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CHLORINATION STUDIES. I. THE REACTION OF AQUEOUS HYPOCHLOROUS ACID
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SUMMARY

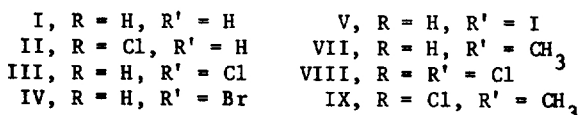
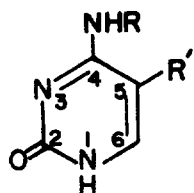
Cytosine, 5-chloro- and 5-methylcytosine react with one equivalent of HOCl to yield the corresponding 4-N-chloro derivatives (II, VIII and IX). Two or three equivalents of HOCl with cytosine yield mixtures of II and VIII accompanied by two unstable compounds identified as a di- and trichlorocytosine respectively. Five equivalents of HOCl when reacted with cytosine produce unstable tri- and tetrachloro-derivatives. Acidification of II with dilute HCl, HBr or HI affords 5-Cl, 5-Br and 5-iodocytosine respectively. An aqueous suspension of II partially converts phenylalanine into phenylacetaldehyde.

Despite our dependence for many decades on the treatment of water by chlorine¹ little is known about the mechanism by which it kills bacteria.² The toxic effect of active chlorine might involve modification or destruction of bacterial DNA. Accordingly we have studied the action of "hypochlorous acid"³ on some DNA bases. The stable end products of halogenation of these bases are well known. However, only one serious attempt has been directed at labile intermediates which might be generated in these reactions.⁴ On the basis of our own contemporaneous studies we now discuss further aspects of the action of hypochlorous acid on cytosine under physiological conditions.

When cytosine (I) was reacted with one equivalent of HOCl 4-N-chlorocytosine (II) was obtained in 76% yield. The compound contained one active chlorine; its mass spectrum displayed parent ions at m/e 145, 147 while fragment ions of diagnostic utility were recorded at m/e 110 (M minus Cl)⁺

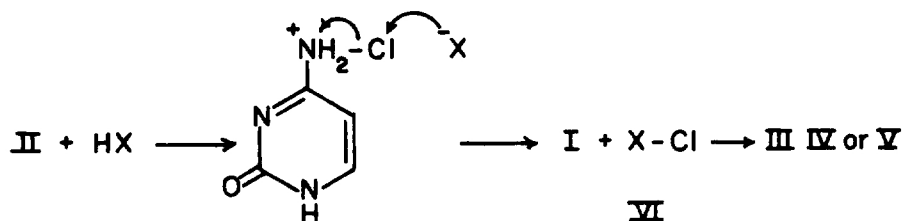
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and m/e 95 (M minus NHCl)⁺. The olefinic protons (C-5, 6) evoked NMR doublets at δ 6.35 and 7.65 ($J = 7\text{Hz}$).



Dilute aqueous HCl, HBr or HI converts II into III, IV and V respectively.

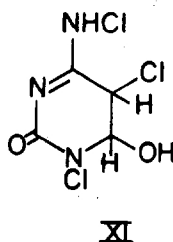
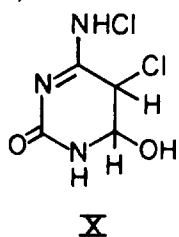
Protonation of the N-chloro-group of II followed by halogen ion attack would yield cytosine plus VI. This in turn halogenates⁵ cytosine to give a simple direct synthesis of any of the three 5-halocytosine derivatives.



Treatment of 5-chloro- or 5-methylcytosine (III and IV) with one equivalent of HOCl yielded VIII and IX respectively. The mass spectrum of 4-N, 5-dichlorocytosine (VIII) contained structurally significant ions corresponding to M^+ , (M minus Cl)⁺, (M minus NHCO)⁺ and (M minus NHCl)⁺.

Cytosine gave, with two equivalents of aqueous HOCl, a precipitate, (in variable yield depending on the rate of addition of reagent), identified as a mixture of II and VIII. TLC indicated two other products; these were more abundant with three equivalents of HOCl. The residues in the ether and water phases, following ether extraction of the reaction mixture, were examined by mass spectrometry. Molecular ion clusters were observed (aqueous and ether phases) corresponding to the molecular ions of X and XI. Both products contained N-Cl groups as demonstrated by their positive reaction with starch-KI spray on TLC. These compounds proved too unstable to isolate as they readily decomposed to VIII and III. The mass spectrum of X contains ions corresponding to (M minus H_2O)⁺, (M minus Cl)⁺, (M minus NHCHOH)⁺ and

(M minus HOCl)⁺. The "trichlorocytosine" structure is less well established; it plausibly may be produced from X by reaction with one equivalent of HOCl. The mass spectrum of XI contains ions equivalent to (M minus HOCl)⁺ and (M minus NCl=CHOH)⁺.



Five equivalents of HOCl converted cytosine (I) into a mixture (molecular ions determined by high resolution mass spectrometry) of XI ($C_4H_4N_3O_2Cl_3$) and $C_4H_3N_3O_2Cl_4$. On standing at room temperature the tetrachloro-compound decomposed within one hour (no molecular ion visible by mass spectrometry) and the only stable end products isolated were III and VIII.

4-N-Chlorocytosine (II) oxidized a solution of phenylalanine to phenylacetaldehyde, a known product from the reaction of the amino acid with NaOCl.⁶ This reaction is believed to proceed through initial formation of an N-chloro amino acid derivative⁶ possibly formed by direct transfer of chlorine between II and phenylalanine.⁷

The facile formation of N-chloro-derivatives of cytosine from its reaction with HOCl has been demonstrated. The isolation⁸ of III from DNA treated with NaOCl probably involved initial formation of N-chloro compounds which were converted into III during the hydrolytic work-up employed.

EXPERIMENTAL

Aqueous solutions of HOCl were prepared by the method of Higuchi and Hasegawa.⁹ Low and high resolution mass spectra were obtained with Finnigan 1015 Quadrupole and Varian MAT 711 instruments operating at 70 eV. Chlorination reactions were conducted at room temperature by the dropwise addition of HOCl (1 hour per gm equivalent) to a stirred suspension of the base in water. UV spectra were recorded in water. TLC was carried out

with cellulose MN300 CM plates using water as the mobile phase. NMR spectra were recorded by a Varian HA 100 instrument using $D_2O/NaOD$ as solvent.

4-N-Chlorocytosine (II) was obtained in 76% yield, m.p. 175-200° (decomp.). Found C, 32.99; H, 2.85; N, 29.20; Cl, 24.14. $C_4H_4N_3OCl$ requires: C, 33.00; H, 2.77; N, 28.90; Cl, 24.14%. λ_{max} 226 nm (log ϵ = 3.88) and 276 nm (log ϵ = 3.87). Acetic acid (100°) for 1 hour converted II into 5-chlorocytosine (95% yield).

4-N-5-Dichlorocytosine (VIII), m.p. 210° (decomp.) (lit.⁴ m.p. 192° decomp.). Found C, 26.47; H, 1.79; N, 23.15; Cl, 39.20. $C_4H_3N_3OCl_2$ requires: C, 26.63; H, 1.67; N, 23.35; Cl, 39.35%. λ_{max} 226 nm (log ϵ = 3.94) and 280 nm (log ϵ = 3.94). The NMR spectrum contained a singlet at δ = 7.87.

4-N-Chloro-5-methylcytosine (IX) m.p. 210-220° (decomp.). Found: C, 37.87; H, 3.77; N, 26.10; Cl, 22.60. $C_5H_6N_3OCl$ requires: C, 37.60; H, 3.76; N, 26.35; Cl, 22.22%. λ_{max} 223 nm (log ϵ = 3.92) and 271 nm (log ϵ = 3.97).

Conversion of 4-N-Chlorocytosine (II) to 5-Halocytosines (III, IV and V)

Water (1 ml.) plus II was treated with aqueous HX (X = Cl, Br, I) (1 ml. 1N) for 10 minutes, made alkaline (NH_4OH) and the solid collected. 5-Chlorocytosine (III), 60% yield, m.p. 289-292° (lit.¹⁰ m.p. 289-292°), 5-bromocytosine (IV) 55% yield, m.p. 250-255° (lit.¹¹ m.p. 245-255°), 5-iodocytosine (V) 45% yield, m.p. 224-225° (lit.¹² m.p. 225-245°).

Reaction of Cytosine with 3 and 5 Equivalentents of HOCl.

The aqueous phase from these reactions was partitioned between ether and the aqueous phase lyophilized overnight and then mass spectra were immediately recorded. X had an R_f = 0.76; XI R_f = 0.68.

Reaction of 4-N-Chlorocytosine with Phenylalanine.

II (130 mg.) was added to phenylalanine (140 mg.) in water (10 ml.) and the reaction stirred for 1 hour under a N_2 atmosphere. Ether extraction followed by gas chromatography-mass spectrometry identified phenylacetaldehyde.

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