diated with a 350-nm light source in a Pyrex vessel for 48 h. The photolysate was concentrated to 5 mL, the precipitated triphenylene was filtered, and the filtrate was concentrated to dryness. The NMR of the residue exhibited a multiplet at δ 3.2 and signals at δ 5.28 and 6.3 characteristic of **3b**. When the same experiment was repeated with 100 mg of triphenylene in 100 mL of acetonitrile, the NMR spectrum of the product was identical with that of the starting **3a**. A similar experiment in which 40 mg of benzophenone and 60 mg of **3a** in 100 mL of acetonitrile were irradiated for 40 h by using a 350-nm light source and a Pyrex vessel led only to the recovery of **3a**. No photolysis was observed when 50 mg of **3a** was irradiated at 350 nm for 50 h in a Pyrex vessel in 100 mL of methanol in the absence of the sensitizer.

Attempted Quenching of the Photochemical Conversion of 3a to 4a. Solutions of **3a** (10^{-4} M) in acetonitrile were irradiated with a 254-nm light source in the presence of piperylene in the concentration range 4×10^{-4}–2×10^{-3} M. The conversion of **3a** to **4a** was not inhibited.

Attempted Reaction of 3a with $NaOCH_3$. (a) A saturated solution of $NaOCH_3$ in CH_3OH which contained 10^{-4} M **3a** was monitored by UV for 20 h. There was no change in the absorption spectrum.

(b) A solution of 10 mg of **3a** in 10 mL of CH_3OD saturated with CH_3ONa was stirred at room temperature for 1 h. The ^1H NMR obtained on evaporating the solution to dryness indicated that both α and β anomers (**3a** and **3b**) were present but that H-1′ was not exchanged with deuterium.

Attempted Reduction of Photobleached Product (6) with $NaBH_4$. A solution of 100 mg (0.33 mmol) of **3a** in 60 mL of CH_3OH was degassed and irradiated at 254 nm for 7.5 h. The solvent was removed by evaporation, and the oily residue was dissolved in 5 mL of ethanol and was stirred with 15 mg (0.4 mmol) of $NaBH_4$ overnight. The solvent was distilled, and the oily residue was dissolved in $CHCl_3$, washed with water, dried, and concentrated to an oil. The NMR spectrum of the oil exhibited NMR signals characteristic of both **3a** and **3b**.

Low-Temperature Photolyses. Samples were cooled with liquid nitrogen and irradiated through quartz windows in the apparatus of Richtol and Klappmeier.[2,18] An IR band was detectable at 2030 cm^{-1} on irradiation of **3a** with a 254-nm source for 1 h in a KBr matrix. Irradiation of glasses, prepared by cooling a neat sample of **1d** or a solution of it in 4:1 ethanol:methanol, with a 254-nm light source for 1 h also resulted in the formation of an infrared band at 2030 cm^{-1}. In all the above experiments the absorption at 2030 cm^{-1} disappeared within 10 min after evaporation of the liquid nitrogen. Mixtures of triphenylene and **1d** or benzophenone and **1d**, in which the IR absorption bands of the sensitizer were roughly equal in intensity to that of **1d**, were cooled with liquid nitrogen and irradiated with a 350-nm light source. No new IR bands were detectable after irradiation. Irradiation of 10^{-4} M **3a** or **1d** in 4:1 ethanol:methanol, after cooling with liquid nitrogen, with a 254-nm source for 1–2 h resulted in the loss of the absorption of the enamino nitriles. Their absorption maxima were partially regenerated after the mixture was warmed to room temperature and maintained there for 24 h.

Acknowledgment. We thank P. Dudgeon for performing some of the experiments and G. Brooks for technical assistance. This work was supported by Grant No. CA-14511 from the National Cancer Institute, a Project Seed Grant from the American Chemical Society to G. Brooks, and NASA Grant No. NGR 33-018-148 for the support of P. Dudgeon.

Registry No. **1a**, 15595-71-8; **1c**, 71734-94-6; **1d**, 57090-86-5; **2c**, 26751-12-2; **3a**, 71734-86-6; **3b**, 71734-87-7; **4a**, 71734-88-8; **6a**, 71734-95-7; **6b**, 71734-96-8; **10**, 1118-61-2; 2-oxocyclohexanecarbonitrile, 4513-77-3; benzylamine, 100-46-9.

(18) Richtol, H. H.; Klappmeier, F. H. *J. Chem. Phys.* **1966**, *44*, 1519–23. Richtol, H. H.; Klappmeier, F. H. *Appl. Spectrosc.* **1964**, *18*, 113.

Bromination of Cytosine Derivatives

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The reactions of cytosine (**6a**), 5-bromocytosine (**8a**), cytidine (**6b**), 5-bromocytidine (**8b**), 1-methylcytosine (**18a**), 5-bromo-1-methylcytosine (**8c**), 1,N^4-dimethylcytosine (**18b**), 5-bromo-1,N^4-dimethylcytosine (**18c**), 5-bromo-1,3-dimethylcytosine (**38**), 5-methylcytosine (**39**), N^4,5-dimethylcytosine (**40**), and 1,4,4-trimethylcytosine (**41**) with bromine or *N*-bromosuccinimide in aqueous or methanolic solutions have been studied. Product analyses and UV spectral changes observed at different pHs strongly support an addition–elimination mechanism. This is further substantiated by the synthesis and characterization of the previously unavailable bromohydrin intermediates, 5-bromo-6-hydroxy-5,6-dihydro and the corresponding -6-methoxy derivatives of **6a**, **8a**, **6b**, **8b**, **8c**, **18a**, **18b**, **18c**, **38**, **39**, **40**, and **41**. The final products of bromination were 5-bromocytosine (**8**) or 5-bromouracil (**3**) derivatives. The former resulted from direct dehydration of the bromohydrins and the latter from deamination prior to dehydration. From the UV spectra of these new 5,6-dihydrocytosine compounds and the related cyclobutyl dimers and bisulfite adducts, a scheme for calculation of the UV absorption maxima of hydrocytosine derivatives was deduced. The empirical values for bathochromic shifts due to substituents distinct and characteristic for amino (λ_{max} 239 nm, ϵ ~10 000) and imino (λ_{max} 227 nm, ϵ ~5000) forms are given.

On the basis of experimental data, the following sequence of reactions for the bromination of uracil (Ura) derivatives (**1**) was first suggested.[2] This mechanism was subsequently confirmed and extended.[3] The first step in the sequence resulted in the formation of the unstable bromohydrin derivatives (**2**) which were subsequently converted to the 5-bromouracil (BrUra) derivatives (**3**), probably by spontaneous or acid-catalyzed dehydration. In the presence of an excess of Br_2 or HOBr, however, the prod-

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(2) S. Y. Wang, *Nature (London)*, **180**, 91 (1957); *J. Org. Chem.*, **24**, 11 (1959).

(3) A. M. Moore and S. M. Anderson, *Can. J. Chem.*, **37**, 590 (1959); see also D. J. Brown, "The Pyrimidines", Interscience, New York, 1962, p 174.

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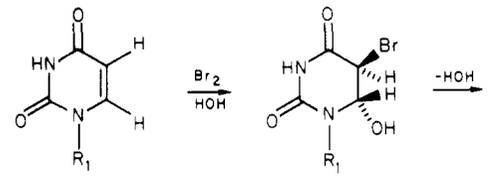
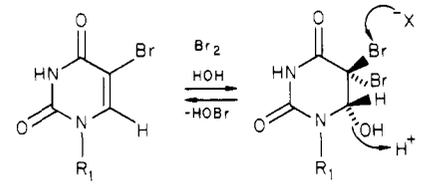


1a, R_1 = H
b, R_1 = ribose
c, R_1 = CH_3

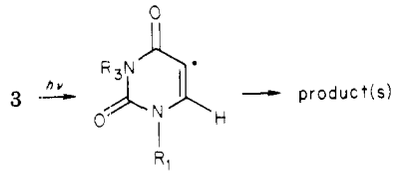
2a, R_1 = H
b, R_1 = ribose
c, R_1 = CH_3



3a, R_1 = H
b, R_1 = ribose
c, R_1 = CH_3

4a, R_1 = H
b, R_1 = ribose
c, R_1 = CH_3

ucts formed were 5,5-dibromo-6-hydroxy-5,6-dihydrouracil derivatives (**4**). Although the products **4** are more stable than **2**, **4** will readily revert to **3** by the nucleophilic- or electrophilic-catalyzed elimination of HOBr. This mechanism can be used to account for the reactions of halogenated Ura, thymine (Thy), and the other 5-substituted pyrimidines with a great number of nucleophiles and can be extended to include other nucleophilic displacements and dehalogenation reactions of pyrimidines.[4] It has also been shown that the replacement of Thy with BrUra in DNA increases the sensitivity of the biological systems to UV light and X- and γ-rays.[5,6] The mechanism responsible for this effect has been attributed to the production of Ura-5· radicals (**5**) by irradiation, and subsequent reactions

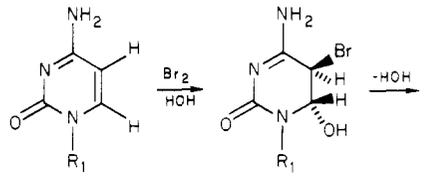


5

of the radicals **5** lead to the formation of lethal products.[5-7]

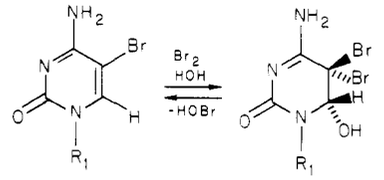
Studies of the chemical modification of viral RNA by bromination and iodination[8] suggest that the reaction sequence proposed for Ura derivatives is also applicable to the halogenation of Cyt derivatives (**6**). Therefore, the following reaction steps (mechanism 1) should also take place with **6** (Scheme I). However, neither **7** nor **9** were isolated, and their existence was inferred on the basis of UV spectroscopic evidence. The facile deamination of hCyt derivatives (such as **7** and **9**) to the respective 5,6-dihydrouracil (hUra) derivatives (such as **2** and **4**) has

Scheme I



6a, R_1 = H
b, R_1 = ribose
c, R_1 = CH_3

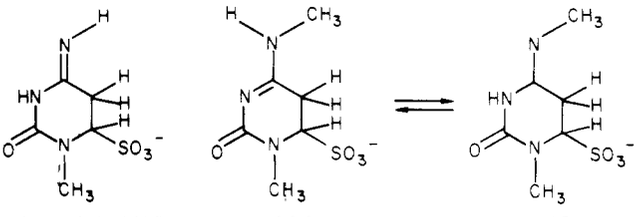
7a, R_1 = H
b, R_1 = ribose
c, R_1 = CH_3



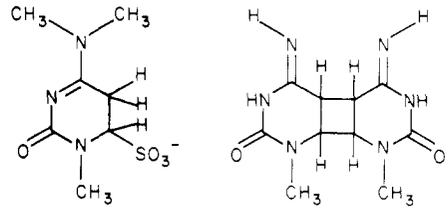
8a, R_1 = H
b, R_1 = ribose
c, R_1 = CH_3

9a, R_1 = H
b, R_1 = ribose
c, R_1 = CH_3

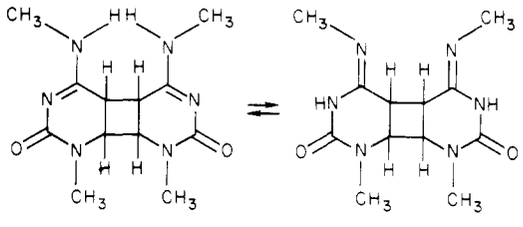
Scheme II



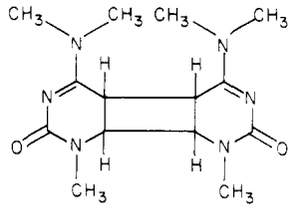
10, 243 (243)[a] 11A 251 (251) 11I



12, 262 (261) 13, 246 (243)



14A 253 (251) 14I



15, 262 (261)

[a] λ_{max} (H_2O) obsd (calcd).

(4) Examples are: K. Wataya, K. Negishi, and H. Hayatsu, *Biochemistry*, **12**, 3992 (1973); G. S. Rork and I. H. Pitman, *J. Am. Chem. Soc.*, **97**, 5559 (1975); F. A. Sedor and E. G. Sander, *ibid.*, **98**, 2314 (1976); B. C. Pal, *ibid.*, **100**, 5170 (1978); C. Garrett, Y. Wataya, and D. V. Santi, *Biochemistry*, **18**, 2798 (1979).

(5) F. Hutchinson, *Q. Rev. Biophys.*, **6**, 201 (1973), and references cited therein.

(6) S. Y. Wang in "Photochemistry and Photobiology of Nucleic Acids", Vol. 1, S. Y. Wang, Ed., Academic Press, New York, 1976, pp 295–313 and references cited therein.

(7) The references cited here are related to the formation of coupled products, Ura(5–5)Ura [H. Ishihara and S. Y. Wang, *Nature (London)*, **210**, 1222 (1966); M. Ehrlich and M. Riley, *Photochem. Photobiol.*, **20**, 159 (1974); S. Sasson, S. Y. Wang, and M. Ehrlich, *ibid.*, **25**, 11 (1977); S. Sasson and S. Y. Wang, *ibid.*, **26**, 357 (1977)]; the production of single strand breaks in DNA molecules [M. B. Lion, *Biochim. Biophys. Acta*, **155**, 505 (1968); R. Hewitt, K. Marburger, and T. Lapthisophon, *Radiat. Res.*, **39**, 485 (1969); G. Hotz and R. Walser, *Photochem. Photobiol.*, **12**, 207 (1970); F. Hutchinson and H. B. Hales, *J. Mol. Biol.*, **50**, 59 (1970)] and the formation of DNA–protein cross-links [P. R. Schmmel, G. P. Budzik, S. S. M. Lam, and H. J. P. Schoemaker, "Aging, Carcinogenesis, and Radiation Biology", K. C. Smith, Ed., Plenum Press, New York, 1976, p 123; S. Y. Lin and A. D. Riggs, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 947 (1974); I. Saito, S. Ito, and T. Matsuura, *J. Am. Chem. Soc.*, **100**, 2901 (1978)].

(8) K. W. Brammer, *Biochim. Biophys. Acta*, **72**, 217 (1963); W. Patton, V. Bacon, A. M. Duffield, B. Halpern, Y. Hoyano, W. Pereira, and J. Lederberg, *Biochem. Biophys. Res. Commun.*, **48**, 880 (1972).

made chemical studies rather difficult and the isolation of products of the former type (7 and 9) is frequently precluded.[9]

$$7 \xrightarrow[HOH]{-NH_3} 2 \rightarrow 3 \rightleftarrows 4$$

$$9 \xrightarrow[HOH]{-NH_3} 4 \rightarrow 3$$

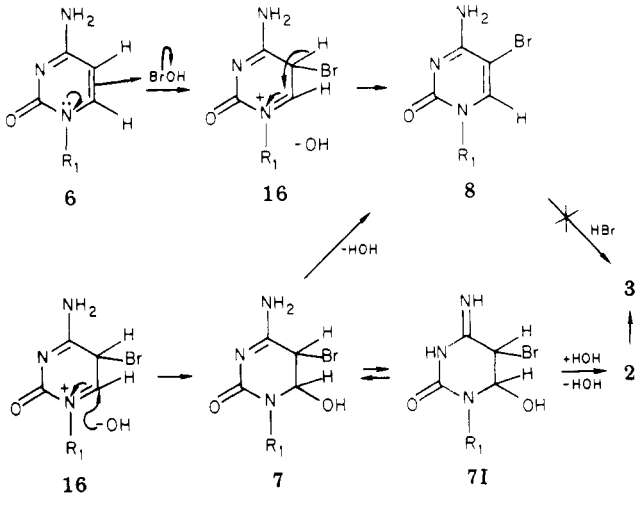
Following our success in the isolation of various Cyt bisulfite adducts (e.g., **10–12**)[10] and the photodimers of methyl Cyt derivatives (e.g., **13–15**),[11] we have reinvestigated the bromination reaction of a number of Cyt derivatives in aqueous solutions. These results show that, contrary to the earlier claim of Hilbert and Jansen,[12] the product identified as 5,5-dibromo-6-hydroxy-5,6-dihydrocytosine (**9**, R_1 = H) was in fact a byproduct, $NH_4Br \cdot H_2O$, and the major product was found to be 5,5-dibromo-6-hydroxy-5,6-dihydrouracil (**4**, $R_1 = R_3$ = H). Under comparable conditions, we have succeeded for the first time in isolating a number of Cyt bromohydrins (**7** and **9**) as "stable" products from their corresponding Cyt derivatives (**6**). Furthermore, these findings enable us to have a better understanding of the characteristics of dihydrocytosine derivatives and the reaction mechanisms of halogenation of pyrimidines.

Results and Discussion

It is well established[10,11] that the N^4-unsubstituted hCyt derivatives (e.g., **10** and **13**) have ultraviolet absorptions λ_{max} (H_2O) at ~245 nm with ϵ ~5000, the N^4-monosubstituted derivatives (e.g., **11** and **14**) at ~250 nm with ϵ ~7500, and the N^4-disubstituted derivatives (e.g., **12** and **15**) at ~260 nm with ϵ ~10000. The observed differences in the ϵ_{max} are attributed to the existence of unsubstituted derivatives predominantly in the imino form (**I**), disubstituted derivatives in the amino form (**A**), and monosubstituted derivatives as a mixture of the two forms (e.g., **11A** ⇄ **11I**, and **14A** ⇄ **14I**). In addition, each N^4-CH_3 group produced a bathochromic shift of 5–7.5 nm. This information should be useful for analyzing the spectral data obtained in this study for the deduction of the molecular structures of reaction intermediates and products and for the suggestion of the plausible reaction mechanisms.

The reaction of Cyt (6a) with 1 molar equiv of bromine yields BrCyt (**8a**) as the major product (89%). The observed UV absorption spectrum within 1 min showed a λ_{max} shift from 267 to 276 nm with a λ_{min} from 247 to 250 nm, and the resulting spectrum was essentially unchanged after 2 h. No increase in absorbance at ~240 nm which would give evidence of transient existence of **7a** was observed. This evidence may be interpreted as being at variance with the addition–elimination mechanism (**6** → **7** → **8**; mechanism 1) and in support of an electrophilic aromatic substitution mechanism (**6** → **16** → **8**; mechanism 2) as shown in Scheme III. The formation of the intermediate **16** is analogous to an ene–amine tautomerization.[13] The loss of a C(5)-H from **16** would give **8**.[14] However, nucleophilic attack of HO^- at C(6) of **16** could occur[15] and

Scheme III



would yield **7** which could, in turn, also produce **8**. Therefore, unequivocal distinction between these two mechanisms cannot be made, and mechanism 1 should not be ruled out, especially since a small amount of BrUra (**3a**) was isolated. The product **3a** was not formed directly from BrCyt (**8a**) by deamination or indirectly from Cyt (**6a**) by bromination of its deamination product, Ura, since no **3a** was detected on treatment of **8a** with aqueous HBr, and no **1a** was detected on similar treatment of **6a**. One plausible alternative could involve the formation of the addition product, **7a**, followed by the elimination reactions with or without deamination. Dehydration of **7a** without deamination would give **8a** as the major reaction product. After deamination, **7a** would be converted to **2a** via the amino–imino equilibrium of hCyt derivatives (**7** ⇄ **7I**), and dehydration of **2a** would yield **3a**, BrUra, as the minor product. Accordingly, the inability to detect the absorbance increase at ~240 nm due to the formation of **7a** has to be explained in terms of rapid spontaneous reactions of **7a** to form other products. To substantiate this notion, the existence of **7a** or its analogues would have to be demonstrated by spectral evidence and, more convincingly, by isolation and identification.

Bromination of BrCyt (**8a**) in a manner analogous to that of **6a** and under the previously described conditions[12] led to the formation of 5,5-dibromo-6-hydroxy-5,6-dihydrouracil (**4a**) and NH_4Br. This latter compound has the same melting point characteristics (i.e., decomposition with sublimation or evolution of gas at 175–180 °C) as that claimed[12] earlier for the "HBr salt of 5,5-dibromo-6-hydroxy-5,6-dihydrocytosine". Earlier identification of the alleged hCyt derivative (**9a**) was based solely on an elemental analysis of N,[12] which also corresponds to $NH_4Br \cdot H_2O$.

Although we were unable to isolate the desired product **9a**, its existence was demonstrated spectroscopically in a 0.1 mM solution. The appearance of a λ_{max} at ~240 nm was observed instantaneously (Figure 1), and this spectrum lasted for at least 2.5 h. This clearly indicated that **9a** was stable under this experimental condition, and the deamination of **9a** yielding **4a**, which exhibits only end absorption, did not occur.

The bromination of cytidine (Cyd, 6b) was performed in dilute aqueous solutions. Figure 2 shows the resulting

(9) H. E. Johns and G. DeBoer, *Biochim. Biophys. Acta*, **204**, 18 (1970); A. J. Varghese and C. S. Rupert, *Photochem. Photobiol.*, **13**, 365 (1971); M. Green and S. S. Cohen, *J. Biol. Chem.*, **228**, 601 (1957).
(10) H. Taguchi and S. Y. Wang, *J. Org. Chem.*, **42**, 2028 (1977).
(11) H. Taguchi, B. S. Hahn, and S. Y. Wang, *J. Org. Chem.*, **42**, 4127 (1977).
(12) G. E. Hilbert and E. F. Jansen, *J. Am. Chem. Soc.*, **56**, 134 (1934).
(13) S. Y. Wang, *Nature (London)*, **184**, 184 (1959); S. Y. Wang in "Excited States in Organic Chemistry and Biochemistry", B. Pullman and W. Goldblum, Eds., D. Reidel Publishing Co., Holland, 1977, p 39.
(14) W. Hauswirth and S. Y. Wang, *Photochem. Photobiol.*, **26**, 231 (1977).
(15) B. S. Hahn and S. Y. Wang, *J. Am. Chem. Soc.*, **94**, 4764 (1972); W. Hauswirth, B. S. Hahn, and S. Y. Wang, *Biochem. Biophys. Res. Commun.*, **48**, 1614 (1972); M. N. Khattak, W. Hauswirth, and S. Y. Wang, *ibid.*, **48**, 1622 (1972).

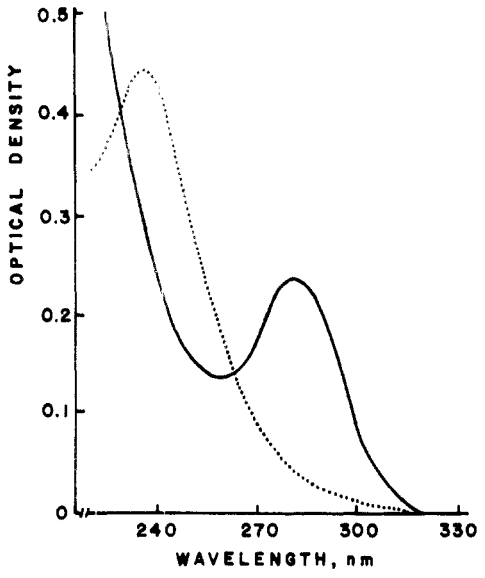


Figure 1. Bromination of 5-bromocytosine (**8a**) in 0.1 mM aqueous solution. The UV spectra of BrCyt (—) and after 1 min to 2.5 h of mixing with bromine-water (···).

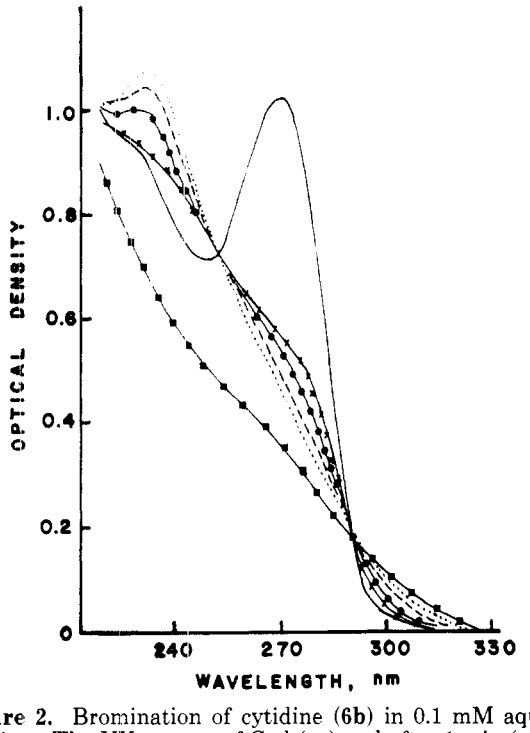


Figure 2. Bromination of cytidine (**6b**) in 0.1 mM aqueous solution. The UV spectra of Cyd (—) and after 1 min (···), 30 min (- - -), 120 min (●), 4 h (×), and 12 h (■) of mixing with bromine-water.

spectra at various time intervals. Again, the λ_{max} at ~240 nm appeared instantaneously with the concomitant disappearance of the characteristic λ_{max} of Cyd at 271 nm. However, the absorbance at the 240-nm region gradually decreased until only end absorption remained after the solution had stood for 12 h. While these observations indicate that 5-bromo-6-hydroxy-5,6-dihydrocytidine (**7b**) may be formed as the major product analogous to the reaction of **8a** or **6a**, this product is prone to deamination under the same conditions as the corresponding hCyt derivatives, **8a** and **6a**.

The bromination of 5-bromocytidine (BrCyd, 8b) was also investigated. For the purposes of investigating the effects of pH on this reaction and its products, *N*-bromosuccinimide (NBS) was used in place of bromine

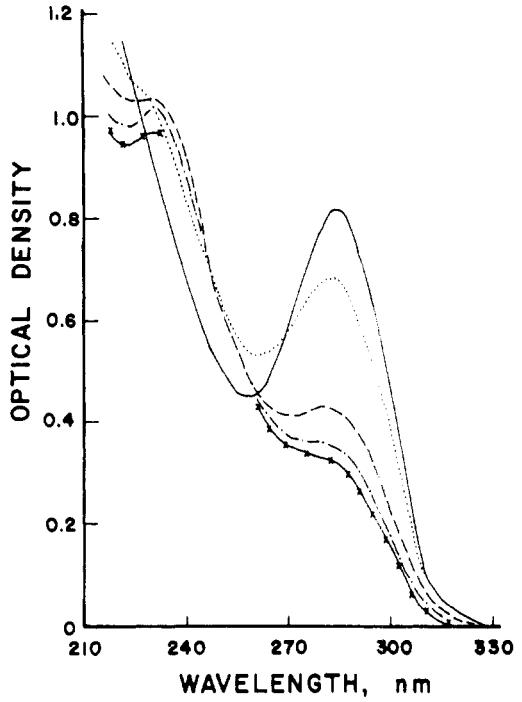
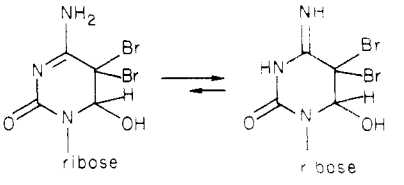


Figure 3. Bromination of 5-bromocytidine (**8b**) in 0.1 mM aqueous solution. The UV spectra of BrCyd before (—) and after 5 min (···), 30 min (- - -), 2 h (-·-), and 8 h (×) of mixing with NBS in water.

since this reagent can be used with neutral conditions. Figure 3 shows a pattern of spectral changes which resemble those described previously using bromine, except that the changes occur more slowly. After the solution had stood for 5 min, a shoulder appeared in the region 240 nm and a λ_{max} became apparent which reached a maximum at approximately 30 min. During this time, a decrease of only ~50% of the original λ_{max} at 279 nm occurred. After 30 min, a gradual decrease was observed for both maxima. These spectral changes indicate that the formation of 5,5-dibromo-6-hydroxy-5,6-dihydrocytidine (**17**) reached a maximum at approximately 30 min. Subsequent changes imply that there was no further net increase in the production of **17** nor did it react to form other products by the loss of HOBr.

Therefore, the 30-min samples were used to examine the effects of pH on the reaction (Figure 4). These samples contain **17** in a yield >90% on the basis of the comparison



17A 237 (237) 17I

of the absorbance at 240 nm with that of analogous compounds (see below). This absorption maximum remained in pH 4 and 6 buffered solutions, indicating that **17** was reasonably stable under these conditions, whereas at pH 2 the solution displayed only a shoulder in this spectral region with decrease in ϵ_{max} which was expected from the spectral behavior of a hCyt derivative. In addition, a new absorption maximum appeared in the 290-nm region, corresponding to that of BrCyd (λ_{max} 300 nm).[16] On the basis of the results of some foregoing reactions, this observation

(16) P. K. Chang and A. D. Welch, *Biochem. Pharmacol.*, **6**, 50 (1961).

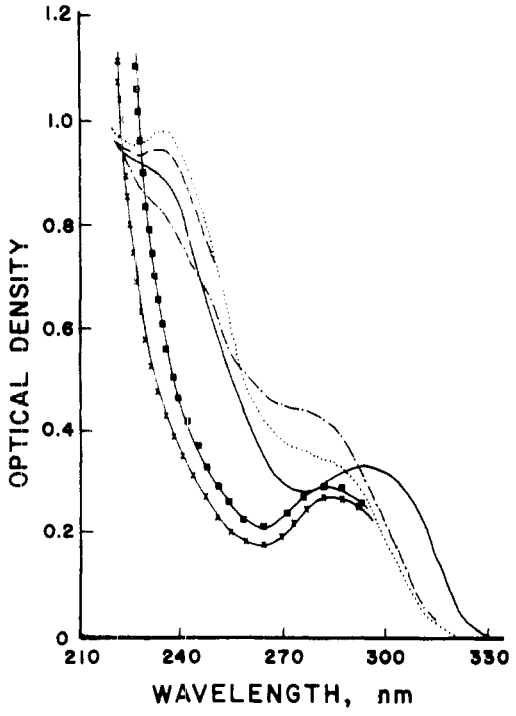
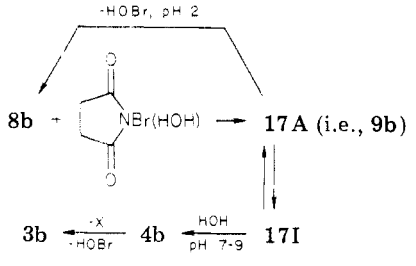


Figure 4. Effect of pH of solution on 5,5-dibromo-6-hydroxy-5,6-dihydrocytidine (17). The UV spectra in pH 2 (—), pH 4 (···), pH 6 (- - -), pH 7 (-·-), pH 8 (×), and pH 9 (■).

is not unexpected. If 17 reverts to BrCyd (**8b**) at low pH conditions, it would suggest that acid-catalyzed loss of BrOH must have occurred. At pH 7 a slight shoulder with reduced ϵ_{max} was observed without the appearance of a distinct new absorption maximum. In more basic media (pH 8–9), an absorption maximum at 280 nm appeared, and only the end absorption remained. Since hCyt derivatives generally exhibit λ_{max} in the 240–260-nm region at these pHs, the lack of this absorption implies that deamination leading to the formation of **4b** has occurred, and this product should display only end absorption. Although the 280-nm peak does not correspond to the λ_{max} of BrCyd (**8b**), it conforms with that observed for BrUrd (**3b**).[17] The product **3b** might be formed by the base-catalyzed elimination of HOBr from **4b**, which will result from deamination of **17** or **17I**.

The following reaction scheme analogous to that for Ura derivatives summarizes the above discussion.



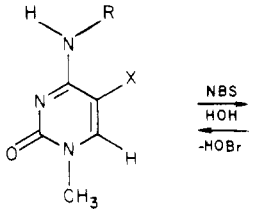
Thus, the apparent rate and extent of reaction using NBS are slower and reduced as compared with those with Br_2, albeit similar spectral and thus, by inference, chemical changes take place in both.

The bromination of three methylcytosine derivatives was subsequently studied in an attempt to isolate and to identify the elusive bromohydrin derivatives of these methylcytosines. The patterns of UV spectral changes in dilute aqueous solutions of 1-methylcytosine (**18a**), 1,N^4-dimethylcytosine (**18b**), and 5-bromo-1,N^4-dimethylcytosine (**18c**) on bromination were analogous to those observed for the reactions **6** → **7** and **8** → **9**. The first step in these reactions was attended by the instantaneous disappearance of the characteristic λ_{max} for the starting pyrimidine with the simultaneous appearance of an absorption maximum which can be attributed to the corresponding bromohydrin. The subsequent spectral changes were varied. The new absorption maximum observed at 235 nm indicated the formation of 5-bromo-6-hydroxy-1-methyl-5,6-dihydrocytosine (**19**) from **18a**.

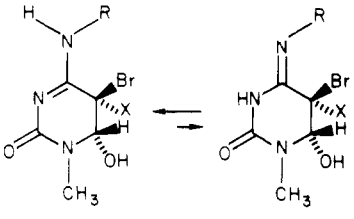
Table I. UV Spectral Data of 5,5-Dibromo-6-hydroxy-1,N^4-dimethyl-5,6-dihydrocytosine (21) in Various Buffered Solutions

buffer	λ_{max}, nm	ϵ_{max}	λ_{min}, nm	ϵ_{min}
0.5 N HCl	229	7730		
0.1 N HCl	229	7340		
pH 2	229	6250		
	~280[a]	~3160[a]		
pH 4	267	6590		
pH 6	267	6610		
H_2O	267	6610		
pH 7	264	6600		
pH 8	252	7710	230	5950
pH 9	251	9490	231	6525

[a] The underline indicates a shoulder in the spectrum.



18a, R = X = H
b, R = CH_3; X = H
c, R = CH_3; X = Br



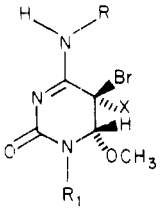
19A	235 (235)	19I
20A	263 (266)	20I
21A	267 (271)	21I

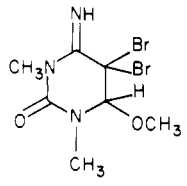
When this solution was allowed to stand, this maximum decreased gradually with the appearance of a shoulder in the 280-nm region which reached a maximum within a 2–8 h period. The spectrum after 24 h showed a λ_{max} at 285 nm which suggested the formation of **8c** in a yield of approximately 30% (estimated from the ϵ_{max}). The other major products were probably **2c** (end absorption only) and **3c** (λ_{max} 278 nm), resulting from deamination. Similarly, UV spectral changes were seen with the formation of 5-bromo-6-hydroxy-1,N^4-dimethyl-5,6-dihydrocytosine (**20**, λ_{max} 263 nm) from **18b**; however, the apparent rate of UV changes of **20** was slower. A broad peak in the 270-nm region and a λ_{max} at ~285 nm were displayed with the absorption spectra after 24 and 48 h, respectively, indicating the formation of **2c** and **3c** by the loss of methylamine and of **18c** in approximately 40% yield by the elimination of HOH. In contrast, the new absorption maximum at 267 nm due to the formation of **21** from **18c** in-

(17) M. Prystas and F. Sorm, *Collect. Czech. Chem. Commun.*, **29**, 2956 (1964).

volved only the gradual decrease of the absorption with no increase either in the end absorption or in the 280-nm region during a 24-h interval. This observation suggested that the reaction was uncomplicated and that the product **21** is relatively stable. In fact the product, 5,5-dibromo-6-hydroxy-1,N^4-dimethyl-5,6-dihydrocytosine (**21**), was isolated and characterized. This is the first example of the isolation of the elusive cytosine bromohydrin. Although the elemental analysis and the NMR spectrum confirmed the assigned structure, the UV λ_{max} (HOH) at 267 nm (Table I) is different from the generally accepted value of 240 nm for hCyt derivatives.

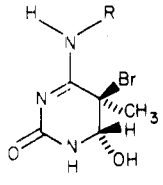
In light of the successful isolation of **21** and for the clarification of the spectral characteristics of hCyt derivatives, a number of additional bromohydrin derivatives (Table II) were synthesized with Br_2 in either water (HO-Br) or methanol (CH_3OBr). The compounds prepared include: 5,5-dibromo-6-methoxy-5,6-dihydrocytosine (**22**);

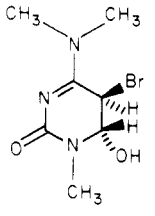




22, R_1 = R = H; X = Br
23, R_1 = CH_3; R = H; X = Br
24, R_1 = R = CH_3; X = H
25, R_1 = R = CH_3; X = Br

26





27, R = H
28, R = CH_3

29

5-bromo-5-methyl-6-hydroxy-5,6-dihydrocytosine (**27**); 5,5-dibromo-6-hydroxy-5,6-dihydrocytosine (**17**); 5,5-dibromo-6-methoxy-1-methyl-5,6-dihydrocytosine (**23**); 5-bromo-6-hydroxy-N^4,5-dimethyl-5,6-dihydrocytosine (**28**); 5-bromo-6-hydroxy-1,N^4-dimethyl-5,6-dihydrocytosine (**20**); 5-bromo-6-methoxy-1,N^4-dimethyl-5,6-dihydrocytosine (**24**); 5,5-dibromo-6-hydroxy-1,N^4-dimethyl-5,6-dihydrocytosine (**21**); 5,5-dibromo-6-methoxy-1,N^4-dimethyl-5,6-dihydrocytosine (**25**); 5-bromo-6-hydroxy-1,N^4,N^4-trimethyl-5,6-dihydrocytosine (**29**); and 5,5-dibromo-6-methoxy-1,3-dimethyl-5,6-dihydrocytosine (**26**). On the basis of the UV spectra of these compounds (**17**, **22–29**, and **33–37**), 1-methyl-5,6-dihydrocytosine (**30**),[18] cyclobutyl dimers of Cyt derivatives (**13–15**, **31**, and **32**),[11] and bisulfite adducts of cytosines (**10–12**),[10] a scheme for the calculation of the UV absorption maxima for hCyt derivatives in neutral aqueous media was deduced. The empirical values for bathochromic shifts due to substituents, distinct and characteristic for amino and imino forms, are given in Table III. The λ_{max} calculated are presented in parentheses following those observed values given under the structures. It may be seen that they are in excellent agreement except for compounds **20** and **21**. These latter experimental values are slightly lower than those calculated for the amino form and suggest an equilibrium mixture of tautomers in which the amino form predominates. Assuming equal values of ϵ for the two tautomers, one can

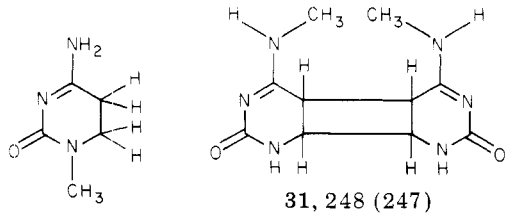
Table II. Reactions of Cytosine Derivatives with NBS in Water or Methanol

reactant	substituent[a]				reaction conditions			substituent[a]				yields and properties			
	R_1	R_{4a}	R_{4b}	X	agent	temp, °C	time, h	product	X_a	X_e	Y_a	Y_e	yield, %	mp, °C	λ_{max}(HOH), nm (ϵ)	λ_{max}(CH_3OH), nm (ϵ)
8a	H	H	H	Br	CH_3OBr	0	24	**22**	Br	Br	CH_3O	H	*b*			241 nm
39	H	H	H	CH_3	HOBr	0	1	**27**	Br	CH_3	OH	H	39[c]		283	
8b	ribose	H	H	Br	HOBr	25	1	**17**	Br	Br	OH	H	*d*		237	
8c	CH_3	H	H	Br	CH_3OBr	0	1	**23**	Br	Br	CH_3O	H	*e*		238	237
40	H	CH_3	H	CH_3	HOBr	0	1	**28**	Br	CH_3	OH	H	89[f]		258	
18b	CH_3	CH_3	H	H	HOBr	0	3	**20**	Br	H	OH	H	75	121–122	263 (7800)	
18b					CH_3OBr	0	5	**24**	Br	H	CH_3O	H	*g*		265	264
18c	CH_3	CH_3	H	Br	HOBr	25	1	**21**	Br	Br	OH	H	90	153–154	267 (6450)	266 (5900)
18c					CH_3OBr	0	2	**25**	Br	Br	CH_3O	H	79	141–142	268 (6400)	266 (6200)
41	CH_3	CH_3	CH_3	H	HOBr	0	2	**29**	Br	H	OH	H	58	112–113	276 (9100)	
38	H			Br	CH_3OBr	0	20	**26**	Br	Br	CH_3O	H	53	122–123	243 (5410)	242 (5320)

[a] They are assigned according to the molecular structures of Table III. [b] Because of low solubility of **8a** in CH_3OH, the pulverized material was suspended in CH_3OH for reaction. After 24 h, the insoluble substance was filtered off, and the product in the filtrate was collected. It was washed with ether, and its UV spectrum was taken. [c] The precipitate was collected by filtration and washed with $CHCl_3$. [d] After reaction, the mixture was lyophilized, and the residue was subjected to TLC (cellulose, 3:1 *n*-propyl alcohol–water). The major band was eluted and its UV spectrum measured. [e] After evaporation until dry, the material soluble in both CH_3OH and ethyl acetate was collected. [f] After lyophilization, the material insoluble in $CHCl_3$ was collected. [g] After evaporation until dry, the residue was dissolved in acetone and reprecipitated by the addition of ethyl acetate.

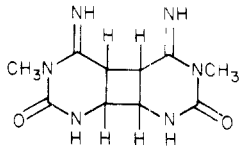
(18) D. M. Brown and M. J. E. Hewlins, *J. Chem. Soc. C*, 2050 (1968).

Chart I

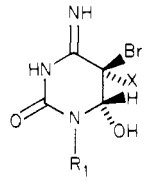


30, 243 (243)

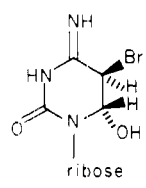
31, 248 (247)



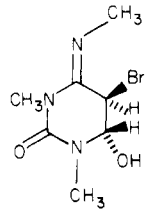
32, 227 (227)



33, $R_1 = CH_3$; X = H; 235 (235)
34, R_1 = H; X = Br; 235 (237)
35, $R_1 = CH_3$; X = Br; 237 (237)

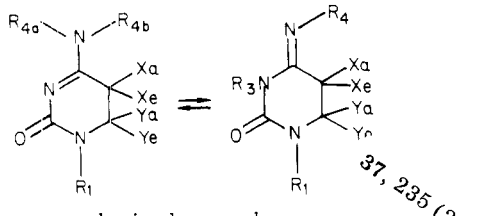


ribose



36, 237 (237)

Table III. The Values of UV Absorption Chromophores and Bathochromic Shifts of Substituents of 5,6-Dihydrocytosine Derivatives



basic chromophore

A, 239 nm ($\epsilon \sim 10\,000$) B, 227 nm ($\epsilon \sim 5000$)

bathochromic substituent shifts

	A	B
$R_1 = CH_3$, ribose	4 nm	0 nm
$R_3 = CH_3$	N/A	0 nm
$R_{4a} = CH_3$	8 nm	N/A
$R_{4a} = R_{4b} = CH_3$	18 nm	N/A
$R_4 = CH_3$	N/A	0 nm
X_a = Br	15 nm	8 nm
$X_a = X_e$ = Br	20 nm	10 nm
$Y_a = Y_e$ = H or substituent	0 nm	0 nm

calculate the percent of amino form (y) from the following simple relationship:

$$(\text{obsd } \lambda_{max}) = (\text{calcd}_{amino}\ \lambda_{max})y + (\text{calcd}_{imino}\ \lambda_{max})(1 - y)$$

The calculated values of y are 89.7% (**20A**) and 87.5% (**21A**) for **20** and **21**, respectively. Although this treatment is rather attractive for the determination of solution composition of a variety of hCyt derivatives on the basis of their UV spectra, it can only be considered as an approximation since the necessary condition of equal ϵ values for exact quantitation is seldom met.

In conclusion, the study of UV spectral changes of reactions of various cytosine derivatives with bromine and *N*-bromosuccinimide in aqueous or methanolic solutions

Table IV

time, min	λ_{max}, nm	ϵ_{max}	λ_{min}, nm	ϵ_{min}
0	267	6190	247	4190
1	276	5360	250	3540
30	276	5127	251	3270
60	277	5200	251	3080
120	278	5250	251	2890

demonstrates that the halogenation of cytosine bases and nucleosides involves the addition–elimination mechanism as the major pathway. This is further corraborated by the isolation and characterization of a number of bromohydrin derivatives. This information has greatly enhanced our understanding of the amino–imino tautomerization and of the UV spectral characteristics of dihydrocytosine derivatives. This knowledge should be relevant to mutagenesis resulting from the chemical modifications of cytosine moieties in nucleic acids[19] and to the mechanism of water disinfection by means of halogenation.[20]

Experimental Section

General Procedures. Ultraviolet and infrared (KBr pellets) absorption spectra were recorded on Beckman Model DK-1 and Perkin-Elmer Model 21 spectrophotometers, respectively. Nuclear magnetic resonance spectra were obtained on a Varian 220-MHz spectrometer in $(CD_3)_2SO$ at 22 °C.

Materials. Cytosine derivatives were prepared in this laboratory according to the methods reported previously.[21] BrCyd was synthesized according to the method of Duval and Ebel.[22]

Reaction of Cyt (6a) with Br_2 in Aqueous Solution. Preparation of 5-Bromocytosine (BrCyt, 8a). Bromine (0.30 mL, 5.5 mmol) was added to a suspension of pulverized **6a** (0.65 g, 5.0 mmol) in 10 mL of water. The mixture was stirred at ambient temperature for 2 h and then refrigerated overnight. The crystalline precipitate was collected by filtration and washed with cold water: 1.06 g (78.6%); mp 255–256 °C dec [BrCyt·HBr, (lit. mp 245–255 °C)];[23] λ_{max} (H_2O) 283 nm (ϵ 5360); λ_{min} (H_2O) 258 nm (ϵ 2810); NMR [$(CD_3)_2SO$] δ 8.18 (s, 1, C(6)H) and 5.6 (v br, NH).

The filtrate was neutralized with 5% sodium bicarbonate solution and was concentrated to approximately 10 mL. The precipitate (0.13 g) which collected on refrigeration overnight was redissolved in water and applied on Whatman No. 3 paper for chromatography with *n*-propyl alcohol–water (10:3 (v/v)) as eluent. **6a** (R_f 0.26, 8 mg), **8a** (R_f 0.43, 110 mg), and **3a** (R_f 0.64, 9 mg) were obtained. Thus, the total yield of **8a** was 89.2%, with 0.8% converted to **3a** and 1.2% recovered as starting material, **6a**.

Conversion of BrCyt·HBr to the free base was best achieved by treating a suspension of the pulverized salt (0.38 g, 1.4 mmol) in water (7 mL) with concentrated NH_4OH (1.1 mL, 14 mmol), and **8a** was isolated as described above in a total yield of 98% (253 and 32 mg). After recrystallization from water, the purified product (0.24 g, 83.2%) melted at 170–171 °C: NMR [$(CD_3)_2SO$] δ 7.77 (s, 1, C(6)H); λ_{max} (H_2O) 282 nm (ϵ 4700); λ_{min} (H_2O) 259 nm (ϵ 2570).

UV Spectral Study of the Reaction of Cyt (6a) with Bromine in Water. Equal volumes of 0.2 mM aqueous solutions of Cyt and Br_2 were mixed, resulting in a 0.1 mM solution of Cyt. The UV spectral changes in the 210–360 nm region were recorded in Table IV.

Stability of Cyt (6a) and BrCyt (8a) in Hydrobromic Acid. One millimole of Cyt (129 mg) in 2 mL of 0.57 M HBr (1.1 mmol) was stirred at room temperature for 2 h. After the solution was neutralized with $NaHCO_3$ and left standing at ~5 °C, Cyt was

(19) C. O. Doudney in "Photochemistry and Photobiology of Nucleic Acids", Vol. 2, S. Y. Wang, Ed., Academic Press, New York, 1976, pp 309–374 and references cited therein.
(20) V. P. Olivieri, C. W. Kruse, Y. C. Hsu, A. C. Griffiths, and K. Kawata in "Disinfection Water and Wastewater", J. D. Johnson, Ed., Ann Arbor Science, Ann Arbor, Mich., 1975.
(21) See references cited in ref 10 and 11.
(22) J. Duval and J. P. Ebel, *Bull. Soc. Chim. Biol.*, **46**, 1059 (1964).
(23) H. L. Wheeler and T. B. Johnson, *Am. Chem. J.*, **31**, 591 (1904).

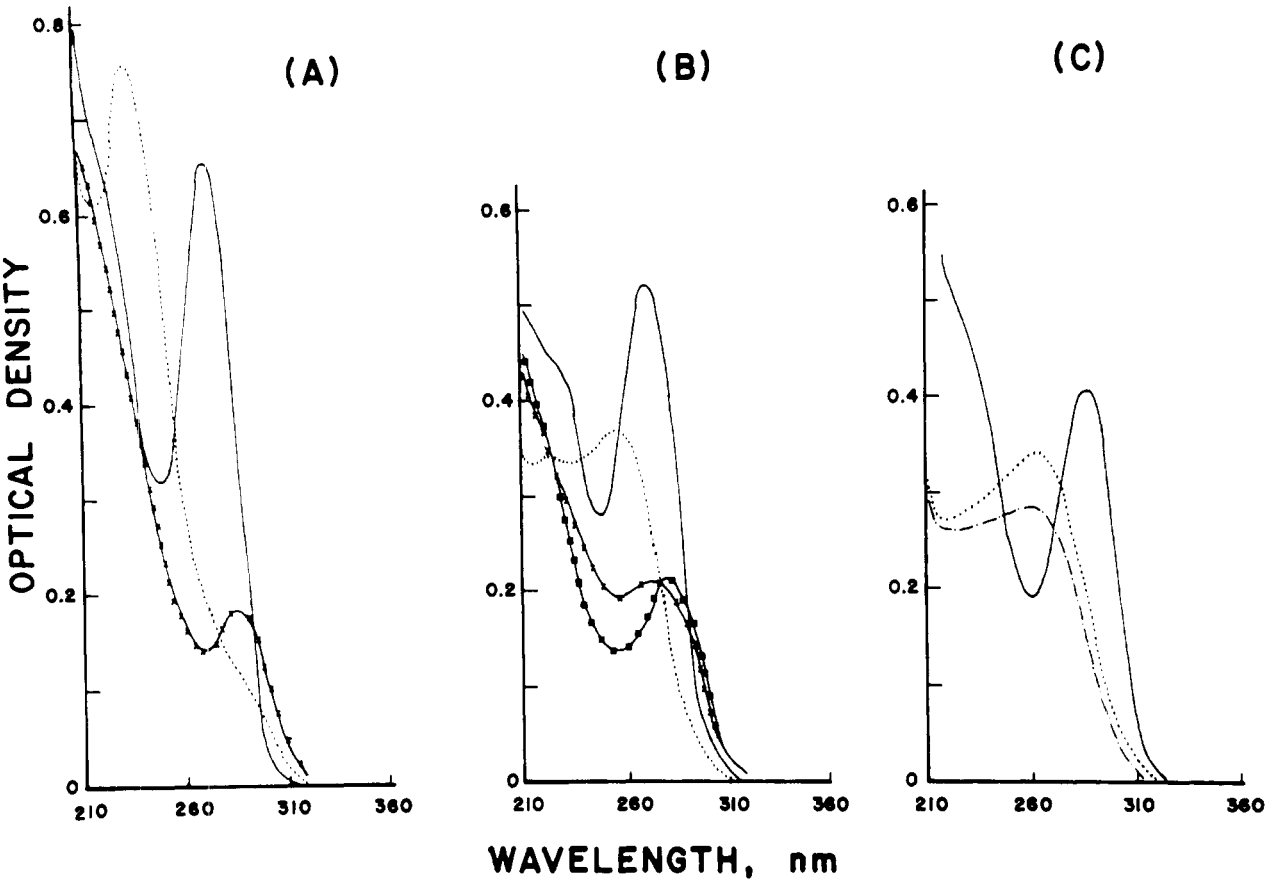


Figure 5. Bromination of 1-methylcytosine (A), 1,N^4-dimethylcytosine (B), and 5-bromo-1,N^4-dimethylcytosine (C) in 0.1 mM aqueous solutions. The UV spectra before (—) and after mixing with bromine–water: (A) 1 min (···) and 24 h (×); (B) 1 min (···), 22 h (×) and 48 h (■); and (C) 1 min (···) and 21 h (---).

recovered in 96.9% yield (125 mg). Only one spot corresponding to Cyt was detected on TLC of the mother liquor.

BrCyt (190 mg) was treated in a similar manner, and 83.4% (227 mg) was precipitated as the hydrobromide. The filtrate, after neutralization, gave two crops totalling 23 mg (12%). Only one spot corresponding to BrCyt was detected on TLC of the various fractions.

Reactions of BrCyt (8a) with Br_2 in Aqueous Solution. Bromine (0.06 mL, 1.1 mmol) was added to a suspension of **8a** (0.19 g, 1.0 mmol) in 5 mL of water. A clear solution resulted after the solution was stirred at room temperature for 2 h, and it was lyophilized at −12 °C. The residue was extracted with acetone, and the insoluble material was found to be ammonium bromide (94.7 mg, 96.7%) [see Discussion]. The acetone extract was concentrated, and a crystalline product appeared on addition of petroleum ether (30–60 °C). This compound (249 mg, 86.5%) melted at 209–211 °C with strong effervescence and then resolidified as observed for an authentic sample of 5,5-dibromo-6-hydroxy-5,6-dihydrouracil (**4a**).[2] The IR spectra were also identical, and the mixed melting point showed no depression.

UV Spectral Study of the Reaction of Various Cyt Derivatives with Br_2 in Water. A 0.1 mM solution of the Cyt derivative was prepared by mixing equal volumes of 0.2 mM aqueous solutions of the base and of Br_2. The UV spectra were recorded at various time intervals and are shown in Figure 1 for BrCyt (**6a**), Figure 2 for Cyd (**6b**), Figure 5a for 1-methylcytosine (**18a**), Figure 5b for 1,N^4-dimethylcytosine (**18b**), and Figure 5c for 5-bromo-1,N^4-dimethylcytosine (**18c**), respectively.

Reaction of 5-Bromocytidine (BrCyd, 8b) with *N*-Bromosuccimide (NBS). BrCyd (32.2 mg, 0.10 mmol) and NBS (19.6 mg, 0.11 mmol) were dissolved in 100 mL of water, and a 3.0-mL portion of this solution was diluted to 50 mL with water. The spectra at various time intervals were taken and are shown in Figure 3.

Preparation of 5-Bromo-1,N^4-dimethylcytosine (18c). To an ice-cooled solution of **18b** (557 mg, 4 mmol) in $CHCl_3$ (20 mL) was added 0.24 mL of bromine (4 mmol). The resulting yellow-orange crystals were collected, washed with chilled chloroform, and dried (1.015 g). After treatment with 1 g of $NaHCO_3$ in 20 mL of water for 1 h, the **18c** obtained was extracted with five 10-mL portions of $CHCl_3$. The residue after evaporation of the dried $CHCl_3$ extracts (660 mg) was recrystallized twice from chloroform–petroleum ether: mp 175–176 °C; λ_{max} (H_2O) 287 nm (ϵ 7670); λ_{min} (H_2O) 259 nm (ϵ 3545). Anal. Calcd for $C_6H_8ON_3Br$: C, 33.04; H, 3.70; N, 19.27; Br, 36.65. Found: C, 33.26; H, 3.80; N, 19.22; Br, 36.88.

To obtain the hydrobromide salt of **18c**, the initial precipitate was dissolved in water, and the solution was extracted with $CHCl_3$ and concentrated. The hydrobromide crystallized as white needles, dec >256 °C without melting. Anal. Calcd for $C_6H_9ON_3Br_2$: C, 24.10; H, 3.03; N, 14.06; Br, 53.46. Found: C, 24.30, H, 3.06; N, 14.29; Br, 53.02.

Preparation of 5,5-Dibromo-1,N^4-dimethyl-6-hydroxy-5,6-dihydrocytosine (21). **18c** (0.44 g, 210 mmol) and NBS (0.39 g, 2.2 mmol) in 20 mL of water were stirred at room temperature for 4 h and then allowed to stand at 5 °C overnight. The crystals were collected by filtration, washed with cold water, and dried. A second crop was obtained from the concentrated mother liquor. The combined crops were recrystallized from benzene–methanol: 0.57 g (91%); mp 154–155 °C; NMR [$(CD_3)_2SO$] δ 5.07 (d, J = 6 Hz, 1, collapses to singlet with D_2O, C(6)H), 7.30 (d, J = 6 Hz, 1, disappears with D_2O, OH), 2.85 (s, 3, CH_3), and 2.93 (s, 3, CH_3); UV λ_{max} (H_2O) 267 nm (ϵ 6450); λ_{max} (CH_3OH) 266 nm (ϵ 5820). Anal. Calcd for $C_6H_9O_2N_3Br_2$: C, 22.94; H, 2.89; N, 13.38; Br, 50.60. Found: C, 22.70; H, 2.72; N, 13.47; Br, 50.18.

Preparation of 5,5-Dibromo-6-methoxy-5,6-dihydrocytosine (22), 5,5-Dibromo-6-methoxy-1-methyl-5,6-dihydrocytosine (23), 5-Bromo-6-methoxy-1,N^4-dimethyl-5,6-dihydrocytosine (24), 5,5-Dibromo-6-methoxy-1,N^4-dimethyl-5,6-dihydrocytosine (25), and 5,5-Dibromo-6-methoxy-1,3-dimethyl-5,6-dihydrocytosine (26). Silver carbonate (305 mg, 1.1 mmol) was added to a cooled solution of Br_2 (0.03 mL, 0.55 mmol) in 0.7 mL of MeOH. The reaction mixture was stirred for 30 min at <5 °C and then filtered. The filtrate should contain ~1 mmol of MeOBr.

To this solution, 0.5 mmol of a Cyt derivative, **8a**, **8c**, **18b**, **18c**, or 5-bromo-1,3-dimethylcytosine (**38**), in 0.3 mL of MeOH was added. This admixture was stirred for a period of time (see Table II) and then evaporated until dry under reduced pressure at 22 °C. The residue was dissolved in acetone, and the product was precipitated by the dropwise addition of ethyl acetate. The precipitate was collected and redissolved in acetone. Ethyl acetate was again added to the solution just prior to it becoming permanently cloudly. This clear solution was refrigerated, and the crystalline product was collected. Reaction conditions, results, and product properties are given in Table II.

Preparation of 5-Bromo-6-hydroxy-5-methyl-5,6-dihydrocytosine (27) and 5-Bromo-6-hydroxy-N^4,5-dimethyl-5,6-dihydrocytosine (28). 5-Methylcytosine (**39**, 1 mmol) in 3 mL of water was treated with 1.1 mmol of NBS. The mixture was stirred for 3 h and then refrigerated overnight. The crystalline product was collected. N^4,5-Dimethylcytosine (**40**) was treated in a similar manner. However, 1 mL of water was used as solvent, and the reaction time required was ~1 h. After lyophilization, the residue was triturated with $CHCl_3$ and the product isolated. Further information regarding the reactions and the products is presented in Table II.

Preparation of 5,5-Dibromo-6-hydroxy-5,6-dihydrocytidine (9b), 5-Bromo-6-hydroxy-1,N^4-dimethyl-5,6-dihydrocytosine (20), 5,5-Dibromo-6-hydroxy-1,N^4-dimethyl-5,6-dihydrocytosine (21), and 5-Bromo-6-hydroxy-1,N^4,N^4-trimethyl-5,6-dihydrocytosine (29). One millimole of 5-bromocytidine (**8b**), 1,N^4-dimethylcytosine (**18b**), 5-bromo-1,N^4-dimethylcytosine (**18c**), and 1,N^4,N^4-trimethylcytosine (**41**) in 10, 2, 1, and 0.3 mL of water, respectively, was treated with 1.1 mmol of NBS. The solution was stirred at room temperature for a period of time and then refrigerated overnight. The crystalline product was collected. [See Table II for additional infomation.]

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Registry No. 3a, 51-20-7; **4a**, 1124-83-0; **6a**, 71-30-7; **6b**, 65-46-3; **8a**, 2240-25-7; **8a**·HBr, 71647-17-1; **8b**, 3066-86-2; **8c**, 65567-60-4; **9b**, 71647-18-2; **18a**, 1122-47-0; **18b**, 6220-49-1; **18c**, 71647-19-3; **18c**·HBr, 71647-20-6; **20**, 71647-21-7; **21**, 71647-22-8; **22**, 71647-23-9; **23**, 71647-24-0; **24**, 71647-25-1; **25**, 71647-26-2; **26**, 71647-27-3; **27**, 71647-28-4; **28**, 71647-29-5; **29**, 71647-30-8; **38**, 64236-15-3; **39**, 554-01-8; **40**, 62006-34-2; **41**, 2228-27-5; bromine, 7726-95-6; ammonium bromide, 12124-97-9; NBS, 128-08-5.

Supplementary Material Available: Expanded version of Figure 5 showing additional time curves (2 pages). Ordering information is given on any current masthead page.

Phosphinimines as Useful Intermediates in the Synthesis of 3-(Acylamino)-β-lactams

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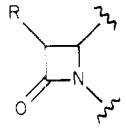
N-Substituted 3-azido-4-(methylthio)-2-azetidinones **9** (trans) and **10** (cis) reacted with triphenylphosphine to give the corresponding 3-phosphinimino-β-lactams **11** and **12**. Treatment of these iminophosphoranes with phenoxyacetyl chloride afforded respectively the *trans*- and *cis*-3-(acylamino)-β-lactams **13** and **14**. The *cis*-β-lactam **14** was obtained also from the *trans*-β-lactam **11**: condensation of **11** with *p*-nitrobenzaldehyde gave the Schiff base **19**; kinetically controlled epimerization of **19**, followed by hydrolysis and acylation, afforded the β-lactam **14**.

The azido group has been used as a progenitor of the acylamino side chain in some total syntheses of penicillins,[1] cephalosporins,[1] and various analogues of these β-lactam antibiotics.[2-12] In these papers, 3-azidoazetidinones, represented by the partial structure **1**, were reduced by catalytic hydrogenation, hydrogen sulfide in the presence of ammonia or triethylamine, or zinc in acetic acid to the corresponding 3-aminoazetidinones **2**. Compounds **2** were subsequently converted into 3-(acylamino)azetidinones **3**.



1, R = N_3
2, R = NH_2
3, R = R′CONH
4, R = $Ph_3P{=}N$
5, R = *p*-$O_2NC_6H_4CH{=}N$

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