Preparation of 1-β-D-Ribofuranosyl-2-thiopyrimidines by the Mercuri Condensation*

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ABSTRACT: Condensation of the mercury salt of 2-thiouracil with 2′,3′,5′-tri-*O*-benzoylribofuranosyl chloride produces a mixture of products. Most covalent bond formation with the C-1′ position of the sugar is found at an exocyclic atom and the N-3 position of the pyrimidine moiety. The reactivity of the N-1 position of the 2-thiopyrimidine is enhanced, however, if the compound is acetylated prior to formation of the mercury salt. Thus, it is possible to prepare 2-thiouridine and 2-thiocytidine in good yield by acetylation followed by the mercuri condensation. A spectrophotometric titration of 2-thiouridine gave a pK_a value of 8.8. The ribofuranosyl-2-thiopyrimidines are identified by desulfurization to 1-β-D-ribofuranosyl-4-oxopyrimidine with a partially deactivated Raney nickel catalyst. The distinctive ultraviolet absorption spectra of 1-β-D-ribofuranosyl-4-oxopyrimidine and the N-3 glycosyl of 4-oxopyrimidine provide a confirmation of the structural assignments of the ribofuranosyl-2-thiopyrimidines.

The sulfur analogs of uridine and cytidine may afford a comparison of the nucleophilic reactivity of sulfur and oxygen atoms in enzymatic reactions. The enzymatic synthesis of 2-thiouridine (Strominger and Friedkin, 1954) and a chemical preparation have been reported (Shaw *et al.*, 1958). The treatment of a 2,5′-*O*-cyclouridine derivative with H_2S and triethylamine yields 2-thiouridine (Brown *et al.*, 1958; Chambers and Kurkov, 1963), and 2,4-dithiouridine with ammonia gives 2-thiocytidine (Ueda *et al.*, 1966).

The condensation of the mercury salt of a pyrimidine derivative with a blocked ribofuranosyl chloride should be a flexible and simple route to the ribofuranosyl-2-thiopyrimidines. In a preliminary experiment, the direct reaction of 2-thiouracil with mercuric chloride was performed by the procedure of Fox *et al.* (1956). The low yield of an N-1 reactive mercury salt indicates that the sites of highest electron density on the pyrimidine are favored in bond formation with the mercury atom; most of the mercury is bonded to the exocyclic sulfur or oxygen atom and to the N-3 position of 2-thiouracil. Thus, the subsequent condensation with a blocked ribofuranosyl chloride gives the 2-*S*-ribofuranosyl-2-thiouracil (or 4-*O*-ribofuranosyl-2-thiouracil) and the 3-*N*-ribofuranosyl-2-thiouracil derivatives as the predominant products.

From these considerations it seemed probable that the N-1 reactive mercury salt of 2-thiouracil would be required to yield 2-thiouridine from the condensation with a blocked ribofuranosyl chloride. The 1-acetyluracil, prepared with acetic anhydride by Spector and Keller (1958), was found to participate in the transfer of the acetyl group to a sulfhydryl or an amino group in aqueous solution. The synthesis of 1-acetyluracil suggested that 2-thiouracil could be acetylated at the N-1 position; the displacement of the acetyl group of acetyl-2-thiouracil by mercuric ion would provide an N-1 reactive mercury salt. An examination of the melting points, stoichiometry, and ultraviolet spectra show that the N-1 reactive di-2-thiouracilmercury is not identical with the product of the direct salt reaction.

Results

The direct reaction of equimolar quantities of 2-thiouracil and $HgCl_2$, in an aqueous solvent, gave the 2-thiouracil·HgCl salt in 80% yield. The assignment of a 1:1 stoichiometry to the 2-thiouracil·HgCl salt, prepared in this manner, is supported by the fact that the yield and the physical properties of the thiouracilmercury chloride are not altered when a 2:1 molar ratio of pyrimidine to $HgCl_2$ is used in the synthetic procedure. (Thiouracilmercury chloride melts at a temperature above 320°, ultraviolet spectrum λ_{max} (ethanol) 2720 Å.)

The acetyl group of acetyl-2-thiouracil and acetyl-4-acetamido-2-thiopyrimidine is rapidly removed at 25° in aqueous alkali. The reaction of $HgCl_2$ with the acetylated pyrimidines was performed, therefore, with anhydrous ethanol as the solvent. The apparent stoichiometry of the mercury salts, prepared from the acetylthiopyrimidines, is 2 equiv of pyrimidine to 1 of mercury. From acetyl-2-thiouracil (Ia) (Scheme I), an 83% yield of N-1 reactive di-2-thiouracilmercury was obtained: mp 282–286° dec, ultraviolet spectrum λ_{max} (ethanol) 2940 Å. When equimolar amounts of the pyrimidine and $HgCl_2$ were introduced into the reaction mixture, the yield and the physical properties of the product were unchanged. The $HgCl_2$ treatment of acetyl-4-acetamido-2-thiopyrimidine (Ib), with exclusion of moisture, gave a 70% yield of the N-1 reactive di(4-acetyl-2-thiocytosine)mercury.

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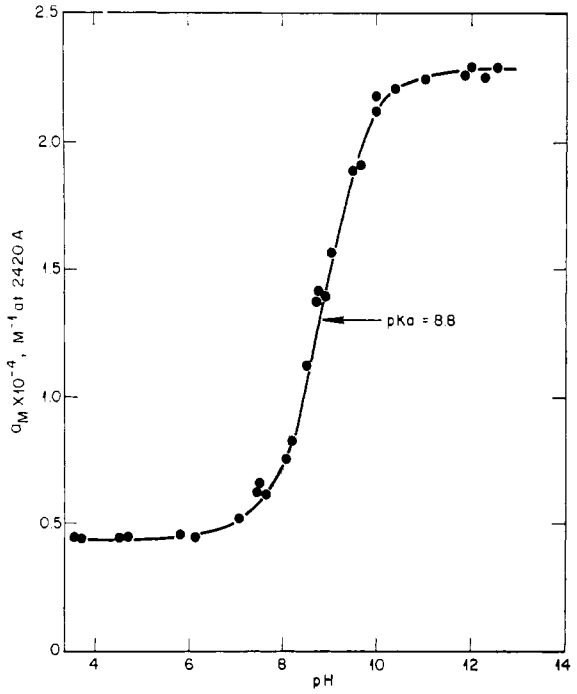


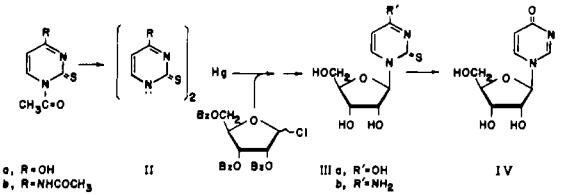
FIGURE 1: The effect of pH, at a constant ionic strength of 0.1, on the a_M of 2-thiouridine at 2420 Å. The solutions were prepared with tetraethylammonium chloride. The curve was calculated with eq 1 and a pK_a of 8.8.

Condensation of the N-1 reactive mercury salt of 2-thiouracil with 2′,3′,5′-tri-*O*-benzoyl-1-D-ribofuranosyl chloride and removal of the benzoyl groups led to 1-β-D-ribofuranosyl-2-thiouracil (IIIa). Similarly, the mercury salt of 4-acetyl-2-thiocytosine condensed with the ribofuranosyl chloride and subjected to alkaline hydrolysis provided 1-β-D-ribofuranosyl-2-thiocytosine (IIIb). The hydrogenation of 2-thiouridine (IIIa), catalyzed by acetone-deactivated Raney nickel in ethanol, gave 1-β-D-ribofuranosyl-4-oxopyrimidine (IV). The latter compound was also obtained from 2-thiocytidine (IIIb) by hydrogenation followed by mild alkaline hydrolysis of the ribofuranosyl-4-iminopyrimidine.

The pK_a of 2-thiouridine, at an ionic strength of 0.1, was determined from the molar absorbancy (a_M) at 2420 Å from pH 3.7 to 12.5 in tetraethylammonium chloride. The experimental points of Figure 1 were fitted to a curve calculated from eq 1.

$$a_M = \frac{a_A}{1 + K_a/(H^+)} + \frac{a_B}{1 + (H^+)/K_a} \qquad (1)$$

SCHEME I



In eq 1, a_A is the molar absorbancy of the neutral species and a_B is the molar absorbancy of the anionic species of 2-thiouridine; the derivation may be found in Wigler (1963). Equation 1 and a pK_a value of 8.8 gave the best fit to the experimental points (see Figure 1).

Discussion

The preparation of 1-β-D-ribofuranosyl-2-oxopyrimidines, from the condensation of a ribofuranosyl chloride with dithyminylmercury (Fox *et al.*, 1956) and mono-4-acetylcytosinemercury (Fox *et al.*, 1957), has proven to be a convenient procedure. If the oxygen atom at position 2 of the pyrimidine is replaced by hydrogen or sulfur, however, the N-1 glycosyls are not obtained in good yield from the mercuri procedure. Funakoshi *et al.* (1961) attempted to prepare 1-β-D-ribofuranosyl-4-oxopyrimidine from di-4-oxopyrimidinemercury and the benzoylated ribofuranosyl chloride, but the resultant product was found to be 3-β-D-ribofuranosyl-4-oxopyrimidine. The condensation with the 2-thiouracilmercury chloride, prepared from a direct salt reaction, gave almost a threefold yield of the N-3 glycosyl in comparison with the N-1 glycosyl. When di-2-methylthiouracilmercury was used in the condensation, the N-3 glycosyl was the only detectable product.

The position of the mercury bonds in 2-thiouracilmercury chloride was not determined directly on the salt, but the structure may correspond to the bonds observed in the nucleosides obtained from the subsequent condensation reaction. The N-1 and N-3 glycosyls are probably derived from the corresponding N-1 bonded and N-3 bonded pyrimidinemercury species. The formation of a mercury bond with one of the exocyclic atoms is suggested by the facile hydrolysis of fraction 1 to 2-thiouracil in dilute acid or alkali. Thus, the C-1′ atom of the sugar moiety is probably bonded to one of the exocyclic atoms on the pyrimidine at the S-2 position or the O-4 position ofthe nucleoside. Spectral analysis could be used to determine the position of the glycosidic bond, but the absorbance of the benzoyl groups interferes with this determination. We were unable to confirm the structure of 2′,3′,5′-tri-*O*-benzoyl-2-*S*-ribofuranosyl-2-thiouracil because debenzoylation destroys the glycosidic linkage.

The utility of mercuric bromide in the prevention of exocyclic *O*-glycosyl formation has been reported by Ukita *et al.* (1964). To extend this observation, the $HgBr_2$ procedure was incorporated into the synthesis of 2-thiouridine and 2-thiocytidine. In one preliminary experiment, the $HgBr_2$ was omitted from the condensation of 2-thiouracilmercury chloride with the blocked ribofuranosyl chloride. This resulted in the preparation of fraction 1, but no N-1 or N-3 glycosyl compounds could be found in the reaction mixture.

The ionization constant of 2-thiouridine was determined for a comparison with 1-ethyl-2-thiouracil (Shugar and Fox, 1952a). The same authors observed that the pK_a value of uridine is approximately 0.5 pH unit lower than the pK_a of 1-methyluracil (Shugar and Fox, 1952b). The acid-strengthening effect of the

ribose moiety of uridine may be due to the interaction of the 2′-hydroxyl group (or the 5′-hydroxyl group) with the 2-oxopyrimidine group (Fox *et al.*, 1953; Wierzchowski and Shugar, 1957). The pK_a shift is not observed for the N-1 bonded 2-thiouracil derivatives; however, the pK_a of 2-thiouridine is almost identical with the pK_a of 1-ethyl-2-thiouracil (Shugar and Fox, 1952a).

The availability of 1-β-D-ribofuranosyl-4-oxopyrimidine suggests some biochemical experiments that may provide valuable new information. This nucleoside lacks an active hydrogen atom on the pyrimidine ring and will not undergo Watson and Crick (1953) base pairing. Furthermore, this nucleoside is devoid of a polar substituent at the 2 or 6 position of the pyrimidine ring, which precludes intramolecular interaction with groups on the ribose moiety (Gassen and Witzel, 1967). Our first attempts to prepare the ribonucleoside of 4-oxopyrimidine were unsuccessful because the acetic anhydride treatment of 4-oxopyrimidine does not provide an acetyl derivative. Since the N-1 reactive mercury salt could not be prepared, the mercury condensation gave the N-3 glycosyl compound (Funakoshi *et al.*, 1961) and no N-1 glycosyl derivative.

The Raney nickel hydrogenolysis of 2-thiouracil to 4-oxopyrimidine in aqueous ammonium hydroxide for 2 hr under vigorous reflux was reported by Brown (1950). When 2-thiouridine was treated in this manner, however, the product was devoid of selective ultraviolet absorption. Although the further characterization of this product was not performed, in all probability it is the 2,3,5,6-tetrahydropyrimidine derivative. The complete reduction of 4-thiouridine, under similar experimental conditions, has been known for some time (Fox and Van Praag, 1960).

The hydrogenation of 2-thiouridine was repeated under milder conditions. Aqueous ammonium hydroxide at 60° and ammonia in ethanol under reflux showed a transient formation of a dihydropyrimidine derivative, but no ribofuranosyl-4-oxopyrimidine; the only product after 2 hr was the tetrahydropyrimidine. Apparently, the reduction of the pyrimidine ring occurs almost as fast as desulfurization when a fully activated nickel catalyst is used in an alkaline solvent. A partially deactivated catalyst must be used, therefore, in the preparation of the N-1 glycosyl of 4-oxopyrimidine.

The desulfurization of a diformoxythiolcholanate derivative to the aldehyde with acetone-deactivated Raney nickel, in a neutral solvent, has been demonstrated by Spero *et al.* (1948). These authors also showed that the W-4 catalyst produces further reduction of the thiol ester to the alcohol. Our results show that the desulfurization of 2-thiouridine and 2-thiocytidine catalyzed by acetone-deactivated Raney nickel can be controlled to prevent reduction of the pyrimidine ring. This partially deactivated W-4 Raney nickel is a reliable catalyst for the synthesis of 1-β-D-ribofuranosyl-4-oxopyrimidine in an alkali-free ethanol solvent.

The ultraviolet absorption spectra of the derivatives of 4-oxopyrimidine show some characteristic bands that are very useful in the identification of different positional isomers. In aqueous alkali, 3-methyl-2-thiouracil (Shugar and Fox, 1952a) and the N-3 glycosyl analogs show bands near 2600 and 3140 Å (Sano, 1962); the 3140-Å band is missing for the 2-thiouracil anion substituted in the N-1 position. Shugar and Fox (1952a) observed a very strong band near 2390 Å for the 1-methyl-2-thiouracil anion; the same band was observed for anionic 2-thiouridine (Brown *et al.*, 1958). The 2390-Å band was used for the spectrophotometric titration of the latter compound. When derivatives of 2-thiouracil are desulfurized to the corresponding 4-oxopyrimidine, the 3140-Å band is no longer found, but the 2600–2690- and the 2390-Å bands are still prominent spectral characteristics.

The structure of the free base, 4-oxopyrimidine, suggests a potential proton tautomerism between the N-1 and the N-3 positions. A solution of 3.0×10^{-5} M 4-oxopyrimidine in dry cyclohexane gave ultraviolet spectrum λ_{max} 2690 Å (a_M 3700); this band closely duplicates the aqueous spectrum of 3-methyl-4-oxopyrimidine (ultraviolet spectrum λ_{max} 2690 Å (a_M 3900); Brown *et al.*, 1955). The spectral data obtained with cyclohexane solvent provide support for the conclusion of Brown *et al.* (1955) that the proton of 4-oxopyrimidine is located on the N-3 position.

In water solution (pH 5–6) the 3-methyl-4-oxopyrimidine (Brown *et al.*, 1955) and the 3-β-D-ribofuranosyl-4-oxopyrimidine (Funakoshi *et al.*, 1961) reveal an ultraviolet band close to 2690 Å. An intense band near 2390 Å is observed for 1-methyl-4-oxopyrimidine (Brown *et al.*, 1955) and 1-β-D-ribofuranosyl-4-oxopyrimidine at pH 6 in water. Brown *et al.* (1955) suggested that the high excitation energy (2390 Å is equivalent to 120 kcal mole^{-1}) of the N-1-substituted 4-oxopyrimidine may be correlated with a large electric dipole moment. The excitation energy of the N-3-substituted 4-oxopyrimidines (2690 Å is equivalent to 106 kcal mole^{-1}) is lower than the corresponding value for the N-1 analogs. It is of interest to note that the ultraviolet excitation of 1-methyl-4-oxopyridine in methanol requires 14 kcal mole^{-1} more than the excitation of 1-methyl-2-oxopyridine (Berson, 1953).

In an extension of this investigation, to be reported elsewhere, 2-thiouridine was phosphorylated at the 2′ or 3′ positions, converted into the nucleotide of 4-oxopyrimidine by hydrogenolysis, and finally cyclized to prepare 2′,3′-cyclic phosphoro-1-β-D-ribofuranosyl-4-oxopyrimidine. This compound was found to be inactive as a substrate for pancreatic ribonuclease A; the 2-oxopyrimidine or 6-oxopyrimidine group is required for enzymatic activity (Gassen and Witzel, 1967).

Experimental Section

Direct Mercury Salt Formation of 2-Thiouracil and Condensation with 2′,3′,5′-Tri-O-benzoyl-D-ribofuranosyl Chloride. 2-Thiouracil (8 mmoles) in aqueous NaOH was treated with 8 mmoles of $HgCl_2$ in ethanol by the method of Fox *et al.* (1956). After the removal of chloride ion, the salt was dried and suspended in 300 ml of hot xylene; a solution of 16 mmoles of the blocked ribofuranosyl chloride in benzene was added,

and the suspension was heated with $HgBr_2$ under reflux for 3 hr. The reaction mixture was cooled and filtered, and petroleum ether (bp 30–60°) was added to the filtrate to precipitate the glycoside mixture. The precipitate was suspended in hot ethanol, and a white precipitate (fraction 2) was removed by filtration at 50° (fraction 2 was kept for hydrolysis in alkali). The filtrate (fraction 1) was cooled slowly, dissolved in $CHCl_3$, and placed on a 1.2 × 45 cm column of silica gel powder (J. T. Baker Chemical Co.). A compound, tentatively identified as 2′,3′,5′-tri-*O*-benzoyl-2-*S*-ribofuranosyl-2-thiouracil (18% yield), was eluted with 200 ml of $CHCl_3$ and crystallized from ethanol (mp 108–112° dec). The product was quantitatively converted into 2-thiouracil in 1.0 N HCl or 1.0 N NaOH at 50° for 1 hr.

Fraction 2 was dissolved in 30 ml of NH_3 in dry methanol and stored at 20° for 3 days. The solvent was removed under vacuum, and the residue was dissolved in water and extracted with $CHCl_3$. The aqueous fraction was concentrated, dissolved in ethanol, and applied to a 1.5 × 50 cm column of Avicel microcrystalline cellulose (American Viscose Division, FMC Corp., Marcus Hook, Pa.). A sample of 2-thiouridine (5% yield) was eluted from the column with 60 ml of 1-butanol–ethanol–water (5:1:4). When 200 ml of solvent was eluted from the column, a sample of 3-β-D-ribofuranosyl-2-thiouracil was obtained in 13% yield. The N-3 glycosyl is readily identified from its ultraviolet spectrum (λ_{max} (0.1 N NaOH) 3160 Å; see Shugar and Fox, 1952a). Treatment of this compound with hot aqueous chloroacetic acid (Shaw *et al.*, 1958) gave uracil and 2-thiouracil as the only ultraviolet-absorbing products. Desulfurization of 3-β-D-ribofuranosyl-2-thiouracil with a partially deactivated Raney nickel catalyst gave 3-β-D-ribofuranosyl-4-oxopyrimidine, identical with the compound (a gift from Dr. T. Ukita) prepared by the method of Funakoshi *et al.* (1961).

Preparation of 2-Thiouridine by the Mercuri Procedure. Pyridine (10 ml) and 6.4 g of 2-thiouracil were heated under reflux in 200 ml of dry acetic anhydride for 6 hr (Spector and Keller, 1958). The solution was concentrated and stored at 4° for crystal formation. The crystals were washed with CCl_4 and dried to give 6.2 g of acetyl-2-thiouracil, mp 242–243°, sublimed at 306–308°.

A warm solution of 5.1 g (30 mmoles) of acetyl-2-thiouracil in 30 ml of 1.0 N NaOH in dry ethanol was stirred, and 15 mmoles of $HgCl_2$ in 150 ml of ethanol was slowly added. The solution was brought to 80° for 5 min, cooled to 4°, and stored for crystal formation. The precipitate was washed with water and ethanol and dried under vacuum to yield 5.6 g of the N-1 reactive mercury salt.

The mercury salt (4.8 g contains 21 mmoles of 2-thiouracil) was suspended in 300 ml of hot xylene, and 22 mmoles of 2′,3′,5′-tri-*O*-benzoyl-D-ribofuranosyl chloride in benzene was gradually added. The mixture was heated under reflux for 30 min and cooled to 100°, and 2.8 g of $HgBr_2$ was added. After an additional period of 120 min under reflux, the mixture was cooled to 25°, stored for 16 hr, and filtered. The filtrate was concentrated, treated with petroleum ether, and stored at 4° for crystal formation. Chloroform was used to dissolve the crystals; this solution was extracted with 30% KI in H_2O. The $CHCl_3$ fraction was dried with Na_2SO_4 and the solvent was removed under vacuum. The product was crystallized from ethyl acetate and petroleum ether to give 5.2 g, mp 147–149°.

The 2′,3′,5′-tri-*O*-benzoyl-2-thiouridine was dissolved in 100 ml of 1.0 N NaOH in 50% aqueous ethanol and heated under reflux for 1 hr. The solution was neutralized with acetic acid and the solvent was removed under vacuum. The residue was dissolved in water, treated with an excess of Bio-Rad AG 50W (H^+) resin, and extracted with ethyl ether. The aqueous fraction was concentrated and the residue was crystallized from ethanol to give 1.85 g of 2-thiouridine: mp 214–215°, $[\alpha]_D^{25}$ +30.8° (*c* 1.4, H_2O), ultraviolet spectrum λ_{max} (0.1 N NaOH) 2380 Å. The yield of 2-thiouridine was 20% from 2-thiouracil and 32% from the tribenzoylribofuranosyl chloride. Uridine was formed from 2-thiouridine in the hot chloroacetic acid reaction (Shaw *et al.*, 1958).

Preparation of 2-Thiocytidine by the Mercuri Procedure. The conversion of 2,4-dithiopyrimidine into 2-thiocytosine was accomplished by the method of Hitchings *et al.* (1949). The latter compound (2.6 g) was acetylated and crystallized from acetic anhydride to yield 2.8 g (13 mmoles) of acetyl-4-acetamido-2-thiopyrimidine, mp 237–239°, sublimed at 324–326°. The N-1 reactive mercury salt of 4-acetyl-2-thiocytosine (mp 280–285°) was prepared with 6.5 mmoles of $HgCl_2$ in dry ethanol by the procedure used in the synthesis of 2-thiouridine. The mercury salt (2.5 g contains 9.2 mmoles of 2-thiocytosine) was treated with 10 mmoles of tribenzoylribofuranosyl chloride and $HgBr_2$. 2,′3′,5′-Tri-*O*-benzoyl-4-acetyl-2-thiocytidine (2 g) was hydrolyzed at 100° in 1.0 N NaOH in 50% ethanol for 30 min. The sodium ion was removed and the product was purified by passage through an Avicel column. The 2-thiocytidine was crystallized from ethanol to yield 750 mg, $[\alpha]_D^{25}$ +64.2° (*c* 1.8, H_2O). Chloroacetic acid treatment of 2-thiocytidine gave cytidine, and Raney nickel hydrogenolysis followed by mild alkaline hydrolysis gave 1-β-D-ribofuranosyl-4-oxopyrimidine.

Preparation of 1-β-D-Ribofuranosyl-4-oxopyrimidine. The type W-4 Raney nickel (Augustine, 1965) was heated in acetone under reflux, cooled, and stored in ethanol (Spero *et al.*, 1948). 2-Thiouridine (1 g) was dissolved in 30 ml of 50% ethanol in water, and 0.8 g (wet weight) of nickel catalyst was added. The suspension was kept at the reflux temperature for 3 hr and cooled, and the catalyst was removed by filtration. The catalyst was resuspended in 10 ml of hot water three times, and the combined filtrate was concentrated under vacuum. The residue was crystallized from ethanol and dried to give 300 mg of 1-β-D-ribofuranosyl-4-oxopyrimidine: mp 170–172° dec, ultraviolet spectrum λ_{max} (H_2O) 2390 Å (a_M 11,300), infrared spectrum (KBr) 1.64 × 10^3 cm^{-1}. The absorption bands are very similar to those reported for 1-methyl-4-oxopyrimi-

dine by Brown *et al.* (1955).

Anal. Calcd for $C_9H_{12}N_2O_5$: C, 47.37; H, 5.30; N, 12.28. Found: C, 47.10; H, 5.12; N, 12.23.

A Gilford Model 240 spectrophotometer, and cells with a path length of 10 mm, were used to obtain the results of Figure 1. The pH measurements were performed with a Radiometer TTT1 titrator and a Model 630T scale expander.

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