Organic Chemistry, Santa Cruz, CA, 1980, p 148; (b) C. Verny-Doussin, Thesis, University of Paris, 1979.

(19) The corresponding equation (eq 3) in ref 1 unfortunately contains an error: the denominator terms should be multiplied by K_1.

(20) J. W. Bunting, *Adv. Heterocycl. Chem.*, **25**, 1 (1979).

(21) M. Eigen, *Angew. Chem., Int. Ed. Engl.*, **3**, 1 (1964).

In addition, attributing the observed rate data to eq 4 yields values of $k_2K_{R^+}$ = 13–60 s^{-1} (i.e., the same as k_2K_1 in Table IV). For $k_2 \approx 10^{10}$ M^{-1} s^{-1} (vide supra) this means $K_{R^+} = 1.3 \times 10^{-9} - 6 \times 10^{-9}$ M (pK_{R^+} = 8.9–8.2), and hence values of $k_1 \geq$65–300 s^{-1}. Again these required values seem unreasonable on considering that for 2-pyrimidinone cations **15** pK_{R^+} = 5.7–6.6 and k_1 = 4.9–10 s^{-1}.[15] In particular, the attack of water on the more stable cytosine cations **6** should be much slower than upon **15**, rather than much faster.

An entirely different piece of evidence against the covalent hydrate mechanism in Scheme V is the much slower rate of iodination of cytosine. The highly reactive enamine **13** should react with bromine and iodine at the same rate ($k_2 \approx 10^{10}$ M^{-1} s^{-1}),[18] and thus according to eq 4 both iodination and bromination should show essentially the values of k_2^{obsd} if the mechanism in Scheme V is operative. In fact, the iodination of cytosine is about 1000 times slower than its bromination, much more in keeping with the direct attack of halogen as shown in Scheme III.

As a final point we return to the question of the reactive tautomer of cytosine. Earlier we noted that the very similar reactivities of cytosine, 1-methylcytosine (**1b**), and cytidine (**1c**) suggest that the 1*H* tautomer **1a** is the reactive form. However, 3-methylcytosine **5b** is 420 times more reactive toward bromine than **1b**. If the reactivities of the tautomers **5a** and **1a** have a similar relationship, then these two tautomers may well compete for bromine since the tautomeric ratio of [**1a**]/[**5a**] is 400.[6c] Reaction upon other tautomers is probably insignificant.[22]

Conclusions

Simple cytosines react with bromine in aqueous solution to yield observable adducts **2** (observed in the present work as cations **7**). Slow dehydration of these adducts leads to overall substitution and the production of a 5-bromocytosine.

Kinetic studies of the initial fast reaction with bromine revealed that the adducts arise from bromine attack followed by hydration, rather than the reverse. For the 1*H* tautomer of cytosine and for 1-substituted cytosines the mechanism in Scheme III is suggested. However, in the case of cytosine itself the 1*H* tautomer **1a** and the 3*H* tautomer **5a** may well compete for bromine. Reaction upon **5a** requires a slightly different mechanism, similar to that shown in Scheme IV for 3-methylcytosine.

The observed adducts **7** have the particular stereochemistry of bromine trans to hydroxyl. This implies preferential nucleophilic attack of water anti to the bromine in cation **11** (Scheme II) and in the corresponding cation in Scheme IV.

The present work reveals the high reactivity of cytosine and cytidine toward bromine in aqueous solution ($k_2 \approx 10^6$ M^{-1} s^{-1}). Likewise, earlier work showed that uracil ($k_2 \approx 10^5$ M^{-1} s^{-1}) and its anion ($k_2 \approx 10^{10}$ M^{-1} s^{-1}) are very reactive.[1] For the analogous chlorinations we anticipate rate constants equal to (uracil anion) or greater than (uracil and cytosine) these values. Therefore, there is a very real possibility of adventitous halogenation, particularly chlorination, of uracil and cytosine in the environment.

Experimental Section

Cytosine, cytidine, and 3-methylcytosine were obtained from commercial sources and recrystallized before use.

1-Methylcytosine (1b) was prepared by modification of a literature procedure[24] originally designed to yield only small amounts. This method involves methylation of the bis(trimethylsilyl) derivative of cytosine.

Cytosine (4.8 g, 43 mmol) and chlorotrimethylsilane (4 mL, 32 mmol) were mixed together in 200 mL of hexamethyldisilazane and then refluxed for 1 h. After the mixture cooled, iodomethane (40 mL, 643 mmol) was added, and the mixture was refluxed for 2 h and then stirred overnight. Rotary evaporation of the volatiles gave yellow crystals which were dissolved in 10% aqueous acetic acid (110 mL). The resultant two-phase mixture was rotary evaporated until only one phase remained, and when this was cooled, yellowish crystals were deposited which were filtered off (5.6 g). Concentration of the filtrate to half its volume yielded a second crop (0.9 g). These materials proved to be the hydroiodide salt of **1b**: yield 60%; later preparations gave yields up to 77%; mp 265–270 °C.

A suspension of pulverized **1b** hydroiodide (14.9 g, 59 mmol) in 47 mL of concentrated aqueous ammonia was stirred for 2 h at room temperature and then cooled at 0 °C for 1 h. The white precipitate was **1b** (6.05 g, 82% yield) which after recrystallization from ethanol gave had a melting point of 290 °C dec (lit. mp 285 °C dec,[24] 300 °C[25]).

Proton NMR spectra (Table I) were obtained on Varian A-60 and T-60 spectrometers. Chemical shifts were obtained relative to internal DSS.

The equipment, techniques, solutions, and methods of analysis of the data employed in the kinetic studies followed those of other recent studies from this laboratory.[1,11,15]

Iodination experiments were carried out under pseudo-first-order conditions with a tenfold excess of substrate. Solutions were 0.1 M in potassium iodide, and iodine disappearance was monitored at 350 nm where triiodide ion absorbs strongly. Second-order rate constants were corrected for the fraction of iodine existing as such by using K = 0.001 30 for the dissociation constant of triiodide ion.[26]

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Registry No. **1a**, 71-30-7; **1b**, 1122-47-0; **1b**·HI, 6749-83-3; **1c**, 65-46-3; **5b**, 4776-08-3; **6a**, 20791-98-4; **6b**, 80375-48-0; **6c**, 63600-30-6; **6d**, 80375-49-1; **7a**, 80375-50-4; **7b**, 80375-51-5; **7c**, 80375-52-6; **7d**, 80387-06-0; **9a**, 80375-53-7; **9b**, 80375-54-8; **9c**, 80375-55-9; **9d**, 80375-56-0; **10a**, 80387-07-1; **10b**, 80375-57-1; **10d**, 80375-58-2.

(22) 2-Methoxypyridine is 2000 times less reactive toward bromine than 1-methyl-2-pyridone.[23] Therefore, reaction with the hydroxy tautomer of cytosine, even if present to the extent of 0.1%, must be negligible compared to that with **1a** and **5a**.

(23) O. S. Tee and M. Paventi, unpublished results.

(24) T. T. Sakai, A. L. Pogolotti, and D. V. Santi, *J. Heterocycl. Chem.*, **5**, 849 (1968).

(25) G. W. Kenner, C. B. Reese, and A. R. Todd, *J. Chem. Soc.*, 855 (1955). Using an older nomenclature, they refer to **1b** as "3-methylcytosine".

(26) M. Davies and E. Gwynne, *J. Am. Chem. Soc.*, **74**, 2748 (1952).