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Oxidation of Nucleic Acid Bases by Potassium Peroxodisulfate in Alkaline Aqueous Solution[1]

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The kinetics and products of peroxodisulfate oxidation of the common nucleic acid bases have been investigated in alkaline aqueous solution. The oxidations are first order in peroxodisulfate and first order in nucleic acid base. Relative rates in 1 *N* KOH at 40° follow: adenine, 1; thymine, 5.5; uracil, 5.2; cytosine, 8.6; guanine, 338. Values for the apparent second-order rate constants decrease with decreasing hydroxide ion concentration at constant ionic strength, suggesting involvement of the ionized base as the kinetically significant reactant. No significant reaction between peroxodisulfate and any nucleic acid base is observed under neutral or acidic conditions at 40°. Radical traps have no effect on either the rate or extent of peroxodisulfate disappearance or on the rate or extent of product formation. Uracil and cytosine were oxidized to uracil 5-sulfate and cytosine 5-sulfate, respectively. Urea was the only identified product of adenine and thymine oxidation. Oxidation of guanine produced ammonia, carbon dioxide, urea, guanidine, and 2,4-diamino-*s*-triazine-6-carboxylic acid. Triazinecarboxylic acid formation was also observed in 8-hydroxyguanine oxidation.

Chemical oxidation of the nucleic acid bases guanine, cytosine, adenine, uracil, and thymine has received considerable attention.[2] A partial list of the reagents employed to oxidize these materials includes potassium permanganate,[3-11] osmium tetroxide,[12-16] hydrogen peroxide,[17-19] and various organic peracids.[20-23] Potassium peroxodisulfate (persulfate, peroxydisulfate), a potent oxidant whose reactivity and utility are quite varied,[24-26] has received only limited use as an oxidant of purines or pyrimidines of biological origin. Biltz and Schauder[27,28] document its use as an oxidant of uric acid in acetic acid solution and more recently Hull[29] studied its reactions with substituted pyrimidines under alkaline conditions.

Work in our laboratory has recently been concerned with the investigation of reactions between nucleic acid components and a number of reagents[16,19,21,30] with the intention of devising or elaborating particular reactions which show promise as selective modifiers of nucleic acids. We therefore undertook a survey of the reactivity of the nucleic acid bases with peroxodisulfate. We report here the results of our observations on the kinetics and products of these reactions.

Results

Kinetics. Under pseudo-first-order conditions with all bases except thymine, semilog plots for the disappearance of peroxodisulfate with time showed good linearity for at least 2 half-times. Initial concentrations were varied by at least a factor of 4 (Table I). Neither EDTA nor acrylamide, a sulfate radical ion trap,[19,31,32] had any significant effect on the rate of peroxodisulfate disappearance.

Semilog plots of peroxodisulfate disappearance in reaction with thymine in 1.0 *N* KOH at 40° showed satisfactory linearity for only approximately 10% of the reaction. Values for the apparent second-order rate constant for thymine oxidation were evaluated from these linear regions. Following this "induction" period, semilog plots of peroxodisulfate disappearance became concave downward. EDTA (1×10^{-4} *M*) and acrylamide (5×10^{-4} *M*) had no effect on the rate or extent of peroxodisulfate disappearance under these reaction conditions.

The kinetics of peroxodisulfate disappearance in reaction with thymine in 0.1 *N* KOH were also measured at 60°. Semilog plots of peroxodisulfate concentration *vs.* time were again concave downward, the induction periods varied depending on the reagents employed, and satisfactory linearity in the initial slopes of peroxodisulfate *vs.* time plots was generally not observed.

Oxygen exerted a significant retarding effect on the rate of peroxodisulfate disappearance in the presence of thymine in 0.1 *N* KOH at 60°. Both a lengthening of the observed induction period and a decrease in the maximum slope of semilog plots of peroxodisulfate concentration *vs.* time were observed. In contrast to the lack of linearity observed in its absence, thymine oxidations carried out in the presence of EDTA showed reasonable first-order dependence on peroxodisulfate for at least 1 half-time. These results suggest that metal ion catalyzed decomposition of peroxodisulfate is significant at this temperature in the absence of a sequestering agent such as EDTA.

While no definitive conclusion can be drawn as to the mechanism of thymine oxidation at 60° in 0.1 *N* KOH, the effects of EDTA and oxygen imply significant involvement by free radicals and metal ions in peroxodisulfate disappearance and present evidence that the mechanism of thymine oxidation at 40° is different from that at 60°.

The curvature observed in plots of peroxodisulfate disappearance *vs.* time for the oxidation of thymine at 40° in 1 *N* KOH is attributed to further oxidation of an initially formed product and not to free-radical decomposition. There are precedents in the literature for kinetics of this

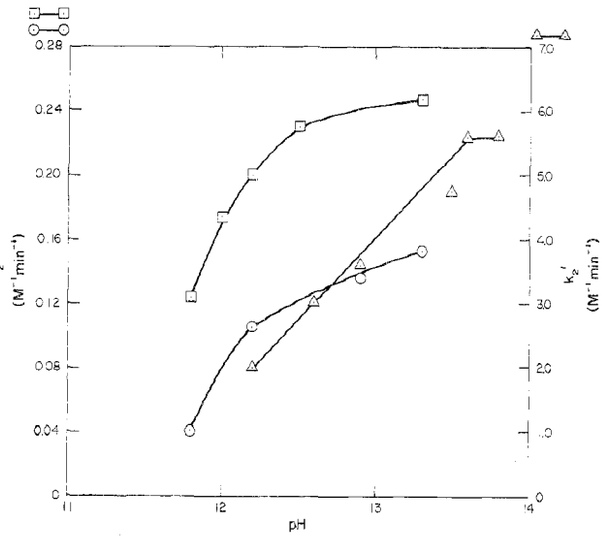


Figure 1. Apparent second-order rate constants as a function of pH: ⊙—⊙, uracil, 40°; ⊡—⊡, cytosine, 40°; △—△, guanine, 25°.

type.[26] This conclusion is consistent with the lack of effect EDTA and acrylamide on the rate of peroxodisulfate disappearance and with the observed stoichiometry of thymine oxidation, which will be presented in an accompanying section.

Substrate Dependence. Linearity in semilog plots of the concentration of peroxodisulfate *vs.* time suggests that peroxodisulfate disappearance may be described by the relationship $-d[S_2O_8^{2-}]/dt = k\psi[S_2O_8^{2-}]$, where $k\psi$ is the pseudo-first-order rate constant. The data of Table I suggest that $k\psi$ is a linear function of substrate concentration. The rate law for the disappearance of peroxodisulfate which satisfies these results is given by $-d[S_2O_8^{2-}]/dt = k_2'[\text{substrate}]_{\text{total}}[S_2O_8^{2-}]$, where $k_2' = k\psi/[\text{substrate}]_{\text{total}}$. For all cases considered, the disappearance of peroxodisulfate was first order in peroxodisulfate and first order in substrate. The rate law held for a minimum of 2 half-lives for each substrate. Guanine is the most reactive substrate with peroxodisulfate under these reaction conditions. The nucleic acid pyrimidines are oxidized at similar rates. Adenine is the least reactive substrate.

pH Dependence. Figure 1 shows the variation in the apparent second-order rate constant as a function of pH for the oxidation of uracil and cytosine at 40° and guanine at 25°. The data for cytosine and guanine show a plateau in the pH range 12.5–13 and 13.5–13.8, respectively. Half-maximal rates are observed near pH 11.8 for cytosine and 12.6 for guanine. A pK_a of 11.7 for proton loss from cytosine was calculated for 40° using the heat of ionization for cytosine presented by Izatt and Christensen.[33] Izatt, Christensen, and Rytting[34] include a pK_a of 12.62 for the second proton loss from guanine at 20°.

Uracil shows no well-defined plateau over the pH range investigated. The pK_a for the second proton loss from uracil is greater than 13[34] at 25° and Shapiro and Kang[35] conclude it is probably nearer 14. These data, together with our observed two-fold rate increase for uracil oxidation over the pH range 12.2–13.3, suggest that the uracil dianion is more reactive toward peroxodisulfate than the singly ionized form. Similarly, only the cytosine anion is significantly reactive. These conclusions are supported by our observations[36] that the nucleosides of these bases do not react with peroxodisulfate at a significant rate in 1 *M* Na_2CO_3 solution.

Figure 1 shows that the guanine dianion is more reactive than the singly ionized form, although our findings[36] for guanosine (the nucleoside) suggest that the singly ionized form is appreciably reactive.

More limited data for thymine and adenine also suggest that it is the di- and monoanion, respectively, which are the reactive species. None of the substrates showed any detectable reaction with peroxodisulfate under neutral or acidic conditions at 40°.

Table I
Kinetics of the Peroxodisulfate Oxidation of Nucleic Acid Bases[a]

Substrate	Concn range, *M*	k_2', M^{-1} min^{-1}	Relative rate
Adenine	0.025–0.100	0.029 ± 0.005	1
Thymine	0.0099–0.100	0.16 ± 0.03[b]	5.5
Uracil	0.025–0.114	0.15 ± 0.006	5.2
Cytosine	0.0107–0.100	0.25 ± 0.02	8.6
Guanine	0.03	9.8 ± 0.3	338
	0.005–0.0200	5.16 ± 0.4[c]	

[a] General conditions: [substrate]/[$K_2S_2O_8$] = 10, T = 40°, 1.0 *N* KOH. [b] Evaluated from linear region of semilog plots of [$K_2S_2O_8$] *vs.* time. [c] 25°.

Table II
Peroxodisulfate Oxidation of the Nucleic Acid Bases. Temperature Dependence[a]

Substrate	Temp, °C	k_2', M^{-1} min^{-1}	E_a, kcal mol^{-1}	ΔS^*, cal mol^{-1} deg^{-1}
Guanine	40	9.8	9.3 ± 0.3	−34 ± 1
	25	5.2		
Cytosine	50	0.49	13.5 ± 1.4	−28 ± 5
	40	0.25		
Uracil	50	0.30	13.9 ± 1.1	−28 ± 4
	40	0.15		
Thymine	40	0.16[b]	11 ± 1.5	−28 ± 5
	30	0.09[b]		
Adenine	50	0.058	13.9 ± 1.4	−31 ± 4
	40	0.029		

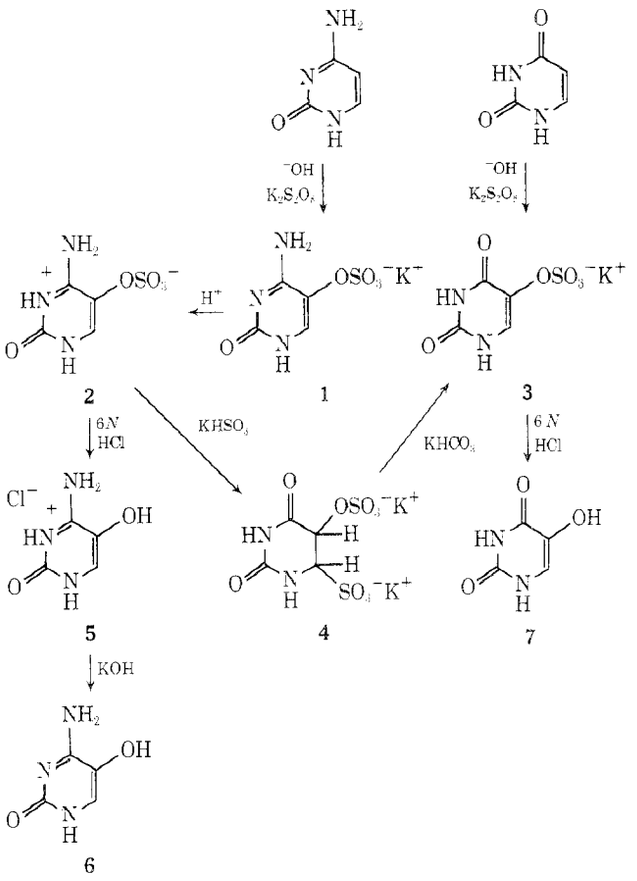
[a] General conditions: [substrate]/[$K_2S_2O_8$] = 10, 1 *N* KOH. Values are averages of two runs. [b] Evaluated from initial slopes of semilog plots of peroxodisulfate concentration *vs.* time.

Ionic Strength Dependence. The rates of oxidation of all of the bases increase with increasing ionic strength as expected for reaction between ions of like charge. Plots of the logarithm of the rate constants *vs.* the square root of ionic strength are linear in spite of the fact that the ionic strengths involved are far in excess of those for which the Debye–Hückel limiting law was derived.[37] The magnitude of the ionic strength effect is illustrated by the following data: guanine, 1.0 *M* KOH, $k_2' = 5.5\ M^{-1}$ min^{-1}, $\mu = 1$; same conditions but μ increased to 2.3 by the addition of KCl, $k_2' = 10.6\ M^{-1}$ min^{-1}.

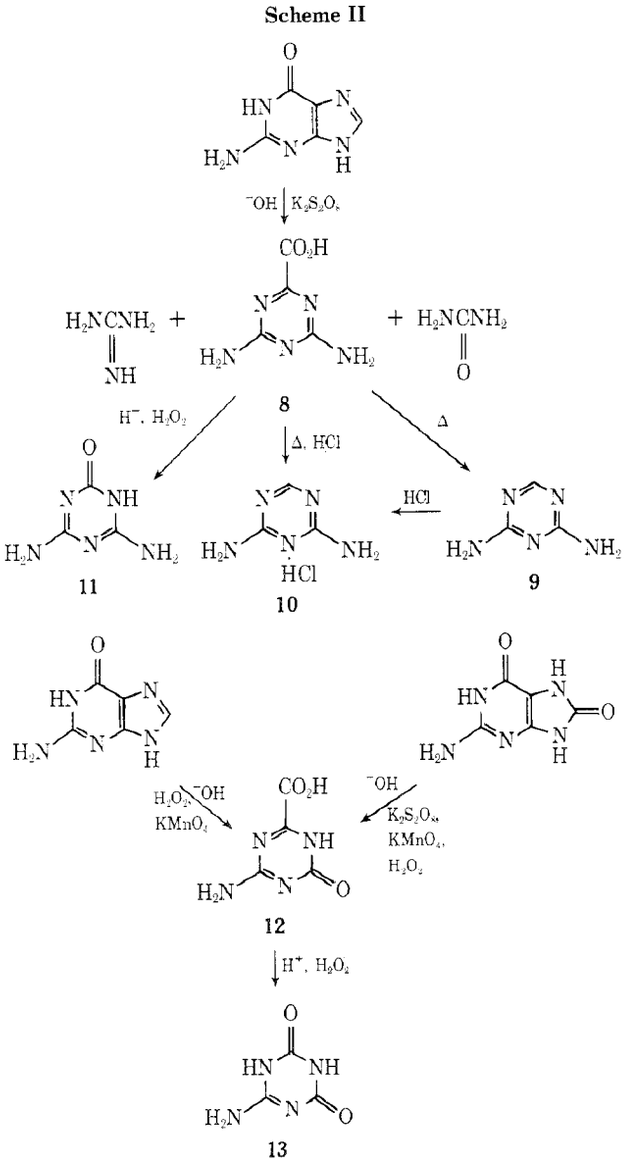
Temperature Dependence. The variation of the apparent second-order rate constants with temperature for adenine, thymine, uracil, cytosine, and guanine is presented in Table II along with the derived activation parameters.

Products. Cytosine and Uracil. Cytosine and uracil react with peroxodisulfate in 1 *N* KOH to yield the potassium salts of cytosine 5-sulfate (1) and uracil 5-sulfate (3), respectively (Scheme I). Paper chromatography of the reaction mixtures in solvent I showed starting material and the corresponding 5-sulfate to be the only ultraviolet-absorbing materials present in both reactions. No other products were detected when chromatograms were sprayed with either Ehrlich's reagent or the nitroprusside–ferricyanide–hydroxide spray. The 5-sulfates are the expected products based on the work of Hull.[29] At a substrate/peroxodisulfate ratio of 10 (the kinetic conditions), the yields of the 5-sulfates were 87 (cytosine) and 72%

Scheme I



Scheme II



(uracil). The yields dropped to 62 (cytosine) and 41% (uracil) at a substrate/peroxodisulfate ratio of 1. Cytosine 5-sulfate was isolated both as the free acid (2) and as the corresponding potassium salt (1). Uracil 5-sulfate was isolated as the potassium salt (3). The structures were assigned on the basis of ir spectra, nmr spectra in DMSO-d_6 and D_2O, elemental analyses, and hydrolysis of each material in 6 N HCl to known compounds, namely, 5-hydroxycytosine (6)[38] and 5-hydroxyuracil (isobarbituric acid, 7).[39,40] Further proof of the structures of these sulfates was obtained by the conversion of cytosine 5-sulfate to uracil 5-sulfate through the intermediacy of the bisulfite adduct, dipotassium 5-sulfo-6-sulfono-5,6-dihydrouracil (4). This material was prepared by incubating cytosine 5-sulfate (2) in 1 M $KHSO_3$ solution at 40° for 15 hr. The solid collected at the end of this incubation showed only end absorption in the ultraviolet. Its nmr spectrum in D_2O showed the absence of the characteristic H-6 resonance typical of 5-substituted pyrimidines. The spectrum showed instead a pair of doublets centered at 4.80 and 5.55 ppm, J = 6 Hz, resulting from coupling of two adjacent carbon-bound protons at positions 5 and 6 of a saturated pyrimidine ring. On addition of NaOD the doublets disappeared and a single H-6 resonance appeared. The elemental analysis for the product was correct for $C_4H_4N_2O_9S_2K_2$. On heating this material in 1 M $KHCO_3$ solution, a solid precipitated on cooling whose ir, uv, and nmr spectra and whose chromatographic behavior in solvent I were indistinguishable from those of the potassium salt of uracil 5-sulfate. These transformations are consistent with the known chemistry of bisulfite addition to pyrimidine rings.[41]

Thymine. Thymine reacts with peroxodisulfate in 1 N KOH to give ring-cleavage products. Paper chromatography of oxidation solutions showed the presence of three products. Two of the products were detected by Ehrlich's reagent. One of the Ehrlich-positive spots was identified as urea by its chromatographic mobility (R_f in solvent I, 0.58), by its color development with Ehrlich's reagent, and by its destruction by the enzyme urease. The second Ehrlich-positive material (R_f in solvent I, 0.18) became pink shortly after spraying with Ehrlich's reagent and became blue on standing in air for 3–4 hr. While this material was not identified, it should be pointed out that a compound with identical chromatographic mobility and color development properties can be prepared by incubation of *cis*-thymine glycol in 1 N KOH for 1 hr, conditions sufficient for complete degradation of thymine glycol.[9] The third component detected chromatographically appeared as a dark spot when viewed with an ultraviolet light source (R_f in solvent I, 0.05). This material was not identified. Ultraviolet maxima at pH 1, 7, and 14 were obtained following elution of this material from paper chromatograms: λ_{max} (pH 1) 273, λ_{max} (pH 7) 270, λ_{max} (pH 14) 291 nm.

Although hydroxyacetone and pyruvic acid have been observed as products of thymine oxidation in other systems,[3,19] we failed to detect either material. Control experiments showed that neither pyruvic acid nor hydroxyacetone survives incubation in 1 N KOH at 40° in the presence or absence of peroxodisulfate.

Adenine. The reaction of adenine with peroxodisulfate in 1 N KOH is complex and has not been elucidated here.

The reaction solutions are deep red in color and were shown chromatographically to be mixtures of a number of colored materials. Unchanged adenine was detected in reaction mixtures after 24-hr incubation in the presence of 2 equiv of peroxodisulfate in 1 *N* KOH at 40°. The red color of the alkaline reaction mixtures is immediately discharged by the addition of sodium dithionite or by acidification of the alkaline solutions but no readily recognizable materials were identified. Only urea was identified unambiguously.

Guanine. Products characterized in the alkaline peroxodisulfate oxidation of guanine were guanidine, urea, ammonia, carbon dioxide, and a material which is proposed to be 2,4-diamino-*s*-triazine-6-carboxylic acid. Guanidine was identified by its chromatographic mobility (R_f in solvent I, 0.52), by its color development with the nitroprusside–ferricyanide–hydroxide spray, and by isolation as its crystalline picrate.[5] Ammonia was detected by its odor. Carbon dioxide was characterized by its liberation from acidified reaction solutions and by isolation as barium carbonate in a barium hydroxide trap.

A precipitate having properties consistent with the structure 2,4-diamino-*s*-triazine-6-carboxylic acid (**8**) (Scheme II) is obtained on acidification of guanine–peroxodisulfate oxidation mixtures. While neither this material nor those resulting from its various chemical transformations were isolated in adequate purity to provide acceptable elemental analyses, the infrared and mass spectra of the materials produced showed excellent agreement with known compounds.

The impure solid (**8**) which precipitates on acidification of guanine–peroxodisulfate product mixtures is sparingly soluble in acidic or neutral aqueous solution at room temperature but freely soluble in neutral buffered aqueous solution at pH greater than 6. It does not melt and shows no signs of decomposition at temperatures below 310°. At temperatures above 310° it decomposes with the liberation of dense white vapor. This vapor can be condensed on a cold-finger. The white sublimate shows a parent peak in its mass spectrum at m/e 111 and an ir spectrum identical with that of formoguanamine (guanamine, 2,4-diamino-*s*-triazine, **9**).

The impure, acid-precipitable solid (**8**) dissolves in hot 1 *N* HCl with the liberation of 50% of the theoretical amount of carbon dioxide. The ir spectrum of solid recovered following decarboxylation is virtually identical with that of authentic formoguanamine hydrochloride (**10**).

Oxidative decarboxylation of the guanine–peroxodisulfate precipitate (**8**) in HCl–H_2O_2 solution produces a material whose mass and ir spectra[42] are virtually identical with those of ammeline (2,4-diamino-6-hydroxy-*s*-triazine, **11**).

J00-28-1

Experimental Section

Chemicals.---Adenine (6-aminopurine), uracil (2,4-dioxypyrimidine), thymine (2,4-dioxy-5-methylpyrimidine), cytosine (2-oxy-4-aminopyrimidine), and guanine (2-amino-6-oxypurine) were purchased from the Sigma Chemical Company, St. Louis, Missouri. P and L Biochemicals Inc., Milwaukee, Wisconsin, Calbiochem, La Jolla, California, and Schwarz/Mann, Orangeburg, New York in pure form as determined by their ultraviolet spectra[55]. Guanidine hydrochloride was obtained from Helco, Inc., Delaware Water Gap, Pennsylvania. Dimethylsulfoxide, tetramethylsilane, 8-hydroxyguanine (2-amino-6,8-dioxypurine) and 2,4-diamino-6-chloro-*s*-triazine were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. Dimethylsulfoxide was rendered anhydrous by storing over type 4-A molecular sieves obtained from Fisher Chemical, Fair Lawn, New Jersey. Deuterated dimethylsulfoxide (99.5% D) was purchased from Diaprep, Inc., Atlanta, Georgia, Prochem Ltd., Lincoln Park, New Jersey, and Norell Chemical Company, Inc., Landing, New Jersey. Deuterium oxide (99.8% D) was obtained from Norell Chemical Company, Landing, New Jersey. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate was obtained from Merck, Sharp and Dohme of Canada, Ltd., Montreal, Canada. Sodium deuteroxide (99% D) was purchased from E. Merck, Darmstadt, Germany. Potassium bromide (Optronic Grade) and phosphonium iodide were products of Alpha Inorganics-Ventron, Beverly, Massachusetts. Potassium peroxodisulfate was a Baker Analyzed Reagent, Phillipsburg, New Jersey and was recrystallized from water for use in kinetic experiments. All other inorganic chemicals were Baker Analyzed Reagents and were used without further purification.

Ammeline[59] (2,4-diamino-6-hydroxy-*s*-triazine) was prepared from 2,4-diamino-6-chloro-*s*-triazine by alkaline hydrolysis. Formoguanamine[55] (guanamine) (2,4-diamino-*s*-triazine) was prepared by reduction of 2,4-diamino-6-chloro-*s*-triazine using the hydriodic acid-phosphonium iodide method of Dreis[50].

J00-28-2

Instrumentation.---Ultraviolet absorption spectra were measured using a Perkin-Elmer Model 202 Spectrophotometer. Colorimetric measurements were carried out on a Klett-Summerson Colorimeter. Infrared spectra were recorded on a Perkin-Elmer Model 237-B Grating Infrared Spectrophotometer using potassium bromide discs as sample supports. Mass spectra were obtained on a Finnegan Model 1015SL Quadrupole Mass Spectrometer at 75 eV. Nuclear magnetic resonance spectra were obtained on a Varian Associates T-60 Spectrometer (60 MHz) at 35°. Tetramethylsilane was used as an internal standard in deuterated dimethylsulfoxide and sodium 2,2-dimethyl-2-silapentane-5-sulfonate in deuterium oxide.

Methods.---Kinetic runs were carried out in a water bath held to within 0.1° of the indicated temperature. Reactions were followed by measuring the disappearance of peroxodisulfate with time using a modification of the iodometric method of Kolthoff and Carr[51,61]. Blank corrections were in the range of 0.20 to 1.20 ml of 0.001 N thiosulfate. Kinetics were run under pseudo-first-order conditions by maintaining a substrate to peroxodisulfate ratio greater than or equal to 10. Values for the pseudo-first-order rate constants (k_1^1) were obtained from the slopes of semi-log plots of the concentration of peroxodisulfate *vs.* time. Apparent-second-order rate constants (k_2^1) (no correction for per cent ionized) were obtained by division of the appropriate k_1^1 values by total substrate concentration.

The variation in the determination of rate constants for reactions involving peroxodisulfate at concentrations of 10^{-5} *M* were generally of the order of ± 6%.

Urea was determined by the colorimetric method of Coulombe and Favreau[62]. Guanidine was determined by the colorimetric method of Marston[63] as presented by Snell and Snell[64]. In both cases, batchwise pretreatment of neutralized reaction aliquots with excess anion exchange resin (Bio-Rad AG 1-X8, 200-400 mesh, chloride form) to remove unreacted peroxodisulfate was required since peroxodisulfate interfered with both determinations.

The yields of cytosine-5-sulfate and uracil-5-sulfate were determined by reaction with the Folin phenol reagent[51] following hydrolysis in 3*N* HCl to the corresponding 5-hydroxy compounds. Uracil and cytosine do not interfere.

Paper chromatography (ascending) was performed on Whatman 3 MM paper in the machine-cut direction at 25° using isopropanol/ammonium hydroxide (55%)/water 7:1:2 v/v as solvent I. Dried chromatograms were sprayed with Ehrlich's reagent[65] for the visualization of urea and urea derivatives and the nitroprusside–ferricyanide–hydroxide spray[59] for the visualization of guanidine.

Elementary analyses were carried out by Galbraith Laboratories, Inc., Knoxville, Tennessee and Heterocyclic Chemical Company, Harrisonville, Missouri.

J00-28-3

Hydrogen cytosine-5-sulfate monohydrate (2).---To a solution of 3.0 g cytosine (0.018 mole) in 100 ml 1.0 *N* KOH was added 7.3 g $K_2S_2O_5$ (0.027 mole) as a solid. The solution was stirred at room temperature for 18 hours. The pale yellow solution was brought to approximately pH 2 by the addition of 9 ml concentrated hydrochloric acid. When the solution was cooled a pale yellow precipitate formed. The solid was filtered, washed with cold water, acetone, and ether to afford 3.2 g (89%) of crude hydrogen cytosine-5-sulfate (2). One crystallization from 45 ml water afforded 2.6 g (70%) of pure product. Rf in Solvent I, 0.23; cytosine, 0.40. uv $\lambda_{max}^{pH\,1}$ 288 nm (ϵ 8950), $\lambda_{max}^{pH\,6.8}$ 277 nm (ϵ 5400), $\lambda_{max}^{pH\,14}$ 290 nm (ϵ 7700); ir (KBr) 3525, 3400, 3200, 3100, 1710, 1670, 1600, 1295, 1200, 1050, 850, 712 cm^{-1}; nmr (DMSO-d_6) δ 7.3 (broad, 4) 7.4 (s, 1, 6-H).

J00-28-4

Anal. Calcd for $C_4H_5N_3O_5S \cdot H_2O$: C, 21.33; H, 3.14; N, 18.66; S, 14.24. Found: C, 21.09; H, 2.30; N, 18.40; S, 14.23.

Potassium cytosine-5-sulfate monohydrate (1).---To a rapidly stirred suspension of 2 g hydrogen cytosine-5-sulfate (2) (0.01 mole) in 40 ml H_2O was added 4 *N* KOH dropwise until the pH of the solution remained constant at pH 7. The solution was warmed to insure complete dissolution of all suspended solid and the pH readjusted to pH 7 by the additon of 4 *N* KOH. The resulting warm solution was diluted with 100 ml boiling ethyl alcohol and allowed to cool slowly to room temperature. The white solid which precipitated was filtered, washed with ethanol and ether and dried under vacuum over P_2O_5 for 15 hours to yield 2 g (88%) of potassium cytosine-5-sulfate monohydrate (1). Ir (KBr) 3575, 3450, 3150, 1675, 1650, 1270, 1245, 1220, 1050, 840, 725 cm^{-1}; nmr (DMSO-d_6) δ 7.2 (s, 1, 6-H), 7.5 (broad, 3).

Anal. Calcd for $C_4H_4N_3O_5SK \cdot H_2O$: C, 18.25; H, 2.30; N, 15.96; S, 12.18. Found: C, 18.54; H, 2.10; N, 16.14; S, 12.58.

Dipotassium-5-sulfo-6-sulfono-5,6-dihydrouracil (4).---Hydrogen cytosine-5-sulfate (2), 0.5 g (0.0024 moles), was heated in 30 ml 1 *M* $KHSO_3$ solution until it dissolved completely. The solution was stored at 40°. Any solid which settled out of solution during the first 4 hours of incubation was redissolved by heating. Incubation at 40° was continued for a total of 15 hours. A white solid which was present at the end of this time was collected, washed with 10 ml water and acetone and dried under vacuum over P_2O_5 for 4 hours. The weight of dry solid was 0.35 g (40%). An analytical sample of dipotassium-5-sulfo-6-sulfono-5, 6-dihydrouracil (4) was prepared by one crystallization from water. Ir (KBr) 3475, 3175, 3100, 1725, 1250, 1230, 1200, 1145, 1115, 1080, 1050, 1035, 855, 755, 700 cm^{-1}; nmr (D_2O) δ 4.80 (d, 1, J = 6 Hz, 5-H), 5.55 (d, 1, J = 6 Hz, 6-H); nmr (D_2O-NaOD) δ 7.6 (s, 1, 6-H).

Anal. Calcd for $C_4H_3N_3O_8S_2K_2$: C, 13.11; H, 1.10; N, 7.53; S, 17.50. Found: C, 13.12; H, 1.10; N 7.68; S, 17.67.

J00-28-5

Potassium uracil-5-sulfate (3).---To a solution of 2 g uracil (0.016 moles) in 100 ml 1 *N* KOH was added 9.6 g $K_2S_2O_5$ (0.036 moles) as a solid and the solution was stirred at 40° for 24 hours. The resulting colorless solution was neutralized with concentrated sulfuric acid and diluted to 200 ml by the addition of 100 ml methyl alcohol. The precipitated K_2SO_4 was removed by suction filtration and the methanol removed by evaporation under reduced pressure at 40°. The volume of the aqueous condensate was restored to 100 ml with water and a solution of 0.7 g $BaCl_2 \cdot 2H_2O$ in 20 ml of water was added with constant stirring. The precipitate was removed by gravity filtration and the filtrate condensed to dryness under reduced pressure at 40°. The solid residue was dissolved in 25 ml hot distilled water and was diluted to 100 ml with boiling acetone with rapid stirring. A semi-solid separated at once, and the hot supernatant suspension was decanted immediately and allowed to cool slowly to room temperature. The amorphous white solid which separated was redissolved in 20 ml hot water and reprecipitated by the addition of 80 ml boiling acetone. The isolated yield of crude potassium uracil-5-sulfate (3) was 0.7 g (14%). An analytical sample was prepared by recrystallization from water. Rf Solvent I, 0.125; uracil 0.43; uv $\lambda_{max}^{pH\,1}$ 266 nm (ϵ 7650), $\lambda_{max}^{pH\,6.8}$ 259 nm (ϵ 7650), $\lambda_{max}^{pH\,14}$ 283 nm (ϵ 7230); ir (KBr) 3180, 3090, 1730, 1710, 1515, 1290, 1270, 1245, 1230, 1065, 875, 730, 665 cm^{-1}; nmr (DMSO-d_6) δ 7.4 (s, 1, 6-H), 10.9 (broad, 2, 1 and 3 NH); nmr (D_2O-NaOD) 7.4 (s, 1, 6-H).

Anal. ---Calcd for $C_4H_3N_2O_5SK$: C, 19.51; H, 1.23; N, 11.38; S, 13.02. Found: C, 19.52; H, 1.25; N, 11.35; S, 13.22.

Potassium uracil-5-sulfate (3) from dipotassium-5-sulfo-6-sulfono-5, 6-dihydrouracil (4).---A solution prepared by heating 0.2 g dipotassium-5-sulfo-6-sulfono-5, 6-dihydrouracil (4) (0.0006 moles) in 2 ml 1 *M* KHCO$_3$ was kept warm in a boiling water bath with continuous stirring for approximately 5 minutes until all effervescence ceased. One drop of glacial acetic acid was added and the solution was allowed to cool slowly to room temperature and then chilled on ice until no further setting of white solid was evident. The solid was filtered, washed with 2 ml of distilled water, acetone, ether, and air dried to afford 0.05 g (57%) of potassium uracil-5-sulfate (3).

J00-28-6

5-hydroxyuracil (isobarbituric acid) (7).---To a hot solution containing 1.2 g (0.005 moles) of crude potassium uracil-5-sulfate (3) in 7 ml water was added an equal volume of concentrated hydrochloric acid. A solid separated immediately from the hot solution and heating was continued for an additional 5 minutes. The solid was collected by filtration, washed with water until the odor of HCl was no longer detectable and dried over P_2O_5-KOH for 15 hours to yield 0.40 g (64%) of 5-hydroxyuracil (7). uv $\lambda_{max}^{pH\,1}$ 278 nm (ϵ 6550), $\lambda_{max}^{pH\,5.8}$ 278 nm (ϵ 6300), $\lambda_{max}^{pH\,14}$ 305 nm (ϵ 4750); ir (KBr) 3460, 3100, 3000, 2900, 2810, 1690, 1670, 1280, 1240, 1135, 830 cm^{-1}; nmr (DMSO-d_6) δ 6.8 (d, 1, 6-H), 7.2 (broad, 1, 5-OH), 10.1 (broad, 2, 1-NH), 11.1 (broad, 1, 3-NH).

Anal. Calcd for $C_4H_4N_2O_3$: C, 37.50; H, 3.15; N, 21.87. Found: C, 37.32; H, 2.98; N, 21.76.

5-hydroxycytosine (6).---A solution of 3 g hydrogen cytosine-5-sulfate (2) (0.014 moles) in 7 ml 5 *N* HCl was heated in a boiling water bath for 15 minutes. The resulting solution was chilled on ice to afford 2 g (85%) of water soluble 5-hydroxycytosine hydrochloride (5). This solid was dissolved in 30 ml warm distilled water and the pH of the stirred solution adjusted to pH 7 by the dropwise addition of 4 *N* KOH. The free base precipitated, was washed with water, acetone, and ether and dried under vacuum over P_2O_5 for 15 hours to afford 1.2 g (77%) of 5-hydroxycytosine (6). uv $\lambda_{max}^{pH\,1}$ 300 nm (ϵ8000), $\lambda_{max}^{pH\,6.8}$ 288 nm (ϵ3000), $\lambda_{max}^{pH\,14}$ 322 nm (ϵ8950); ir (KBr) 3175, 2850, 1725, 1650, 1475, 1445, 1200, 812 cm^{-1}.

Anal. Calcd for $C_4H_5N_3O_2$: C, 37.79; H, 3.97; N, 33.06. Found: C, 37.51; H, 3.74; N, 32.91.

These data and the analogies between the chemical transformations observed for the guanine-oxidation product and for the triazinecarboxylic acid isolated in the alkaline oxidation of uric acid[43-48] provide compelling evidence that 2,4-diamino-*s*-triazine-6-carboxylic acid is a product of guanine oxidation under the conditions employed here.

Peroxodisulfate consumption is virtually complete in the reaction with guanine over a period of 7 hr at 40° in 1.0 *N* KOH with 0.05 *M* peroxodisulfate and 0.01 *M* guanine. One mole of guanine consumes 2.4 mol of peroxodisulfate. This figure is unchanged if the reaction is carried out in 1 *M* sodium carbonate.

The molar ratios of product formed per mole of guanine oxidized follow: urea, 0.25; guanidine, 0.55; and 2,4-diamino-*s*-triazine-6-carboxylic acid, 0.13. Neither urea nor guanidine is oxidized by peroxodisulfate under the reaction conditions. If we assume that these three products are formed by independent routes, then they account for 93% of the guanine oxidized.

Among the bases, the largest overall consumption of $K_2S_2O_8$ is observed in the reaction with thymine in 1 *N* KOH. The ratio of peroxodisulfate consumed per mole of substrate approaches 4 over a 24-hr period. In the presence of a five-fold molar excess of peroxodisulfate, adenine consumes 2 equiv of peroxodisulfate over the same time period. Under these conditions, 0.37 mol of urea is formed per mole of thymine while 0.25 mol of urea is formed per mole of adenine.

Acrylamide had no significant effect on the rate or extent of product formation in these reactions though the overall consumption of peroxodisulfate was greater in the presence of acrylamide than in its absence. Control experiments showed that peroxodisulfate is consumed in the presence of acrylamide alone in 1 *N* KOH at 40° over a 24-hr period. No significant loss of peroxodisulfate in 1 *N* KOH at 40° was observed over the same time period in the absence of acrylamide.

Oxidations with Some Related Oxidants and Substrates. The permanganate oxidation of guanine in 1 *N* KOH at 40° produces guanidine, urea, and 2-amino-4-hydroxy-*s*-triazine-6-carboxylic acid (**12**). The amount of urea and guanidine produced was not measured. The yield of triazinecarboxylic acid as determined by the weight of acid-precipitable solid following guanine oxidation is approximately 20%. Oxidation of guanine by hydrogen peroxide in 1 *N* KOH at 40° affords 2-amino-4-hydroxy-*s*-triazine-6-carboxylic acid (**12**) and urea as identified products. The yield of triazinecarboxylic acid with this latter oxidant is approximately 11%. Unreacted guanine (70%) is

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2,4-diamino-*s*-triazine-6-carboxylic acid (**8**) (Method I).---To a solution of 1 g guanine (0.0066 mole) in 100 ml of 1 *N* KOH was added 5.3 g $K_2S_2O_8$ (0.02 mole) as a solid and the mixture was stirred at room temperature until all the $K_2S_2O_8$ dissolved. The homogeneous solution was then stored at 40° for 18 to 24 hours. At the end of this time the resulting pale yellow solution was acidified to pH 2 by the addition of concentrated hydrochloric acid. Acidification was accompanied by the evolution of CO_2 and the precipitation of a pale yellow amorphous solid. This solid was collected by suction filtration, washed with water, acetone, and ether and dried under vacuum over P_2O_5 for 4 hours to afford 0.27 g of dry solid. Uv: 12.6 mg of solid was dissolved in 100 ml hot water. After cooling, aliquots of solution were diluted 1:10 with either 0.1 *N* HCl, pH 6.8 buffer or 1 *N* KOH; $\lambda_{max}^{pH\ 1}$ 210 nm (broad end absorption), $\lambda_{max}^{pH\ 6.8}$ 256 nm; $\lambda_{max}^{pH\ 14}$ 254 nm.

Anal. Calcd for $C_4H_5N_5O_2$: C, 30.97; H, 3.23; N, 45.16. Found: C, 26.12; H, 4.61; N, 41.84.

2,4-diamino-*s*-triazine (formoguanamine) (**9**).---Dry, finely powdered pH 2 precipitate (**8**) was heated with a burner flame in a sublimation apparatus below a cold finger cooled by running water. The flame was removed at the first appearance of white vapor. When vaporization ceased, heat was again applied until the formation of additional vapor began. This process was cautiously repeated until no additional white vapor appeared after brief heating. A dark brown non-volatile residue (16 mg) (20%) remained. A white sublimate (6 mg) (**9**) was recovered. Mass spectrum: m/e 111; ir (KBr) 3440, 3370, 3120, 1670, 1590, 1550, 1475, 1370, 1325, 1270, 1160, 1060, 1000, 815, 720 cm^{-1}.

Decarboxylation of 2,4-diamino-*s*-triazine-6-carboxylic acid (**8**).---To 6 ml of 1 *N* HCl in a 12 ml test tube was added 0.060 g of the pH 2 precipitate (**8**). The stoppered tube was connected by tubing to two 12 ml test tubes in series each containing 10 ml 6% $Ba(OH)_2 \cdot 8H_2O$ solution. The entire system was purged with nitrogen prior to and during the decarboxylations. The tube containing the suspended solid sample was supported in a boiling water bath and the evolved CO_2 collected until all of the solid sample had dissolved in the acid solution (45 minutes). No additional CO_2 could be collected if heating was continued after all solid had dissolved. By this method, 0.036 and 0.039 g $BaCO_3$ were obtained from two 0.060 g portions of sample. The average weight of $BaCO_3$ collected corresponded to 50% of theoretical if all the solid sample were pure 2,4-diamino-*s*-triazine-6-carboxylic acid. Therefore, assuming that the quantitation of evolved CO_2 provided an accurate measure of the triazine carboxylic acid present, then approximately 50% (0.15 g) of the total dry weight of the pH 2 precipitate was 2,4-diamino-*s*-triazine-6-carboxylic acid. This weight corresponds to an overall yield of 13% based on the amount of guanine oxidized.

2,4-diamino-*s*-triazine-6-carboxylic acid (**8**) (Method II).---To a solution of 4 g guanine (0.027 moles) in 400 ml 1 N KOH was added 21.2 g $K_2S_2O_8$ (0.079 moles) as a solid and the resulting mixture stirred at room temperature until all $K_2S_2O_8$ had dissolved. The solution was stored at 40° for 24 hours. At the end of this incubation, the alkaline reaction solution was mixed with 9 g Norit-A (acid washed) charcoal and the mixture stirred for 10 minutes. The charcoal was filtered by suction filtration. The collected charcoal was slowly eluted on the same filter with 150 ml hot distilled water. The eluent was filtered through celite to remove any suspended charcoal and the pH of the resulting filtrate adjusted to pH 2 by the addition of concentrated HCl. A white precipitate formed and the solution was chilled on ice until no further settling of solid was detected. The precipitate was collected by filtration, washed with water, acetone, and ether and air dried to afford 0.30 g of white powder. Mass spectrum: m/e 44 (CO_2) and m/e 111 (P-44); ir (KBr) 3700-2500 (broad), 1650, 1575, 1480, 1380, 1350, 1330, 1075, 990, 940, 780 cm^{-1}.[27] Decarboxylation of 0.0898 g of this material produced 0.0391 g of $BaCO_3$ or 77% of theoretical.

Anal. Calcd for $C_4H_5N_5O_2$: C, 30.97; H, 3.23; N, 45.15. Found: C, 29.95; H, 5.41; N, 41.15.

2,4-diamino-*s*-triazine hydrochloride (formoguanamine hydrochloride) (**10**).---To 5 ml of 1 *N* HCl in a 12 ml test tube was added 0.05 g of crude charcoal-eluted pH 2 precipitate. The suspension was heated in a boiling water bath for 45 minutes, withdrawn, and cooled to room temperature. A solid (0.008 g) precipitated, was filtered and discarded. The filtrate was evaporated twice to dryness with water and the white residue was dried for 12 hours under vacuum over P_2O_5-KOH to afford 0.05 g of solid whose ir spectrum was essentially identical to the spectrum of 2,4-diamino-*s*-triazine hydrochloride (**10**). Ir (KBr): 3250, 3120, 2750, 1675, 1630, 1600, 1550, 1500, 1440, 1370, 1220, 1160, 1040, 1000, 960, 842, 790, 675 cm^{-1}.

2,4-Diamino-6-hydroxy-*s*-triazine (Ammeline) (**11**) by Oxidative Decarboxylation of (**8**).---To 3 ml 1 *N* HCl in a 12 ml test tube was added 0.03 g of charcoal-eluted pH 2 precipitate (**8**) (Method II) and 0.5 ml 30% H_2O_2. The mixture was heated on a boiling water bath for 30 minutes. The pH of the resulting homogeneous colorless solution was adjusted to pH 4 by neutralization with 0.3 ml 10 N KOH and 1 drop of glacial acetic acid. A white precipitate formed immediately and was filtered, washed with water, acetone, ether and air dried to yield 0.016 g of white powder. The powder was dissolved in 2 ml hot 0.5 *M* Na_2CO_3 solution and allowed to cool slowly to room temperature. The solid which separated on cooling was filtered, washed with water, acetone, and ether and air dried to afford 0.008 g of white powder. Mass spectrum: m/e 111, 127. The mass spectrum of authentic ammeline showed the same prominent peaks. The ir spectrum was in agreement with that presented by Padgett and Hamner[42].

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2,4-Diamino-6-hydroxy-*s*-triazine (Ammeline) (**11**) by Alkaline Hydrolysis of 2,4-Diamino-6-chloro-*s*-triazine.---To a solution of 100 ml 1.0 *N* KOH was added 1 g (0.007 moles) of 2,4-diamino-6-chloro-*s*-triazine. The solution was boiled with vigorous stirring until all the solid had dissolved and allowed to cool slowly to room temperature. When cool, the pH of the resulting solution was adjusted to pH 6 by the addition of concentrated hydrochloric acid. The white solid which precipitated was collected by filtration and washed with water, acetone, and ether to afford 0.78 g (89%) of ammeline. The infrared spectrum of this material was in good agreement with the spectrum presented by Padgett and Hamner[42] although the spectrum of ammeline prepared by the above method showed greater resolution.

2-Amino-4-hydroxy-*s*-triazine-6-carboxylic acid hemihydrate (**12**) by Hydrogen Peroxide Oxidation of Guanine.---To a solution of 100 ml 1 *N* KOH was added 2 g guanine (0.013 moles) and 9 ml 30% hydrogen peroxide (0.08 moles). The homogeneous solution was kept at 40° for 24 hours. At the end of the incubation the excess hydrogen peroxide was destroyed with MnO_2 and the alkaline solution filtered. The pH of the resulting filtrate was adjusted to pH 6 by the addition of concentrated HCl. Unreacted guanine precipitated and was filtered. The pH of the resulting filtrate was adjusted to pH 2 by the addition of concentrated HCl. The white solid which precipitated was collected by filtration, washed with water, acetone, and ether and air dried to afford 0.23 g (11%) of solid. Ir (KBr) 3600-2000 (broad) 3150, 1730, 1675, 1620, 1525, 1455, 1375, 1350, 1220, 1120, 1055, 1010, 925, 830, 780, 770 cm^{-1}.

Anal. Calcd for $C_4H_4N_4O_3 \cdot 1/2\ H_2O$: C, 29.09; H, 3.03; N, 33.94. Found: C, 29.39; H, 3.12; N, 34.50.

2-Amino-4,6-dihydroxy-*s*-triazine (Ammelide) (**13**) by Oxidative Decarboxylation of (**12**).---To a solution of 3 ml 1 *N* KOH in a 12 ml test tube was added 0.08 g (**12**) and 0.5 ml 30% hydrogen peroxide. The mixture was heated on a boiling water bath until all material dissolved (15 minutes), removed, and allowed to cool slowly to room temperature. The pH of the colorless solution was adjusted to pH 4 by neutralization with 0.3 ml 10 *N* KOH and 1 drop of glacial acetic acid. A solid separated immediately, and the solution was allowed to stand at room temperature until no further settling of solid was apparent. The precipitate was collected by filtration, washed with water, acetone, and ether and air dried to afford 0.043 g (61%) of white powder whose ir spectrum was in excellent agreement with the spectrum for ammelide (**13**) (2-amino-4,6-dihydroxy-*s*-triazine) presented by Padgett and Hamner[42].

Anal. Calcd for $C_3H_4N_4O_2$: C, 28.12; H, 3.12; N, 43.75. Found: C, 27.92; H, 3.10; N, 43.83.

2-Amino-4-hydroxy-*s*-triazine-6-carboxylic acid (**12**) by Permanganate Oxidation of Guanine.---Oxidation of 2 g (0.013 moles) guanine in 100 ml 1.0 N KOH by 2 equivalents of potassium permanganate for 24 hours at 40° produced 0.4 g (20%) of (**12**).

2-Amino-4-hydroxy-*s*-triazine-6-carboxylic acid (**12**) by Oxidation of 8-hydroxyguanine with Potassium Peroxodisulfate, Potassium Permanganate, and Hydrogen Peroxide.---To 100 ml of 1 N KOH was added 2 g of 8-hydroxyguanine (0.012 moles) and 6.5 g $K_2S_2O_8$ (0.024 moles). The solution was stirred at room temperature until all the $K_2S_2O_8$ had dissolved. The homogeneous solution was stored at 40° for 24 hours. Following the incubation, the pH of the pale-green solution was adjusted to pH 6 with concentrated HCl. Any precipitated solid was filtered and the pH of the filtrate adjusted to pH 2 by the addition of concentrated HCl. A white solid precipitated immediately and the solution was allowed to stand at room temperature until settling of solid was complete. The white solid was collected by filtration, washed with water, acetone, and ether and air dried to afford 0.33 g (17%) of (**12**) as confirmed by its ir spectrum.

Oxidation of 2 g (0.012 moles) 8-hydroxyguanine in 100 ml 1.0 *N* KOH by 2 equivalents of potassium permanganate for 24 hours at 40° produced 0.7 g (37%) of 2-amino-4-hydroxy-*s*-triazine-6-carboxylic acid

Oxidation of 2 g 8-hydroxyguanine by 4 equivalents of hydrogen peroxide under identical reaction conditions produced 0.5 g (32%) of the same triazine carboxylic acid.

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67. This ir spectrum is identical to the ir spectrum of authentic material prepared according to Brüning's method[27] by $KMnO_4$ oxidation of acetoguanamine (2,4-diamino-6-methyl-*s*-triazine).

We thank the American Cyanamid Co. for a generous supply of acetoguanamine.

recovered after 24 hr of hydrogen peroxide oxidation even in the presence of a fourfold molar excess of peroxide.

Urea and 2-amino-4-hydroxy-*s*-triazine-6-carboxylic acid are products of 8-hydroxyguanine oxidation in 1 *N* KOH at 40° when either potassium peroxodisulfate, potassium permanganate, or hydrogen peroxide is used as oxidant. Unreacted 8-hydroxyguanine is recovered when hydrogen peroxide is used as an oxidant. A schematic summary of these transformations is included in Scheme II. Oxidative decarboxylation of 2-amino-4-hydroxy-*s*-triazine-6-carboxylic acid produced a material whose ir spectrum is in excellent agreement with that presented for ammelide[42] (2-amino-4,6-dihydroxy-*s*-triazine, **13**). The elemental analysis is also correct for ammelide.

Discussion

A reasonable mechanism for the formation of uracil 5-sulfate and cytosine 5-sulfate from the reactions of uracil and cytosine with peroxodisulfate in 1 *N* KOH involves bimolecular nucleophilic displacement by the cytosine anion or the uracil dianion on the peroxide oxygen of peroxodisulfate. A similar mechanism has been invoked in the peroxodisulfate oxidation of phenols[49,50] and aromatic amines[51] in alkaline solution. Nucleophilic displacements on peroxide oxygen are well known and have been reviewed by Edwards,[52] Curci and Edwards,[53] and Behrman and Edwards.[54]

Neither cytosine 5-sulfate or uracil 5-sulfate has been previously described. The hydrolysis of cytosine 5-sulfate in 6 N HCl provides a convenient method for the synthesis of 5-hydroxycytosine, a material available previously in low yield through a multistep procedure.[38] Cier, *et al.*,[55] report this material as a product formed from the reaction of the Fenton reagent on cytosine. Ekert and Monier[56] suggest that it is one of the products formed from cytosine in aerated aqueous solution under the influence of X-rays, although no evidence was given.

We suggest that the peroxodisulfate oxidations of guanine, thymine (at 40°), and adenine also proceed *via* initial bimolecular nucleophilic displacement on the peroxide oxygen of peroxodisulfate. The site of attack by peroxodisulfate cannot be described with certainty for any of these substrates. Neither the rate of peroxodisulfate consumption nor the rate or extent of ring-cleavage product formation is affected by the presence of acrylamide, a known free-radical trap. This suggests that for at least the major part of these reactions a free-radical mechanism is not involved. If any of the product-forming steps in these reactions involved a significant free-radical contribution, then the introduction of a radical trap known to react with sulfate radical ions ($SO_4\cdot^-$) or hydroxyl radicals ($HO\cdot$) would result in a decrease in both the yield and rate of formation of the ring-cleavage products in these reactions. The increases observed in the overall consumption of peroxodisulfate in the presence of acrylamide and nucleic acid bases in 1 *N* KOH indicates that peroxodisulfate reacts with acrylamide under these reaction conditions but the reaction between peroxodisulfate and nucleic acid base is not significantly altered.

The observed activation energies for the oxidation of all the nucleic acid bases are in the range of 9–14 kcal mol^{-1}. The entropies of activation are in the range −28 to −34 cal mol^{-1} deg^{-1}. These values are consistent with a large number of activation energies and entropies of activation for reactions involving nucleophilic displacement on peroxide oxygen[52-54] and are very similar to the values obtained for the peroxodisulfate oxidation of phenols[49,50] and aromatic amines[51] in alkaline solution. Activation energies for reactions involving formation of sulfate-anion radicals ($SO_4\cdot^-$) by homolysis of peroxodisulfate in the rate-limiting step are commonly of the order of 25 kcal mol^{-1}.[26]

Guanine reacts more rapidly with peroxodisulfate than any of the other nucleic acid bases under the conditions employed in this investigation. The identified products are urea, guanidine, and 2,4-diamino-*s*-triazine-6-carboxylic acid.

2-Amino-4-hydroxy-*s*-triazine-6-carboxylic acid has evidently not been previously described. 2,4-Diamino-*s*-triazine-6-carboxylic acid has been reported in the patent literature.[57] The structural assignment for 2,4-diamino-*s*-triazine-6-carboxylic acid is based on evidence that it decarboxylates in hot acidic solution to formoguanamine (2,4-diamino-*s*-triazine). It is oxidatively decarboxylated under the same conditions in the presence of hydrogen peroxide to ammeline (2,4-diamino-6-hydroxy-*s*-triazine). These transformations are analagous to those reported for oxonic acid (2,4-dihydroxy-*s*-triazine-6-carboxylic acid).[43-48] Oxonic acid decarboxylates in acid solution to allantoxaidin (2,4-dihydroxy-*s*-triazine). Oxidative decarboxylation in the presence of hydrogen peroxide affords cyanuric acid (2,4,6-trihydroxy-*s*-triazine). Thus, the oxidation of uric acid,[43-48] guanine, and 8-hydroxyguanine in alkaline solution afford triazinecarboxylic acids as oxidation products.

The formation of 2,4-diamino-*s*-triazine-6-carboxylic acid by peroxodisulfate oxidation of guanine in alkaline solution is particularly interesting since the permanganate and hydrogen peroxide oxidation of guanine produce 2-amino-4-hydroxy-*s*-triazine-6-carboxylic acid under the same reaction conditions. We failed to detect any deamination of 2,4-diamino-*s*-triazine-6-carboxylic acid to the 2-amino-4-hydroxy compound after 24-hr incubation in 1 *N* KOH at 40° and conclude that the formation of the former triazinecarboxylic acid by peroxodisulfate oxidation of guanine must proceed by a pathway which differs from the pathway of hydrogen peroxide and permanganate oxidation.

There is little justification for presentation of a mechanism for the peroxodisulfate oxidation of guanine analagous to Brandenberger's proposed mechanism for the alkaline oxidation of uric acid.[47] We can, however, rely on the previous investigations of Brandenberger[44-46] and Cannelakis and Cohen[43] to lend support to our contention that the mechanisms for the oxidation of either purine are probably similar.

Brandenberger and Cannelakis and Cohen demonstrated that carbons 2, 4, and 8 of uric acid were incorporated in the triazine ring of the oxonic acid formed as a result of the alkaline oxidation of uric acid using either hydrogen peroxide or potassium permanganate as an oxidant. It seems reasonable that the same carbons of guanine are incorporated in the triazine ring of 2,4-diamino-*s*-triazine-6-carboxylic acid as a result of the alkaline oxidation of guanine by potassium peroxodisulfate.

This conclusion is based on the following evidence. Both urea and guanidine are liberated in the peroxodisulfate oxidation of guanine, although the molar ratio of urea or guanidine produced per mole of guanine oxidized is less than 1. Control experiments indicate that neither urea nor guanidine is attacked by peroxodisulfate in 1 *N* KOH or in 1 *M* Na_2CO_3 solution. Guanidine, however, is degraded by prolonged incubation in 1 *N* KOH in the absence of peroxodisulfate. Paper chromatograms of 1% guanidine hydrochloride solutions incubated at 40° for 24–48 hr in 1 *N* KOH reveal the presence of guanidine and at least two materials which are detected by Ehrlich's reagent. Although one of the Ehrlich-positive spots is urea, we point out that the production of urea from guanidine degrada-

tion is not significant over the time period used to measure the complete oxidation of guanine in 1 M Na_2CO_3 solution and since the same stoichiometries are observed for the degradation of guanine in both 1 N KOH and 1 M Na_2CO_3, we conclude that the urea liberated in both cases is not exclusively due to the alkaline degradation of guanidine. Hence, guanidine liberation represents destruction of the pyrimidine portion of the guanine molecule while urea production must represent oxidative degradation of the imidazole ring.

The molar ratio of urea and guanidine liberated per mole of guanine oxidized is 0.25 and 0.55, respectively. Under the assumption that the urea liberated contains carbon 8 of the guanine molecule and that the guanidine liberated contains carbon 2, then no more than a 20% yield of 2,4-diamino-*s*-triazine-6-carboxylic acid could be produced if carbons 2 and 8 of the guanine skeleton were incorporated into the triazine ring of 2,4-diamino-*s*-triazine-6-carboxylic acid. The measured yield of this material by determination of the amount of carbon dioxide liberated from weighed samples of acid-precipitable guanine oxidation product is about 13%.

While the measured amounts of guanidine and urea are identical for the peroxodisulfate oxidation of guanine in either 1 N KOH or 1 M Na_2CO_3 solution, no solid can be collected on acidification of reactions following oxidation in 1 M Na_2CO_3 solution. We conclude that the formation of triazinecarboxylic acid by guanine oxidation under these conditions must either not take place or takes place to a lesser extent than in the case of guanine oxidation in 1 N KOH. Similarly, no 2-amino-4-hydroxy-*s*-triazine-6-carboxylic acid can be isolated from acidic solution when 8-hydroxyguanine is oxidized by potassium peroxodisulfate in 1 M Na_2CO_3 solution. It is reasonable to conclude that solutions of higher alkalinity are required for the formation of triazinecarboxylic acid by oxidation of either purine.

Urea production in the case of thymine oxidation by potassium peroxodisulfate in 1 N KOH at 40° indicates destruction of the pyrimidine ring but again the molar ratio of urea produced per mole of substrate oxidized is less than 1. Urea production in the alkaline oxidation of adenine represents degradation of the original molecule, but the structure of the intact adenine ring system presents at least two possible sites for oxidative release of urea under alkaline conditions.

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