Acid and Micellar Catalysis of the Decomposition of 9-Alkylpurine-6-diazotates[1,2]

Clifford A. Bunton* and Barry B. Wolfe

Contribution from the Department of Chemistry, University of California, Santa Barbara, California 93106. *Received March 4, 1974*

Abstract: At pH >1, the decomposition of 9-propylpurine-6-diazotate (Ia) in aqueous solution gives 9-propylhypoxanthine (IIa), but at lower pH, and especially in the presence of chloride ion, Ia gives 9-propyladenine (IIIa) by denitrosation. For the protonation of Ia, pK_a = 5.32, and for protonation of the diazohydroxide, it is 1.34. At pH >1, the reaction is general acid catalyzed with a slow proton transfer to the diazohydroxide, in agreement with a small, but normal, deuterium solvent isotope effect for hydronium ion catalysis. The rate increases steadily with decreasing pH up to 1, where a plateau is observed, and at pH <0, the rate again increases because of incursion of denitrosation, and the pH–rate profile can be fitted quantitatively up to pH 0. The spectra of the intermediate diazohydroxide and its conjugate acid have been measured. Anionic micelles of sodium lauryl sulfate catalyze, and cationic micelles of cetyltrimethylammonium bromide inhibit, the decomposition of 9-octylpurine-6-diazotate (Ib).

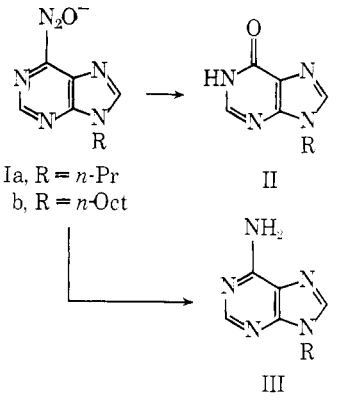
Nitrous acid can change the base sequence of a nucleic acid by deaminating purine and pyrimidine residues,[3] and the mutagenesis can be related to the way in which deamination changes the hydrogen bonding between base pairs. Reactions of nitrous acid are of additional importance, because nitrites are used as food preservatives,[4] and nitrogen oxides are smog constituents.[5]

The first step of deamination of primary amines is N-nitrosation[6] which is followed by a series of rapid reactions. Aromatic diazonium ions can be isolated, but aliphatic diazonium ions have at the most only a transient existence. However, secondary *N*-nitrosamines are stable and are active carcinogens,[4] and their formation from nitrous acid is reversible.

We have examined part of a deamination sequence using the decomposition of pyridine-2- and -4-diazotates,[9] and we now report an extension of this investigation using adenine derivatives, because related compounds could be involved in nitrous acid deaminations in a nucleic acid.

Decomposition of pyridine-2- and -4-diazohydroxide gives a diazonium ion which is very short lived for the 2-compound, but relatively long lived for the 4- compound, and we determined the rate constants for decomposition and trapping by phenoxide ions of the pyridine-4-diazonium ion.[9] At pH <1, the decomposition of pyridine-4-diazohydroxide is complex, and the products depend on the reaction conditions. The pyridinediazotates behave somewhat similarly to the analogous benzene compounds, except that the 2-diazonium ion is very unstable, and protonation of the pyridine nitrogen atom can be important in reactions of the 4-diazotate. We expected that diazotates of purines would be intermediate between pyridine and alkyl diazotates in their behavior, and we used an adenine derivative, 9-alkylpurine-6-diazotate (Ia,b), which at pH >1.5 gives 9-alkylhypoxanthine (IIa), but in more acidic media gives 9-alkyladenine (III) by denitrosation.

We also examined micellar catalysis of the decomposition of 9-octylpurine-6-diazotate (Ib). Many biological reactions occur readily at water–lipid interfaces, and micelles provide a simple model for such interfaces. For discussions of micellar catalysis and inhibition, see ref 10–14.



Ia, R = *n*-Pr
b, R = *n*-Oct

II

III

Experimental Section

Materials. 9-Propyladenine (IIIa)[15] was prepared from adenine (15 mmol) in dry DMF (40 ml) with K_2CO_3 (15 mmol) and *n*-PrBr (30 mmol). The mixture was stirred at 38°, and the reaction was followed by tlc (Sigel, EtOH:$CHCl_3$, 1:9). When the reaction was complete (2 days), water (75 ml) was added, and the volatiles were removed in a rotary evaporator. This procedure was repeated several times, and the crude product was extracted with hot methyl ethyl ketone. Several crystallizations from the same solvent gave fine white needles, mp 173.5–174° (lit.[16] 168°). 9-Octyladenine (IIIb) was prepared in the same way except that the crude product was obtained as an oil by pouring the reaction mixture into water; it was extracted into EtOAc and dried (K_2CO_3). After evaporation of EtOAc, the oil solidified on treatment with petroleum ether, and after recrystallization (methyl ethyl ketone) it had mp 126–127.5° (lit.[17] 131°); Ia and Ib were homogeneous by tlc. 9-Propylhypoxanthine (IIa)[18] was prepared by deaminating 9-propyladenine (IIIa, 0.33 mmol) with 4 *M* $NaNO_2$ (2 ml) in 3 *M* acetate buffer (1:1, 10 ml). After 16 hr, the water was pumped off, and IIa was extracted with methyl ethyl ketone and was recrystallized from methyl ethyl ketone, mp 255–260° (lit.[17] 259–260°). The diazotates were prepared by standard methods.[19] 9-Propyladenine (IIIa, 3 mmol) was treated with $NaNH_2$ (3 mmol) and isoamyl nitrite (3.2 mmol) in dry THF (130 ml) under N_2. After 15 days, a flocculent green solid was recovered by centrifugation and was washed several times with dry THF and vacuum dried. It had λ_{max} 304 nm, and a portion was purified in solution by chromatography (TEAE-cellulose, $EtN^+H_3HCO_3^-$ 0.05 *M*).[9] A portion of the solid was acidified (dilute HCl), and gave 9-propylhypoxanthine (IIa); λ_{max} 249 and 255 nm in base (pH 12). Sodium 9-octylpurine-6-diazotate (Ib) was similarly prepared. It had λ_{max} 302 nm, and on acidification this peak slowly shifted to λ_{max} 250 nm.

Sodium lauryl sulfate (NaLS) was recrystallized from aqueous EtOH, and cetyltrimethylammonium bromide (CTABr) was recrystallized several times from acetone–EtOH.[20]

Kinetics. The reactions were followed spectrophotometrically, at 25.0°; *cf.* ref 9. The observed first-order rate constants, k_ψ, were calculated using the integrated first-order rate equation.

Spectra. The spectra of the stable species were measured on a Cary 11 or 15 spectrophotometer.

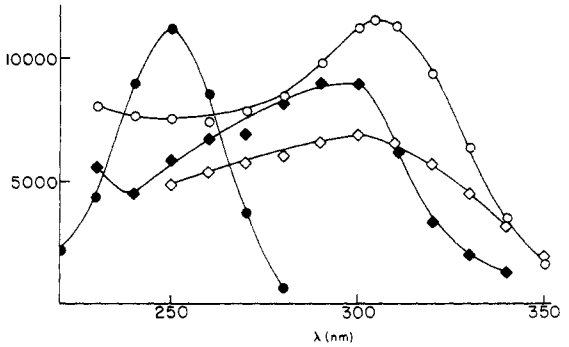


Figure 1. Spectra of 9-propylhypoxanthine (●) and 9-propylpurine derivatives: diazotate (○), diazohydroxide (◇), and protonated diazohydroxide (◆).

Table I. Rate Constants for Decomposition of 9-Propylpurine-6-diazotate in Strong Acid[a]

C_{H^+}, M	10^3k_ψ, sec^{-1}	C_{H^+}, M	10^3k_ψ, sec^{-1}
4.90[b]	51.5	0.110[e]	10.3
3.44	364	0.101	10.8
3.44[c]	50.9	0.094	10.6
3.37[d]	92.1	0.0803	10.1
3.08[b]	17.1	0.0615	9.58
2.24	98.0	0.0411	7.44
2.12[e]	18.6	0.214	5.11
1.60	51.2	0.0117	3.59
1.17[d]	17.0	0.010[f]	3.28
1.10[e]	10.3	0.0087[g]	2.93
1.06[e]	10.4	0.0073	2.32
1.03[e,e]	72.7	0.0048	1.68
1.04[b]	14.9	0.0032	1.21
0.98[b]	13.4	0.0019	0.80
0.94	23.4	0.0014	0.61
0.31[e]	10.2	0.0010	0.47
0.115[e,f]	6.29	0.00036	0.29
0.115[e]	10.2		

[a] HCl unless specified. [b] $MeSO_3H$. [c] $HClO_4$. [d] H_2SO_4. [e] With 2 M NaCl. [f] With 1 M sodium salt. [g] With 0.1 M NaCl.

The spectrum of 9-propylpurine-6-diazohydroxide was measured by adding the diazotate Ia to 0.1 M formate buffer (pH 3.8) and scanning the spectrum in a Cary 15 spectrophotometer within 108 sec (under these conditions $t_{1/2}$ for the decomposition is *ca.* 40 min).

The spectrum of protonated 9-propylpurine-6-diazohydroxide was determined by adding the diazotate Ia to 1.15 M $HClO_4$ and following the absorbance at various wavelengths for *ca.* 30 sec. The absorbances were extrapolated back to the mixing time using a near-linear plot.

The spectra are shown in Figure 1. They all show a red shift relative to the parent purine, which is consistent with the presence of a diazo group. However, the structure of the diazohydroxide is assumed.

Results and Discussion

Reaction in Aqueous Solution. Aromatic diazonium ions are relatively stable, and pyridine-4-diazonium ion can be trapped, but the 2-ion cannot.[9,21] We expected that a diazonium ion derived from Ia would be very short lived, and it was not trapped by alkaline solutions of phenols or naphthols under conditions used with pyridine-4-diazonium ion,[9,21] and we assume that a diazonium ion is either not formed in the decomposition of I, or that it rapidly gives the products.

The pH–rate profile for the decomposition of 9-propylpurine-6-diazotate (Ia) is shown in Figure 2. The reaction is catalyzed by general acids, and the rate constants, k_0, in Figure 2 are the values of k_ψ obtained by using strong acid (Table I) or by using buffers at given pH values and extrapolating the first-order rate constants to zero buffer concentration[22] (Table II). The different methods for determining k_0 in the absence of buffers agreed, except that at low pH the reaction is faster in HCl than in $HClO_4$, because of catalysis by chloride ion (Figure 2 and Table II), and added chloride ion speeds the reaction. The different values of k_ψ in $HClO_4$, H_2SO_4, and CH_3SO_3H are probably due to different electrolyte effects of the anions.

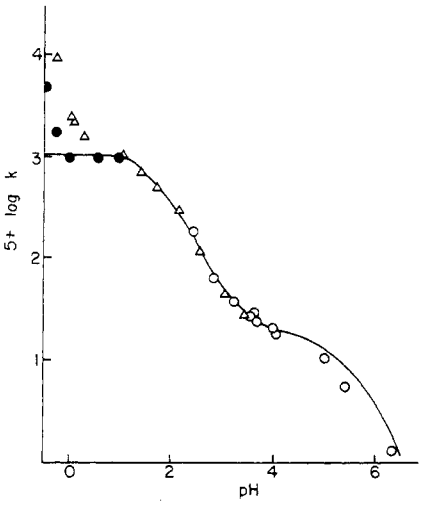


Figure 2. pH–rate profile for decomposition of 9-propylpurine-6-diazotate at 25.0°: HCl (△), $HClO_4$ (●), calculated using buffer dilution (○). The line is calculated using the derived rate constants.

Table II. Rate Constants for Decomposition of 9-Propylpurine-6-diazotate in Buffers

Acid	pK_a	pH	10^3k_{HA}, l. mol^{-1} sec^{-1}	10^4k_0, sec^{-1}
$(CO_2H)_2$	1.23[a]		~110	≈12
$CHCl_2CO_2H$	1.29[b]		~200	≈67
CH_2CNCO_2H	2.43[b]	2.39	19.0	19.1
CH_2ClCO_2H	2.86[b]	2.78	21.4	7.1
$CHOCO_2H$	3.32[c]	3.19	25.4	3.9
$EtOCH_2CO_2H$	3.60[b]	3.55	7.28	3.0
HCO_2H	3.77[a]	3.64	5.63	2.5
$(CH_2CO_2H)_2$	4.19[a]	4.02	3.42[d]	1.8
$CH_2(CH_2CO_2H)_2$	4.34[a]	3.53	3.51[d]	2.9
CH_3CO_2H	4.76[b]	3.97	2.13	2.1
Me_3CCO_2H	5.01[b]	4.98	1.57	1.1
succinate	5.48[a]	5.42	0.56	0.58

[a] H. C. Brown in "Determination of Organic Structures by Physical Methods," E. A. Braude and F. C. Nachod, Ed., Academic Press, New York, N. Y., 1955. [b] J. F. J. Dippy, S. R. C. Hughes, and A. Rozanski, *J. Chem. Soc.*, 2492 (1959). [c] R. M. C. Dawson, D. C. Elliott, W. H. Elliott, and K. M. Jones, "Data for Biochemical Research," Clarendon Press, Oxford, 1959. [d] Corrected for the number of ionizable hydrogens.

General Acid Catalysis. The slopes, k_{HA}, of plots of k_ψ against concentration of the buffer acids are given in Table II together with the intercepts, k_0. The values of K_{HA} are calculated taking into account the number of acidic hydrogen atoms in the catalyzing acid. They fit a reasonably good Brønsted plot (Figure 3), except for the stronger acids (Table II) where the buffering was ineffective, and the pH varied during reaction. The value of the Brønsted exponent, $\alpha = 0.5$, suggests that the proton is partially transferred in the transition state.[23]

General acid catalysis is rarely observed in the decomposition of diazotates,[24] although Machackova and Sterba have observed it in decomposition of 2,4-dinitrobenzene and 2,6-dichloro-4-nitrobenzenediazotate.[25]

Reaction Products. The products which were determined

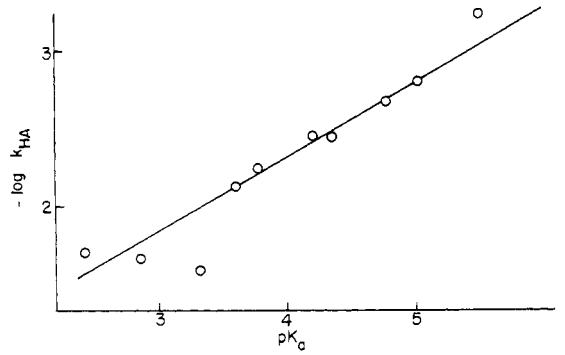


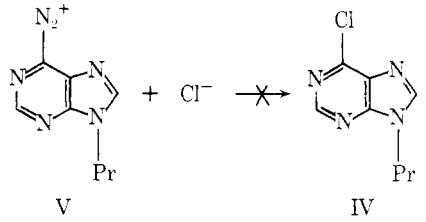
Figure 3. Bronsted plot for decomposition of 9-propylpurine-6-diazotate at 25.0°.

Table III. Products of Decomposition of 9-Propylpurine-6-diazotate

Acid	λ_{max}, nm[a]	Product
6 *M* HCl	260	9-Propyladenine
5.5 *M* $HClO_4$	259	9-Propyladenine
1.15 *M* $HClO_4$	255	9-Propyladenine + 9-propylhypoxanthine[b]
0.115 *M* $HClO_4$	253	9-Propylhypoxanthine + 9-propyladenine[b]
0.05 *M* HCl	250	9-Propylhypoxanthine

[a] The possible products have the following λ_{max}, nm: 9-propyladenine, 260; 9-propylhypoxanthine, 250; 9-propylchloropurine, 233 and 263. [b] Minor product.

spectrophotometrically at complete reaction (Table III) depend on the nature and concentration of the acid. We found no 6-chloro-9-propylpurine (IV) from attack of chloride on a (hypothetical) diazonium ion V, although this reaction is important in the decomposition of pyridine-4-diazotate,[9] where the diazonium ion has a finite life, suggesting that if V exists it has a very short life.



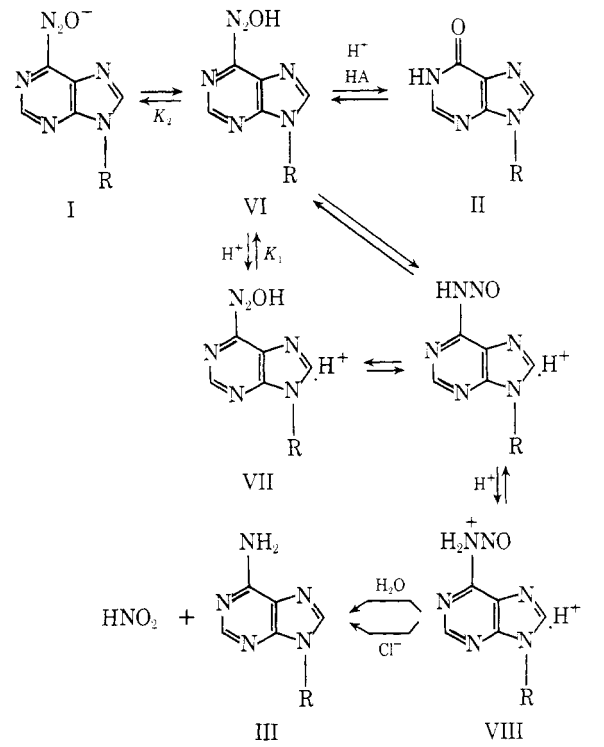
V IV

On the basis of the product composition (Table III), we postulate Scheme I for the overall decomposition of I, and it is consistent with the kinetic evidence. The positions of the protons in some of the structures in this scheme are assumed, for example, in the conjugate acid VII.

Calculation of Rate and Equilibrium Constants. The values of pK_2 were determined spectrally by measuring the absorbance of a solution of chromatographed 9-propylpurine-6-diazotate (Ia) at 300 nm in 10^{-3} *M* NaOH and HCl where diazotate and diazohydroxide are present. The respective absorbances were 0.585 ± 0.002 and 0.382 ± 0.008, and at pH 5.25 the absorbance was 0.475 ± 0.009 (means of eight values), giving pK_2 = 5.32. The value of pK_1 was similarly determined from the absorbances at 285 nm in 10^{-3} *M* HCl and 1.15 *M* $HClO_4$ where the diazohydroxide and its conjugate acid are present, and the absorbances were 0.363 ± 0.005 and 0.507 ± 0.008, respectively, and in 6×10^{-2} *M* HCl where the absorbance was 0.445 ± 0.008 (means of eight values), giving pK_1 = 1.34. Where necessary we used the extrapolation procedure described in the Experimental Section.

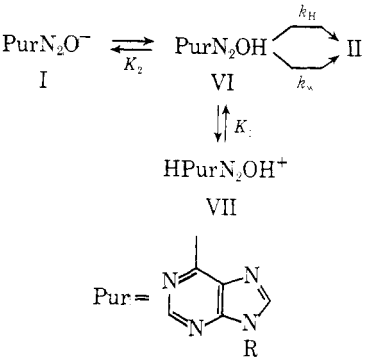
In treating th rate data, we first consider the values of k_0 determined at pH >1 in the region in which we assume that

Scheme I



the conjugate acid VII is unreactive and in which the product is wholly 9-propylhypoxanthine (II), formed from the diazohydroxide VI. (We do not know the position of the proton in VI and VII.) The kinetic scheme is given in Scheme II and neglecting activity coefficients eq 1 applies.

Scheme II



$$k_0 = \frac{k_w + k_H C_{H^+}}{1 + (K_2/C_{H^+}) + (C_{H^+}/K_1)} \quad (1)$$

At pH <3.5, $C_{H^+} \gg K_2$, and eq 1 gives eq 2. A plot of the

$$k_0(1 + C_{H^+}/K_1) = k_w + k_H C_{H^+} \quad (2)$$

left-hand side of eq 2 against C_{H^+} should be linear (Figure 4), and the slopes and intercept calculated using a least-squares program give k_H and k_w (Table IV).

Table IV. Equilibrium and Rate Constants for Decomposition of 9-Propylpurine-6-diazotate[a]

Constant	Solvent: H_2O	Solvent: D_2O[b]
K_1	4.53×10^{-2}	1.73×10^{-2} (2.6)
K_2	4.78×10^{-6}	
$k_H(k_D)$	0.345	0.302 (1.14)
k_w	1.89×10^{-4}	$\sim 10^{-4}$ (1.9)

[a] At 25.0°. [b] Values in parentheses are the isotope effects K_{H_2O}/K_{D_2O} or k_{H_2O}/k_{D_2O}.

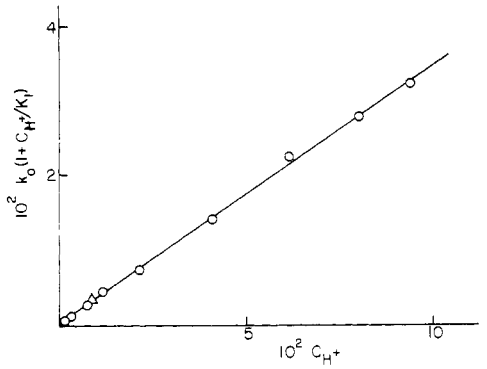


Figure 4. Determination of k_H and k_w for decomposition of 9-propylpurine-6-diazotate: HCl (○), HCl + 0.1 *M* NaCl (△).

The values of k_0 calculated using eq 2 and the rate and equilibrium constants given in Table IV fit the experimental results except in highly acidic media where there is an acid-catalyzed component of reaction (Figure 2), or where salt effects are large.

Solvent Deuterium Isotope Effects. The observation of a general acid catalyzed decomposition suggests that slow proton transfers are kinetically important,[22,23] and therefore we examined the kinetic solvent deuterium isotope effects (Table V). [For the more acidic media, we used perchloric or methanesulfonic acid because of denitrosation by chloride ion (Tables I and III).]

Table V. Deuterium Solvent Isotope Effects on the Reaction of 9-Propylpurine-6-diazotate[a]

Acid	$10^4 k_0^D$, sec^{-1} Obsd	Calcd	k_0^H/k_0^D
4.90 *M* $MeSO_3D$	746		0.69
3.08 *M* $MeSO_3D$	155		1.1
1.53 *M* DCl	1060		0.44
1.09 *M* $DClO_4$	53.7	51.1	1.9
1.04 *M* $MeSO_3D$	53.7	51.1	2.5
1.00 *M* $DClO_4$ + 2 *M* NaCl	2280		0.3
0.519 *M* $DClO_4$	42.7	50.3	
0.14 *M* $DClO_4$	36.3	46.4	
0.111 *M* DCl	49.6	45.1	
0.098 *M* $MeSO_3D$	43.7	44.3	
0.0735 *M* DCl	41.9	42.3	
0.0646 *M* $DClO_4$	38.8	41.2	
0.0255 *M* $DClO_4$	29.2	31.4	
0.025 *M* DCl	33.1	31.1	
0.0165 *M* DCl	26.9	25.9	
0.0122 *M* DCl	21.5	22.2	
0.0083 *M* DCl	18.4	17.6	
0.00628 *M* DCl	15.0	14.6	
0.0042 *M* DCl	11.3	11.1	
0.002 *M* DCl	6.32	6.24	
0.000725 *M* DCl	3.80	3.06	
0.000234 *M* DCl	1.98	1.69	
pD 4.55[b]	1.19	1.1	

[a] At 25.0°. [b] Acetate buffer extrapolated to zero buffer anion $C_{D^-} = 2.82 \times 10^{-5}$ *M*.

For the region pD 1–4, the rate constant, k_0^D, for reaction in the absence of general acids is given by eq 3.

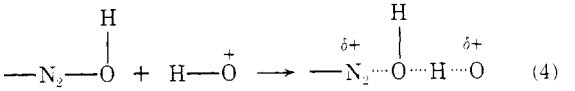
$$k_0^D = \frac{(k_D C_D) + k_{D_2O}}{1 + C_D/K_1^D} \quad (3)$$

The obvious spectral method for determination of K_1^D would have required large volumes of D_2O because of the number of determinations needed (Experimental Section), and two approaches were used in treating the isotope effect. Equation 3 predicts that a plot of $k_0^D(1 + C_{D^+}/K_1^D)$ against C_{D^+} should be linear with slope k_D and intercept k_{D_2O}. The results for reaction of 9-propylpurine diazotate (Ia) in D_2O containing strong acid but no added salt were treated in this way, choosing arbitrary values of K_1^D, and linear plots were obtained when $10^2 K_1^D = 1.7 \pm 0.3$. The intercept k_{D_2O} was relatively independent of the arbitrary value of K_1^D and was approximately 1×10^{-4} sec^{-1}, which is the value of the rate constant in D_2O–acetate buffer pD 4.55 extrapolated to zero acetate (Table V), but the slope depended on the arbitrary value of K_1^D.

Using this value of $k_{D_2O} = 1 \times 10^{-4}$ sec^{-1}, we solved eq 3 to obtain K_1^D and k_D, and a simple least-squares program gave the values in Table IV. They appear to be satisfactory because they give values of k_0^D calculated using eq 3 which are in reasonable agreement with experiment up to pD 1 (Table V).

There is uncertainty in the values given in Table IV because they are measured indirectly. The values of k_H/k_D and k_{H_2O}/k_{D_2O} are both greater than unity as expected for reactions which involve slow proton transfers, but they are much smaller than the value of *ca.* 6 which is traditionally associated with slow proton transfers.[26] However, smaller values are often observed, and have been explained in terms of conservation of zero-point energy for a transition state in which there is either a large or a small degree of proton transfer.[27,28] In addition Swain and his coworkers have suggested that proton transfers between electronegative atoms may give unusually low deuterium isotope effects because the proton in the transition state may lie in a potential energy well,[29] and the traditional treatment of primary deuterium isotope effects for proton transfer assumes a linear transition state; this may be incorrect.[30] There will also be secondary solvent deuterium isotope effects in our reaction because the acids are D_3O^+ (or its hydrate) and D_2O, and there are exchangeable hydrogen atoms in the diazohydroxide VI.

If we assume that the decomposition of the diazohydroxide VI is an S$_E$ displacement on oxygen[31] (eq 4), the transition state could be very unsymmetrical and hence give a small isotope effect. However the value of $\alpha \sim 0.5$ for the general acid catalyzed reaction (Figure 3) suggests that there is extensive proton transfer in this transition state,[22,23] but there are problems in precisely relating α to the extent of proton transfer in the transition state.[23b]



(4)

The value of $K_1^H/K_1^D = 2.6$ is not unreasonable because proton acids in water are stronger than deuterio acids in D_2O,[33-35] and the isotope effect decreases with increasing strength of the weak acid.

Equation 3 only applies to reactions at pH >1, and a new acid-catalyzed reaction appears at lower pH, where the protonated diazohydroxide VII is the bulk species. This reaction becomes especially important in the presence of high concentration of chloride ion. The overall solvent deuterium isotope effect also changes (Table V), and the reaction becomes faster in D_2O than in H_2O suggesting that this new mechanism involves equilibrium protonation followed by nucleophilic attack of chloride ion or water (by analogy) upon a diprotonated species (VIII), although we lack further mechanistic information on the reaction under these conditions, except that denitrosation becomes important (Scheme I), and it is unreasonable to believe that reaction is a single step process.

Micellar Effects. Anionic micelles of NaLS have no ef-

fect on the decomposition of 9-propylpurine-6-diazotate (Ia). Probably Ia is not hydrophobic enough to be incorporated into the micelles, and the reaction of the 9-octyl compound Ib was therefore examined.

The decomposition of Ib in 10^{-2} *M* HCl is inhibited by cationic micelles of CTABr, but the effect is relatively small (Table VI). This observation was expected because,

Table VI. Effect of CTABr on the Decomposition of 9-Octylpurine-6-diazotate[a]

C_{CTABr}, *M*	$10^4 k_\psi$, sec^{-1}
	28.2
0.01	10.6
0.02	7.92
0.05	7.08

[a] At 25.0° in 10^{-2} *M* HCl.

although protons should be excluded from the cationic micelle, the substrate should be taken up by it.[10-14]

The reaction of Ib is catalyzed by anionic micelles of NaLS, and the catalysis increases markedly with increasing pH as shown by the values of k_ψ/k_0, where k_0 is the rate constant in the absence of micelles (Figure 5). The maximum rate constants (k_{max}) for reaction in the presence of NaLS and the rate constants (k_0) in the absence of micelles are given in Table VII. The rate enhancements in-

Table VII. Effect of Anionic Micelles on the Decomposition of 9-Octylpurine-6-diazotate[a]

C_{H^+}, *M*	$10^3 k_0$	$10^3 k_{max}$	k_{max}/k_0
10^{-3} [b]	0.375	6.99	18.6
10^{-2} [b]	2.73	17.7	6.5
0.1	9.59	16.0	1.7
1.0	10.6	22.3	2.1
3.0	38.9	57.5[c]	

[a] In $HClO_4$ unless specified. [b] In HCl. [c] Value obtained with 2×10^{-2} *M* NaLS.

crease with decreasing acidity, as expected in terms of our postulated mechanism. In 10^{-3} *M* HCl, the substrate is present largely as the uncharged diazohydride (VIb) which should be taken up into the micelle, and hydrogen ions should be attracted out of solution into the Stern layer. The transition state is cationic with a structure close to that of the (hypothetical) diazonium ion and should be stabilized by the anionic micelle. However, with increasing acidity the initial state becomes the protonated diazohydroxide (VII), and then the reaction rate in water is almost independent of pH in the range 0-1.5 (Figure 5). The protonated diazohydroxide should be stabilized by the anionic micelle, and this initial state stabilization reduces the micellar catalysis, which is very small for 0.1 *M* perchloric acid, although it increases again as a new acid-catalyzed reaction appears in more acidic solution. (The lability of long chain alkyl sulfates in strongly acid solution causes problems when NaLS is a micellar catalyst in such systems).[36]

As is generally observed with micellar catalyzed bimolecular reactions, there are maxima in plots of rate constant against surfactant concentration.[10-14] These maxima are most pronounced at the lowest acid concentration which is consistent with our earlier suggestion that these maxima are related to the depletion of hydrogen ions in the body of the solution by attraction into the Stern layer of the anionic micelle.[20,37] Micellar catalysis of acetal hydrolysis was treated in terms of this hypothesis,[20] but this simple treatment can-

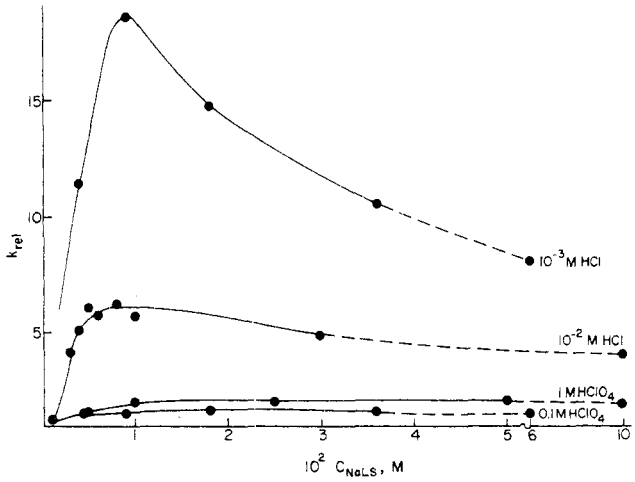
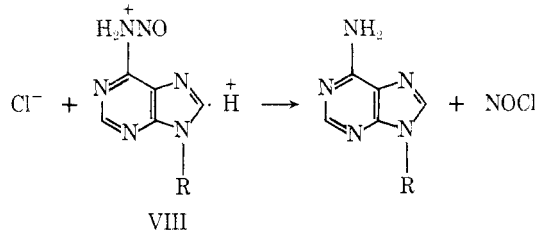


Figure 5. Rate enhancement of the decomposition of 9-octylpurine diazotate by anionic micelles of NaLS at 25.0°.

not be applied to the present reaction because of changes in the charge of the substrate with increasing acidity.

Acid Catalysis. Although the kinetic form for pH > 0.5 can be treated satisfactorily in terms of reactions I → VI → II (Scheme I), additional reactions appear at higher acid concentrations, involving denitrosation rather than deamination (Table III). Denitrosation presumably involves formation of the diprotonated nitrosamine VIII in equilibrium with protonated diazohydride which is the initial state at pH <0.5 (Table IV). Because chloride ion speeds reaction, we have to assume that the rate-limiting step at low pH is bimolecular, at least for chloride ion catalysis, and, because we find no chloropurine (Table III), attack must be on the nitrosoamino group. Water could act in the same way, al-



VIII

though there is no compelling evidence for this assumption. The postulated equilibrium proton transfer is consistent with the deuterium solvent isotope effect of $k_0^H/k_0^D \sim 0.69$ in moderately concentrated methanesulfonic acid (Table V), because in less acidic media where 9-propylhypoxanthine (IIa) is the product, a normal deuterium isotope effect is always observed (Tables I and V). The value of $k_0^H/k_0^D = 0.69$ is in the range expected for an A2 reaction with attack of water upon a conjugate acid.[38] The isotope effect of 0.3-0.4 for reactions in the presence of large amounts of chloride ion is very reasonable because k_H/k_D should decrease when chloride ion rather than water is the nucleophile.

The behavior of pyridine-4-diazotate[9] is quite different from that of 9-propylpurine-6-diazotate, especially for reaction in acidic solution. With pyridine-4-diazotate, we observed relatively slow nucleophilic attack by chloride or aryloxide ions, which probably attacked the diazonium ion, whereas we saw no such reaction with the purine diazotate but nucleophilic attack by chloride ion upon the protonated nitrosamine. (Moreover we observed no effect of 10^{-4} *M* ascorbic acid in 0.115 *M* $HClO_4$ on the reaction rate or products of Ia although ascorbic acid reduced pyridine-4-diazohydroxide under these conditions.[9])

Reaction of Ia at pH >1 appears to be a general acid catalyzed decomposition of the diazohydroxide with a slow proton transfer to oxygen by an SE2 mechanism. At lower pH, a dicationic species builds up, and denitrosation becomes important when protonating power as measured by an acidity function,[39] *e.g.*, h_0, increases rapidly.

Anti-Syn Equilibria. We do not know the configuration of our diazotates, and it is possible that an anti-syn interconversion could be kinetically important, as has been suggested for the decomposition of other diazotates to diazonium ions.[24,25] Several pieces of evidence suggest that equilibration is not kinetically important in our reactions. (1) There is no change in the absorbance of 9-propylpurine-6-diazotate in 10^{-3} *M* NaOH when the solution is heated to 70° for 30 min, provided that correction is made for thermal expansion, and the rates of reaction at 25° in 0.115 *M* $HClO_4$ are identical for the two solutions. In addition there was no deviation from linearity of a first-order rate plot when a small amount of the heated material at 70° was used to start the reaction at 25° (the first absorbance reading was made within 8 sec). These observations suggest either that *syn*- and *anti*-diazotates are in equilibrium or that only one isomer is present. In acid solution, the isomers should be equilibrated *via* the nitrosamine (Scheme I). (2) The deuterium solvent isotope effects are consistent with the postulated kinetic and equilibrium proton transfers but are hard to rationalize in terms of a slow anti-syn isomerization which did not involve acid catalysis.

Our evidence does not of course exclude an acid-catalyzed conversion of an *anti*- into a *syn*-diazohydroxide which very rapidly decomposes to the products,[24,25] and this is an alternative mechanism to direct decomposition.

References and Notes

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