# Decomposition of Pyridine-2- and -4-diazotates[1]

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Abstract: The pH dependence of the decomposition of pyridine-4-diazotate is complex. At pH >6, there is second-order dependence on hydroxide ion concentration due to equilibria between pyridine-4-diazonium ion, -4-diazo-hydroxide, and -4-diazotate. The rate is independent of pH between 4 and 6, and the rate constants for formation of pyridine-4-diazonium ion, its decomposition to 4-pyridone, and its back reaction with hydroxide have been measured. There is a rate minimum at pH 1.5 due to formation of protonated pyridine-4-diazohydroxide, with an acid-catalyzed decomposition in more acid solution. The pK values for the acid dissociations of protonated pyridine-4-diazohydroxide to diazohydroxide and then to diazotate are 4.15 and 6.9, respectively. The products of the acid-catalyzed decomposition depend upon the medium. The normal product is 4-pyridone, but there is considerable denitrosation in more concentrated acid to give 4-aminopyridine, and in addition an intermediate can be trapped by chloride ion giving 4-chloropyridine or by ascorbic acid giving 4-aminopyridine, but without affecting the over-all rate. Kinetic solvent deuterium isotope effects are consistent with the proposed mechanisms. The 4-diazonium ion can be trapped by phenoxide ions, and the rate constants have been estimated, and with the anion of 2-methyl-1-naphthol they approach diffusion control. The decomposition of pyridine-2-diazotate has a pH dependence which can be explained in terms of equilibrium protonation followed by decomposition to 2-pyridone.

Nitrosation by nitrous acid or its derivatives is an important step in the formation of carcinogenic secondary N-nitrosamines[3] and in chemical mutagenesis caused by deamination of a purine or pyrimidine residue in DNA.[4] The object of the present work was to examine one step of the deamination sequence using simple heterocyclic compounds. We chose the aminopyridines because they should have properties in between those of the anilines and the aminopurines or pyrimidines. Deamination is a stepwise process.

$$RNH_2 \xrightarrow{NOX} RNHNO \longrightarrow R\overset{+}{N_2} \longrightarrow products$$
$$\updownarrow$$
$$RN_2O^-$$
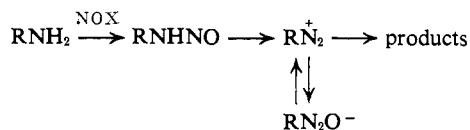
With most aromatic primary amines the diazonium ion is stable, but it is, at the most, a very short-lived reaction intermediate in the deamination of primary aliphatic amines.[5]

There is little information on the reactions of nitrous acid with primary amino derivatives of heterocyclic amines. Purines and pyrimidines have been deaminated,[4] but there is little evidence for long-lived diazonium ions in these reactions. However, Kalatzis has demonstrated the existence of 4-pyridinediazo compounds and showed that the analogous 2-pyridine derivatives were very reactive at low pH.[7] However, the nature of these diazo compounds was not well established except for the diazotates. It seemed, therefore, that the decomposition of the pyridine-2- and -4-diazo-
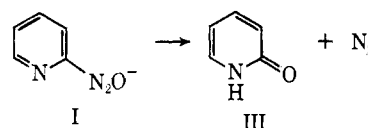
tates (I) and (II) would throw light on the reactions

I   II
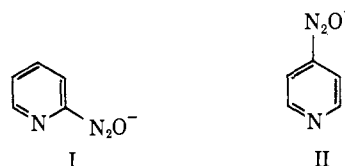
involved in the chemical modification of nucleic acid bases by nitrous acid and on the diazo compounds involved in those reactions.

Results

Decomposition of Pyridine-2-diazotate (I). Decomposition of the diazotate (I) to the pyridone (III)[8] had the

I   III

rate-pH profile shown in Figure 1, where k_ψ is the observed first-order rate constant of reaction, which follows simple first-order kinetics, and we could not trap an intermediate diazonium ion (cf. ref 7).

This pH profile is very similar to those found for the decomposition of benzene diazotates to diazonium ions,[9,10] and Lewis and Hanson reported that the decomposition of pyridine-2-diazotate had this pH profile.[9b] Our values of pK = 6.2 for the acid dissociation of pyridine-2-diazohydroxide and 6×10^{-2} sec^{-1} for the rate constant of its decomposition at 25.0° agree well with theirs, and for pH >6.5 a plot of log k_ψ against pH has a slope of −1.05, so that the reaction sequence

(1) Support of this work by the National Science Foundation and the Arthritis and Metabolic Diseases Institute of the U.S.P.H.S. is gratefully acknowledged.
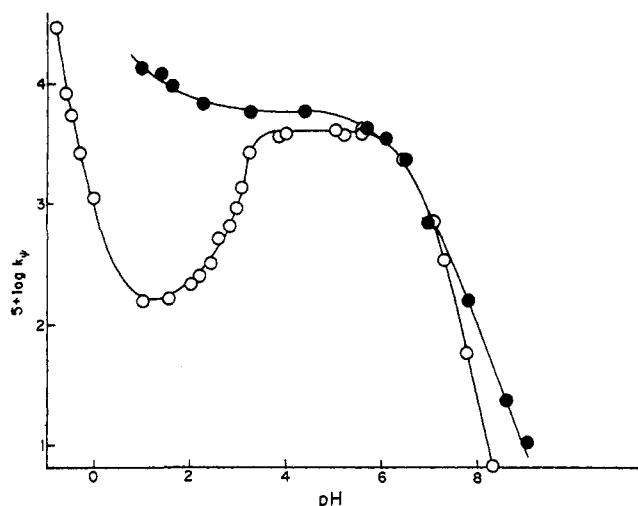(2) U.S.P.H.S. Postdoctoral Fellow.
(3) I. A. Wolff and A. E. Wasserman, *Science*, 177, 15 (1972), and references cited.
(4) H. Schuster and G. Schramm, *Z. Naturforsch. B*, 13, 697 (1958); W. Vielmutter and H. Schuster, *ibid.*, 15, 304 (1960); L. E. Orgel, *Advan. Enzymol. Relat. Subj. Biochem.*, 27, 289 (1965); E. Freese in "Molecular Genetics," J. H. Taylor, Ed., Academic Press, New York, N. Y., 1963, Chapter 5.
(5) For a review of deamination and diazotisations see ref 6.
(6) J. H. Ridd, *Quart. Rev., Chem. Soc.*, 15, 418 (1961).
(7) E. Kalatzis, *J. Chem. Soc. B*, 273, 277 (1967).

(8) Because the 2- and 4-pyridones are in tautomeric equilibria with the hydroxy pyridines, we write the product in whichever structure is convenient.
(9) (a) E. G. Lewis and H. Suhr, *J. Amer. Chem. Soc.*, 80, 1367 (1958); (b) E. G. Lewis and M. P. Hanson, *ibid.*, 89, 6268 (1967).
(10) For a general discussion, see ref 11.
(11) H. Zollinger, "Azo and Diazo Chemistry," Interscience, New York, N. Y., 1961.

Figure 1. Variation of the rate constants for decomposition of pyridine-2-diazotate (solid circles) and pyridine-4-diazotate (open circles) as a function of pH.

can be written as
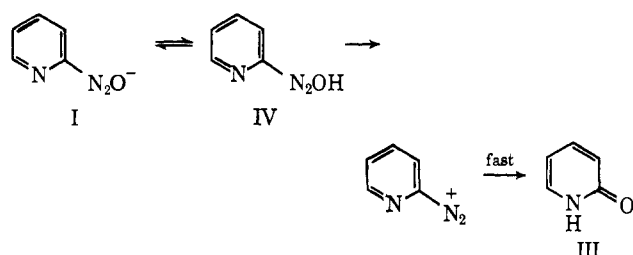
(Here, and elsewhere, we use the term "diazohydroxide" to indicate the conjugate acid of the diazotate, although for convenience we draw the structures as IV and VII.)

Decomposition of Pyridine-4-diazotate (II). The diazotate II is stable in alkali but decomposes rapidly at lower pH giving 4-pyridone (V) except in strong acid where the product depends on the anion of the acid. The pH profile for the rate constant of the over-all reaction to pyridone is shown in Figure 1, and the over-all rate constants are given in Table I. (They are de-

**Table I.** Rate Constants for Decomposition of Pyridine-4-diazotate[a]

pH	$10^4 k_\psi$, sec^{-1}	pH	$10^4 k_\psi$, sec^{-1}
8.40[a]	0.28	3.25[h]	267
7.76[a]	0.50	3.07[h]	135
7.28[a]	33.6	2.97[h]	91.0
7.08[b]	71.2	2.83[h]	64.0
6.46[c]	230	2.57[h]	50.9
5.62[d]	394	2.43[h]	32.5
5.62[a]	382	2.20[h]	24.9
5.61[c]	432	2.02[h]	21.4
5.29[e]	366	1.55[h]	16.0
5.05[e]	410	1.0[h]	15.3
4.02[f]	381	0.0[h]	114
3.88[g]	371		

[a] At 25.0° in aqueous buffers, 0.05 M phosphate. [b] 0.03 M phosphate. [c] 0.1 M phosphate. [d] 0.01 M phosphate. [e] 0.1 M acetate. [f] 0.4 M borax. [g] 0.01 M acetate. [h] Dilute HCl.

termined in some cases from the latter part of a plot of absorbance against time.) Because the rates and products depend upon the nature of the acid, reactions at low pH are discussed later.

There are striking differences between the pH profiles for the decomposition of the 2- and 4-diazotates (Figure 1 and ref 9b), and a diazonium ion (VI) can be trapped in reactions of pyridine-4-diazotate (II) but not in reactions of the 2-diazotate. Therefore, whereas the rate-limiting step for decomposition of pyridine-2-diazotate is conversion of the 2-diazohydroxide (IV) into a very reactive 2-diazonium ion or direct decomposition to products, the decomposition of the 4-diazotate could be a stepwise reaction with build-up of a diazonium ion (VI). At pH 4–6 the overall reaction does not follow first-order kinetics during the first half-life, and this kinetic form is discussed in detail later in the paper (Appendix), the pH profile for decomposition of the 4-diazotate (Figure 1) uses data at pH 4–6 obtained from the latter part of the reaction which followed a first-order kinetic form because at pH 4.5–6 the initial part of the reaction was not first order. It soon became clear that the reaction was indeed stepwise with build-up of at least one intermediate, but by following the decomposition at different wavelengths, and examining the detailed kinetic form of the overall reaction, we attempted to determine the nature of the intermediates and the rate constants for their interconversion. We postulate a series of reactions (Scheme I) for pH 1–8 and consider reaction at pH <1 separately.

Scheme I
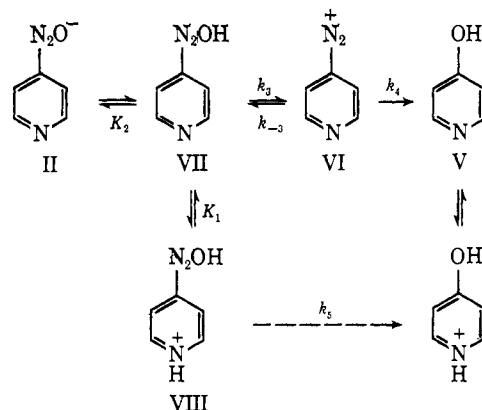
Scheme I is qualitatively consistent with the pH profile (Figure 1). (i) At high pH the 4-diazotate (II) is the predominant species and k_ψ for formation of pyridone increases with decreasing pH and formation of the 4-diazohydroxide (VII) and its conversion into the 4-diazonium ion (VI). On this hypothesis a plot of log k_ψ against pH should have a slope of -2. The actual slope of this plot is -1.95 at high pH (Figure 1). At pH 4–6 the 4-diazohydroxide (VII) is the predominant species and its decomposition and that of the diazonium ion (VI) to products are pH independent. (ii) At pH <4 the 4-diazohydroxide (VII) becomes protonated to give the less reactive conjugate acid (VIII). (iii) At pH <1 new reactions appear which probably involve proton transfer to the diazohydroxide group.

We then determined the various kinetic and equilibrium constants in Scheme I.

Determination of K_2. The acid dissociation constant, K_2, was determined using a stopped-flow spectrophotometer because of the high reactivity of the 4-diazohydroxide (VII) (Experimental Section). In 0.1 M phosphate buffer we calculated $pK_2 = 6.9$ at 25.0°.

Determination of k_3 and the Spectrum of the Diazonium Ion (VI). When repetitive scans were carried out as rapidly as possible at 25.0° on the reaction mixture at pH 4–6 using a Cary 15 spectrophotometer, we found that the diazohydroxide (VII), λ_{max} 313 nm (Experimental Section), disappeared within 20 sec and that a new species was formed which slowly ($t_{1/2} \approx 16$ sec) decomposed to 4-pyridone (V). We assumed that this new species was the diazonium ion (VI) and determined its uv spectrum using a stopped-flow spectrophotometer. The extinction coefficients were calculated indirectly (Experimental Section).

There is an isosbestic point at 273 nm between 4-pyridone and what we assume to be the 4-diazonium ion (VI) (Experimental Section), and if the reaction is followed at this wavelength we follow the disappearance of the 4-diazohydroxide (VII). This approach was used at pH <5.8 where the 4-diazohydroxide is the bulk component, and the reaction then followed a first-order kinetic form for up to 3.1 half-lives. The rate constant k_{obsd} so determined (Table II) is inde-

**Table II.** Rate Constants of Decomposition of Pyridine-4-diazohydroxide[a]

pH	$10k_{obsd}$, sec^{-1}
4.66	1.47
5.05	1.66
5.45	1.62
5.77	1.66

[a] At 25.0° in 0.1 M acetate buffer for pH 4.66–5.45. 0.1 M phosphate buffer was used at pH 5.77.

pendent of pH from pH 5 to 5.8. These k_{obsd} values are much larger than the values of k_ψ for formation of pyridone determined at 254 nm from the final first-order part of the rate plot (Appendix) where all the 4-diazohydroxide has decomposed. We therefore identify k_{obsd} with k_3 in Scheme I.

These observations suggest that under these conditions the reverse reaction of diazonium ion with hydroxide ion is much slower than the forward reaction, otherwise k_{obsd} should be pH dependent. Additional experiments were made to test this point and the possibility that the 4-diazonium ion (VI) might be attacked by both hydroxide ion and water, as in Scheme II.

Scheme II
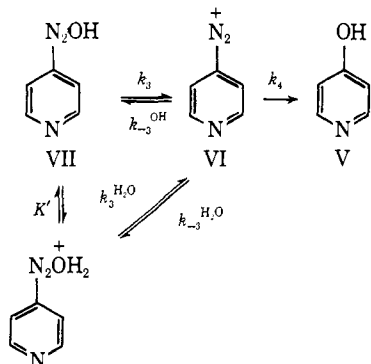
We concluded that $k_{-3}^{H_2O}$ (and $k_{-3}^{OH}[OH^-]$) is much smaller than k_3 because the reaction is first order, when followed at the isosbestic point at 273 nm; whereas, if $k_{-3}^{H_2O}$ was not small relative to k_3, the reaction should
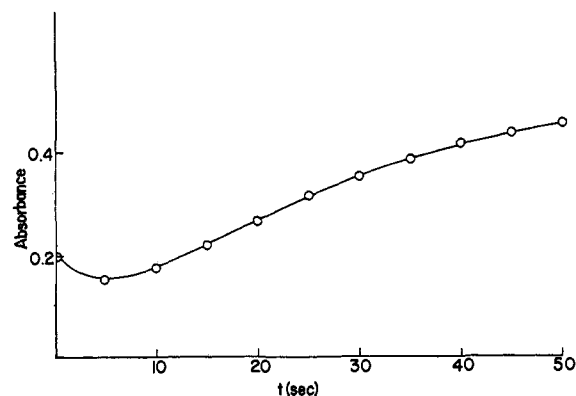
Figure 2. Variation of absorbance at 254 nm with time for the decomposition of pyridine-4-diazotate at pH 5.45. The points were determined experimentally and the line was computed (Appendix).

deviate from a first-order kinetic form. (It should be noted that the equilibrium represented by K' in Scheme II should be in favor of the 4-diazohydroxide (VII) at pH 5.) The question of deviation from first-order kinetics was tested by writing a program for the analog computer for the reaction[12]
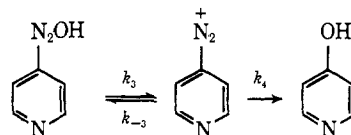
Values of $k_3 = 1.65 \times 10^{-1}$ and $k_4 = 4.1 \times 10^{-2}$ sec^{-1} were used. (The determination of k_4 is discussed in the following section.) For the computed rate plot to be linear for up to 2 half-lives $k_3/k_{-3} > 20$. It is therefore safe to assume that at pH <6 the forward reaction is much faster than the reverse in Scheme II. However, this reverse reaction is important at higher pH.

Conversion of the 4-Diazonium Ion (VI) into 4-Pyridone (V). When the reaction was followed at 254 nm (λ_{max} for 4-pyridone) and pH 4–6, we found that the absorbance decreased for ca. 5 sec and then increased, following a first-order kinetic form (Figure 2). We assume that the initial decrease of absorbance is due to build-up of 4-diazonium ion (VI, λ_{max} 305 nm) and that the rate constant calculated after ca. 20 sec is k_4 for the decomposition of 4-diazonium ion to 4-pyridone (Scheme I).[13] The value of k_4 determined in this way was 4.10×10^{-2} sec^{-1} at pH 4–6 and 25.0°. This is the value of k_ψ at this pH determined by following the over-all reaction conventionally in a Cary 15 spectrophotometer and ignoring the initial curved part of the first-order rate plot. In an Appendix we show that our values of k_3 and k_4 and the absorbances of the reacting species lead to a plot of absorbance vs. time of the form which we observed, and the solid line in Figure 2 was computed.

Evidence for the Reversion of 4-Diazonium Ion (VI) to 4-Diazohydroxide (VII) at High pH. In the preceding section we showed that the 4-diazonium ion went forward to products, rather than reverting to 4-diazohydroxide, at pH 4–6. However, at higher pH the

(12) B. B. Wolfe, Ph.D. Thesis, University of California, Santa Barbara, Calif.
(13) The half-life for decomposition of diazohydroxide is ca. 4 sec., so after 20 sec ca. 95% of it had disappeared.

4-diazonium ion is captured by hydroxide ion faster than it goes to product and is then in equilibrium with 4-diazohydroxide and 4-diazotate ion (II), Scheme III

Scheme III
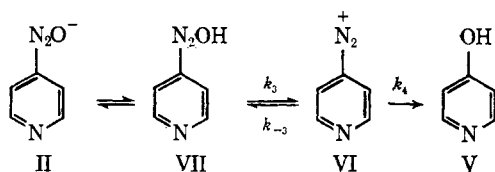
and

$$k_{-3} = \frac{k_3 k_4 [H^+]}{k_4([\overset{+}{H}] + K_2)} - k_4$$

The values of k_{-3} (Table III) show that the stationary

Table III. Reaction of Pyridine-4-diazotate at pH > 6.4

pH[a]	k_{-3}, sec^{-1}	$k_{-3}/[OH^-]$, l. mol^{-1} sec^{-1}
6.46	0.168	5.84×10^6
7.08	0.325	2.71×10^6
7.28	0.533	2.79×10^6
7.76	1.55	2.70×10^6
8.40	7.13	2.84×10^6

[a] Phosphate buffers: 0.1 M at pH 6.46; 0.03 M at pH 7.08; and 0.05 M elsewhere. The reaction was followed at 25.0°.

state treatment is satisfactory for pH >7 because k_{-3} varies linearly with hydroxide ion concentration[14] and the second-order rate constant, $k_{-3}^{OH} = 2.76 \times 10^6$ l. mol^{-1} sec^{-1}. These data confirm that the reaction of the diazonium ion with water is unimportant, and we cannot reliably estimate its rate constant, although it is probably less than 10^{-2} sec^{-1}.

Determination of K_1 and Decrease of Reaction Rate at pH <3.5. Our initial hypothesis was that protonation of the pyridine nitrogen atom of the 4-diazohydroxide (VII) gave the less reactive VIII, and it was necessary therefore to determine the dissociation constant K_1 (Scheme I) and to find evidence for the protonated species VIII. Because of the high reactivity of these species we determined their absorbances at various wavelengths and pH values using a stopped-flow spectrophotometer, following the method used for the determination of K_2. The absorbances were measured from 270 to 320 nm and pH 1.4 to 4.37 in 0.1 M formic or acetic acid buffer. From these absorbances and those of the 4-diazohydroxide (VII) in 0.1 M acetate buffer pH 5.48 and its conjugate acid VIII in 0.05 M hydrochloric acid we calculated $pK_1 = 4.15$, and we confirmed this value kinetically. At pH 2–5 the reversion of 4-diazonium ion to 4-diazohydroxide is slow, and the postulated reactions are
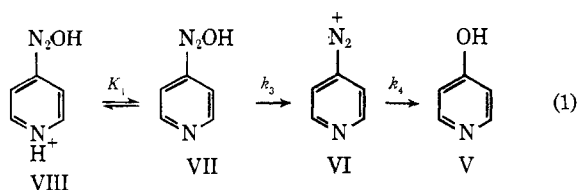
(1)

The differential equations for these consecutive reactions have been solved[15] and give

$$[ArN_2OH] = [ArN_2OH]_0 e^{-k_1 t/a} \tag{2a}$$

$$[ArN_2^+] = (k_3[ArN_2OH]_0/b)(e^{-k_1 t/a} - e^{-k_4 t}) \tag{2b}$$

$$[ArOH] = \frac{[ArN_2OH]_0\{ak_4(1 - e^{-k_1 t/a}) + k_3(e^{-k_4 t/a} - 1)\}}{b} \tag{2c}$$

where

$a = 1 + [\overset{+}{H}]/K_1$; $b = k_4 - k_3/a$; and $[ArN_2OH]_0$ is the initial concentration of pyridine-4-diazohydroxide (VII). The absorbance of the reaction mixture is given by

$$Abs = \epsilon[HArN_2\overset{+}{O}H] + \epsilon'[ArN_2OH] + \epsilon''[Ar\overset{+}{N_2}] + \epsilon'''[ArOH] \tag{3}$$

All the extinction coefficients are known (Experimental Section), and a computer program was written to calculate the concentrations of the various reacting species using eq 2 and 3 and therefore the absorbance at any given time, with various values of K_1.

Plots of ln $(Abs_\infty - Abs_t)$ against time were linear, and the observed and calculated values of the slopes of these plots are given in Table IV for experiments

Table IV. Determination of pK_1 of Pyridine-4-diazotate

pH	pK_1[a]	d ln $(Abs_\infty - Abs_t)/dt$ Calcd	d ln $(Abs_\infty - Abs_t)/dt$ Obsd[b]
2.43	4.00	4.33	3.25
	4.10	3.46	
	4.20	2.76	
3.07	4.00	17.1	13.5
	4.10	14.0	
	4.20	11.3	

[a] Arbitrary values of pK_1. [b] The reaction was followed at 234 nm.

at pH 2.43 and 3.07. The best fit between the observed and calculated slopes is given by $pK_1 \sim 4.15$.

Rate and Equilibrium Constants for Experiments at pH >2. The rate and equilibrium constants for reactions of pyridine-4-diazotate (II) at 25.0° are in Table V. The values of pK_1 and pK_2 are reasonable in terms of known acid dissociation constants for similar compounds.

Solvent Isotope Effects. Scheme I predicts the following kinetic deuterium solvent isotope effects. (i) At pH (pD) >6 the reaction should be faster in D_2O. (ii) At pH (pD) 4–6 the solvent isotope effect should be small. (iii) At pH (pD) 1–4 the reaction should be slower in D_2O.

The results fit the predictions (Table VI), and the values of k_{H_2O}/k_{D_2O} are reasonable in terms of deuterium

(14) The stationary state approximation requires that $k_{-3} \gg k_4$. This condition is not fulfilled at pH 6.46 because $k_4 = 0.041$ sec^{-1}.

(15) E. A. Coddington, "An Introduction to Ordinary Differential Equations," Prentice Hall, New York, N. Y., 1961, p 40.

Table V. Rate and Equilibrium Constants
for Reactions of Pyridine-4-diazotate

Constants	Value
pK_2	6.9^a
pK_1	4.15
k_3	$0.165\ sec^{-1a}$
k_4	$0.041\ sec^{-1}$
$k_{-3}{}^{OH}$	$2.76 \times 10^6\ 1.\ mol^{-1}\ sec^{-1}$
$k_{-3}{}^{H_2O}$	Small

^{a} For pyridine-2-diazotate $pK = 6.2$, and the rate constant for decomposition of the diazohydroxide is $0.06\ sec^{-1}$ at $25.0°$, cf. ref 9b.

Table VI. Kinetic Solvent Deuterium Isotope
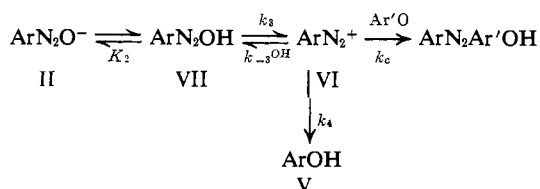Effects for Decomposition of Pyridine-4-diazotate

pH (pD)^a	$10^2k_{D_2O}$	$10^2k_{H_2O}$	k_{H_2O}/k_{D_2O}
7.34	1.45	0.275	0.19
5.20	3.68	3.66	1.0
2.99	2.68	0.933	3.45

^{a} T. H. Fife and T. C. Bruice, *J. Phys. Chem.*, **65**, 1079 (1961).

solvent isotope effects on acid dissociation,[16,17] which appear to be the important factors; although there probably are small effects due to kinetic solvent isotope effects and to secondary structural isotope effects because some of the reacting species (Scheme I) have exchangeable hydrogen atoms.

Trapping of the 4-Diazonium Ion. Kalatzis found that the treatment of 4-aminopyridine with nitrous acid gave an unstable species which coupled with phenols.[7] We have confirmed his observations and also observed coupling reactions starting with pyridine-4-diazotate (II) and determined the rates of reaction of the 4-diazonium ion (VI) with phenoxide and naphtholate ions. We did not observe these reactions with pyridine-2-diazotate (I), in agreement with our assumption that diazonium ion does not build up during decomposition of I.

Rates of Diazo Coupling. At pH 8–10 the 4-diazonium ion (VI) is in equilibrium with 4-diazotate ion (II) and gives 4-pyridone in a rate-limiting step. In the presence of reactive phenols and naphthols over-all reaction to azo dye is zero order with respect to phenol or naphthol so that the rate-limiting step is formation of the 4-diazonium ion, and the coupling reaction (k_c) with $Ar'O^-$ is faster than both the back reaction with

$$\underset{II}{ArN_2O^-} \underset{K_2}{\overset{k_3}{\rightleftharpoons}} \underset{VII}{ArN_2OH} \underset{k_{-3}{}^{OH}}{\overset{}{\rightleftharpoons}} \underset{\downarrow VI}{ArN_2{}^+} \overset{Ar'O}{\underset{k_c}{\longrightarrow}} ArN_2Ar'OH$$

$$\underset{V}{\overset{\downarrow k_4}{ArOH}}$$

hydroxide ion $(k_{-3}{}^{OH})$ and the forward reaction to 4-pyridone (k_4).

However, the anion of *p*-hydroxybenzoate ion (IX) couples relatively slowly with the diazonium ion, though the coupling and reaction with OH^- are still much faster than formation of 4-pyridone (V). Decarboxylation accompanies coupling (Experimental Section).

(16) C. K. Rule and V. K. La Mer, *J. Amer. Chem. Soc.*, **60**, 1974 (1938); C. A. Bunton and V. J. Shiner, *ibid.*, **83**, 42, 3207 (1961).
(17) K. Wiberg, *Chem. Rev.*, **55**, 1713 (1955); V. Gold, *Advan. Phys. Org. Chem.*, **7** 259 (1969).
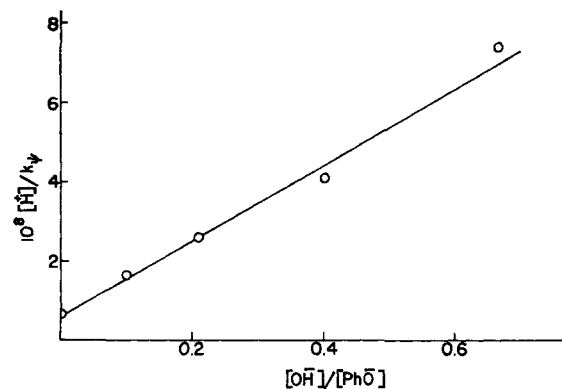
Figure 3. Determination of the rate constant of coupling of pyridine-4-diazonium ion and the anion of *p*-hydroxybenzoate ion.
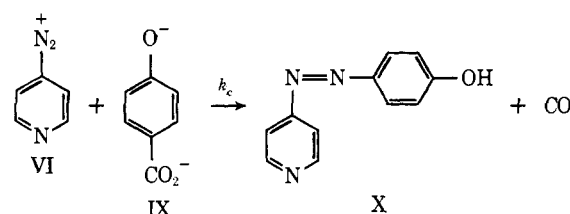
Under these conditions, at pH >8, the rate constant (k_ψ) for the formation of X fits eq 4 and 5; at pH 8

$$k_\psi = \frac{k_c k_3[Ar'O^-]}{(k_{-3}{}^{OH}[OH^-] + k_c[Ar'O^-])(K_2 + [H^+])} \quad (4)$$

$[H^+] \ll K_2$ and

$$\frac{[H^+]}{k_\psi} = \frac{K_2 k_{-3}{}^{OH}[OH^-]}{k_c k_3[Ar'O^-]} + \frac{K_2}{k_3} \quad (5)$$

Plotting $[H^+]/k_\psi$ against $[OH^-]/[OAr^-]$ for reaction with *p*-hydroxybenzoate ion gives a linear plot with slope $K_2 k_{-3}{}^{OH}/k_c k_3$ and therefore the value of k_c. This plot is shown in Figure 3 for reaction followed at 438 nm, and results for the individual runs are in Table VII.

Table VII. Azo Coupling of Pyridine-4-diazonium
Ion and *p*-Hydroxybenzoate Ion^a

$10^5[Ar'OH]$, M	pH	$10^5 k_\psi$, $sec^{-1\ b}$
6.80	9.33	6.30
9.10	9.08	20.2
18.0	9.13	28.2
45.5	9.31	29.6

^{a} $pK_a = 9.39$: L. P. Hammett, *Chem. Rev.*, **16**, 67 (1935). ^{b} At 25.0°.

Knowing $k_c = 1.3 \times 10^5\ 1.\ mol^{-1}\ sec^{-1}$ for reaction of the ion IX we carried out competition experiments between it and more reactive phenoxide ions and analyzed the azo dyes spectrophotometrically. This competition was extended to a series of phenoxide and naphtholate ions (Experimental Section). The second-order rate constants are calculated from eq 6.

$$\frac{k_c{}^R}{k_c} = \frac{K_a[Ar'OH]}{K_a + [H^+]} \cdot \frac{K_a{}^R + [H^+]}{K_a[Ar'OH^R]} \cdot \frac{[Azo]^R}{[Azo]} \quad (6)$$

In eq 6 the superscript R denotes the reference phenoxide ion, $Ar'O^-$. The second-order rate constants,

k_c, for reaction of phenoxide ions with the diazonium ion (VI) in Table VIII approach the diffusion-controlled limit.[18,19]

Table VIII. Second-Order Rate Constants for Azo Coupling of Pyridine-4-diazonium Ion[a]

| Coupling agent	k_c, 1. mol^{-1} sec^{-1}
p-Hydroxybenzoate	1.3×10^5
o-Chlorophenol	1.0×10^5
Phenol	1.8×10^6
2-Naphthol	1.1×10^7
o-Methylphenol	1.2×10^7
o-Methoxyphenol	1.2×10^8
2,6-Dimethylphenol	2.4×10^8
1-Naphthol	1.9×10^9
2-Methyl-1-naphthol	7.2×10^9

[a] At 25.0°.

Reaction in Acidic Solutions. The rates of decomposition of both pyridine-4- and -2-diazotate increase at low pH (Figure 1); however, the rate enhancement for the 2-diazotate (I) is small, and therefore we examined the reaction of the 4-diazotate (II) in detail.

Products. The products of decomposition of II (determined spectrophotometrically) depended upon the nature and concentration of the mineral acid (Table IX). With 1.15 M perchloric acid 4-pyridone

**Table IX.** Products of Reaction of Pyridine-4-diazotate in Acid

Acid	Product[a]
0.1 M HClO$_4$	ArOH (major) + ArNH$_2$[b]
1 M HClO$_4$	ArOH (major) + ArNH$_2$[b]
1 M HCl	ArOH (major) + ArNH$_2$[b]
1 M HClO$_4$ + 3 M NaCl	ArOH + ArCl
2 M HCl	ArOH (major) + ArNH$_2$[b]
4 M HCl	ArCl
4 M HClO$_4$	ArOH + ArNH$_2$[b]
5.5 M HClO$_4$	ArNH$_2$[b]
6 M HCl	ArCl

[a] Ar ≡ N⟨⟩—
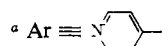
[b] This product was spontaneously renitrosated and slowly gave pyridone.

(λ_{max} 234 nm in acid) was the major product, but in 5.5 M perchloric acid the product had λ_{max} 262 nm in acid and 241 nm in base and was 4-aminopyridine (XI). This product slowly decomposed in the reaction mixture, giving 4-pyridone. The obvious explanation of these observations is that the 4-diazohydroxide (VIII) is denitrosated in 5.5 M perchloric acid, probably through an intermediate nitrosamine (XII) or its conjugate acid, Scheme IV. Nitrous acid then nitrosates

Scheme IV

VIII   XII   XI
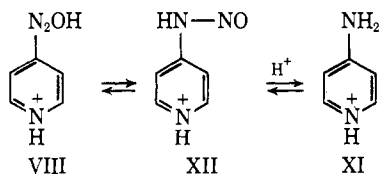
(18) The rate constant for the diffusion-controlled reaction of *p*-nitrobenzenediazonium ion and azide ion is slightly lower than 10^{10} l. mol^{-1} sec.$^{-1}$.[19]

(19) C. D. Ritchie, *Accounts Chem. Res.*, **5** 348 (1972).

and deaminates the kinetically controlled 4-aminopyridinium ion (XI) so that 4-pyridone is the thermodynamically controlled product. The results show that denitrosation and decomposition to 4-pyridone have different acid dependencies.

Denitrosation occurs readily with secondary N-nitrosoamines, and there is no evidence that primary N-nitrosoamines have a more than transitory existence, although they or their conjugate acids could well lose NO$^+$, rather than rearrange to diazo compounds. Kalatzis has reported, although without giving the evidence, that nitrosation of 4-aminopyridine "is reversible" in highly acidic solutions.[7]

These observations suggest that the reactions in Scheme IV are reversible and that it might be possible to trap the intermediates in acid. Ascorbic acid reacts rapidly with nitrous acid and its derivatives[20] and inhibits the formation of secondary alkyl nitrosoamines.[3] When excess ascorbic acid was added to pyridine-4-diazotate (II) in 0.115 M perchloric acid, over 50% of the product was 4-aminopyridine (detected spectrophotometrically) although 4-pyridone was formed in the absence of ascorbic acid. However, ascorbic acid did not increase the over-all reaction rate so that its intervention must be after the rate-limiting step.

Chloride ion, in relatively high concentration, also changes the product composition (Table VIII), giving 4-chloropyridine (XIII) (λ_{max} 228 and 257 nm), presumably by nucleophilic attack upon the 4-diazonium ion or other diazo species.

XIII
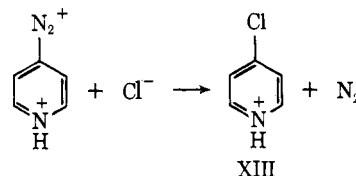
Rates of Acid-Catalyzed Reactions. The rate of decomposition of pyridine-4-diazotate increases sharply as the acid concentration increases (Table X). Although

Table X. Rate Constants for Decomposition of Pyridine-4-diazotate in Aqueous Acid[a]

[H$^+$], M	$10^3 k_\psi$, sec^{-1}	[H$^+$], M	$10^3 k_\psi$, sec^{-1}
0.1	1.53	3.0	55.5
1.0	11.4	4.0	83.4
1.15[b]	19.5	4.0[b]	150
1.15[c]	16.0	6.0	275
2.0	28.6		

[a] At 25.0° and HCl unless specified. [b] HClO$_4$. [c] DClO$_4$ in D$_2$O.

the products are different for reactions in hydrochloric and perchloric acids (Table IX), there is no great difference in the rates, especially in view of the greater protonating power of perchloric acid,[21] and therefore the products must be formed after the rate-limiting step.

(20) C. A. Bunton, H. Dahn and L. Loewe, *Nature (London)*, **183**, 163 (1959); H. Dahn, L. Loewe, and C. A. Bunton, *Helv. Chim. Acta*, **43**, 303, 317, 320 (1960).

(21) R. H. Boyd in "Solute-Solvent Interactions," J. F. Coetzee and C. D. Ritchie, Ed., Marcel Dekker, New York, N. Y., 1969, Chapter 3.

There is a small deuterium solvent isotope effect for reaction in 1.15 M perchloric acid with $k_{H_2O}/k_{D_2O} = 1.22$ suggesting slow proton transfer but that the transition state for this transfer is unsymmetrical. Probably proton transfer to the –OH group of the protonated 4-diazohydroxide (VIII) is involved,[22] because at low pH the pyridine ring of the substrate is fully protonated ($pK_2 = 4.15$). We found no evidence for build-up of any intermediates, and the reactions were cleanly first order, except when denitrosation gave 4-aminopyridine which was itself deaminated (Table IX). A reasonable reaction sequence is shown in Scheme V.

Scheme V
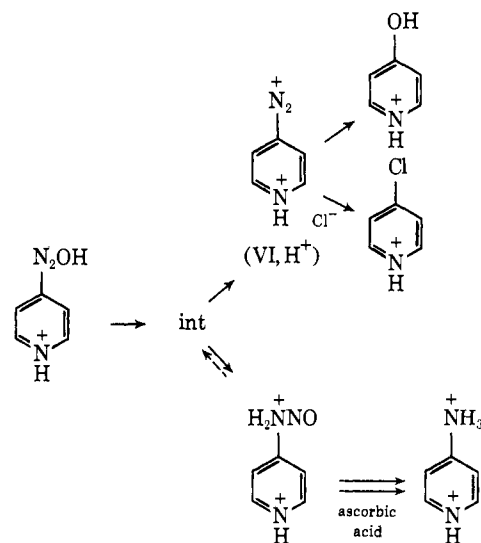
In Scheme V we assume that an intermediate (int) is formed slowly in an acid-catalyzed reaction and decomposes rapidly and that increasing acidity favors its conversion into nitrosoamine. In view of the deuterium isotope effect we expect formation of int to be general acid catalyzed, though it is difficult to test this point at such acidity. The decomposition to 4-pyridone or 4-chloropyridine is shown involving a diazonium ion intermediate, but direct decomposition would fit the evidence just as well, because we could not trap a diazonium ion in acid. If we assume that the substrate is the conjugate acid of the *anti*-diazohydroxide, int could be the protonated *syn*-diazohydroxide (*syn*-diazohydroxides are much less stable than anti).[9,10,25] However, the diazohydroxides and *N*-nitrosoamines, or their conjugate acids, could interconvert by reversible prototropic rearrangements, so that we could be dealing with a tautomeric system. In addition, int should be on the reaction path for the diazotization of 4-aminopyridine in strongly acidic media,[7] and we note that rate-determining proton transfers appear to be important in the diazotization of weakly basic amines.[6]

The rate of decomposition of pyridine-2-diazotate increases slightly at low pH (Figure 1), possibly be-

(22) For discussions of isotope effects in proton transfers, see ref 17, 23, and 24.

(23) C. A. Bunton and V. J. Shiner, *J. Amer. Chem. Soc.*, **83**, 3214 (1961).

(24) C. G. Swain, D. A. Kuhn, and R. L. Schowen, *J. Amer. Chem. Soc.*, **87**, 1553 (1965); *cf.* L. L. Ingraham, *Biochim. Biophys. Acta*, **279**, 8 (1972).

(25) (a) J. S. Littler, *Trans. Faraday Soc.*, **59**, 2296 (1963); (b) O. Machackova and V. Sterba, *Collect. Czech. Chem. Commun.*, **37**, 3313, 3467 (1972).
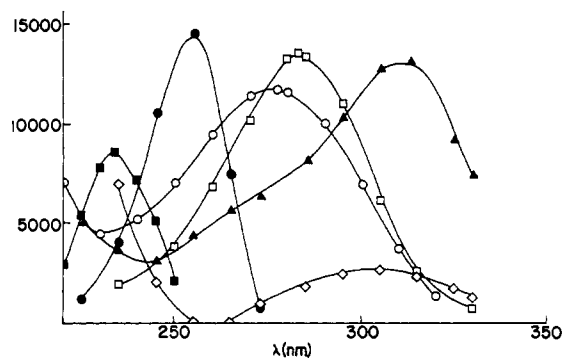
Figure 4. Spectra of reactants, products, and intermediates in the decomposition of pyridine-4-diazotate: (●) 4-pyridone; (■) 4-hydroxypyridinium ion; (○) pyridine-4-diazotate; (▲) pyridine-4-diazohydroxide; (□) pyridinium-4-diazohydroxide; (◇) pyridine-4-diazonium ion.

cause of incursion of an acid-catalyzed dehydration to the diazonium ion.

Possible Anti–Syn Interconversion. We discussed the decomposition of the pyridine diazotates at pH >1 without considering the possibility of a kinetically significant anti → syn interconversion. Lewis and Hanson found a minimum in Hammett plots for decomposition of benzene diazohydroxides to diazonium ions[9] and suggested that, with strongly electron-attracting substituents, the rate-limiting step was conversion of the *anti*- into *syn*-diazohydroxide but that for other substituents it could be direct conversion into diazonium ion, and the pH dependence of the decomposition of diazotates to diazonium ion has been explained in terms of anti–syn interconversions[9,25b] or direct decomposition.[9]

We heated a portion of pyridine-4-diazotate in 0.1 M sodium hydroxide for 1 day and cooled it rapidly but found essentially identical rate constants for both samples at pH from 1 to 7. These observations are consistent with the assumption that our sample was predominantly anti under all conditions,[9] and it could give diazonium ion directly, or the slow step could be conversion of *anti*- into *syn*-diazohydroxide. It is difficult to believe that the intermediate which we draw as the diazonium ion (VI) in Scheme I could be *syn*-diazohydroxide, because *syn*-diazotates (and therefore *syn*-diazohydroxides) are very much less stable than anti,[9,10,25] but it is possible that anti–syn interconversions are important in highly acidic solutions where the pyridine group is protonated, for example the unstable int (Scheme V) could be a syn isomer.

Spectra of the Reaction Intermediates. In studying the reaction of nitrous acid with 4-aminopyridine Kalatzis observed rapid formation of an intermediate with λ_{max} at 285 nm, which he assumed was the diazonium ion.[7] We conclude, however, that this intermediate is pyridine-4-diazohydroxide (Experimental Section). The uv spectra of the reactants, products, and reaction intermediates are shown in Figure 4, and λ_{max} and ϵ_{max} are in Table XI. The spectra of the 4-diazohydroxide and its conjugate acid are consistent with these compounds containing the diazo group.

We made a few observations on the pyridine-2-diazotate (I) which had λ_{max} 285 nm and ϵ 3.4 × 10⁴ in dilute alkali (*cf.* ref 9b). When the solution was acidified (dilute acetic acid), a new absorption λ 293 nm

Table XI. Spectra of Diazo Compounds

Material	λ_{max}, nm	ϵ
(structure) N⟨ ⟩—N₂O⁻	277	11,800
(structure) N⟨ ⟩—N₂OH[a]	313	13,200
(structure) HN⁺⟨ ⟩—N₂OH[b]	283	13,600
(structure) N⟨ ⟩—N₂⁺	305	2,720
(structure) HN⟨ ⟩=O	254	14,680
(structure) HN⁺⟨ ⟩—OH[c]	234	8,630

[a] pH 5.77 in 0.1 M phosphate buffer. [b] 0.05 M HCl. [c] 0.5 M HCl.

(ϵ 2.2 × 10⁴) appeared, and the spectrum then slowly changed to that of 2-pyridone (III). We assume that the intermediate is the diazohydroxide (IV),[26] but because of the problem of identifying intermediates in the decomposition of pyridine-2-diazotate we did not pursue this question.

Reactivities of Pyridine-2- and -4-Diazo Derivatives. There are marked differences between the 2 and 4 derivatives in that the cationic intermediates generated in reactions of the 2-diazotate (I) are short lived and go forward to products rather than reverting to reactants. For example neither we nor Kalatzis[7] observed build-up of pyridine-2-diazonium ion (V) during reaction, probably because the strong inductive effect of the pyridine nitrogen atom speeds attack by solvent. In addition the rate minimum in the reaction of pyridine-4-diazotate (II) at pH 1.5 was unexpected (Figure 1). It was explained in terms of protonation of pyridine-4-diazohydroxide (VII) (pK_a = 4.15), but pyridine-2-diazohydroxide (IV) should be much less basic and therefore unprotonated in our pH range.[27]

The rate constant (k_3) for conversion of 4-diazohydride (VII) into 4-diazonium ion (VI) is 0.165 sec⁻¹ (Table V) and is only slightly larger than the rate constant of 0.06 sec⁻¹ for over-all decomposition of pyridine-2-diazohydroxide in the plateau region pH 3–5 (Figure 1). These values are reasonable in terms of the known rate constants for decomposition of para-substituted benzene diazohydroxides to diazonium ions.[9]

However, our failure to observe a diazonium ion in reactions of pyridine-2-diazotate (I) shows that the 2-diazonium ion (IV) loses nitrogen extremely readily. This assumption is supported by the variations of log k_ψ with pH at pH >6.5 (Figure 1). With pyridine-2-diazotate (I) the slope is −1.05 because of the equilibrium between diazotate and diazohydroxide, but with pyridine-4-diazotate (II) it is −1.95, because two proton equilibria are involved between diazotate and diazo-

(26) Kalatzis observed a new absorption, λ_{max} 283 nm, when pyridine-2-diazotate was acidified with 0.02 M HCl[7] and assumed that it was that of pyridine-2-diazonium ion (V), but it could have been that of protonated diazohydroxide.

(27) For 2- and 4-methoxypyridine pK_a = 3.28 and 6.62, respectively,[28] and pyridine-2- is a stronger acid than pyridine-4-diazohydroxide.

(28) A. Albert and J. N. Phillips, *J. Chem. Soc., London,* 1294 (1956).

hydroxide and diazohydroxide and diazonium ion (Scheme I).

Experimental Section

Materials. The diazotates were prepared by adding isoamyl nitrite (12 mmol) in dried THF (35 ml) to a stirred slurry of NaNH₂ (13.1 mmol) and the aminopyridine (13.6 mmol) in dried THF (105 ml) under N₂.[29] The light yellow precipitate was washed several times with TMF or dried Et₂O and vacuum dried. The products which were obtained in 50–70% yield had nmr and ir spectra consistent with the expected structure.

Pyridine-4-diazotate (II) was further purified by chromatography over triethylaminoethyl cellulose using aqueous 0.01 M Et₃N/CO₂ as eluent. Portions were assayed spectrophotometrically, but the trimethylamine diazotate decomposed when the solution was concentrated *in vacuo*. The concentration of the eluted solutions was determined spectrophotometrically by decomposing the diazotate to 4-pyridone.

Sodium pyridine-4-diazotate was also purified by dissolving it in freshly distilled DMF and an equal volume of dried THF. Dried Et₂O was then added slowly to precipitate the diazotate fractionally. Both methods of purification gave λ_{max} 277 nm and ϵ 11,800 (chromatography) and 11,700 (precipitation): nmr (60 MHz) δ 7.06 (m), 8.17 (m); ir (Nujol) 1601, 1310, 1255, 1210, 1005, 930, 830, 720, 695 cm⁻¹. The rate constants for the over-all decomposition were the same using crude or purified samples. By analogy we assume that the products were the *anti*-diazotates.[9]

Kinetics. The over-all rates of decomposition were generally followed in a Gilford spectrophotometer with a thermostated cell block at 25.0°, at 254 nm (pH > 3.5) or 234 nm (pH < 3.5) for pyridine-4-diazotate and 255 nm for pyridine-2-diazotate. For reactions of the 2-diazotate the ionic strength was 0.1 (with NaCl).

The fast reactions were followed using a Durrum-Gibson stop-flow spectrophotometer. All rate measurements were at 25.0° and except where noted the plots of ln (Abs_∞ − Abs_t) against time were linear for at least 3 half-lives, provided that freshly made up solutions were used.

Spectral Measurements. The uv absorption spectra of the stable compounds (diazotates and pyridones) were measured in water using Cary 11 or 15 spectrophotometers. The absorption spectra of pyridine-4-diazohydroxide and its conjugate acid were measured using the stop flow-spectrophotometer, which was calibrated using potassium hydrogen phthalate,[30] because there were discrepancies between it and the Cary spectrophotometers due to dark current and perhaps to stray light.

Solutions of pyridine-4-diazotate and the appropriate buffer were mixed rapidly in the stop-flow spectrophotometer and the transmittance was measured at various wavelengths.

The spectrum of pyridine-4-diazonium ion (VI) was measured indirectly. At pH 4–6 the reaction is

$$B \underset{k_{-3}^{OH}}{\overset{k_3}{\rightleftharpoons}} C \overset{k_4}{\longrightarrow} D$$
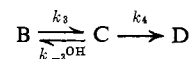
where B = ArN_2OH, C = ArN_2^+, and D = 4-pyridone.

The differential equations have been solved and give[31]

$$C_B = C_B^0 \left(\frac{k_{-3}^{OH}[OH^-] + k_4 - \gamma_1}{\gamma_2 - \gamma_1} e^{-\gamma_1 t} + \frac{k_{-3}^{OH}[OH^-] + k_4 - \gamma_2}{\gamma_1 - \gamma_2} e^{-\gamma_2 t} \right)$$

$$C_C = C_B^0 k_3 \left(\frac{1}{\gamma_2 - \gamma_1} e^{-\gamma_1 t} + \frac{1}{\gamma_1 - \gamma_2} e^{-\gamma_2 t} \right)$$

$$C_D = C_B^0 k_3 k_4 \left(\frac{1}{\gamma_1 \gamma_2} - \frac{1}{\gamma_1(\gamma_2 - \gamma_1)} e^{-\gamma_1 t} - \frac{1}{\gamma_2(\gamma_1 - \gamma_2)} e^{-\gamma_2 t} \right)$$

$$-\gamma = (-(k_3^{OH}[OH^-] + k_3 + k_4) \pm \{(k_{-3}^{OH})^2[OH^-]^2 + 2k_{-3}^{OH}[OH^-](k_3 + k_4) + k_3^2 + k_4^2 - 2k_3k_4\}^{1/2})/2$$
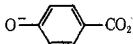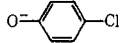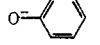
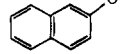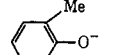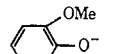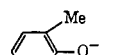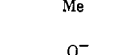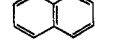(29) A. E. Chichibabin and M. D. Ryazoncev, *J. Russ. Phys. Chem.,* **46,** 1571 (1915).

(30) "U. V. Atlas of Organic Compounds," Vol. III, Plenum Press, New York, N. Y., 1967.

(31) N. M. Rodiguin and E. N. Rodiguina, "Consecutive Chemical Reactions," Van Nostrand, New York, N. Y., 1964, p 24.

Table XII. Extinction Coefficients of Azo Dyes[a]

Trapping agent	pK$_a$[b]	400	425	445	475	540
O⁻–C₆H₄–CO₂⁻	9.39[c]	12,122	14,300	14,639		667
O⁻–C₆H₄–Cl	8.48[c]	13,769	17,077	17,308		247
O⁻–C₆H₅	9.95[d]	13,962	17,217	17,515		581
2-naphtholate (O⁻)	9.51[e]	4,961	7,172	8,837	10,593	5,295
2-Me, O⁻-phenol	10.28[f]	10,879	16,243	19,137		2,379
2-OMe, O⁻-phenol	9.93[f]	7,669	13,125	16,815	19,772	4,447
2,6-diMe, O⁻-phenol	10.58[g]	7,098	12,513	16,318	20,232	6,330
1-naphtholate (O⁻)	9.34[e]	2,068	4,868	7,633		20,337
2-Me-1-naphtholate (O⁻)	9.68[h]			6,600		20,534

[a] Values of ϵ. [b] The phenol or naphthol. [c] L. P. Hammett, *Chem. Rev.*, **16**, 67 (1935). [d] C. M. Judson and M. Kilpatrick, *J. Amer. Chem. Soc.*, **71**, 3110 (1949). [e] "Handbook of Chemistry and Physics," Chemical Rubber Publising Co., Cleveland, Ohio, 1969. [f] D. R. Boyd, *J. Chem. Soc., London*, 1538 (1915). [g] G. W. Wheland, R. M. Brownell, and E. C. Mayo, *J. Amer. Chem. Soc.*, **70**, 2492 (1948). [h] Determined in the course of this work.

Therefore, the concentrations of each species can be calculated at any given time. A solution of pyridine-4-diazotate (6.42 × 10⁻⁵ M) was rapidly prepared (7 sec) in 0.1 M acetate buffer (pH 5.4) and the spectrum was scanned at 25.0° on a Cary 15 spectrophotometer so that the absorbance was known at various wavelengths and times (more than one determination had to be made). From these data we calculated the contribution of pyridine-4-diazonium ion to the total absorbance.

Azo Coupling. Solutions of the azo coupling products were prepared from pyridine-4-diazotate and several phenols under conditions in which all the diazonium ion is trapped. The extinction coefficients of the azo dyes were then calculated at pH 11.05 (Table XII). In one reaction
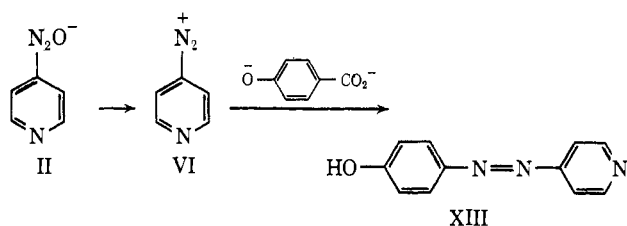
we isolated XIII and purified it by solution in EtOH, treatment with activated charcoal, and recrystallization. It had no ir absorbance at 1600–2000 cm⁻¹: (60 mHz) δ 8.40 (d, J = 3 Hz); 7.56 (d, J = 4.5 Hz); 7.32 (d, J = 3 Hz), 6.54 (d, J = 4.5 Hz).
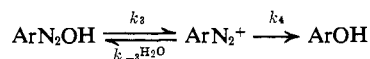
Using these extinction coefficients and pK_a for the phenols and naphthols (Table XII) we used competition between pairs of trapping agents to determine the relative reactivities of the aryloxide ions toward pyridine-4-diazonium ion.

The pH for the coupling experiments was 8–8.5 and the concentrations of the trapping agents were in the range 10⁻⁴–2 × 10⁻² M so that easily measurable amounts of each azo dye were formed. Pairs of trapping agents were chosen so that the concentrations of the azo dyes could easily be calculated from the final spectra.

Appendix

Analog Simulation of the Reaction at pH 4–6. If the formation of 4-pyridone is followed at λ 254 nm the absorbance *decreases* for 5–10 sec, but after 20 sec the change of absorbance follows the normal first-order rate equation (Figure 2).

We assume that the reaction can be represented as

$$\text{ArN}_2\text{OH} \underset{k_{-3}^{H_2O}}{\overset{k_3}{\rightleftharpoons}} \text{ArN}_2^+ \overset{k_4}{\longrightarrow} \text{ArOH}$$

We used the values of k_3 and k_4 given in Table V ($k_{-3}^{H_2O}$ is small, >10⁻² sec⁻¹), and using an analog computer, we fitted the experimental plot of absorbance against time,[12] taking the following extinction coefficients at 254 nm, ϵ_{ArN_2OH} 3980, $\epsilon_{Ar^+N_2}$ ∼0, ϵ_{ArOH} 14,680 (Figure 4).

The points in Figure 2 were taken from a Polaroid photograph of the oscilloscope trace from the stopped-flow spectrophotometer, and the solid line was obtained by putting the output from the analog computer into an X–Y plotter.