interactions clearly correspond to hydrogen bonds N(1)–H(1)···O(2) and N(3)–H(3)···O(2), the distances N(1)–O(2) and N(3)–O(2) being 2.884 and 2.855 Å, respectively. The bonds are nearly linear and coplanar with the planar part of the molecule (the angles at H(1) and H(3) being 174.4 and 172.6°) and hold the molecules together in approximately planar infinite ribbons running in the z direction (Figures 2 and 3). In the x direction there is π-electron contact between successive ribbons, which are about 3.3 Å apart, and in the y direction between the methyl groups (H–H distances of 2.49 and 2.51 Å). The strong bonding in only one direction explains the cleavage properties of the crystal and also the vibrational anisotropy of the atoms. The O(4) is not taking part in hydrogen bonds, its shortest separation from a hydrogen atom in a neighboring molecule (H(5)) being 2.60 Å. It is noteworthy that both D and L molecules can be accommodated in the same site without coming too close to any atom in either D or L molecules in neighboring sites. However, the occupancies differ significantly from 0.50, being close to 0.60 and 0.40, and the packing of D and L molecules is therefore not random. It is more favorable for a molecule to obey the symmetry operations of the crystal than not, although the energy differences must be small (the Boltzmann equation gives an energy difference of 0.2 kcal/mol for 3:2 occupancies at room temperature).

The crystals of dihydrothymine represent an unusual and complicated form of racemate, in which both enantiomorphs occupy all sites in a centrosymmetrical space group. It can probably only occur when the difference in space requirement between the enantiomorphs is small and the packing mainly is governed by the common, "nonenantiomorph," part of the molecule.

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Reaction of Ninhydrin with Cytosine Derivatives

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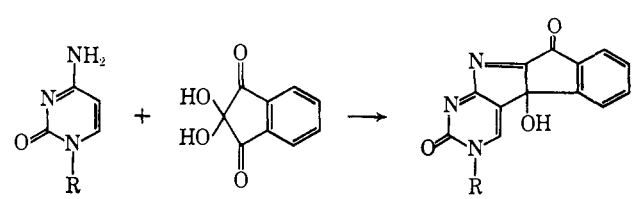
Contribution from the Department of Chemistry, New York University, New York, New York 10003. *Received August 18, 1967*

Abstract: Ninhydrin (1,2,3-indantrione monohydrate) reacted with cytosine, cytidine, and cytidine nucleotides to form products (**3a–d**) in which the amino nitrogen and 5-carbon of cytosine had formed bonds with two adjacent carbons of the five-membered ring of ninhydrin. The use of this reaction is suggested for the modification of cytosine residues of nucleic acids. Bovine pancreatic ribonuclease cleaved the reaction product of ninhydrin and cytidine 2′,3′-cyclic phosphate (**3c**) to the corresponding open 2′(3′)-phosphate **3d**. The structure of **3a**, the adduct of ninhydrin and cytosine, was deduced from its spectroscopic properties and from its conversion to a number of degradation products. Compound **3a** was ultimately converted to 5-(3-phthalamidyl)uracil (**12a**). The properties of the transformation products of **3a** and the mechanisms of the reactions are discussed.

There appears to be a considerable need for organic reagents for the modification of nucleic acids, especially those with specificity for a particular heterocyclic component.[1] Such reagents are of value as mutagens,[2] as markers for electron microscope studies,[3] as aids to the determination of the structure[4] and biological function[5] of nucleic acids, and for the purpose of increasing the specificity of enzymatic cleavage of nucleic acids.[6]

The color reaction of ninhydrin (1,2,3-indantrione monohydrate) with amino acids and peptides has been known for more than half a century,[7] and it has been termed "one of the most valuable of all biochemical reagents."[8] Virtually nothing is known, however, of its reactions with aromatic and heterocyclic amines. We recently reported a reaction between ninhydrin and guanine derivatives in which a five-membered ring was formed by addition of the 1 and N-2 positions of guanine across two adjacent carbonyl groups of ninhydrin.[9] This reaction was also observed with glyoxal and guanine derivatives. The adduct of ninhydrin and guanine was labile, and decomposed into its components under mildly alkaline conditions. We wish to report now that ninhydrin (**2**), unlike glyoxal, also reacts with cytosine derivatives (**1**), to form adducts of structure **3**. Adenine and uracil derivatives do not



1a, R = H **2** **3**
b, R = β-D-ribofuranosyl
c, R = β-D-ribofuranosyl 2′,3′-cyclic phosphate
d, R = β-D-ribofuranosyl 2′(3′)-phosphate

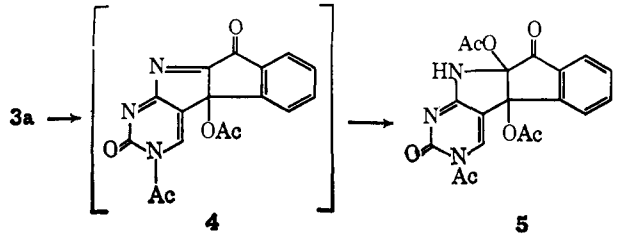
(1) P. Cerutti, K. Ikeda, and B. Witkop, *J. Am. Chem. Soc.*, **87**, 2505 (1965).
(2) R. M. Herriot, *Cancer Res.* **26**, Part 1, 1971 (1966).
(3) E. N. Moudrianakis and M. Beer, *Biochim. Biophys. Acta*, **95**, 23 (1965).
(4) K. Burton in "Essays in Biochemistry," Vol. 2, P. N. Campbell and G. D. Greville, Ed., Academic Press Inc., New York, N. Y., 1965, p 57.
(5) J. H. Weil, N. Befort, B. Rether, and J. P. Ebel, *Biochem. Biophys. Res. Commun.*, **15**, 447 (1964).
(6) P. T. Gilham, *J. Am. Chem. Soc.*, **84**, 687 (1962).
(7) S. Ruhemann, *J. Chem. Soc.*, **97**, 2025 (1910).
(8) R. West, *J. Chem. Educ.*, **42**, 386 (1965).
(9) R. Shapiro and J. Hachmann, *Biochemistry*, **5**, 2799 (1966).

react with ninhydrin. The cytosine reaction involves the formation of a new carbon–carbon bond, and is not reversed by alkaline treatment. Thus, reaction with ninhydrin followed by exposure to mild alkali (pH 9) should prove a method for the specific modification of the cytosine residues of nucleic acids.

The reaction proceeds readily at neutral pH, without heat. Thus, cytidine 2′,3′-cyclic phosphate (**1c**) was converted completely to **3c** in 18 hr at room temperature. Under the same conditions, cytidine (**1b**) and cytidine 2′(3′)-phosphate (**1d**) were about half converted to **3b** and **3d**. In preparative runs, which were routinely conducted at higher temperatures (65–90°), cytidine and cytosine were seen to react completely. A 4- to 20-fold excess molar amount of ninhydrin was used. Reaction with ninhydrin should not, however, serve as a method for converting cytosine residues in a nucleic acid to a form resistant to bovine pancreatic ribonuclease, as that enzyme converted the modified 2′,3′-cyclic phosphate **3c** to the corresponding open phosphate **3d**.

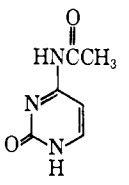
The assignment of structure **3** to the products is based upon a study of the chemical and physical properties of the cytosine derivative **3a**, and of the degradation products described below. The structures of **3b–d** are based upon analogy and upon a comparison of ultraviolet spectra (and infrared spectrum for **3b**) with **3a**. The analysis and molecular weight (mass spectrum) of **3a** are consistent with the indicated structure. The nmr spectrum of **3a** contains peaks assigned to the four benzenoid protons, and a singlet for H-6 of cytosine, but no peak for H-5 of cytosine. The ultraviolet spectra (at various pH values) of **3a** show absorption at longer wavelength than is present in cytosine and the existence of two maxima above 250 mμ. They do not represent a simple addition of cytosine and benzenoid absorption. This suggests that the cytosine chromophore has been altered. The involvement of the amino group is suggested by its absence from the nmr spectrum in hexadeuteriodimethyl sulfoxide. This is not definitive, as it could be due to broadening of the peak. Compound **3a** is inert to nitrous acid, which also indicates the absence of the amino group. The fact that various 1-substituted cytosines undergo reaction with ninhydrin implies that the 1 position of cytosine is not involved. A degradation product of **3a** described below (**8**) decarboxylates readily under a variety of conditions. The products still contain the benzene and cytosine rings, and have a new proton bound to a nonaromatic carbon atom. This indicates that the central carbonyl group of the ninhydrin moiety was lost and that the 5 position of cytosine was therefore attached to one of the carbons of ninhydrin adjacent to the benzene ring.

Acetylation of **3a** by boiling acetic anhydride, followed by an aqueous work-up, afforded a triacetyl derivative to which structure **5** has been assigned.



4 **5**

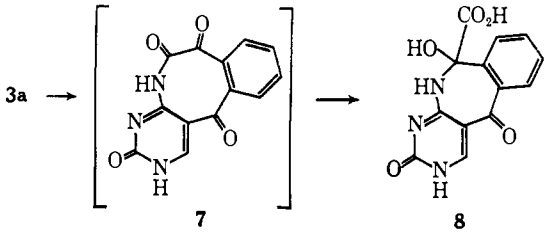
The nmr spectrum of **5** contains peaks for H-6 of the cytosine ring, four benzenoid protons, and three methyl groups (τ 7.40, 7.84, and 7.87). Triacetate **5** was very readily hydrolyzed back to **3a** by dilute hydrochloric acid or sodium bicarbonate. These are conditions under which the acetyl group of acetylcytosine (**6**) was found to be stable. Acetylcytosine was also inert to nitrous acid, while triacetate **5** was partly converted



6

to a new product by this reagent. These facts indicated that the amino group of cytosine in **5** was not acetylated. Compound **5** showed no dissociation between pH 1 and 8, which suggested acylation of a ring nitrogen of the cytosine. This was supported by the ultraviolet spectra of **5**, which differed from those of cytosine and **3a**. The assignment of the acetyl group to the 1 position of the cytosine ring, rather then the 3 position, is arbitrary. One possible route for the formation of **5** from **3a** is *via* the diacetate **4**, which could add acetic acid during the work-up to give **5**.

Sodium metaperiodate cleaved **3a** to give a carboxylic acid to which we have assigned structure **8**. The product was insoluble in water but dissolved in ammonium bicarbonate solution, pH 7.5. It moved as an anion



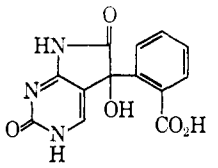
7 **8**

upon paper electrophoresis in phosphate buffer, pH 6.9. Its infrared spectrum exhibited broad absorption from 2.75 to 4.4 μ and a carbonyl band at 5.72 μ. The mass spectrum did not reveal the expected molecular ion at 287, but had its peak of greatest mass at 243, suggesting loss of CO_2. The nmr spectrum of **8** (CF_3CO_2H) showed one exchangeable proton close to the position expected for amino protons of cytosine.[10] The ultraviolet spectra of **8** (at various pH values) differed from those of cytosine and the ninhydrin–cytosine adduct **3a**. This was particularly true for the spectrum of the dianion of **8** (pH 11) which showed a bathochromic shift, with a maximum at 313 mμ. Spectrophotometric titration of **8** revealed pK_a values of approximately 3 and 9.5. Carboxylic acid **8**, unlike **3a**, was attacked by nitrous acid to yield a mixture of products which were not identified. These properties are all consistent with the structure assigned to **8**. This compound could be formed by cleavage by periodate between the hydroxyl and C=N bonds of **3a**. The presumed intermediate would be the eight-membered lactam **7**, which by hydrolysis followed by ring–chain tautomerism could give **8**. The bathochromic shift

(10) O. Jardetsky, P. Pappas, and N. G. Wade, *J. Am. Chem. Soc.*, 85, 1957 (1963).

in the ultraviolet at pH 11 is explained by increased conjugation of N-1 of the cytosine ring with the carbonyl group after ionization of the proton attached to it. This also explains the increased acidity of this proton compared to that in cytosine (pK_a = 12.2).[11]

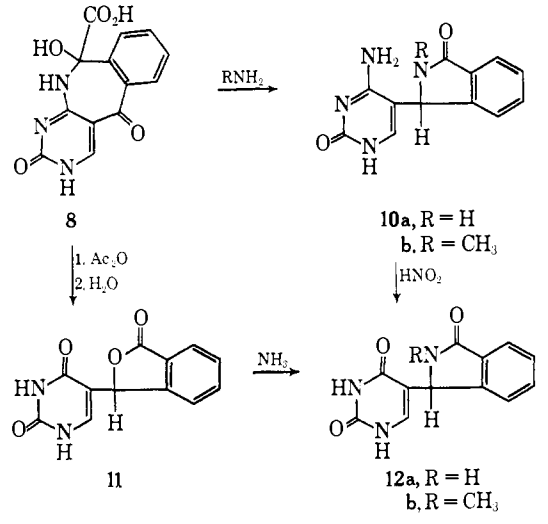
An alternative structure, **9**, was considered for the product of periodate cleavage of **3a**. This would result from cleavage between the ketone and C=N



9

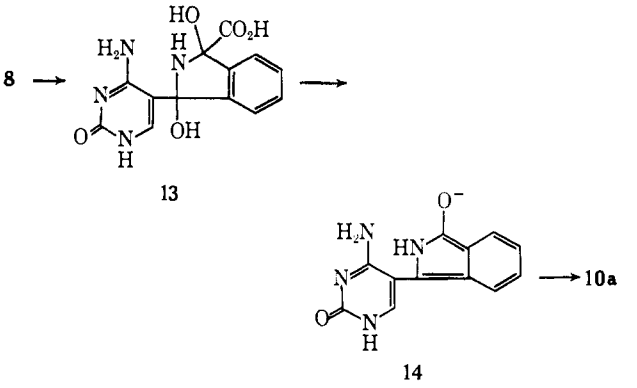
bonds of **3a**, followed by the tautomeric shift of a proton. Acetylcytosine (**6**) was examined as a model for structure **9**. It had rather different ultraviolet spectra than the periodate cleavage product, however, and exhibited a shift to shorter wavelengths at pH 11. The protons bound to nitrogen in acetylcytosine did not appear in the nmr (CF_3CO_2H) at room temperature, while at $-10°$ a singlet appeared at τ -1.28, considerably downfield from the chemical shift of the exchangeable proton of **8**. Furthermore, acetylcytosine resisted attack by nitrous acid. Structure **9** was ruled out on these grounds.

When carboxylic acid **8** was treated with aqueous ammonia at 5°, the carboxyl group was lost, and 5-(3-phthalimidyl)cytosine (**10a**) was produced. The anal-



8 — **10a**, R = H; **b**, R = CH_3 — **11** — **12a**, R = H; **b**, R = CH_3

ysis, molecular weight (mass spectrum), and spectroscopic properties of **10a** were all consistent with this structure. The nmr (CF_3CO_2H) contained a broad exchangeable peak assigned to the amino group and a new singlet associated with the proton bound to the central carbon atom of **10a**. The ultraviolet spectra of **10a** showed benzenoid absorption below 250 mμ and the characteristic absorption of a cytosine derivative above 250 mμ. A possible mechanism for the formation of **10a** involves condensation of ammonia with the masked 1,4-dicarbonyl system of **8** to give intermediate **13**, decarboxylation of this with loss of water to give **14**, and protonation of **14** to form **10a**.

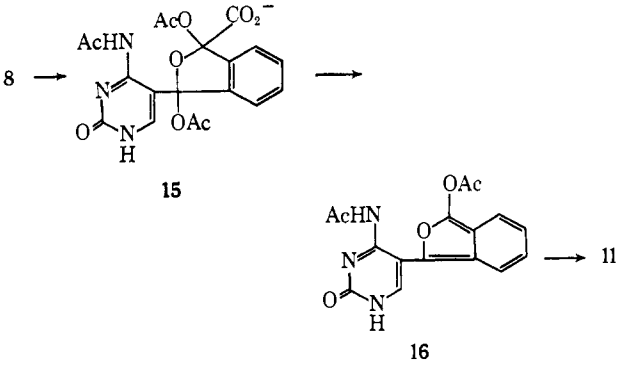


13 — **14** — **10a**

Nitrous acid smoothly deaminated **10a** to form 5-(3-phthalimidyl)uracil (**12a**). This structure was supported by the analysis and nmr and ultraviolet spectra of the product. This included the characteristic shift to higher wavelength in the ultraviolet in alkali shown by uracil derivatives with an unsubstituted 1 position.[12]

An analogous series of reactions was conducted with the carboxylic acid **8** and methylamine. The product of reaction was identified as 5-(2-methyl-3-phthalimidyl)cytosine (**10b**), and this was deaminated by nitrous acid to form 5-(2-methyl-3-phthalimidyl)uracil (**12b**). This eliminated the mechanistic possibility that the amino group of **10a** was derived from ammonia.

When **8** was heated in refluxing acetic anhydride, then subjected to an aqueous work-up, 5-(3-phthalidyl)-uracil, **11**, was isolated in low yield. The analysis, molecular weight (mass spectrum), and ultraviolet and nmr spectra all supported structure **11**. The infrared spectrum showed a band at 5.64 μ, which we consider to be the stretching frequency of the lactone carbonyl group. Finally, **11** was converted by ammonia to **12a**. A number of mechanisms can be written to rationalize the formation of **11** from carboxylic acid **8**. One possibility involves the formation of **15** from **8** and decarboxylation of this with loss of acetate to give intermediate **16**. The conversion of the isobenzofuran group to a substituted phthalide moiety and the loss of the acetamido group of the pyrimidine ring could occur during the work-up. It has been reported that acylcytosines are readily deaminated to uracil derivatives in aqueous acetic acid.[13]



8 — **15** — **16** — **11**

Experimental Section

Ultraviolet spectra were taken using a Perkin-Elmer 202 spectrophotometer. The spectrum was taken in aqueous solution,

(11) G. H. Beaven, E. R. Holiday, and E. A. Johnson in "The Nucleic Acids," Vol. 1, E. Chargaff and J. N. Davidson, Ed., Academic Press Inc., New York, N. Y., 1955, p 493.

(12) I. Wempen and J. J. Fox, *J. Am. Chem. Soc.*, 86, 2474 (1964).

(13) D. M. Brown, A. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2384 (1956).

except where otherwise noted. Maxima above 220 mμ are reported. Infrared spectra were obtained in KBr with a Perkin-Elmer Infracord spectrophotometer. Only prominent bands in the area 2.8–7.0 μ are listed. Nuclear magnetic resonance spectra were determined using a Varian A-60 instrument and are reported on the τ scale with tetramethylsilane (τ 10.00) as standard. Mass spectra were obtained from the Morgan Schaffer Corp., Montreal, Quebec. They used a Hitachi Perkin-Elmer RMU-6D instrument. The sample was introduced through a direct inlet. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Microanalyses were performed by Mr. George I. Robertson, Florham Park, N. J. Thin layer chromatography was carried out on 1-mm-thick layers (2 mm for preparative purposes) of Avicel microcrystalline cellulose (American Viscose Co., Marcus Hook, Pa.). After development of the chromatograms, ultraviolet-absorbing materials were located with the aid of an ultraviolet lamp equipped with a short-wavelength filter. The solvent systems employed were: solvent 1, 1-butanol–water (86:14); solvent 2, 2-propanol–water (70:30); solvent 3, 2-propanol–ammonia–water (7:1:2); solvent 4, 95% ethanol–1 *M* sodium acetate (7:3). Cytosine was purchased from the Mann Research Laboratories, Inc., New York, N. Y. Cytidine and cytidine nucleotides (as the sodium salts) were obtained from Schwartz BioResearch, Inc., Orangeburg, N. Y. These substances were found to be chromatographically homogeneous in several solvent systems.

Reaction of Ninhydrin with Cytosine. Formation of 3a. Ninhydrin (16.0 g) and cytosine (2.5 g) were combined in 60 ml of water, and the reaction mixture was heated at 90° for 48 hr, with magnetic stirring. The crystals of **3a** that separated out on cooling were collected by filtration and washed with water. The filtrate was evaporated to dryness and the residue treated with acetone. The acetone-insoluble material was separated by filtration, and washed again with acetone to give a second crop of product. The combined crops of **3a** weighed 2.9 g (43%). The compound was recrystallized from water to give colorless needles which showed no melting point below 300°; infrared absorption at 2.79, 2.90–4.00 (broad, with maxima at 3.12 and 3.60), 5.79, 5.98 (shoulder), 6.06, 6.31, and 6.87 μ; ultraviolet maxima (pH 1) 226 mμ (ε 16,700), 258 mμ (ε 11,800), and 293 mμ (ε 9300); (pH 7) 257 mμ (shoulder) (ε 12,100) and 287 mμ (shoulder) (ε 7700); (pH 12) 240 and 295 mμ; molecular weight (mass spectrum) 253; thin layer chromatography, R_f 0.44 in solvent 1; R_f 0.46 in 2-propanol–water (82:18), R_f 0.72 in solvent 2. The nmr spectrum (CF_3CO_2H) showed a singlet (one proton) at τ 1.39 and a multiplet (four protons) at 1.69–2.05.

Anal. Calcd for $C_{13}H_7N_3O_3 \cdot H_2O$: C, 57.57; H, 3.34; N, 15.49. Found: C, 57.27; H, 3.55; N, 14.75.

Reaction of Ninhydrin with Cytidine. Formation of 3b. Ninhydrin (3.5 g) and cytidine (0.24 g) were heated together in 60 ml of H_2O with stirring at 65° for 18 hr. The solution was cooled and evaporated to dryness under vacuum. The residue was extracted with acetone, and the undissolved solids were purified by dissolving in acetone and precipitating with a mixture of acetone and ether. The yield of crude product was 0.23 g (53%). This was further purified of small amounts of ninhydrin by preparative thin layer chromatography in solvent 1 (the ninhydrin ran near the solvent front) and precipitation from acetone as described above. The white solid (**3b**) melted with decomposition from 192 to 195°; infrared absorption at 2.75–3.90 (broad), 5.80, 6.01, 6.37, and 6.86 μ; ultraviolet maxima (pH 1) 233 (shoulder), 263, and 298 mμ; (pH 7) 256 (shoulder) and 292 mμ; thin layer chromatography, R_f 0.52 in solvent 1.

Anal. Calcd for $C_{18}H_{15}N_3O_7 \cdot 2H_2O$: C, 51.31; H, 4.55; N, 9.97. Found: C, 51.51; H, 4.52; N, 9.97.

Reactions of Ninhydrin with Cytidine 2′,3′-Cyclic Phosphate and Cytidine 2′(3′)-Phosphate. Formation of 3c and 3d. Enzymatic Cleavage of 3c. To each of the above nucleotides (10 mg), dissolved in 3 ml of H_2O, was added ninhydrin (100 mg), and each reaction was allowed to stand at room temperature for 18 hr. The reactions were worked up by preparative thin layer chromatography in solvent 1. Three runs were necessary to separate the spots effectively. The reaction with the cyclic phosphate had gone to completion, as no cytidine 2′,3′-cyclic phosphate (R_f 0.11) was seen. The product (**3c**) had R_f 0.21 and was homogeneous upon rechromatography in solvents 3 (R_f 0.68) and 4 (R_f 0.80); ultraviolet maxima (pH 1) 235, 262, and 293 mμ; (pH 5) 254 (shoulder) and 298 mμ. The reaction with the 2′(3′)-phosphate had proceeded only partially, and the product (R_f 0.12) was separated from the starting material (R_f 0.05) as well as excess ninhydrin by preparative thin layer chromatography. The product (**3d**) was homogeneous upon rechromatography in solvents 3 (R_f 0.30) and 4 (R_f 0.75); ultraviolet maxima (pH 1) 233 (shoulder), 264, and 293 mμ; (pH 5) 255 (shoulder) and 288 mμ.

A portion of the solution containing **3c** was adjusted to pH 7.4 with ammonium bicarbonate, treated with 0.5 mg of bovine pancreatic ribonuclease A (CalBiochem, Los Angeles, Calif.), and allowed to stand at room temperature overnight. A control solution containing cytidine 2′,3′-cyclic phosphate (**1c**) was treated in the same manner. Thin layer chromatography in solvents 1, 3, and 4 indicated that both cyclic phosphates **1c** and **3c** had been converted to the corresponding open phosphates **1d** and **3d**.

Acetylation of the Ninhydrin–Cytosine Adduct 3a. Formation of 5. To 600 mg of **3a** were added 30 ml of acetic anhydride, and the reaction mixture was heated at reflux for 2 hr. It was cooled to room temperature, and water was added to decompose the acetic anhydride. The resulting solution was evaporated to dryness under vacuum, and then allowed to stand in a vacuum desiccator containing KOH, to remove traces of acetic acid. The solid residue was warmed with absolute ethanol and filtered, yielding 457 mg of insoluble brownish crystals. These were further purified by recrystallization (with charcoal) from an ethanol–water mixture. A yield of 350 mg (38%) of triacetate **5** was obtained, as colorless needles, mp 158–160°; infrared absorption at 2.90–3.90 (broad), 5.71, 6.02, 6.35, and 6.85 μ; ultraviolet maxima (pH 1 and 7) 226, 236 (shoulder), and 303 mμ; (pH 11) 240 and 298 mμ; molecular weight (mass spectrum) 397; thin layer chromatography, R_f 0.92 (fluorescent spot) in solvent 3. The nmr spectrum (CD_3SOCD_3) showed a singlet at τ 1.36 (one proton), a multiplet at 1.89–2.28 (four protons), a singlet at 7.40 (three protons), and a doublet at 7.84 and 7.87 (six protons).

Anal. Calcd for $C_{19}H_{15}N_3O_7 \cdot H_2O$: C, 54.94; H, 4.13; N, 10.12. Found: C, 55.10; H, 4.40; N, 9.77.

Triacetate **5** was hydrolyzed completely to **3a** on standing in dilute HCl for 2 hr at room temperature. The crystals that separated were collected by filtration. Their infrared and ultraviolet spectra and R_f in solvent 1 coincided with those of **3a**. It was possible to establish, by thin layer chromatography, that the same hydrolysis took place in 3 hr at room temperature in dilute sodium bicarbonate solution. Treatment with sodium nitrite in acetic acid–sodium acetate buffer, pH 4, for 4 hr, converted **5** partly to **3a**, and partly to a new substance, which appeared as a fluorescent spot (R_f 0.03) in solvent 1. Triacetate **5** was unaffected by treatment with sodium metaperiodate solution.

Reaction of 3a with Sodium Metaperiodate. Formation of Carboxylic Acid 8. To a solution of 500 mg of sodium metaperiodate in 15 ml of water was added 220 mg of ninhydrin–cytosine adduct **3a**, and the reaction mixture was stirred at room temperature. The initial suspension dissolved to form a clear solution, and a new precipitate appeared. After 24 hr of reaction, this was collected by filtration and washed repeatedly with water until the washings gave a negative test with starch–iodide paper. A yield of 200 mg (83%) of carboxylic acid **8**, as a white solid, was obtained. This was purified by dissolving it in a cold dilute solution of ammonium bicarbonate and precipitating it with cold dilute HCl. Carboxylic acid **8** did not melt below 300°, but sintered and became dark brown at 218–220°; infrared absorption at 2.75–4.40 (broad, with maxima at 3.02 and 3.36), 5.71, 6.00, 6.19, 6.35 (shoulder), 6.48, and 6.79 μ; ultraviolet maxima (pH 1) 251 and 286 mμ; (pH 5) 228 and 288 mμ; (pH 11) 260 and 313 mμ; pK_a (spectrophotometric titration) at approximately 3 and 9.5; thin layer chromatography, R_f 0.16 in solvent 1. The mass spectrum did not show the expected molecular ion at *m/e* 287, but rather one at 243. The nmr spectrum (CF_3CO_2H) showed a broad peak at τ 0.82 (one proton), a singlet at 1.45 (one proton), and a multiplet from 1.5 to 2.35 (four protons). On running the spectrum in CF_3CO_2D, the peak at τ 0.82 was not observed.

Anal. Calcd for $C_{13}H_9N_3O_5 \cdot H_2O$: C, 51.15; H, 3.63; N, 13.77. Found: C, 51.06; H, 3.90; N, 13.41.

Carboxylic acid **8** (50 mg) was allowed to react with 100 mg of sodium nitrite in 5 ml of 1 *N* acetic acid–sodium acetate buffer, with stirring, for 15 hr. The solution was neutralized with ammonium bicarbonate and evaporated to dryness under vacuum. The solid was extracted with absolute ethanol and the extract concentrated and worked up by preparative thin layer chromatography in solvent 1. A number of bands were seen, including a dark one of R_f 0.48. This was eluted with water and evaporated to give 2 mg of a bright yellow solid; infrared absorption at 2.95, 3.10–3.70 (broad), 4.58, 6.07, 6.20, 6.42, and 6.88 μ; ultraviolet maxima (pH 1 and 7) at 258 and 268 mμ. The ultraviolet spectrum

of this material was unchanged upon standing at pH 1 for 48 hr and being heated at 80° at that pH for 1 hr.

Reaction of 8 with Ammonia. Formation of 5-(3-Phthalimidyl)-cytosine (10a). Carboxylic acid **8** (75 mg) was dissolved in 10 ml of 1 *N* aqueous ammonia and allowed to stand for 72 hr at 5°. White crystals of **10a** had separated out and were collected by filtration. The yield was 32 mg (54%). The material was recrystallized by dissolving in cold dilute HCl and neutralizing with cold dilute ammonia. The substance did not exhibit a melting point below 300°; infrared absorption at 2.91, 3.05–4.00 (broad, with a maximum at 3.14), 6.00, 6.55, 6.80, and 6.90 (shoulder) μ; ultraviolet maxima (pH 1) 285 mμ; (pH 5) 273 mμ; (pH 11) 281 mμ; molecular weight (mass spectrum) 242; thin layer chromatography, R_f 0.46 in solvent 1; R_f 0.67 in solvent 3; R_f 0.73 in saturated aqueous ammonium bicarbonate. The nmr spectrum (CF_3CO_2H) showed a broad doublet at τ 1.70 (two protons), a singlet at 2.44 superimposed upon a multiplet from 2.15 to 2.88 (five protons), a broad singlet at 3.14 (one proton), and a singlet at 4.40 (one proton). When the spectrum was run in CF_3CO_2D, the peaks at τ 1.70 and 3.14 were not observed.

Anal. Calcd for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13. Found: C, 58.90; H, 4.42; N, 22.87.

Deamination of 5-(3-Phthalimidyl)cytosine (10a) to 5-(3-Phthalimidyl)uracil (12a) by Nitrous Acid. Compound **10a** (180 mg) was added to 3 ml of 80% aqueous acetic acid, and 250 mg of sodium nitrite was introduced. The solution was allowed to stand at room temperature for 16 hr and neutralized by addition of ammonium bicarbonate. It was worked up by preparative thin layer chromatography in solvent 1. Only one new substance, R_f 0.56, was observed. The aqueous eluate from the cellulose, on concentration, yielded **12a** as a white solid, weighing 90 mg (50%). Recrystallization from water gave **12a** as white needles, melting at 202–203°; infrared absorption at 2.80–3.60 (broad, with maxima at 3.10 and 3.25), 5.68 (shoulder), 5.80 (shoulder), 5.95, 6.20 (shoulder), and 6.78 μ; ultraviolet maxima (pH 1) 263 mμ; (pH 11) 287 mμ. The nmr (CD_3SOCD_3) showed a multiplet from τ 2.09 to 2.58 (four protons), a singlet at 2.65 (one proton), and a singlet at 4.40 (one proton). No exchangeable protons were observed; thin layer chromatography, R_f 0.77 in solvent 1; R_f 0.67 in 1-butanol–30% aqueous acetic acid (2:1).

Anal. Calcd for $C_{12}H_9N_3O_3$: C, 59.26; H, 3.73; N, 17.28. Found: C, 59.10; H, 4.01; N, 17.13.

Reaction of 8 with Methylamine. Formation of 5-(2-Methyl-3-phthalimidyl)cytosine (10b) and Its Deamination to 12b by Nitrous Acid. A solution of 250 mg of carboxylic acid **8** in 10 ml of 40% aqueous methylamine was allowed to stand at 5° for 16 hr. The solution was concentrated under vacuum to a small volume and a white crystalline material separated out. This was separated by filtration and washed with water to yield 185 mg (88%) of **10b**, which showed no melting point below 300°. A sample was purified for analysis by recrystallization from absolute ethanol; infrared absorption at 2.90, 3.00–4.30 (broad, with a maximum at 3.20), 5.91, 6.09, 6.55, and 6.80 μ; ultraviolet maxima (pH 1) 285 mμ; (pH 5) 275 mμ (shoulder); (pH 11) 281 mμ; thin layer chromatography, R_f 0.63 in solvent 1. The nmr spectrum (CD_3SOCD_3) showed a multiplet from τ 2.20 to 2.55 (five protons), a singlet at 4.26 (one proton), and a singlet at 7.03 (three protons). No exchangeable protons were observed.

Anal. Calcd for $C_{18}H_{12}N_4O_2$: C, 60.93; H, 4.72; N, 21.86. Found: C, 60.42; H, 4.65; N, 21.67.

A sample of **10b** (5 mg) was dissolved in 3 ml of 1 *N* acetic acid–sodium acetate buffer, pH 4, and 50 mg of sodium nitrite was added. The solution was allowed to stand at room temperature for 4 hr and worked up by preparative thin layer chromatography in solvent 1. Complete conversion to a new product, R_f 0.76, was observed. The material (**12b**) eluted from the cellulose showed ultraviolet maxima (pH 1 and pH 5) at 261 and (pH 11) 289 mμ.

Reaction of 8 with Acetic Anhydride. Formation of 5-(3-Phthalidyl)uracil (11). Carboxylic acid **8** (110 mg) was heated in 30 ml of acetic anhydride at reflux for 2 hr. The solution was cooled, water was added to it, and it was allowed to stand for 16 hr at room temperature. The solvents were removed under vacuum. The glassy residue was dissolved in absolute ethanol, treated with a small amount of charcoal, and filtered. Ether was added to the filtrate, and the brownish gummy impurities which precipitated were discarded. The solution, on concentration, deposited white crystals. These were purified by recrystallization from aqueous ethanol or an ethanol–methanol mixture. Long needles of **11** were obtained (105 mg, 12%) which melted with decomposition from 296 to 298°; infrared absorption at 2.80–3.70 (broad, with maxima at 3.10 and 3.38), 5.64, 5.79, 5.95, 6.36, 6.75, 6.85, and 6.94 μ; ultraviolet maxima (dilute aqueous ethanol) (pH 1 and 7) 235 and 265 mμ; (pH 11) 289 mμ; molecular weight (mass spectrum) 244; thin layer chromatography, R_f 0.74 in solvent 1. The nmr spectrum of **11** (CD_3SOCD_3) showed a broad singlet at τ −1.12 (two protons), a multiplet at 1.80–2.45 (five protons), and a singlet at 3.53 (one proton). On addition of D_2O, the peak at τ −1.12 disappeared.

Anal. Calcd for $C_{12}H_8N_2O_4$: C, 59.02; H, 3.30; N, 11.47. Found: C, 58.62, H, 3.36; N, 11.27.

A small amount of **11** was dissolved in dilute aqueous ammonia and allowed to stand at room temperature for 16 hr. The solution was evaporated to dryness under vacuum, and the solid remaining was washed with water. This corresponded in its infrared spectrum, ultraviolet spectra at pH 1 and 11, and thin layer chromatography in solvents 1 and 3 and in 1-butanol–30% aqueous acetic acid (2:1) to 5-(3-phthalamidyl)uracil (**12a**).

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