2-oxide (VIII), and the infrared spectra of the two samples were identical.

Attempted Nuclear Chlorination of II under More Drastic Conditions.—Sulfur monochloride (15 ml., 25 g., 0.19 mole) was added to a mixture of 3.0 g. (0.016 mole) of II and 4 ml. of glacial acetic acid. This mixture was stirred for 13 hr. at room temperature and then heated at 70–75° for 22 hr. Dry benzene was added to the resulting dark mixture, and the precipitate was collected by filtration. After washing and drying as before, 2.8 g. of dark cinders were obtained.

A mixture of 1.6 g. of these cinders was stirred with 50 ml. of water for 90 sec. The resulting orange solution was rapidly filtered from the dark insoluble material. After approximately 2 min., a light-colored solid began to separate out of the orange filtrate. After the mixture had been allowed to stand overnight, the precipitate was collected and dried to give 0.21 g. (14%) of light purple solid. After purifying as usual, 0.10 g. (7%) of light yellow plates were obtained which melted at 123–124° dec. The infrared spectrum of this product was identical with the spectrum of VIII.

The other Herz compounds and their hydrolysis products were made in a similar manner and the important experimental data for them is given in Table I.

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Cytosine 3-N-Oxide and Its Rearrangement on Acetylation[1]

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Cytosine and cytidine (I) have been oxidized to their N-oxides (II) by *m*-chloroperbenzoic acid. Acetylation of cytosine 3-N-oxide (IIa) resulted in a rearrangement to N^4-acetoxycytosine (III). This was proven by an unambiguous synthesis from 4-methylthiouracil (V).

A variety of purine N-oxides have been studied, some of which have chemotherapeutic[2,3] and oncogenic[4] activities, but until recently most of the known pyrimidine N-oxides have been either alkyl or alkoxy derivatives.[5] Pyrimidine N-oxides bearing only amino or hydroxy functions, or both, have just recently been reported.[6–9] Cramer[10–12] indicates that N-oxides of this type may be useful in determining the base sequence of polynucleotides.

In 1963 Cramer reported the direct oxidation of cytosine, cytidine, and cytidylic acid to the corresponding N-oxides using monoperphthalic acid.[6] However, the scale of those reactions was too small to permit isolation of pure products; identification was made on the basis of spectral data (Table I).

In our hands that method has not been successful, even on a larger scale, but we have accomplished the oxidation of cytosine and cytidine (I) to N-oxides (II), with the commercially available *m*-chloroperbenzoic acid. The product from the cytosine oxidation (IIa), obtained in 21% yield, was assumed to have the 3-N-oxide[13] structure on the basis of its similarity in spectrum to cytidine N-oxide. IIa was obtained as a monohydrate and, to exclude the possibility of a di-N-oxide, was reduced with Pd–C. The uptake of 1 equiv. of hydrogen and the isolation of cytosine confirmed this assumption.

Klötzer, in a very significant series of contributions, has reported total and unequivocal syntheses of several pyrimidine N-oxides,[7–9] including uracil 1- and 3-N-oxides[8] and cytosine 1- and 3-N-oxides.[9] We have now compared our cytosine N-oxide with Klötzer's cytosine 3-N-oxide, both paper chromatographically in several solvent systems and spectrally at three pH values, and established their identity.

Cytosine 3-N-oxide (IIa) is stable to alkali. From acid, it was recovered as its hydrochloride. Treatment of IIa with acetic anhydride in glacial acetic acid produced a compound which had an elemental analysis in agreement with a monoacetyl derivative, $C_6H_7N_3O_3$. Its ultraviolet absorption spectra indicated that the product was not an acetyl derivative of the N-oxide. The compound did not show any strong absorption in the 220–230-mμ region nor give a red-orange color with ferric chloride, as does cytosine 3-N-oxide.

From the spectral similarity of the product to that of N^4-acetylcytosine[14] and to N^4-hydroxycytidine derivatives,[15] and the slow production of a deep blue color with ferric chloride, a hydroxylamine was suspected. We have now established that this reaction had led to a rearrangement to N^4-acetoxycytosine (III). This conversion, IIa → III, is analogous to the Dimroth rearrangement which has recently been reviewed,[16] although the conditions employed are more nearly comparable with a reverse rearrangement reported by Ueda and Fox,[17,18] in which an aminomethyl group is rearranged to a ring N-methyl group.

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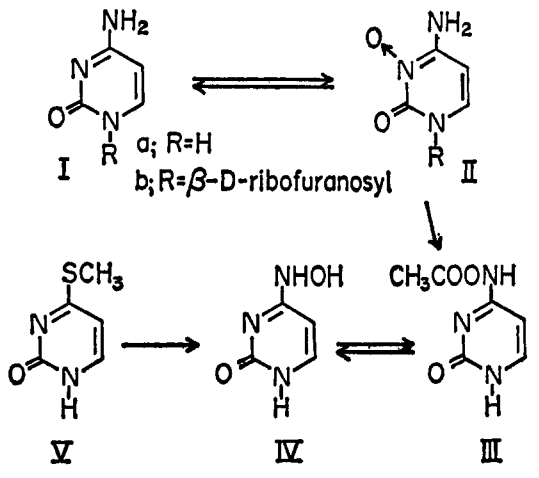
TABLE I

Compound	Spectral data: pH (species)	Spectral data: λ_{max}, mμ ($\epsilon \times 10^{-3}$)			Chromatographic data: Solvent systems A	B	C	D	E	F
Cytosine 3-N-oxide (IIa)	1 (cation)	270			0.33	0.84	0.42	0.31	0.44	0.21
	7 ("neutral")	270 (4.6)	271[a]	270[b]	0.38[a]					
		221 (19.0)	219	221						
	11–12 (anion)	287								
		217								
Cytidine 3-N-oxide (IIb)	1 (cation)	275			0.37	0.87	0.39	0.26	0.38	...
	7 ("neutral")	272 (6.6)	271[a]		0.41[a]	0.81[a]				
		225 (22.2)	224							
	11–12 (anion)	270								
		228								
N^4-Acetoxycytosine (III)	1 (cation)	271			0.91	0.71	0.78	...	c	0.85
		232								
	7 ("neutral")	271								
		232								
	11–12 (anion)	Unstable[c]								
N^4-Hydroxycytosine (IV)	1 (cation)	276			0.88	0.68	0.58	...	0.79	0.76
	7 ("neutral")	218								
		272								
		232								
	11–12 (anion)	Unstable								
Cytosine (Ia)					0.63	0.77	0.45			
Cytidine (Ib)						0.83	0.34			

[a] See ref. 6. [b] See ref. 9. [c] III is hydrolyzed to IV in this solvent.

N^4-Hydroxycytosine (IV) has been reported by several investigators.[9,19,20] We have prepared IV in an alternate manner from 4-methylthiouracil (V) and hydroxylamine (Scheme I). Acetylation of IV with acetic anhydride in glacial acetic acid gave III, which was proved by comparison of ultraviolet and infrared spectra, paper chromatography, and color reaction with ferric chloride. That III gives this deep blue color reaction only after prolonged standing may be accounted for by the slow hydrolysis of the acetyl group to give IV. In fact, we have converted III into IV by gentle treatment with alkali at pH 9, or in solvent E (*ca.* pH 8, see Experimental). The hydrolysis is immediate at this pH and leads to only one product. In stronger alkali (0.1 *N*) the molecule is destroyed and cannot be regenerated by the addition of acid.

SCHEME I



The rearrangement of the N-oxide to an exocyclic hydroxylamine may proceed through either of two pathways. The most logical of these involves a ring opening between C-2 and N-3 and reclosure with the former exocyclic amino group, as in the reverse rearrangement of cytosine[21] and N-4 alkylcytosines.[17,18] A similar ring cleavage is also observed with adenine 1-N-oxide, although it does not reclose. In that case the intermediate carboxamidoxime leads to an oxadiazole. Substituents in the 2-position have been shown to affect greatly the ring cleavage in purine 1-N-oxides.[22]

No rearrangement of an N-oxide oxygen to an adjacent amine group is reported, although rearrangements involving migrations of N-oxide oxygens to adjacent ring carbons or to methyl groups are well known.[23] However, this possibility cannot be excluded on the basis of the present data.

Cytidine 3-N-oxide[13] was obtained in 41% yield by oxidation with *m*-chloroperbenzoic acid. Here, the N-1 is blocked by the sugar moiety and the question of which nitrogen bears the N-oxide function is unambiguous since N-4 is excluded on the basis of spectra. We have been unable to hydrolyze IIb to IIa. When perchloric acid was used to remove the sugar, only uracil was shown to be present by paper chromatographic and spectral data.

We also have paper chromatographic and spectral evidence to support the formation of the N-oxide of 5-methylcytosine, though uracil and thymine are unaltered under these conditions. Similar experiments indicate that 2-acetylaminopyrimidine, N^4-acetylcytosine, 5-bromocytosine, and 4,5,6-triaminopyrimidine were destroyed under these conditions.

Oxidation by means of trifluoroperoxyacetic acid has also been investigated. Neither cytosine nor cytidine was oxidized by this reagent at room temperature under either aqueous or anhydrous conditions. Both of these compounds were slowly destroyed by any attempt to heat the reaction mixture.

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m-Chloroperbenzoic acid is a very useful reagent not only from the point of view of its commercial availability and stability but also because of the various types of solvents which may be employed. We have found that the oxidation of cytidine, for example, proceeds in H_2O, CH_2Cl_2, and EtOH as well as in glacial acetic acid. The solubility of the base is the determining factor in this case, hence acetic acid was chosen. The direct oxidation of cytosine to its 3-N-oxide is the simpler method of obtaining that particular isomer,[9] and this procedure should also be useful in the preparation of 3-N-oxides of naturally occurring glycosyl cytosines.

The paper chromatographic and ultraviolet absorption data are given in Table I. From the spectral changes, the p*K* values of IIa were found to be 4.82 and 10.3, as compared with 4.60 and 12.16 for cytosine.

Experimental

Analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Melting points were obtained on a calibrated Fisher-Johns apparatus unless otherwise noted. Chromatograms were developed by the ascending technique with Whatman No. 1 paper. The solvent systems employed were A, 1% aqueous ammonium sulfate–isopropyl alcohol (1:2); B, 5% disodium hydrogen phosphate–isoamyl alcohol (3:2); C, *t*-butyl alcohol–methyl ethyl ketone–88% formic acid–water (40:30:15:15); D, *n*-butyl alcohol–acetic acid–water (4:1:1); E, isopropyl alcohol–water–28% ammonium hydroxide (7:2:1); and F, *n*-propyl alcohol–water (3:1).

Cytosine 3-N-Oxide (IIa).—To a solution of 10 g. (0.09 mole) of cytosine in 150 ml. of glacial acetic acid was added dropwise, with stirring, a suspension of 31 g. (0.15 mole) of *m*-chloroperbenzoic acid[24] in 125 ml. of glacial acetic acid. The mixture was then heated at 60° for 1.5 hr. and poured into 1500 ml. of cold water which was stirred. The insoluble organic acids were filtered and the filtrate was evaporated to dryness. The 3 g. of residue was washed with ether and recrystallized from 100–125 ml. of boiling water with charcoal. Upon cooling, 1.7 g. of the cytosine 3-N-oxide precipitated, and concentration of the filtrate to half volume yielded an additional 0.7 g.; total 2.4 g. (21%). It was recrystallized from water as white needles, m.p. >260° dec. It gives a red color with ferric chloride.

Anal. Calcd. for $C_4H_5N_3O_2 \cdot H_2O$: C, 33.10; H, 4.86; N, 28.96. Found: C, 33.12; H, 4.83; N, 28.70.

A picrate was prepared and recrystallized from absolute ethanol, m.p. 250–251° dec. (sealed evacuated capillary).

Anal. Calcd. for $C_{10}H_8N_6O_9$: C, 33.72; H, 2.26; N, 23.59. Found: C, 33.73; H, 2.46; N, 24.24.

Hydrogenation of Cytosine 3-N-Oxide.—Cytosine 3-N-oxide (50 mg., 0.00039 mole) was dissolved in 5 ml. of water in a 50-ml. erlenmeyer flask with a side arm. In the side arm was placed 25 mg. of 10% Pd–C and 2 ml. of water. The flask was connected to the hydrogenation apparatus and swept briefly with hydrogen. Then the catalyst and N-oxide were mixed and stirred at room temperature with hydrogen at atmospheric pressure for 6.5 hr. At this point 1 mole equiv. of hydrogen had been absorbed. The catalyst was removed and the filtrate was evaporated to a small volume and allowed to crystallize.

The crystalline cytosine weighed 37 mg. (75%) and was identified by comparison of R_f and ultraviolet spectrum with an authentic sample.

Reaction of Cytosine 3-N-Oxide with Acetic Anhydride.—In a 25-ml. ground-glass erlenmeyer flask was placed 200 mg. (0.0015 mole) of cytosine 3-N-oxide, 8 ml. of glacial acetic acid, and 4 ml. of acetic anhydride. The reaction mixture was stirred at room temperature for 20 hr. During this period, a white solid precipitated. The mixture was chilled and the product was filtered. The yield was 130 mg. Evaporation of the mother liquors gave further product. Both fractions were combined and recrystallization from water gave 170 mg. (67%) of white, matted needles, m.p. 259–263° dec. The product (III) does not give an immediate ferric chloride reaction, but slowly turns blue.

Anal. Calcd. for $C_6H_7N_3O_3$: C, 42.61; H, 4.17; N, 24.84. Found: C, 42.76; H, 4.25; N, 24.58.

4-Methylthiouracil (V).[25]—To a solution of 3.2 g. (0.025 mole) of 4-thiouracil in 40 ml. of water containing 1.0 g. (0.025 mole) of NaOH was added 7.5 g. (0.05 mole) of CH_3I. The reaction mixture was stirred for 1 hr. and the resulting yellow solid was filtered, m.p. 207–209° dec. (lit.[26] m.p. 205°); the yield was 2.2 g. (63%).

N^4-Hydroxycytosine (IV).—A mixture of 4-methylthiouracil[26] (100 mg., 0.0007 mole), hydroxylamine hydrochloride (100 mg., 0.0014 mole), and 10 ml. of anhydrous ethanol was heated under reflux for 4.5 hr. Upon cooling to 0° overnight, a tan solid precipitated. This was filtered to give 30 mg. of the hyrochloride, m.p. 209–211° dec. (lit.[11] m.p. 215–220° dec.). Paper chromatographically IV is homogeneous. It gives an immediate deep blue color with ferric chloride.

N^4-Acetoxycytosine (III).—A mixture of 10 mg. of N^4-hydroxycytosine, 0.2 ml. of glacial acetic acid, and 0.1 ml. of acetic anhydride was heated at 50° for 4 hr. Upon cooling to room temperature, white crystals were deposited. The yield was quantitative; m.p. 250–255° dec., undepressed when mixed with III from acetylation of cytosine 3-N-oxide. The product gives a deep blue color with ferric chloride on prolonged standing. Paper chromatographic data and ultraviolet spectra are given in Table I.

Stability of Cytosine 3-N-Oxide. A. To Alkali.—Cytosine 3-N-oxide was dissolved in 1 *N* NaOH and heated on a steam bath for 18 hr. Cytosine 3-N-oxide was recovered unchanged as shown by R_f values and ultraviolet spectra.

B. To Acid.—Cytosine 3-N-oxide was dissolved in 1 *N* HCl and heated on a steam bath for 24 hr. Paper chromatography of the reaction mixture gave an R_f slightly ahead of the control spot. Therefore, the reaction mixture was evaporated to dryness, dissolved in a minimal amount of water, and streaked on a large sheet of Whatman No. 3 paper. After chromatography with solvent C, the band of R_f 0.32 was eluted with water. This was then evaporated to dryness and the residue was recrystallized from methanol–ethyl acetate. The product was shown to be identical with starting material in its ultraviolet and paper chromatographic behavior. It gives an orange ferric chloride test. However, it contains halogen and the analysis corresponds to a hydrochloride of the N-oxide.

Anal. Calcd. for $C_4H_6N_3O_2 \cdot HCl$: C, 29.19; H, 4.29; N, 25.53. Found: C, 29.63; H, 4.51; N, 25.99.

Cytidine 3-N-Oxide (IIb).—To a solution of 2 g. (0.008 mole) of cytidine in 30 ml. of glacial acetic acid was added 4 g. (0.02 mole) of *m*-chloroperbenzoic acid. The mixture was then heated at 60° for 1 hr. and poured slowly into 400 ml. of water with stirring. The precipitated organic acids were filtered and the filtrate was evaporated to a viscous residue. The residue was dissolved in 90% CH_3OH and then treated with ethyl acetate to precipitate the product: yield 0.9 g. (41%). It gives a dark red-orange color with $FeCl_3$. The solid was boiled in ethanol and the insoluble product was filtered and recrystallized from methanol and ethyl acetate: m.p. >220° dec. It still retained a small amount of water, which could not be removed by heating because of darkening and slow decomposition. The water was determined by a Karl Fisher titration.

Anal. Calcd. for $C_9H_{13}N_3O_6 \cdot 0.25H_2O$: C, 40.95; H, 5.16; N, 15.93; H_2O, 1.7. Found: C, 40.61; H, 5.51; N, 15.89; H_2O, 1.6.

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