[S. Wawzonek, S. C. Wang, and P. Lyons, J. Org. Chem., 15, 593 (1950)].
(12) When 1a was treated with methyl iodide, a methyl-substituted aldehyde also was obtained.
(13) The numbering system used for designating the atoms in 2 is retained for purposes of simplification and ease of comparison.
(14) (a) D. V. Santi and C. F. Brewer, J. Am. Chem. Soc., 90, 6236 (1968); (b) W. J. Wechter, Collect. Czech. Chem. Commun., 35, 2003 (1970), (c) J. A. Rabi and J. J. Fox, J. Am. Chem. Soc., 95, 1628 (1973); (d) M. Remin, E. Darzynkiewicz, A. Dworak, and D. Shugar, ibid., 98, 367 (1976).
(15) The UV spectrum of 10a, which varies with pH, is in good agreement with that of 1b, which has a related chromophore. The only significant difference between the spectra of 10a and the imidazolidone, 1b, is in the extinction coefficients. This difference may well be due to a difference in the stereochemistry of the two chromophores. See ref 5.
(16) The species m/e 125 may be a radical cation or a cation.
(17) T. C. Thurber and L. B. Townsend, J. Org. Chem., 41, 1041 (1976).
(18) D. V. Santi and C. F. Brewer, Biochemistry, 12, 2416 (1973); D. V. Santi, C. S. McHenry, and H. Sommer, ibid., 13, 471 (1974).
(19) See, for example, R. B. Dunlap, N. G. L. Harding, and F. M. Huennekens, Ann. N.Y. Acad. Sci., 186, 153 (1971).
(20) D. Davidson and O. Baudisch, J. Am. Chem. Soc., 48, 2379 (1926).
(21) (a) G. E. Hilbert and T. B. Johnson, J. Am. Chem. Soc., 52, 1152 (1930); (b) ibid., 52, 2001 (1930).
(22) G. E. Hilbert, J. Am. Chem. Soc., 59, 330 (1937).
(23) This area integrates for ca. 3 protons due to the fact that not all water was removed from the original sample by evaporation.
(24) The assignment of the resonances to the syn and anti isomers is arbitrary. These were present in a ratio of 25:75.
(25) Me_2SO was purified by azeotropic distillation with benzene to remove the bulk of the water followed by vacuum distillation from CaH_2.
(26) The value of ε given for 5a is based on 100% conversion of 2a.
(27) Dimethyl sulfone was a ubiquitous minor component of all of these reactions. A control reaction in which the dialkyluracil was omitted did not produce any sulfone.
(28) Fr. Fichter and B. Becker, Chem. Ber., 44, 3481 (1911).
(29) This material was identical with a sample of 1,3-dimethylthymine prepared by the methylation of thymine.[20]
(30) The UV spectra in both 95% EtOH and 0.1 N HCl are reasonable for the structure assigned to 14a. The initial spectrum of this compound in 0.1 N NaOH showed a shoulder at ~290 nm which disappeared in a short time. The spectrum was invariant after 1 h. The value of λ_{max} 265 nm reported is probably due to 2a which is formed by a Michael addition of hydroxide ion to the enol ether carbon atom of 14a, followed by the expulsion of methoxide ion in a reverse Michael addition. The resulting product is 5a (λ_{max} 296 nm in Me_2SO), which then undergoes ring closure to 2a (59% yield).
(31) The assignment of these methyl groups cannot be made with certainty.

Nucleophile and Borate Reactivity with Nicotinamide Adenine Dinucleotide and Its Analogues[1]

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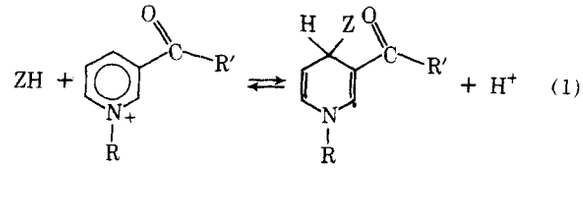
Nucleophilic reactivity for a variety of nucleophiles to the ring addition reaction of pyridine nucleotides has been measured. There is no relationship between rate of reaction and equilibrium affinity for the nucleophile. This situation is similar to the reaction of nucleophiles with the carbonyl carbon or with carbonium ions. The position of equilibrium correlates with γ, the position of equilibrium for nucleophilic reaction with the carbonyl group. However, a better correlation between pyridinium ion addition reactions has been obtained: A new affinity scale for the pyridinium ion, P^+, is defined. Boration of the ribose adjacent to the pyridinium ring reduces the equilibrium constant for nucleophile addition three- to sevenfold, regardless of the structure of the pyridinium moiety.

Nicotinamide adenine dinucleotide (NAD^+)[2] is transformed into ring addition complexes by a variety of agents. Sulfite,[3] hydroxide,[4] cyanide,[5] and enols[6] form favorable complexes. Weaker interactions take place with mercaptans, hydroxylamine, and imidazole.[6,7] A number of stable inhibitory ternary NAD^+–nucleophile–dehydrogenase complexes are formed.[7-11] Though NAD^+ ring addition complexes are formed in the absence of enzyme, complex formation is favored on the enzyme surface when the nucleophile bears a structural resemblance to the natural substrate. Kaplan and Everse[12] have proposed that the ternary complex formed by NAD^+, pyruvate, and lactate dehydrogenase plays a role in metabolite regulation in the living cell.

Borate complexes with the ribose adjacent to the nicotinamide cation in NAD^+ to form NADB, which forms less favorable nucleophilic ring addition complexes at a lower rate, in comparison with NAD^+.[13] The borate complexation with NAD^+ is the cause of the competitive inhibition of a number of dehydrogenases by borate with respect to NAD^+.[14]

In order to understand what chemical properties of the nucleophile favor a stable addition complex, a study of both the rate and equilibrium constants was initiated. This reaction as given in eq 1 is easily followed because the complex is



(1)

chromophoric, absorbing maximally at 310–360 nm (depending upon the nucleophile and the pyridinium ion). Rate and equilibrium constants were obtained as a function of pH, in order to elucidate the overall stoichiometry of the reaction and of the transition state. NAD^+, 3-acetylpyridine adenine dinucleotide ($APAD^+$), and their nucleotide and alkyl analogues were studied. In addition we have measured the boration and phenylboration equilibrium of $APAD^+$, nicotinamide adenine dinucleotide phosphate (NADP), and other analogues of NAD^+, and we have measured the effect of boration on nucleophilic reactivity and affinity. We have compared the scale of reactivity and affinity of nucleophiles to NAD^+ with the reactivity and affinity scales of carbonium ions and carbonyl compounds. A new pyridinium ion affinity scale, P_+, is defined.

Experimental Section

Materials. The pyridine nucleotides were products of Sigma Chemical Co. The concentration of pyridine nucleotide was assayed using horse or yeast alcohol dehydrogenase from Sigma. 1-Benzyl-3-acetylpyridinium chloride ($BzAP^+Cl^-$) was prepared by mixing benzyl chloride with 3-acetylpyridine in benzene at room temperature. Recrystallization from ethanol yielded a white product, mp 189–190 °C (uncorrected). 1-Methyl-3-acetylpyridinium iodide ($MeAP^+I^-$) was prepared by mixing methyl iodide with 3-acetylpyridine at room temperature. Recrystallization from 1-propanol yielded yellow crystals, mp 163–165 °C (uncorrected). 1-Methyl-3-carboxymethylpyridinium iodide ($McCP^+I^-$), *N*-methylnicotinamide iodide ($MeNic^+I^-$), and *N*-methylisonicotinamide iodide ($MeIsonic^+I^-$) were prepared in the same way as $MeAP^+$, using methyl iodide and the corresponding pyridine derivative, mp 258–260, 207–211, and 129–133

°C (all uncorrected), respectively. 1-Benzyl-3-carboxylmethylpyridinium ion ($BzCP^+Cl^-$) was prepared by refluxing methyl nicotinate with benzyl chloride in methanol. After recrystallization from propanol–ether, white crystals were obtained, mp 129–133 °C (uncorrected). 1-Benzylnicotinamide chloride ($BzNic^+Cl^-$) was prepared as above, using nicotinamide as the base, mp 240–242.5 °C (uncorrected). Phenylboronic acid from Sigma was recrystallized from water, vacuum dried, and stored in the desiccator. Fisher reagent grade sodium sulfite and boric acid were used without further purification.

Buffers. To minimize the effect of the slow decomposition of sulfite,[15] fresh sulfite solutions were used for all experiments. Borate buffers were prepared by the neutralization of boric acid with NaOH. The thiol, hydroxylamine, methoxyamine, and hydrazine buffers were prepared just before use, by adding NaOH to the thiol or the amine hydrochloride. Low pH (pH 5–6) sulfite buffers contained acetate/acetic acid to maintain the pH. All the buffer solutions were maintained at constant ionic strength with potassium chloride. Phenylboronic acid buffers were prepared by adding NaOH to phenylboronic acid and filtering. The concentration of phenylboronic acid was determined spectrally at 267 nm.[16] Hydrogen peroxide was determined by titration with $KMnO_4$. The concentration of $KMnO_4$ was standardized by titration with sodium oxalate.

Spectral Measurements. Absorption spectra were obtained with either a Cary Model 14 or a Model 15 recording spectrophotometer, using 1-cm, 3-mL quartz absorption cells. NMR spectra were obtained with a Varian Model T-60 spectrometer. The measurements were made with solutions containing 0.14 M pyridine nucleotide and 0–0.6 M nucleophile in D_2O. The pH was approximately 7–8.

Kinetics. The rate of production of ring addition adducts and their partial decomposition in the presence of borate were measured at 310–360 nm using a Durrum stopped-flow spectrophotometer with an instrument dead time of 2.5 ms. Reactions were performed under pseudo-first-order conditions with nucleophilic concentration in large excess over that of pyridinium ion in a series of Z/ZH buffers of varying total buffer concentration at a constant buffer ratio X. Several buffer ratios were generally used. The transmittance values from the oscilloscope trace were converted to absorbance values; and first-order rate constants, k_{obsd}, were calculated from plots of log $(A_\infty - A_t)$ or log $(A_t - A_\infty)$ against time.[17] All plots were linear to 90% reaction. Second-order rate constants were obtained from the slopes of plots of k_{obsd} against nucleophile concentration. The data were treated using a linear least-squares program on the Olivetti P602 microcomputer.

Equilibrium Constants. A Cary Model 16 or a Beckman DU-2 spectrophotometer, thermostated at 25.0 ± 0.1 °C, was used for equilibrium determinations. The pyridinium salt was introduced with an Eppendorf micropipet into a 1-cm quartz cuvette containing measured amounts of borate buffer solution. The total volume was 3.0 mL and the initial ionic strength was kept constant. Manual measurements were made in the 300–365-nm region, depending upon the substrate and the nucleophile, giving the absorbance value, A, of the cell and its contents, with air as reference. Aliquots of nucleophile were added to the cuvette. The contents of the cuvette were rapidly mixed, and measurements of the absorbance were recorded after each addition of nucleophile. The procedure was repeated for monitoring the pH of the solution before and after addition. The absorbance values were corrected for the volume change due to addition of nucleophile. Double reciprocal plots of absorbance against nucleophile concentrations were made for various concentrations of borate.[18] Assuming that the molar extinction coefficient, ϵ, is the same for the pyridine nucleotide–nucleophile complex and the pyridine nucleotide–nucleophile–borate complex, ϵ was calculated from the average value of the intercepts and the substance concentration, c: $\epsilon = 1/(\text{intercept} \times c)$.[13] The equilibrium constant, K_{app}, was calculated from the slope, substrate concentration, and molar extinction coefficient: $K_{app} = 1/(\text{slope} \times \epsilon \times c)$. For cyanide equilibria which cannot be forced to completion at the cyanide concentrations used, the method of Behme and Cordes[19] was used, where K is the slope of a plot of $A_{obsd}/(CN^-)$ vs. A_{obsd}.

The values of K_{app} and the corresponding values of borate concentration were treated using a nonlinear least-squares curve fitting program on the DEC System-10 computer in order to determine the individual equilibrium constants of Schemes II and III.

pH Determinations. The pHs of the solutions were measured using a Radiometer Type TTT1D pH meter with a Type PHA 630T scale expander. The electrode system employed consisted of a G2222C glass electrode and a K4112 calomel electrode. For the kinetics experiments, the pH of the solutions was measured at the completion of the reactions. For equilibrium studies, the procedure for adding aliquots of nucleophile to a solution of pyridine nucleotide in borate buffer was repeated. The pH was measured before and after each nucleophile addition.

Results

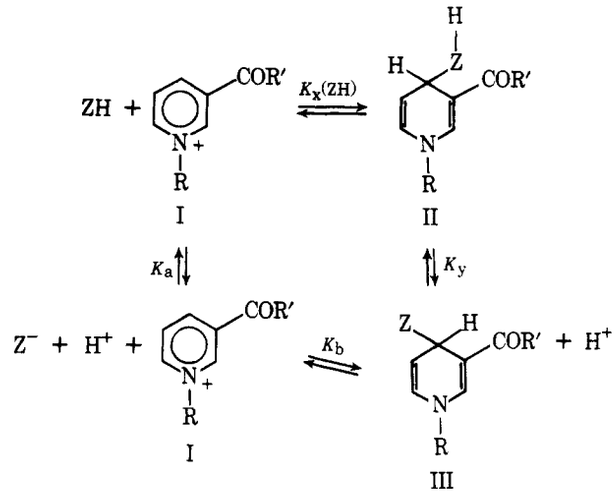
General Treatment. Equilibrium constants were obtained by observing the absorbance of the complex as a function of ZH/Z concentration of constant pH and constant initial concentration of pyridine nucleotide. This process was repeated at a number of pH values. The value of the equilibrium constant K_{tot} is obtained from eq 2, which describes a linear double reciprocal plot of A_{obsd}^{-1} vs. $(Z)_{tot}^{-1}$ for the ring addition of Z. Z_{tot} is [Z + ZH], K_a is the acid dissociation constant of ZH, l is the cell width, ϵ is the extinction coefficient of the complex, C_0 is the initial concentration of pyridinium ion, and A_{obsd} is the observed absorbance. From this plot, ϵ and K_{tot} are both obtained, providing $(Z)_{tot}$ can be varied to concentrations high enough so that the $K_{tot}(Z)_{tot} > 1$. When K_{obsd} is unfavorable, $K_{tot}(Z)_{tot} < 1$ over the entire concentration range of $(Z)_{tot}$, and eq 2 is simplified to eq 3.

$$\frac{lC_0}{A_{obsd}} = \frac{1}{\epsilon}\left(1 + \frac{1}{K_{tot}[Z]_{tot}}\right) \quad (2)$$

$$\frac{A_{obsd}}{l} = K_{tot}\epsilon C_0(Z)_{tot} \quad (3)$$

In this case K_{tot} and ϵ are no longer separable, and only the quantity $K_{tot}\epsilon$ can be obtained by dividing the slope of the A_{obsd} vs. $(Z)_{tot}$ plot at constant C_0 by C_0. Scheme I illustrates

Scheme I



the formation of two complexes, II and III, which are in protonic equilibrium with each other. The value of K_{tot} is given by eq 4, where K_{ab} is K_aK_b, K_{xy} is K_xK_y. Plots of K_{tot} vs. $(H^+)^{-1}$ give K_{xy} as the slope and K_x as the intercept, when $K_a < (H^+)$. If ZH has a measurable ionization constant, K_a, then K_b can be obtained from eq 4.

$$K_{tot} = \frac{K_x[H^+] + K_{ab}}{K_a + [H^+]} = \frac{K_x[H^+] + K_{xy}}{K_a + [H^+]} \quad (4)$$

The equilibrium data for the addition of pyridine nucleotides of sulfite, mercaptide, cyanide, imidazole, hydrazine, and hydroxylamine give K_x values of zero. Only K_{xy} values rather than K_b values can be obtained for most amines, as most amines do not have measurable K_a values. For very weakly interacting systems, ZH, as well as Z, interacts.

Borate buffers were found to profoundly decrease the interaction of nucleophiles with ribose-containing pyridinium compounds, as was found for NAD^+ and nicotinamide adenine mononucleotide (NMN^+) earlier.[13] Scheme II accounts for the effect of borate. The equilibrium constants for boration

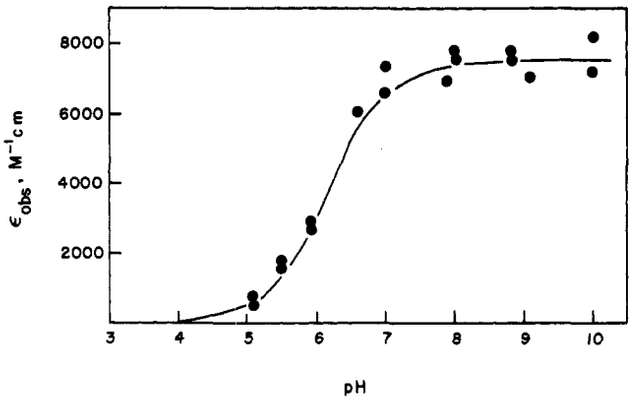


Figure 1. Apparent extinction coefficient for the equilibrium of $[SO_3^{2-} + HSO_3^-]$ with APAD$^+$. Solid line calculated from eq 8 with $\epsilon = 7500\ M^{-1}\ cm^{-1}$, $K_1 = 1590\ M^{-1}$, $K_2' = 240\ M^{-1}$.

Scheme II

$$\begin{array}{ccc} Z^- + NAD^+ & \overset{K_b}{\rightleftharpoons} & NAD\text{-}Z \\ K_{I(borate)} \uparrow\downarrow & & \uparrow\downarrow K_{II(borate)} \\ Z^- + NAD\text{-}B & \overset{K_c}{\rightleftharpoons} & NADZ\text{-}B \end{array}$$

and phenylboration of the nucleotides and nucleotide adducts were obtained from eq 5, which is derived from eq 4.

$$K_{obsd,z} = \frac{K_b + K_I K_c(borate)}{1 + K_I(borate)} \quad (5)$$

$$K_I K_c = K_{II} K_b \quad (6)$$

Sulfite, Cyanide, and Mercaptide Addition. Previous studies on the effect of pH on K_{obsd} have demonstrated that only dianionic SO_3^{2-} and anionic RS^- add to the pyridinium ring.[3,13,20] We now show that in the case of pyridinium rings with aldehyde or acetyl groups in the 3 position, in addition to the pyridinium ring addition equilibrium depending on Z^-, another equilibrium involving the addition of ZH to the carbonyl group is present. The second ZH-dependent equilibrium is apparent from the fact that the apparent equilibrium constant does not follow eq 4, with $K_x = 0$, but falls off more slowly in the acid pH range ($<pK_a$), indicating the presence of a finite K_x term. This is in contrast to the sulfite equilibria with NAD$^+$ and NMN$^+$, where K_x is zero. Another indication of ZH equilibria at the carbonyl group is that ϵ_{obsd} values do not remain constant with pH change, as would be expected for equilibria involving the formation of ring addition products only (Figure 1). The effects of pH on both ϵ and K_{obsd} indicate that at low pH, nonchromophoric HSO_3^- complexes are formed. The complexation reactions of PAAD$^+$ and APAD$^+$ could not be studied at pH values greater than 10, owing to the formation of yellow products, presumably due to the hydroxide ring addition reaction, as in the case of NAD$^+$.[4]

The presence of a second ZH-dependent equilibrium is also manifested by the difference in the number of transients seen in the reaction of sulfite with NAD$^+$ and NMN$^+$ in contrast to APAD$^+$, MeAP$^+$, and BzAP$^+$. Only a single sulfite-dependent transient is observed for NAD$^+$ and other nicotinamide cations at all pH values. This transient is first order in $[SO_3^{2-}]$ and is due to the ring addition reaction.[13] In contrast, for APAD$^+$ and other acetylpyridine cations, at high pH, a single sulfite-dependent transient is seen; and at low pH two transients are seen (Figure 2). The biphasic reaction consists of a rapid sulfite-dependent decrease in transmittance, followed by a slower increase in transmittance (decrease in absorbance), which is independent of sulfite. The second slower reaction has a hyperbolic dependence on sulfite concentration:

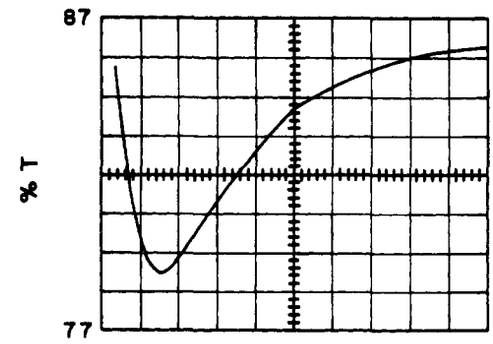


Figure 2. Transmittance vs. time for reaction of APAD$^+$ with 5 mM SO_3^{2-} in a pH 5.1 acetate buffer, $\mu = 0.6$ M. Each horizontal division is 0.2 s.

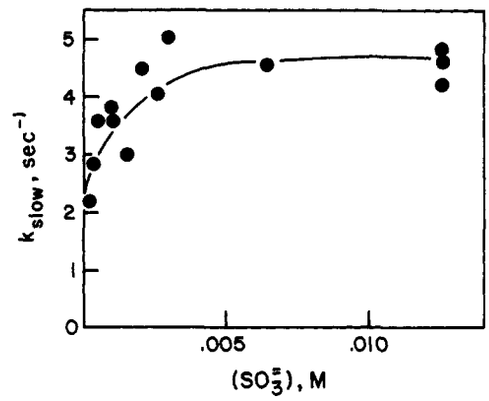
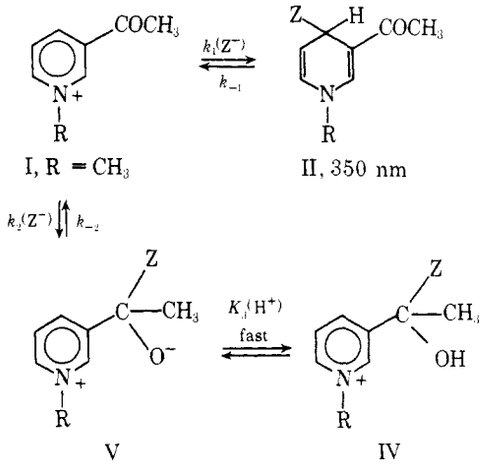


Figure 3. Observed first-order rate constant for the second transient in Figure 2 vs. SO_3^{2-} concentration. The pH range is 4.9–5.3.

at very low sulfite concentrations the rate is sulfite dependent, and at higher sulfite concentrations, the rate becomes sulfite independent (Figure 3). At intermediate pH, 7–9, k_{slow} shows a pH dependence with a 0.7 order in $1/(H^+)$, approximately first order.[22]

Scheme III explains both the rate and equilibrium data. The

Scheme III



pH dependencies for K_{obsd} and ϵ_{obsd} are given by eq 7 and 8

$$K_{obsd,tot} = \frac{[K_a K_b + (H^+)K_2']}{[(H^+) + K_a]} \quad (7)$$

$$\epsilon_{obsd} \approx \frac{K_a K_I}{[K_a K_b + (H^+)K_2']} \quad (8)$$

where $K_{obsd,tot}$ is the dissociation constant with respect to total Z + ZH, K_2' is the product of K_2 and K_3, K_b is k_1/k_{-1}, and K_2

Table I. Rate and Association Constants for the Interaction of Sulfite with Pyridinium Ions[a]

Registry no.	Pyridinium ion	λ_{max}, nm	ϵ, M^{-1} cm^{-1}	K_b, M^{-1}	K_2', M^{-1}	k_1, M^{-1} s^{-1}	k_2, M^{-1} s^{-1}
	β-NAD+ [p]	320	4500 ± 150	41 ± 1		2050 ± 180	
	β-NAD-B[p]	320	4500 ± 150	8.4 ± 0.7		473 ± 46	
62430-84-6	β-NAD-PhB	320	6134 ± 134	3.1 ± 0.4			
62430-85-7	Deamino-NAD+	320	4095 ± 70	30.5 ± 2.2[o]		1390 ± 30[t]	
62475-99-4	α-NAD+	320	1040 ± 240	15.7 ± 0.8[r]		2200[s]	
62430-86-8	α-NAD-B	320	1040 ± 240	2.7 ± 0.6[r]			
62461-57-8	APAD+	340	6900 ± 200[u]	1500 ± 100[u]			
			7500 ± 100[m]	1410 ± 90[m]	240[m]	12 000 ± 400[n]	~2100[l]
62430-87-9	APAD-B	340	7500 ± 70[u]	220 ± 70[u]		3100[q]	
62430-88-0	Deamino-APAD+	340	6176 ± 400	980 ± 140[d]		10 200[r]	
62430-89-1	Deamino-APAD-B	340	6196 ± 410	300		2400[q]	
62430-90-4	NADP+	320	3170 ± 590	22.5 ± 0.5[r]		1400[s]	
62430-91-5	NADP-B	320	3170 ± 590	6.1 ± 0.4[r]		345[s]	
62430-92-6	BzAP+	340	6840 ± 200	108 ± 12[b]	b	4670[j]	~2000[k]
62430-93-7	MeAP+	340	2610 ± 690	19 ± 3[h]	200[h]	1800[i]	~2000[g]
62430-94-8	ThioNAD+	365	4890 ± 180	101 ± 9[f]		2130 ± 180[e]	
62430-95-9	PAAD+	340		2000 ± 130[d]		(26)[c]	
	NMN+[p]	320	4050 ± 13	25 ± 1		1240 ± 50	
	NMN-B[p]	320	4050 ± 13	9.3 ± 0.8		455 ± 30	

[a] Association constants, K, measured at 25.0 ± 0.1 °C, ionic strength 0.6 M; rate constants measured at 25 ± 1 °C, 0.6 M ionic strength. [b] Measured at pH 8.2 and 10.0. The equilibrium constant was not measured in the acid pH range which would allow determination of K_2'. [c] Measured at pH 9.0 and 9.7. The small value represents a rate process other than ring addition. [d] Measured at pH 8.15 and 9.25. The equilibrium constant was not measured in the acid pH range which would allow determination of K_2'. [e] Measured at pH 9.1, 7.2, 6.8, 5.4, and 4.8. [f] Measured at pH 9.1 and 8.0. [g] Measured at pH 6.0–6.5. [h] Measured at pH 6.0, 7.1, 8.1, 8.2, 8.4, 9.1, and 10.1. [i] Measured at pH 8.2, 8.8, and 9.5. [j] Measured at pH 10.1. [k] Measured at pH 4.9–6.5. [l] Measured at pH 4.9–5.3. [m] Measured at pH 5.0–10. [n] Measured at pH 8–10. [o] Measured at pH 7.0, 9.2, and 10.0. [p] From Johnson and Smith.[13] [q] Measured at pH 8.9. [r] Measured at pH 9.1. [s] Measured at pH 9.7–9.8. [t] Measured at pH 7.8, 8.9, and 9.1. [u] Measured in the presence of borate at pH 7.9, and analyzed according to Scheme II. [v] Measured at pH 9.1 and 10.4. [w] Measured by stopped-flow spectrophotometry.

Table II. Association Constants for the Interaction of Mercaptide and Cyanide and Pyridinium Ions[a]

Registry no.	Z^-	Pyridinium ion	μ, M	λ_{max}, nm	ϵ, M^{-1} cm^{-1}	pH	K_b, M^{-1}	K_2', M^{-1}
62430-96-0	ME	β-NAD+	1.0	325	5060	9.5	1.8 ± 0.3[b]	
	ME	β-NAD+	0.5, 50% ethanol	325	6700		9.4 ± 0.5[b]	
62430-97-1	AcCys	β-NAD+	1.0	325	6000	9.0	1.1[b]	
62461-58-9	TG	β-NAD+	1.0	325	8700 ± 2300	9–11[d]	3.56 ± 1.03	
5815-31-6	CN^-	β-NAD+	0.63	325	5270 ± 330	11.4	221 ± 5	
62430-98-2	CN^-	β-NAD-B	0.63	325	5270 ± 330	11.4	41 ± 3	
62430-99-3	TG	APAD	0.63	350	5370 ± 1280	9.1[f]	440 ± 10	
	TG	APAD	1.0	350	5390 ± 1290	5.7–10[e]	171	3
62431-00-9	TG	APAD-B	0.63	350	5370 ±1280	9.1	51 ± 10	
62476-00-0	TG	PAAD	0.63	350	5290 ± 1100	5.7–10[c]	4920 ± 50[a]	88.9 ± 8

[a] Measured at 25.0 ± 0.1 °C unless otherwise noted. [b] Measured at 25 ± 1 °C. [c] Measured at pH 5.7, 6.6, 7.2, 8.0, 8.1, 8.7, 9.7, and 10.0. Fifteen separate measurements were calculated according to eq 7, with $K_a = 2 \times 10^{-10}$ M. [d] Measured at pH 9.0, 10.0, and 11.0. Measured at pH 8.1, 9.0, and 10.0. [f] Measured in the borate system according to Scheme II.

is k_2/k_{-2}. In Scheme III, the HSO_3^--dependent equilibrium is a carbonyl addition reaction, and the SO_3^{2-}-dependent equilibrium is the ring addition reaction. By analogy with sulfite addition to benzaldehyde, the carbonyl addition reaction would be expected to depend on SO_3H^- much more strongly than on SO_3^{2-}. At high pH, only the K_b equilibrium is important, and the only transient seen is due to the k_1 step. At low pH, the K_2' equilibrium becomes more important, but the addition to the pyridinium ring (k_1) is more rapid than the addition to the carbonyl group (k_2). As a result, a metastable K_b equilibrium is established during the fast first transient, resulting in an increase in absorbance as II is formed. The overshoot in the formation of II is then slowly diminished by sulfite addition to the carbonyl group in the k_2 step. As with the rate of sulfite addition to benzaldehyde, the reaction rate depends upon SO_3^{2-} rather than SO_3H^-.[23] According to Scheme III the rate of the low pH second slow step is predicted to be hyperbolic in sulfite, according to eq 9, where K_{OH} is the acid dissociation constant of the OH group.

$$k_{slow} = \frac{k_2(SO_3^{2-})}{1 + K_b(SO_3^{2-})} + \frac{k_{-2}K_{OH}}{(H^+) + K_{OH}} \quad (9)$$

Table I contains equilibrium and rate data for the addition of sulfite to the pyridinium ring. Table II contains equilibrium data for mercaptide and cyanide addition. For those APAD+, BzAP+, MeAP+, and 3-pyridine aldehyde adenine dinucleotide (PAAD+) systems studied over a wide pH range, data from the additional equilibria and rates due to carbonyl addition, analyzed according to eq 7, 8, and 9, are given. The k_2 values are approximate because of saturation of the rate process at very low sulfite concentrations. Rate data for mercaptide reaction with pyridinium ions are not given in Table II, because the second-order rate constant exceeds 10^4

Table III. Association and Rate Constants for the Interaction of Borate and Phenylborate with Pyridine Nucleotides at 25 °C, μ = 0.6 M[a]

Pyridine nucleotide	pH	$K_{B(OH)_4}$, M^{-1}	k_B, k_{BH}, M^{-1} s^{-1}	System	K_{PhB}, M^{-1}	k_{PhB}, k_{PhBH}, M^{-1} s^{-1}
NAD^+[b]	10.4	1500 ± 400	285, 350	SO_3^{2-}	2500 ± 150	170, 7500[d]
	9.0	1700 ± 300		SO_3^{2-}	3400 ± 190	
	8.75	2000 ± 60		Ethanol[c]		
NAD-CN	11.4	150 ± 20		CN^-		
NAD-SO_3^-[b]	10.4	250 ± 90		SO_3^{2-}	150 ± 20	
	9.0	350 ± 70				
NADH[c]	8.75	130 ± 8				
$APAD^+$	9.1	2050 ± 770		SO_3^{2-}		
	7.9	3100 ± 1300		SO_3^{2-}		
	9.1	1960 ± 240		TG		
APAD-TG	9.1	230 ± 50		TG		
APAD-SO_3^-	9.1	560 ± 240		SO_3^{2-}		
	7.9	450 ± 240		SO_3^{2-}		
α-NAD^+	9.0	2270 ± 580		SO_3^{2-}		
α-NAD-SO_3^-	9.0	400		SO_3^{2-}		
NMN^+[f]	9.0	1900 ± 800	440, 690	SO_3^{2-}		
NMN-SO_3^-	9.0	720 ± 300		SO_3^{2-}		
$NADP^+$	9.1	900 ± 120	950, 490[e]	SO_3^{2-}		
NADP-SO_3^-	9.1	240 ± 40		SO_3^{2-}		

[a] pH values at which boration constants were measured are in the first column. [b] See Johnson and Smith.[13] [c] Boration of NADH was determined by the perturbation effect borate has on the equilibrium between NAD^+, ethanol and NADH, acetaldehyde, with the attainment of the equilibrium condition facilitated by the addition of alcohol dehydrogenase.[14] [d] Measured at pH 9.8, 10.5, and 10.8. [e] Measured at pH 9.7–9.8. [f] Registry no., 62461-59-0.

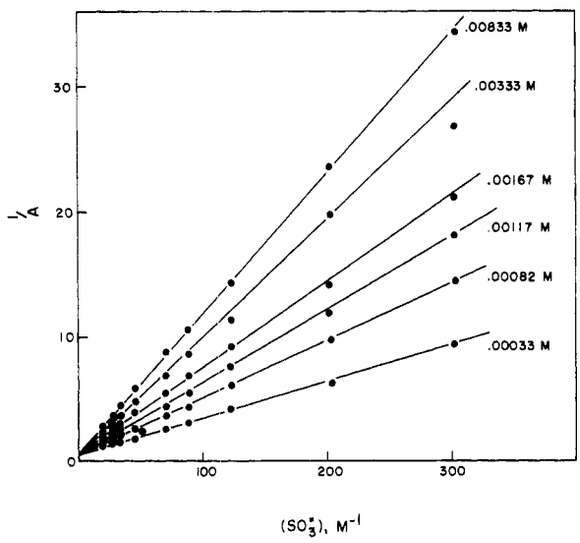


Figure 4. Determination of the perturbation of the equilibrium between sulfite and NAD^+ by phenylboronate. Measurements were made at pH 9.1, μ = 0.63, 25.0 ± 0.1 °C.

M^{-1} s^{-1} in the case of reaction with NAD^+. Using 10^{-3} M NAD^+ and 0.03–0.6 M mercaptide, the reaction was completed within the 2-ms deadtime of the instrument. The unfavorable equilibrium precludes the use of lower concentrations of mercaptide. Therefore only the lower limit of k_1 can be determined. The equilibrium is more favorable with $APAD^+$ and $PAAD^+$, but the kinetics have not been carried out, owing to the multiple equilibria (Scheme III) and the difficulty of identifying the rate constants associated with the multistep process. For example, the second-order rate constant of 26 M^{-1} s^{-1} for the reaction of SO_3^{2-} with $PAAD^+$ (Table I) probably represents a rate of dehydration of the aldehyde or of interaction with carbonyl group, rather than interaction with the ring, because it is so far out of line with the other values of k_1. Weak systems of RSH/RS^-–pyridinium ion interaction will be presented later. That the interaction of RS^- and SO_3^{2-} with pyridine nucleotides represents a covalent reaction is demonstrated by NMR studies of the NAD^+–thioglycolate reaction. Treatment of NAD^+ with increasing concentrations of thioglycolate (TG) buffer results in the progressive broadening of the nicotinamide signals at τ 2.31 (H_2); 2.44 (H_6) doublet, J = 7 Hz; 2.78 (H_4) doublet, J = 8 Hz; 3.48 (H_5) multiplet. The two adenine signals at 3.20 (H_2') and 3.36 (H_8') remain sharp and unperturbed. New signals emerge at τ 4.1, 1 proton (H_2); 5.20 and 5.25, 2 protons (H_5 and H_6); and τ 6.25 (H_4). Treatment of $MeAP^+$ with sulfite results in the progressive broadening of the pyridinium protons τ 0.60 (H_2); 1.05 and 0.09 (H_5 and H_6); and 1.75 (H_4). New broad signals emerge at τ 4.0, 3.0, and 1.85. The NMR spectrum of the product of NAD^+ and SO_3^{2-} has been previously reported.[13]

Borate and Phenylboronate Reactions. The equilibrium of borate and phenylboronate with the nucleotides was studied by the lowering of the apparent nucleophilic association constants in the presence of borate or phenylboronate, as shown in Figure 4. Only the basic forms of borate and phenylboronate are involved in the equilibria. In contrast, both the basic and acidic forms are involved in the rate of reaction with the nucleotides. The rate constants were obtained from the pseudo-first-order rate constants at low borate or phenylboronate concentrations, as before.[13] The rate and equilibrium data are given in Table III.

Weak Nucleophilic Interactions with Pyridinium Ions. The interaction of mercaptans with pyridinium ion is due to the RS^- species only; RSH does not give rise to an absorption band in the 300–360-nm region. Because the interaction is very weak, ϵ and K_{xy} could not be separated; only the product $K_{xy}\epsilon$ could be obtained. The results of RS^- interaction are given in Table IV. The reaction with mercaptans is reversible; lowering the pH reverses the reaction. The solvent effect on the interaction of 2-mercaptoethanol (ME) with $MeNic^+$, changing the solvent from 0 to 50% dioxane, causes a sixfold increase in $K_{xy}\epsilon$ and a dramatic increase in the sharpness of the band (from 130 to 30 nm half-width). In contrast, for

Table IV. Association Constants for Weak Interactions of Pyridinium Ions with Sulfhydryl Anions[a]

Registry no.	RS^-	Pyridine nucleotide	μ, M, and cosolvent[c]	λ, nm	$K_{xy\epsilon} \times 10^{-4}$, M^{-2} cm^{-1}
62431-01-0	TG	MeNic+	1.0	330	0.0415 ± 0.005[b]
	TG	MeNic+	1.0	340	0.030
62431-02-1	ME	MeNic+	0.94	340	0.026
	ME	MeNic+	0.5	340	0.030
	ME	MeNic+	0.5, 20% EtOH	340	0.040
	ME	MeNic+	0.5, 33% EtOH	340	0.061
	ME	MeNic+	0.5, 50% EtOH	340	0.11
	ME	MeNic+	0.5, 50% dioxane	340	0.18
62521-39-5	ME	MeIsonic+	0.97	340	0.02
	ME	MeIsonic+	0.47	340	0.024
	ME	MeIsonic+	0.47, 47% EtOH	340	0.036 ± 0.001
	ME	MeIsonic+	0.31, 63% EtOH	340	0.051
	ME	MeIsonic+	0.47, 47% dioxane	340	0.045
	ME	MeIsonic+	0.31, 63% dioxane	340	0.072 ± 0.03
62521-40-8	AcCys	MeIsonic+	0.94	340	0.0185 ± 0.0005
62521-41-9	TG	MeIsonic+	0.97	340	0.026 ± 0.002
	TG	MeIsonic+	1.0	330	0.025 ± 0.002[b]

[a] Measured at 25 ± 1 °C unless otherwise stated. [b] Measured at 25.0 ± 0.1 °C. [c] Solvent is water unless otherwise specified.

Table V. Rate and Association Constants for the Interaction of Primary Amines with Pyridinium Ions[a]

Registry no.	Pyridinium ion	Amine	k, M^{-1} s^{-1} (pH)	pH range of equilibrium study	λ_{max}, nm	μ, M	K_{xy}	K_x, M^{-1}	$K_{xy}\epsilon$, M^{-1} cm^{-1}	$K_x\epsilon$, M^{-2} cm^{-1}
62430-69-7	NAD^+	NH_2OH	220 (11.1) 110 (10.2)[b]	9.2–11.5	315	2.5	1.7×10^{-11}	0	1.66×10^{-7}	0
62430-70-0		$MeONH_2$		10.8	310	0.5			6.2×10^{-8}	
62430-71-1		NH_2NH_2		8.4–10.5	310	1.0			1.5×10^{-8}	0
62430-72-2		Butylamine		10.8–11.7	300	0.63				310[c]
62430-73-3		Glycine		10.6	300	0.9				380
62430-74-4	NMN^+	NH_2OH		9.1–10.7	313	2.5	1.7×10^{-11}	0	1.6×10^{-7}	0
62430-75-5	$APAD^+$	NH_2OH	73 (8.7)	8.7–9.8	325	2.5	2.1×10^{-9}	3.0	1.9×10^{-5}	0
62430-76-6		NH_2NH_2		8.3–9.4	330	1.0	1.2×10^{-9}	b	5.0×10^{-7}	17 000
62430-77-7	$BzAP^+$	NH_2OH		9.0–10.8	333	2.5	5.7×10^{-10}	0.3	3.7×10^{-7}	0

[a] Equilibrium determinations were made at 25.0 ± 0.1 °C. Rate determinations were made at 25 ± 1 °C at ionic strength 1.25. [b] At pH 9.5 the equilibrium constant could be determined separately as 3.9 ± 1.1. K_{xy} is calculated as $K_{obsd}[H^+]$. [c] Corrected for the ionization of NAD^+ (ref 4).

MeIsonic+ (changing the solvent from 0 to 50% dioxane) causes only a 1.9-fold increase in $K_{xy\epsilon}$ and no change in bandwidth (130-nm half-width).

The reaction of primary amines with pyridinium ions consists of two reactions: (1) a rapid, reversible reaction, (2) a slow, irreversible reaction. The λ_{max} of the products of the second irreversible reaction is greater than that of the first reversible reaction. Though all the reversible amine interactions are weak, the more strongly interacting systems (hydroxylamine and hydrazine) show a pH dependence according to eq 4, and the weakly interacting systems (butylamine) show a pH independence. An example of the pH dependence of hydrazine interaction is shown in Figure 5. In the case of $BzAP^+$ and $APAD^+$, a small non-pH-dependent component is present in the reaction of hydrazine and hydroxylamine. The interaction of butylamine and glycine with $BzAD^+$ was so weak as to be nonmeasurable ($K_{tot\epsilon}$ is less than 0.4). The rate and equilibrium data are collected in Table V.

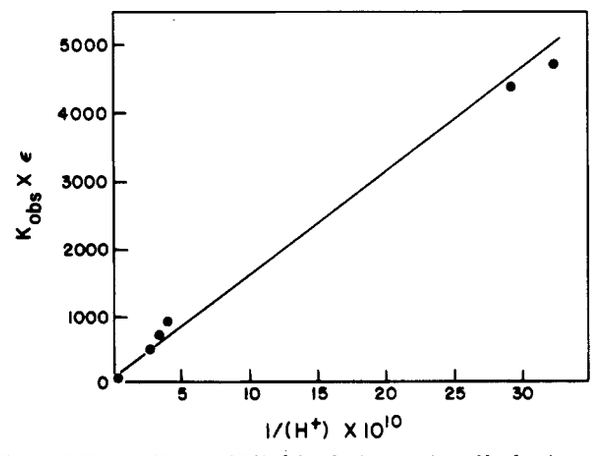


Figure 5. Plot of $K_{tot\epsilon}$ vs. $(H^+)^{-1}$ for the interaction of hydrazine with NAD^+.

Table VI. Association Constants for the Interaction of Imidazole with Pyridinium Ions at 25.0 °C, μ = 0.63 M

Registry no.	Pyridinium ion	λ_{max}, nm	K_{tot}, M^{-1} (pH)[a]	$K_{tot\epsilon}$, M^{-2} cm^{-1} (pH)[a]	$K_{xy\epsilon}$, M^{-1} cm^{-1}	$K_{x\epsilon}$, M^{-2} cm^{-1}
62430-65-3	NAD^+	305	0.15 ± 0.04(10.1)	84.7 (10.1)	6.8×10^{-9}	
62430-66-4	$APAD^+$	325	0.45 (10.1)	3130 ± 170 (10.1)		
				630 (9.5)	2.5×10^{-7}	89
				270 ± 40 (8.0)		
62475-94-9	$PAAD^+$	325	1.15 ± 0.08(10.1)	11 500 (10.1)	8.4×10^{-7}	982
				1070 (8.0)		
62430-67-5	$BzNic^+$	315		111 ± 2 (9.8)		
				112 ± 2 (9.0)		
				100 (8.0)		
62430-68-6	$BzAP^+$	335		110 (9.8)		
				66 (9.5)		87
				110 (9.0)		
				62 (8.0)		
62461-60-3	$BzCP^+$	335		22 ± 6 (10.0)		

[a] pH values at which the measurement was made are given in parentheses.

Table VII. Association Constants for the Interaction of Various Anions with Pyridinium Ions[a]

Registry no.	Nucleophile	Pyridine nucleotide	μ, M	λ_{max}, nm	$K_{tot\epsilon}$, M^{-2} cm^{-1} (pH)
62430-78-8	N_3^-	NAD^+	1.0	300	201 (9.5)[c]
62430-79-9	SCN^-		1.0	300	862 (5.85)
62430-80-2	Thiourea		0.6	315	260 (10.8)
62430-81-3	SCN^-	$APAD^+$	1.0	290	873 (3.6)
	SCN^-		1.0	300	659 (5.8)
62430-82-4	SCN^-	$PAAD^+$	1.0	300	344 (5.9)
62430-83-5	SCN^-	$MeNic^+$	1.0	320	190 (6.2)
	SCN^-		1.0	300	547 (5.8)
62461-61-4	SCN^-	$MeIsoNic^+$	1.0	300	617 (5.9)
			1.0	310	477 (5.9)

[a] Measured at 25.0 ± 0.1 °C.

The interaction of imidazole can be broken down into K and ϵ in the case of the NAD^+, $APAD^+$, and $PAAD^+$ reactions at higher pH. At lower pH only $K_{tot\epsilon}$ could be measured. In the case of $APAD^+$, a pH study was carried out on $K_{tot\epsilon}$ and both pH-independent and OH^--dependent terms $K_{b\epsilon}$ and $K_{xy\epsilon}$, respectively, were found. The $K_{tot\epsilon}$ values for $BzNic^+$ and $BzAP^+$ are independent of pH. A summary of these results is shown in Table VI. In contrast to the reaction of primary amines, the reaction of imidazole, a secondary amine, is entirely reversible.

The interactions of various weakly interacting anions are pH independent. These data are collected in Table VII. An attempt was made to measure the equilibrium of hydroperoxide anion, HO_2^-, with NAD^+. The equilibrium is very slowly established, and because it is necessary to use alkaline solutions (pH 11) to get appreciable concentrations of HO_2^- (pK = 11.5), competing hydrolysis reactions take place. A new band at 320 nm could be seen in alkaline solutions containing H_2O_2, which could not be seen in the control alkaline solutions. The rate of reaction of HO_2^- with NAD^+ is estimated to be 2 M^{-1} s^{-1}.

Those nucleophiles which do not form measurable complexes with NAD^+ ($K_{tot\epsilon} < 250$ M^{-2} cm^{-1}, or no distinct λ_{max}) are thiourea, urea, thiosulfate, nitrite, and phenyl sulfinite.

Discussion

Strong nucleophiles interact with the pyridinium ring to form 1–4 adducts as is indicated by (a) the NMR spectrum of the TG and the SO_3^{2-} adducts[13] and (b) deuterium exchange in the 4 position in heavy water of the cyanide adduct.[24]

In Table VIII are compiled rate and equilibrium data for nucleophilic interactions with NAD^+. The rate data for cyanide were taken from Lindquist and Cordes,[25a] and the rate data for hydroxide were taken from our previous work.[4,33] The order of reactivity follows the series $RS^- > SO_3^{2-} > OH^- > CN^-$, while the order of affinity follows the series $CN^- > SO_3^{2-} > RS^- > OH^-$. The reactivity order does not follow the affinity order, and neither the reactivity nor affinity order follows the basicity order. This situation is analogous to the cases of nucleophilic interactions with organic cations, such as triarylcarbonium ions, tropylium ions, and diazonium ions. The rates of the above reactions are all correlated by eq 10 with N_+, a parameter characteristic only of the nucleophile.[25b] The order of reactivity of the nucleophiles in Table VIII follows the N_+ series $RS^- > SO_3^{2-} > NH_2NH_2 > NH_2OH > OH^- > CN^-$. However, we do not get a linear correlation with N_+ according to eq 10. The points for CN^- and SO_3^{2-} give a slope of 0.96. On the other hand, the points for all the nucleophiles except SO_3^{2-} give a slope of 2.05 with an R^2 value of 0.998.

$$\log k_1 = \log k_0 + N_+ \quad (10)$$

The original interpretation[25b] of the constant selectivity of reactive and unreactive electrophiles toward nucleophiles according to eq 10 was that the transition states for the reactions are very reactant-like, lying somewhere between the solvent-separated and intimate ion pair structures. It was assumed that the cation in the transition state has its solvent shell little perturbed from the ground-state solvent shell. The inherent property of the nucleophile, N_+, was interpreted as the degree of solvation energies of the nucleophiles. If eq 10 is followed by both strong and weak electrophiles, then not

Table VIII. Equilibrium Constants and First-Order Rate Constants for the Interaction of Nucleophiles with NAD+[a]

Nucleophile	pK_a[k]	Log k_1	Log K_b	Log K_{xy}	P_+, log $K_{xy\epsilon}$	N_+[b]	γ	$\gamma + 7.9\sigma_I$
CN^-	9.4	0.74[d]	2.34	−7.06	−3.34	3.67	2.44	7.02
SO_3^{2-}	7.0	3.3	1.61	−5.39	−1.74	7.90	4.02	9.78
OH^-	15.75	1.45[f]	−0.75	−16.50	−12.12	4.75	−3.58	−1.60
TG	10.3	>4	0.55	−9.75	−5.81	9.06[c]	+0.53	2.50
ME	9.50	>4	0.26	−9.24	−5.54		+0.35	2.32
NH_2OH	(5.97)[g]	2.2	*h*	−10.76	−6.78	5.05	+1.24	2.42
$MeONH_2$	(4.60)[g]	1.38	*h*	−11.2[i]	−7.2			
NH_2NH_2	(8.1)[g]	3.3	*h*	−11.8[i]	−7.8	5.66	+0.81	2.00
N_3^-	4.72				2.30	7.6		
SCN^-	0.85				2.94			
Thiourea	*g*				2.42[j]			
ImH	14.52				−8.1	3.66		

[a] Determined at 25 ± 2 °C, ionic strength at 0.6 M, except for hydroxylamine where the ionic strength was 1.2 M. Units of k_N are $M^{-1}\,s^{-1}$. Units of K_b are M^{-1}. [b] N_+ values are from Ritchie.[25c] [c] N_+ value for TG was determined here by observing its rate of reaction with malachite green.[25b] [d] From Lindquist and Cordes.[25a] [e] Estimated value. [f] Calculated from data in Johnson and Morrison,[4] and Guilbert and Johnson.[33] [g] The value given is for ionization from RNH_3^+; the ionization constant of RNH_2 is too small to measure. [h] Cannot measure separately. [i] Estimated. [j] Log $K_{tot\epsilon}$. [k] pK_a values from Jencks.[21]

only is there a constant selectivity of carbonium ions for nucleophiles, but there is also a constant selectivity of nucleophiles (both strong and weak) for carbonium ions. In eq 10 k_0 is the first-order water rate constant, and is a measure of the reactivity of the carbonium ion. For a single nucleophile N_1 reacting with a series of carbonium ions $E_1, E_2, E_3, \ldots, E_x$, eq 11 follows, where $k_{N_1E_x}$ are the rate constants for the interaction of N_1 with the series of $E_1, \ldots, E_x$, $N_+(N_1)$ is the N_+ value for N_1, and k_{0E_x} is the first-order rate constant for the interaction of water with E_x (the reactivity of E_x).

$$\log k_{N_1E_x} = N_+(N_1) + \log k_{0E_x} \qquad (11)$$

The dichotomy of mechanisitic interpretations according to eq 10 and 11 has been clarified by the treatment of Pross,[26a] which takes into account the desolvation of both the cation and the nucleophile. The constant selectivity of electrophiles or nucleophiles is the result of the cancellation of two opposing effects. (a) The selectivity of strongly solvated reactive electrophiles and nucleophiles will be reduced in comparison to weakly solvated, unreactive electrophiles and nucleophiles, owing to the necessity of desolvating the ground state to reach the transition state structure. (b) The selectivity of a strong electrophile or nucleophile will be decreased in comparison to a weak electrophile or nucleophile, due to the more reactant-like transition state for the strong reagents.

It is of interest to look into the nature of the transition state structure as a function of the nucleophile. From linear free energy correlations of k_1 with K_1 the degree of product-likeness can be calculated for various nucleophiles. In the case of the reactions of cyanide and sulfite with nicotinamide cations, these values are 54 and 94%, respectively.[13,25c] For the reaction of hydroxide with quinolinium ions, this value is 36%.[26b] Calculations from the data of the reaction of nucleophiles with cations which follow N_+ give the following product likenesses: hydroxide–tropylium ion, 70%;[26b] cyanide–diazonium ion, 65%;[26c] hydroxide–diazonium ion, 32%;[26c] amines–triaryl carbonium ion, 82%.[26d] Since variations of transition state structure are found in both the cations which follow N_+ and in pyridinium ions, it is unlikely that the marked variation of transition state structure in the pyridinium ion series results in the poor N_+ correlation. Recently, ester carbonyl addition reaction rates have been correlated with N_+.[25c] This means that the interpretations of the reaction mechanism for electrophile–nucleophile interaction can be extended to uncharged electrophiles.

The equilibrium constants for addition to the pyridinium

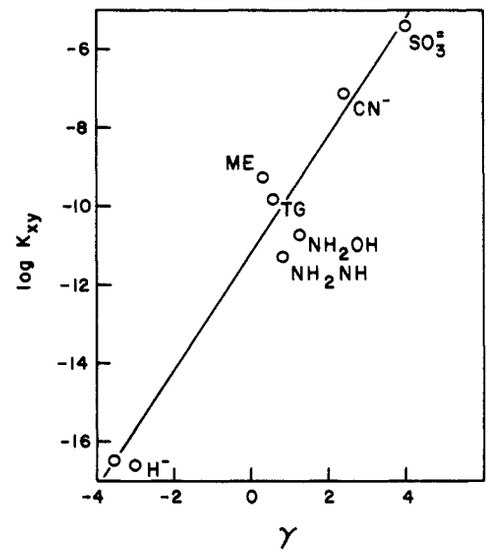
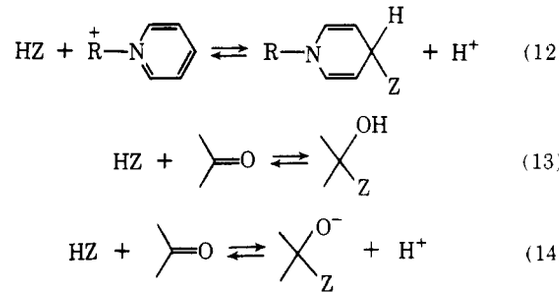


Figure 6. Plot of log K_{xy} vs. γ for nucleophilic addition to NAD^+.

ring, eq 12, can be correlated with the equilibrium constants for addition to the carbonyl carbon, eq 13. The latter reactivity



is related to the γ value.[27] The value of the equilibrium constant for eq 12 is given by K_{xy}. A plot of log K_{xy} vs. γ gives an intercept of −11.2, a slope of 1.45, and an R^2 value of 0.90, as seen in Figure 6. A similar plot of log $K_{xy\epsilon}$ vs. γ gives an intercept of −7.28, a slope of 1.35, and an R^2 value of 0.90. Because the relationship between eq 12 and 13 does not have the same stoichiometry, the relationship between eq 12 and 14, which have the same stoichiometry, was tested. The ionization of alcohols is related to $\Sigma\sigma_I$ values.[28] In the present case of varying the Z substituent only, the free energy of eq 14 is given by $\gamma + 7.9\sigma_I$. Equations 14 and 12 are no better correlated than

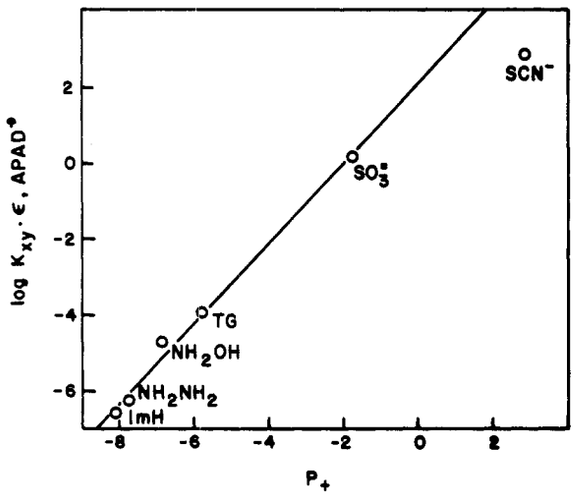


Figure 7. Plot of log $K_{xy\epsilon}$ for APAD$^+$ vs. P_+ (log $K_{xy\epsilon}$ for NAD$^+$).

eq 13 and 12. A plot of log K_{xy} vs. $\gamma + 7.9\sigma_I$ gives an intercept of -13.2, a slope of 0.89, and an R^2 value of 0.87. A plot of log $K_{xy\epsilon}$ vs. $\gamma + 7.9\sigma_I$ gives an intercept of -9.09, a slope of 0.84, and an R^2 value of 0.88.

The equilibrium constants for the addition of nucleophiles to pyridinium ions are better correlated to the equilibrium constants of the same nucleophiles to other pyridinium ions than to the carbonyl carbon. For example, a plot of log $K_{xy\epsilon}$ for APAD$^+$ vs. log $K_{xy\epsilon}$ for NAD$^+$ yields an intercept of 2.05, a slope of 1.05, and an R^2 value of 0.99, when all the nucleophiles except CNS$^-$ in Table VIII are included (Figure 7). Including CNS$^-$, the intercept is 0.86, the slope is 0.87, and the R^2 value is 0.98 (Figure 7). The value log $K_{xy\epsilon}$ is suggested as a better measure of equilibrium nucleophilic activity to the pyridinium ring, and is given the label P_+. Thiocyanate, undoubtedly, interacts by a charge transfer interaction, which is different from the covalent interaction of the other nucleophiles in Table VIII. The negative deviation of CNS$^-$ in Figure 7 suggests a lesser sensitivity to electrophilic character of the pyridinium to charge transfer interaction than to covalent interaction.

The K_{eq} of interaction of a nitrouracil with anionic reagents, Z$^-$, and of quinazolinium ion with HZ both correlate with γ.[30] The rate constants for the latter reaction were found to correlate with basicity, with hydroxide giving a negative deviation. For those nucleophiles in the present study having known N_+ values (5), we found that the reactivity order follows N_+, with a positive deviation for hydroxide. The correlation of K_{eq} with γ in the above examples of heteroaromatic addition reaction and in the present example of pyridinium ion reaction is expected, because γ measures the relative affinity of Z$^-$ for H, as compared to the hydroxy carbon atom (in the case of carbonyl addition) or dihydroheteroaromatic carbon (in the case of heteroaromatic ring addition). The different correlations of the rates of reaction represent a point of difference between our studies and those of Pitman.[30] Too few rate constants were available from either study for a complete description of this process.

There appears to exist a continuum of types of interaction, from covalent interaction in the case of strongly interacting systems to charge transfer complexation in the case of weakly interacting systems. In the case of thiolate complexation with pyridinium compounds, the solvent effect on $K_{obsd\epsilon}$ can be used to diagnose the presence of a contact charge transfer complex or a covalent adduct. It is useful to compare MeNic$^+$ and MeIsonic$^+$, because the latter compound can only form charge transfer complexes and not covalent complexes, while the former can form both types of complexes.[31] A change in solvent from 0 to 50% dioxane causes a sixfold increase in $K_{obsd\epsilon}$ and a dramatic increase in the sharpness of the band for MeNic$^+$. It only causes a 1.9-fold increase in $K_{obsd\epsilon}$, and no change in the bandwidth for MeIsonic$^+$, which cannot undergo ring addition. The weak interaction for MeNic$^+$ in water probably represents a charge-transfer phenomenon, which changes to a covalent complex formation in solvents of lower dielectric constant. In the case of the stronger interaction of thioglycolate with NAD$^+$, a structural study was made by NMR spectroscopy. In the presence of mercaptide, the pyridinium bands of NAD$^+$ decreased somewhat, and new signals typical of the dihydropyridine structure appeared. Weakly basic amines, such as imidazole, which react with NAD$^+$ in a pH-independent reaction, represent another example of the charge transfer interaction.

The effect of ring substituents on the pyridinium ring is to change the transition state structure of the ring addition reaction. In the case of sulfite addition to the nicotinamide ring, it was previously found that the transition state is 94% productlike.[13] In comparing the rate and equilibrium constants for sulfite addition to the acetylpyridine vs. the nicotinamide ring, a larger effect is observed for the equilibrium constant (35- to 120-fold more favorable for the acetylpyridine derivative) than for the rate constant (6- to 7-fold faster for the acetylpyridine derivative). This larger equilibrium-than-rate effect is explicable for the acetylpyridine derivatives, by a less productlike transition state than in the case of the nicotinamide series.[32] The reactivity–selectivity rules of chemistry appear to be operating, in the case of addition to the pyridinium ring.

The effect of boration of the riboside linkage adjacent to the pyridinium ring[13] has about the same effect on ring addition reactions for all of the nucleotides, regardless of the structure of the pyridinium ring on the nucleotide (NAD$^+$ vs. APAD$^+$ or NADP, etc.). In all cases the ring addition equilibrium is reduced three- to sevenfold.

In conclusion, we have found that the affinity of nucleophiles for the pyridinium ring correlates with γ, the affinity of nucleophiles for the carbonyl carbon. A better correlation is obtained with P_+, the affinity of nucleophiles with the pyridinium ring. The rate of nucleophilic addition follows the N_+ order, but does not follow eq 10. These correlations are useful in predicting the inhibition constants and rate of inhibition of pyridine nucleotide requiring dehydrogenases, where the mechanism of inhibition is complex formation with NAD$^+$ or NADP.[34]

Acknowledgment. The work of Mr. Harry Silvis in obtaining the equilibrium constants of the NAD$^+$–RSH system is gratefully acknowledged.

Registry No.—BzAP$^+$Cl$^-$, 5096-12-8; benzyl chloride, 100-44-7; 3-acetylpyridine, 350-03-8; MeAP$^+$I$^-$, 6965-62-4; methyl iodide, 74-88-4; MeCP$^+$I$^-$, 4685-10-3; MeNic$^+$I$^-$, 6456-44-6; MeIsonic$^+$I$^-$, 5613-08-1; methyl nicotinate, 93-60-7; Nic, 59-67-6; IsoNic, 55-22-1; BzCP$^+$Cl$^-$, 7146-29-4; BzNic$^+$Cl$^-$, 5096-13-9; phenylboronic acid, 98-80-6; boric acid, 10043-35-3.

References and Notes

(1) This work was supported by Public Health Service Grant GM-16856.
(2) Abbreviations used follow: ZH, a nucleophile; MeNic$^+$, 1-methylnicotinamide cation; BzNic$^+$, 1-benzylnicotinamide cation; MeIsonic$^+$, 1-methylisonicotinamide cation; PAAD$^+$, pyridine-3-aldehyde adenine dinucleotide; APAD$^+$, 3-acetylpyridine adenine dinucleotide; BzAP$^+$, 1-benzyl-3-acetylpyridinium ion; MeAP$^+$, 1-methyl-3-acetylpyridinium iodide, NAD$^+$, β-nicotinamide adenine dinucleotide; NADB, borate adduct of NAD$^+$; NADSO$_3^-$, sulfite adduct of NAD$^+$; NADBSO$_3^-$, NAD$^+$ complexed with borate and sulfite; NMN, nicotinamide adenine mononucleotide; NMNSO$_3^-$, sulfite adduct of NMN$^+$; NADP, nicotinamide adenine dinucleotide phosphate; deamino APAD$^+$, 3-acetylpyridine deaminoadenine dinucleotide; ThioNAD$^+$, thionicotinamide adenine dinucleotide; deamino-NAD$^+$, nicotinamide deaminoadenine dinucleotide; B, borate; PhB, phenylborate; BzCP$^+$, 1-benzyl-3-carboxymethylpyridinium ion; EtOH, ethanol; ME, 2-mercaptoethanol; TG, thioglycolate; Gly, glycine.

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New Routes to Heterobicyclic Ring Systems via Meta-Bridging. 4. Reactions of Nitroquinoline and Dinitropyridine

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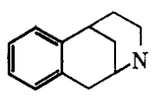
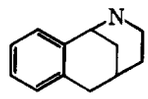
The first examples of heteroaromatic meta-bridging of pyridines and quinolines with amidines and carbanions are described. The effect of aza functionality on the mode of reaction is discussed. Reactions of the corresponding *N*-oxides are also described. The meta-bridged products, highly functionalized aza and diaza bicyclics, result from bis nucleophilic addition of amidines or carbanions to the electron-deficient heterocycles.

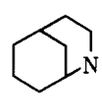
The formation of highly functionalized derivatives of the ring systems **1a, 1b,** and **2** from reaction of electron-deficient



1a



1b



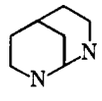
2

naphthalenes and benzenes with amidines has recently been reported.[2,3] Such products are readily formed by cyclization of anionic σ complex (Meisenheimer complex) intermediates which result from nucleophilic addition of amidine to the aromatic, a reaction we have termed "meta-bridging".[13] This type of reaction proceeds in two steps and is distinctly different from the 1,3-dipolar cycloadditions reported by Katritzky[1b] which also yield meta-bridged products.

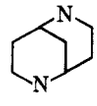
Electron-deficient pyridines form anionic σ complexes[4-7]

3a

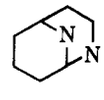
3b



4a



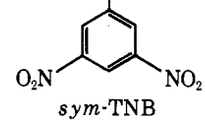
4b



4c

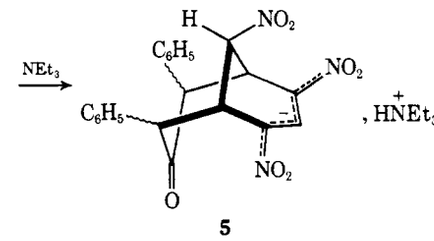
and the activating effect of heterocyclic nitrogen in nucleophilic aromatic substitution has been of interest in this regard.[8] It was thus of interest to investigate the meta-bridging reactions of electron-deficient pyridines. With such substrates, meta-bridging with carbanions could yield either of the ring systems **3a** or **3b,** whereas with amidines **4a, 4b,** or **4c** could result.

The meta-bridging reactivity of dibenzyl ketone with *sym*-trinitrobenzene (TNB) in the presence of triethylamine has been studied in some detail.[9] The reaction occurring has been well characterized and leads to the bridged product **5.** Isomers with both cis and trans phenyls have been isolated,[9]



sym-TNB

+ $(C_6H_5CH_2)_2C{=}O$



5