Secondary Valence Force Catalysis. IV. Rate and Equilibrium Constants for Addition of Cyanide Ion to N-Substituted 3-Carbamoylpyridinium Ions[1]

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Abstract: Rate and equilibrium constants for 1,4 addition of cyanide ion to a series of N-substituted 3-carbamoylpyridinium ions have been measured in aqueous solution at 25°, ionic strength 0.50. All reactions were followed spectrophotometrically near 340 mμ, the absorption maximum for 1,4 adducts of pyridinium ions. In several cases, products absorbing maximally near this wavelength were isolated and shown to be, in fact, the 1,4 adducts by nuclear magnetic resonance spectroscopy or mass spectrometry. The second-order rate constants increase markedly and the K_{diss} values decrease markedly with increases in the electron-withdrawing power of the substituent on the ring nitrogen. Approximate values of ρ^* were obtained for the second-order rate (2.2) and dissociation (−3.7) constants. Nicotinamide mononucleotide and both the α and β isomers of nicotinamide adenine dinucleotide have abnormal affinities for cyanide in view of their reactivities toward this species. The 1,4-cyanide adducts of the 3-carbamoylpyridinium ions are not the thermodynamically stable species in alkaline aqueous cyanide solutions at 25° as shown by the time-dependent loss of the 340-mμ absorption band. On standing for several days, the N-propyl and N-methyl derivatives lose their absorption maxima near 340 mμ, develop a new maximum near 305 mμ, and exhibit increased absorption near 260 mμ. Compounds possessing stronger electron-withdrawing substituents on nitrogen exhibit more rapid spectral changes and, at equilibrium, possess absorption maxima near 260, 320, and 420 mμ. Charge-transfer complexation has little effect on the reactivity or affinity of pyridinium ions toward cyanide as evidenced by rate and equilibrium constants for addition of this species to intramolecularly complexed substrates. An increase in ionic strength in the range 0.5–3.0 *M* of the reaction medium increases dissociation constants and decreases second-order rate constants for the addition of cyanide to the 4 position of N-methyl-3-carbamoylpyridinium ion.

As a result, in part, of the biochemical importance of nucleophilic additions to pyridinium ions, these reactions have received considerable attention.[4,5] Nevertheless, some rather substantial gaps in information and understanding for these reactions exist. This paper reports results of attempts to fill certain of these for one such reaction: addition of cyanide to N-substituted 3-carbamoylpyridinium ions. Experimental efforts have been directed toward three points of interest in this regard. First, what are the thermodynamically and kinetically controlled sites of attack of cyanide ion on these substrates? Second, what is the influence in changes of the nature of the polar substituent on nitrogen on the reactivity and affinity of pyridinium ions toward this nucleophile? And third, what is the effect of charge-transfer complexation of a pyridinium ion on its reactivity and affinity toward cyanide?

Following the proposal by San Pietro[6] that cyanide forms a 1,4 adduct with 3-carbamoylpyridinium substrates, considerable evidence has been accumulated consistent with this view.[7–11] Although much of the evidence is based on comparison of the spectrophotometric properties of the cyanide adducts and the corresponding 1,4-dihydropyridines, several of the former have been isolated and characterized.[8,10,12,13] The ultraviolet spectra of the 1,4- and 1,6-dihydro compounds as well as the difference spectrum of the 1,2-dihydrocompound derived from N-(2,6-dichlorobenzyl)-3-carbamoylpyridinium ion have been recorded by Sund, Diekmann, and Wallenfels.[5] The nmr spectra of a number of 1,3-disubstituted 1,4-, 1,6-, and 1,2-dihydropyridine derivatives have been reported,[11,14–17] and the spectrum of a 4-cyano-1,4-dihydro derivative has been published.[11] Thus there exists considerable information as to the spectral identification of 1,4, 1,2, and 1,6 adducts formed with nucleophilic reagents.

The reversibility of the cyanide addition reactions[18] suggests that the 1,4 adducts are the product of both thermodynamic and kinetic control. However, some doubt as to both the initial position of attack and the thermodynamically stable position of the cyano group was recently created by results of an investigation by Lyle and Gauthier.[19] These workers noted that, in organic solvents, the spectra of some cyanide–pyridinium ad-

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(4) T. C. Bruice and S. Benkovic, "Bioorganic Mechanisms," Vol. II, W. A. Benjamin, Inc., New York, N. Y., 1966, p 301.
(5) H. Sund, H. Diekmann, and K. Wallenfels, *Advan. Enzymol.*, **26**, 115 (1964).
(6) A. San Pietro, *J. Biol. Chem.*, **217**, 579 (1955).
(7) N. O. Kaplan in "The Enzymes," Vol. III, P. D. Boyer, H. Lardy, and K. Myrbäck, Ed., Academic Press Inc., New York, N. Y., 1960, pp 105–169.
(8) K. Wallenfels and H. Schüly, *Ann.*, **621**, 86 (1959).
(9) M. Lamborg, R. Burton, and N. Kaplan, *J. Amer. Chem. Soc.*, **79**, 6173 (1957).
(10) J. C. Powers, *J. Org. Chem.*, **30**, 2534 (1965).
(11) H. Diekmann, G. Englert, and K. Wallenfels, *Tetrahedron*, **20**, 281 (1964).
(12) M. Marti, M. Viscontini, and P. Karrer, *Helv. Chim. Acta*, **39**, 1451 (1956).
(13) A. G. Anderson, Jr., and G. Berkelhammer, *J. Org. Chem.*, **23**, 1109 (1958).
(14) R. F. Hutton and F. H. Westheimer, *Tetrahedron*, **3**, 73 (1958).
(15) H. E. Dubb, M. Saunders, and J. H. Wang, *J. Amer. Chem. Soc.*, **80**, 1767 (1958).
(16) A. F. Sims and P. W. Smith, *Proc. Chem. Soc.*, 282 (1958).
(17) W. L. Meyer, H. R. Mahler, and R. H. Baker, Jr., *Biochim. Biophys. Acta*, **64**, 353 (1962).
(18) K. Wallenfels and H. Diekmann, *Ann.*, **621**, 166 (1959).
(19) R. E. Lyle and G. J. Gauthier, *Tetrahedron Letters*, **51**, 4615 (1965).

ducts changed with time, undergoing a loss of the initial absorption at 260–280 mμ (and in some cases small absorption in the 400–430-mμ range) and a shift in the absorption near 320 mμ to an absorption peak near 340 mμ. These experimental findings suggested[19] that the initial attack was at the 2 and 6 positions but that the thermodynamically controlled product was the adduct at the 4 position.

A series of N-substituted 3-carbamoylpyridinium halides were chosen as substrates in view of their structural similarity to pyridine nucleotide coenzymes and to known cyanide adducts of pyridinium ions, and to permit controlled variation in structure. Some structure–affinity studies have been carried out by Wallenfels and co-workers on the reaction of cyanide with pyridinium ions.[18,20,21] Substrates employed in these studies had various substituents on the 3 position of the pyridinium ring. The electron-withdrawing capacity of these substituents proved to have a pronounced effect on the affinity of these ions for cyanide.

Interest in secondary valence force catalysis for nucleophilic addition reactions of pyridinium ions is heightened by a suggestion that a charge-transfer complex exists between an indole side chain of glyceraldehyde 3-phosphate dehydrogenase and the pyridinium ring of the bound coenzyme, nicotinamide adenine dinucleotide (NAD^+).[22] Complexes of this type (1:1) can also be demonstrated by spectrophotometric means to exist between NAD^+ or NAD^+ models and certain electron-rich compounds such as tryptophan, tyrosine, phenylalanine, and histidine.[9,23–26] Do such interactions alter substantially the reactivity of the pyridinium moiety? Little in the way of a coherent hypothesis has been published about the effects of these charge-transfer complexes on the rates of reactions. Swain[27] found a fourfold higher second-order rate constant in the reaction of sodium phenoxide with benzyldimethylsulfonium ion in water than in the reaction of the stronger base sodium hydroxide with the sulfonium ion species. He attributed the increase in reaction rate to "ancillary molecular bonding of the π complex or charge-transfer type."[27] Conners and Mollica found inhibition by imidazole, possibly due to charge-transfer complexation with the substrate, in the hydrolysis of methyl cinnamate.[28] Other studies of a similar nature have been carried out,[23,29] but no clear understanding as to the role of π complexation on reactivity has yet been realized. An attempt was made in this investigation to detect secondary valence force catalysis in the cyanide addition to N-substituted 3-carbamoylpyridinium substrates which exist as internal charge-transfer complexes.[23]

Experimental Section

Materials. The water used throughout this work was distilled through a Corning all-glass distillation apparatus, Model AG-1a. All acids and inorganic salts used were obtained commercially and were of reagent grade. Spectra of the substrates and products were recorded on a Cary 14 recording spectrophotometer, Perkin-Elmer 137 and 137G infrared spectrophotometers, and a Varian A-60 nuclear magnetic resonance spectrometer. Melting points were taken on a Thomas-Hoover Uni-melt.

N-Benzyl-3-carbamoylpyridinium chloride was prepared from nicotinamide and benzyl chloride[30] and melted at 235–236° dec (lit.[30] mp 236° dec). N-(β-Indolyethyl)-3-carbamoylpyridinium chloride was synthesized according to the method of Lettré, *et al.*,[31] from tryptamine and N-(2,4-dinitrophenyl)-3-carbamoylpyridinium chloride and, after drying the yellow crystals at 78° and 1 mm for 12 hr,[23] the compound melted at 235–236° dec (lit.[31] mp 236° dec). N-(2,4-Dinitrophenyl)-3-carbamoylpyridinium chloride was prepared from nicotinamide and 2,4-dinitrochlorobenzene[31] and was used in impure form with mp 110–115° (lit.[31] mp 115°). N-(4-Nitrobenzyl)-3-carbamoylpyridinium bromide was prepared according to the method described by Wallenfels, *et al.*,[32] from nicotinamide and α-bromo-4-nitrotoluene, mp 245.5–246° dec (lit.[32] mp 246° dec). N-(β-4′-Imidazolylethyl)-3-carbamoylpyridinium chloride was obtained by the method of Shifrin[23] from histamine and N-(2,4-dinitrophenyl)-3-carbamoylpyridinium chloride, mp 202–204° (lit.[23] mp 200–201°). N-Methyl-3-carbamoylpyridinium iodide was prepared according to Kosower[33] from nicotinamide and methyl iodide, mp 206.5–207° (lit.[33] mp 205.5–206°). N-(2,6-Dichlorobenzyl)-3-carbamoylpyridinium bromide was obtained by the procedure of Kröhnke, Ellegast, and Bertram[34] from α-bromo-2,6-dichlorotoluene and nicotinamide, mp 246–248° dec (lit.[34] mp 246–248° dec). α-Bromo-2,6-dichlorotoluene was prepared by bromination of 2,6-dichlorotoluene[34] and melted at 56° (lit.[34] mp 55°). N-Propyl-3-carbamoylpyridinium iodide was synthesized according to Kosower[33] and melted at 182–184° (lit.[33] mp 182°). N-Phenacyl-3-carbamoylpyridinium bromide was prepared according to Wallenfels[32] from phenacyl bromide and nicotinamide and melted at 234.5–236° (lit.[32] mp 234–236°).

α-Bromo-2-chloro-4-nitrotoluene was prepared by bromination of the disubstituted toluene compound. 2-Chloro-4-nitrotoluene (0.2 mol) was dissolved in 50 ml of chloroform and layered with 70 ml of H_2O. This was brought to a boil by heating with a 300-W tungsten-filament lamp and a small uv arc. To this stirred, refluxing solution, a solution of 0.2 mol of bromine in 25 ml of chloroform was dripped in slowly. Refluxing was continued until all of the bromine had been taken up (approximately 1.5 hr), then the cooled mixture was washed with $NaHSO_3$ and H_2O and the separated chloroform layer was dried with sodium sulfate. The chloroform was distilled off under vacuum leaving an oil which was dissolved in methanol and quick frozen in an acetone–Dry Ice bath. The mass of crystals obtained was removed and remained crystalline at room temperature. Recrystallization three times from petroleum ether (bp 90–120°) with charcoal gave the compound with a melting point of 49–50° (lit.[35] mp 49–50°).

N-(2-Chloro-4-nitrobenzyl)-3-carbamoylpyridinium bromide was prepared by refluxing 1.5 mmol of α-bromo-2-chloro-4-nitrotoluene and 1.5 mmol of nicotinamide in acetone for 1 hr. Upon cooling and with addition of ether, white crystals precipitated. These were recrystallized in methanol–ether solution giving crystals which melted at 234–235° dec, yield 84%. *Anal.* Calcd for $C_{13}H_{11}N_3O_3ClBr$: C, 41.85; N, 11.25; H, 2.96. Found: C, 41.94; N, 11.10; H, 3.15.

Kinetic measurements were carried out spectrophotometrically with the aid of a Zeiss PMQ II spectrophotometer equipped with a cell holder thermostated at 25°. All reactions of cyanide with the N-substituted 3-carbamoylpyridinium compounds were conducted in aqueous solution at pH 11.3 and ionic strength 0.5 (maintained with KCl) unless otherwise noted. The reactions were followed by observing the appearance of an absorption band near 340 mμ, the absorption maximum for 1,4 adducts of pyridinium ions,[8] and were initiated by the addition of 0.1 or 0.13 ml of substrate solution (2×10^{-2} *M* to 4×10^{-3} *M*) to 2.90 or 2.87 ml of cyanide solution. In all cases a sufficient excess of cyanide was employed so that pseudo-first-order rate behavior was observed. First-order rate constants were evaluated from the slopes of plots of log

(20) K. Wallenfels and H. Schüly, *Ann.*, **621**, 215 (1959).
(21) K. Wallenfels and M. Gellrich, *ibid.*, **621**, 149 (1959).
(22) G. Cilento and P. Tedeschi, *J. Biol. Chem.*, **236**, 907 (1961).
(23) S. Shifrin, *Biochemistry*, **3**, 829 (1964).
(24) G. Cilento and P. Giusti, *J. Amer. Chem. Soc.*, **81**, 3801 (1959).
(25) S. G. A. Alivisatos, F. Ungar, A. Jibril, and G. A. Mourkides, *Biochim. Biophys. Acta*, **51**, 361 (1961).
(26) S. G. A. Alivisatos, *Biochem. Biophys. Res. Commun.*, **4**, 292 (1961).
(27) C. Swain and L. Taylor, *J. Amer. Chem. Soc.*, **84**, 2456 (1962).
(28) K. Conners and J. A. Mollica, Jr., *ibid.*, **87**, 123 (1965).
(29) F. Menger and M. Bender, *ibid.*, **88**, 131 (1966).
(30) P. Karrer and F. J. Stare, *Helv. Chim. Acta*, **20**, 418 (1937).
(31) H. Lettré, W. Haede, and E. Ruhbaum, *Ann.*, **579**, 123 (1953).
(32) K. Wallenfels, H. Schüly, and D. Hofmann, *ibid.*, **621**, 106 (1959).
(33) E. M. Kosower and S. W. Bauer, *J. Amer. Chem. Soc.*, **82**, 2191 (1960).
(34) F. Kröhnke, K. Ellegast, and E. Bertram, *Ann.*, **600**, 176 (1956).
(35) F. Tiemann, *Chem. Ber.*, **24**, 699 (1891).

($OD_\infty - OD_t$) against time. Second-order rate constants were obtained from measured first-order constants and the expression $k_2 = k_{obsd}/[CN^- + K_{diss}]$. For the two cases of N-(4-nitrobenzyl)-3-carbamoylpyridinium bromide and N-(2-chloro-4-nitrobenzyl)-3-carbamoylpyridinium bromide, slower successive reactions interfered with determinations of infinite-time optical density values. By examining the rate of change of the optical density, the values for OD_∞ for both products were estimated and the approximate rate constants calculated. The interfering reactions were slower for the other substrates and the 4 addition was complete before evidence of secondary reactions appeared. The addition of cyanide to the N-phenacyl compound could not be followed owing to the immediate appearance of yellow color in aqueous solution at pH 11.3.

Equilibrium constants were evaluated, using the method of Behme and Cordes,[36] from the slopes of the plots of $(\epsilon_0 - \epsilon)/N$ against ϵ, where ϵ_0 is the absorbance of the substrate, ϵ the absorbance of the reaction mixture, and N the concentration of cyanide anion. This procedure is necessary since the reactions cannot be forced to completion at attainable concentrations of cyanide. Typical plots using this approach are indicated in Figure 1. In each case the slopes were established by a least-squares analysis of five–seven experimental points. Again as in the rate determination, it was necessary to estimate the OD_∞ values for the N-(4-nitrobenzyl) and N-(2-chloro-4-nitrobenzyl) products due to the interfering side reactions present. The reaction of β-NAD^+ did reach completion above 0.2 M cyanide and the value of K_{diss} was calculated directly in this case. The equilibrium constants were measured at 25°, ionic strength 0.5, and pH 11.3 unless otherwise noted.

The relative equilibrium constants calculated in the ionic strength study (Table I) were obtained from plots of product optical density at infinite times *vs.* cyanide concentrations. The relative K_{diss} values were calculated using the values of (1/slope) of these plots.

Table I. Effect of Ionic Strength on Equilibrium and Rate Constants for Addition of Cyanide Ion to N-Methyl-3-carbamoylpyridinium Ion in Aqueous Solution at 25° [a]

μ, M	K_{diss}^{rel},[b] M	k_2^{rel},[c] M^{-1} min^{-1}
0.5	0.85	5.1
1.0	1.4	3.1
2.0	2.3	2.1
3.0	2.8	1.9

[a] Reactions carried out in 0.05–0.50 M sodium cyanide. Ionic strength made up with sodium perchlorate, pH 11.3. [b] Values of K_{diss} are the (1/slope) values from plots of optical density at infinite time *vs.* cyanide concentration for each ionic strength. [c] Values of k_2^{rel} are obtained from $k_2 \cong k_{obsd}/K_{diss}$ since $K_{diss} \gg [CN^-]$.

Product Analysis. 4-Cyano-1,4-dihydro-N-methylnicotinamide was prepared by the method of Marti, Viscontini, and Karrer[12] from N-methyl-3-carbamoylpyridinium chloride in potassium cyanide solution. Recrystallization from acetonitrile gave cream-colored crystals which turned light yellow upon drying and which decomposed over a 10° range above 125°. The nmr spectrum (Varian A-60) of this compound in deuterated dimethyl sulfoxide showed a singlet at τ 2.88 (H_2), a broad peak at 3.21 ($-CONH_2$), a multiplet centered at 3.88 (H_6) possessing two main peaks, J = 7.5 cps, a complex pattern at 5.2–5.60 (H_4 and H_5), and a large singlet at 7.08 (methyl). This spectrum as well as the nmr spectra for all of the 4-cyano compounds synthesized have corresponding chemical shifts very similar to those reported for the 4-cyano-1,4-dihydro-3-carbethoxy-N-(2,6-dichlorobenzyl) compound.[11] The mass spectrum of this compound, recorded with an AEI MS-9 mass spectrometer, revealed a molecular ion at *m/e* 163 as expected. In addition, major fragments appeared at *m/e* 138, 137, 122, 106, 94, 93, and 78. These findings are consistent with expulsion of acetylenelike fragments from the 4-cyano adduct but cannot be rationalized on the basis of addition at either position 2 or 6.

4-Cyano-1,4-dihydro-N-benzylnicotinamide was prepared by the addition of a solution of 0.08 mol of N-benzyl-3-carbamoylpyridinium chloride in 200 ml of water to a solution of 0.16 mol of potassium cyanide in 20 ml. The resulting sticky precipitate was recrystallized from acetonitrile and gave light yellow crystals which decomposed over a 15° range starting at about 125°, yield 21%. *Anal.* Calcd for $C_{14}H_{13}N_3O$: C, 70.30; H, 5.44; N, 17.58. Found: C, 71.20, H, 6.08; N, 16.90. The ultraviolet spectrum (ethanol) showed a large peak at 340 mμ, suggesting a 1,4-cyanide addition compound. The nmr spectrum (Varian A-60) in deuterated dimethyl sulfoxide exhibited peaks at 2.73 (H_2), 2.76 (aromatic), 3.75 multiplet containing two main peaks, J = 7.5 cps (H_6), 5.18–5.55 complex (H_4 and H_5), and 5.60 (NCH_2–).

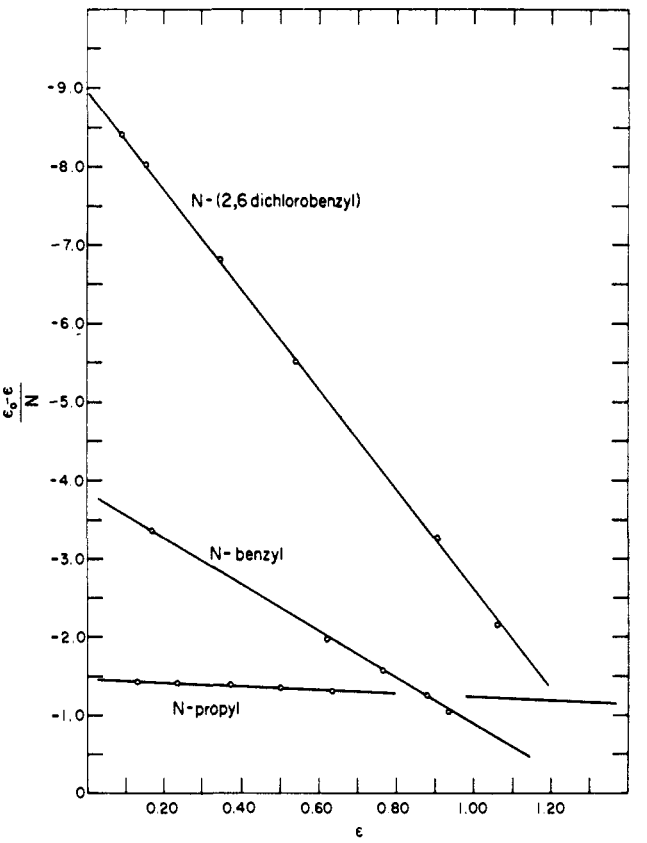

Figure 1. Plots of $(\epsilon_0 - \epsilon)/N$ against ϵ for the addition of cyanide ion to three N-substituted 3-carbamoylpyridinium ions indicating the method of obtaining dissociation constants for the cyanide adducts. See text for details of the method.

4-Cyano-1,4-dihydro-N-(2,6-dichlorobenzyl)nicotinamide was prepared according to the method of Wallenfels and Schüly[8] and after recrystallization in acetonitrile gave a light tan solid which decomposed slowly above 130° (lit.[8] dec pt 135°, gas 146°). The nmr spectrum (Varian A-60) in deuterated dimethyl sulfoxide showed peaks at 2.60 (aromatic), 2.78 (H_2), 3.92 multiplet containing two main peaks, J = 7.5 cps (H_6), 5.18–5.55 complex (H_4 and H_5), and 5.37 (NCH_2–).

4-Cyano-1,4-dihydro-N-(4-nitrobenzyl)nicotinamide was synthesized by adding an aqueous solution of 0.03 mol of potassium cyanide to a solution of 0.015 mol of N-(4-nitrobenzyl)-3-carbamoylpyridinium chloride in 150 ml of water. The sticky dark precipitate was recrystallized from acetonitrile with a small amount of ether added to initiate precipitation and the resulting bright yellow crystals decomposed slowly starting at 135°, gas 148°. *Anal.* Calcd for $C_{14}H_{12}N_4O_3$: C, 59.20; H, 4.38; N, 19.72. Found: C, 58.99; H, 4.42; N, 19.84. The ultraviolet spectra (chloroform) showed a large peak at 333 mμ in addition to a very large peak at 260 mμ.

After standing for various lengths of time, solutions of the N-substituted 3-carbamoylpyridinium ions in concentrated potassium cyanide lost their absorption maxima near 340 mμ characteristic of 4 adducts and developed new maxima which are possibly due to the presence of 2 adducts, 6 adducts, or both. All attempts to extract these adducts employing organic solvents of low dielectric constant resulted in isolation of only the 4 adducts. An effort to extract the former species into dimethyl sulfoxide resulted in decomposition of the products.

(36) M. T. A. Behme and E. H. Cordes, *Biochim. Biophys. Acta*, 108, 312 (1965).

Table II. Rate and Equilibrium Constants for Addition of Cyanide Ion to a Series of N-Substituted 3-Carbamoylpyridinium Ions in Aqueous Solution[a]

Compound	Concn range of cyanide, M	k_2, M^{-1} min^{-1}	K_{diss}, M
1. N-Propyl-3-carbamoylpyridinium iodide	0.05–0.50	0.84	5.4
2. N-Methyl-3-carbamoylpyridinium iodide	0.05–0.50	0.97	4.2
3. N-Benzyl-3-carbamoylpyridinium chloride	0.01–0.50	3.9	0.33
4. N-(2,6-Dichlorobenzyl)-3-carbamoylpyridinium bromide	0.01–0.50	5.8	0.16
5. N-(4-Nitrobenzyl)-3-carbamoylpyridinium bromide	0.02–0.30	14.	0.03
6. N-(2-Chloro-4-nitrobenzyl)-3-carbamoylpyridinium bromide	0.005–0.075	*Ca.* 20	*Ca.* 0.02
7. N-(β-Indolylethyl)-3-carbamoylpyridinium chloride	0.01–0.50	2.5	1.2
8. N-(β-4′-Imidazolylethyl)-3-carbamoylpyridinium chloride	0.02–0.50	2.6	0.93
9. Nicotinamide mononucleotide	0.01–0.50	5.4	0.015
10. Nicotinamide adenine dinucleotide (α isomer)	0.050–0.50	4.5	0.060
11. Nicotinamide adenine dinucleotide (β isomer)[b]	0.005–0.50	11.	0.0061

[a] All reactions followed spectrophotometrically near 340 mμ. Ionic strength maintained at 0.50; temperature 25°. Unless noted otherwise, all measurements made at pH 11.3. [b] pH 10.0.

Several attempts to oxidize the cyanide adducts of pyridinium ions with ferricyanide, in an effort to provide stable species for characterization, failed to yield substantial amounts of workable organic material.

Results

Addition of cyanide ion to alkaline solutions of N-substituted 3-carbamoylpyridinium ions results in formation of compounds exhibiting absorption maxima near 340 mμ. By comparison with spectra of isomeric dihydropyridines and from nuclear magnetic resonance and mass spectral data provided in the Experimental Section, it seems certain that these compounds are the 4 adducts. Second-order rate and equilibrium constants for a series of these reactions have been determined in aqueous solution at 25° and ionic strength 0.50 by methods described above; the results are collected in Table II. All reactions, with the exception of that for the β isomer of NAD^+, were carried out at pH 11.3. Both the rate and extent of these reactions were found to be independent of pH, as expected, as evidenced by the fact that both rate and equilibrium constants for addition of cyanide to N-benzyl-3-carbamoylpyridinium ion in 0.002 and 0.005 M solutions of sodium hydroxide did not vary with hydroxide ion concentration. In the four cases in which comparisons may be made, N-propyl, N-benzyl, N-(2,6-dichlorobenzyl)-3-carbamoylpyridinium ions, and the β isomer of NAD^+, equilibrium constants for cyanide addition are in reasonable agreement with those measured under somewhat different conditions by Wallenfels and Diekmann.[18]

Examination of the data for compounds 1–6 in Table II reveals that both rate and equilibrium constants are marked functions of the nature of the polar substituents on nitrogen. Electron withdrawal in these substituents increases both the reactivity and the affinity of pyridinium ions toward cyanide. Since direct resonance interactions between the pyridinium ring and the substituents on nitrogen are precluded by virtue of the nature of these substituents, one might reasonably expect that both the rate and the equilibrium constants would be correlated by the σ^* substituent constants.[37] Unfortunately, not all of the necessary constants have been determined. For the substituted aromatic substituents, therefore, values of σ^* were calculated from the relationship[38]

$$\sigma^*_{(XC_6H_4CH_2-)} = \frac{0.56}{1.72}\sigma^n_{(X)} + \sigma^*_{C_6H_5CH_2-}$$

and an estimated value of σ^n for *o*-chloro of 0.30 (σ^n for *p*-chloro = 0.24). In view of the uncertainties in the estimated values of σ^* and those in rate and equilibrium constants resulting from interfering secondary reactions in certain cases, plots of log k_2 and log K_{diss} against σ^* yield reasonably straight lines. Least-squares values of ρ^* of 2.2 and −3.7 were obtained for rate and equilibrium constants, respectively (six points, standard deviations 0.13 and 0.21, respectively, and correlation coefficients 0.98 and 0.98, respectively).

The data for nicotinamide mononucleotide and both the α and β isomers of NAD^+ (compounds 9, 10, and 11 in Table II) reveal that these species have an abnormally great affinity for cyanide in view of their reactivity toward this nucleophilic reagent. This fact is particularly pronounced for the β isomer of NAD^+. Thus, this compound has an affinity for cyanide five times as great as that of N-(4-nitrobenzyl)-3-carbamoylpyridinium ion despite the fact that the latter compound is somewhat the more reactive of the two.

Compounds 7 and 8 in Table II exist as internal charge-transfer complexes.[23] Nevertheless, the rate and equilibrium constants for these species are in reasonable accord with predictions based on polar effects of the N substituents alone. Thus, charge-transfer complexation seems to have rather little effect on the reactivity and affinity of pyridinium ions toward cyanide.

The ultraviolet–visible spectra of the products of cyanide addition to compounds 1–6 in Table II were recorded at various time intervals. Examples of these spectra are shown in Figures 2 and 3. For the addition of cyanide to the N-propyl- (Figure 2) and N-methylpyridinium compounds, the peak near 345 mμ slowly decreased in magnitude and at the same time a new absorption peak was formed near 305 mμ together with increased absorption near 260 mμ. No spectral changes were ever observed in solutions at pH 11.3, ionic strength 0.5, when cyanide was absent. Compounds 3–6 (Table I), which possess stronger electron-withdrawing substituents on the nitrogen, exhibited spectral shifts that took place much more rapidly, and at equilibrium showed absorption maxima near 260 mμ, 315–325 mμ, and 405–420 mμ (Figure 3). The spectral shifts ex-

(37) J. Hine, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 97.

(38) C. D. Ritchie and W. F. Sager, *Progr. Phys. Org. Chem.*, 2, 323 (1964).

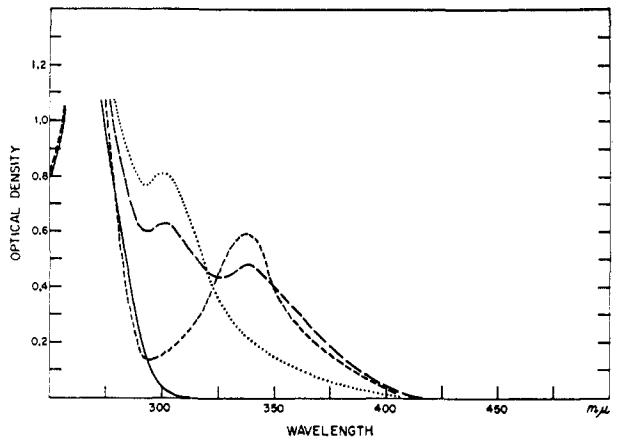

Figure 2. Visible and ultraviolet spectra recorded as a function of time for an aqueous solution containing initially 6.7×10^{-4} *M* N-propyl-3-carbamoylpyridinium iodide and 0.40 *M* cyanide, pH 11.3: ———, no cyanide; - - - - -, 5 min after cyanide addition; – – – –, after 1 day; . . . . , after 5 days.

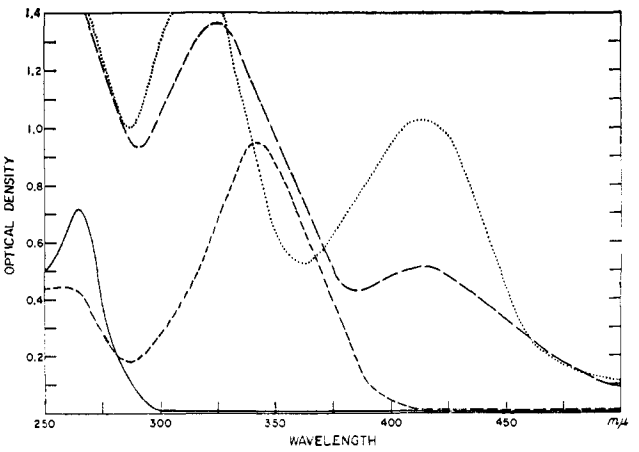

Figure 3. Visible and ultraviolet spectra recorded as a function of time for an aqueous solution containing initially 1.73×10^{-4} *M* N-(2,6-dichlorobenzyl)-3-carbamoylpyridinium chloride and 0.30 *M* cyanide, pH 11.3: ———, no cyanide; - - - - -, 5 min after cyanide addition; – – – –, after 1 day; . . . . , after 5 days.

hibited by these cyanide adducts suggest a possible rearrangement of the 1,4-cyanide adduct to the 1,6- and 1,2-cyanide adducts. After the failure to isolate such adducts (Experimental Section), reversibility studies[18] were carried out to determine if these species could be converted back into the substrates with the loss of cyanide.

The addition of cyanide to the 4 position of the N-benzyl compound is completely reversible. Shortly after the addition of cyanide at pH 11.3 the spectrum exhibited a decrease in the 265-mμ absorption of the substrate and the development of a new peak near 340 mμ. Following acidification to pH 1, which in effect dilutes the cyanide, the spectrum was identical with the spectrum of the N-benzyl compound itself. When base was added bringing the reaction media to pH 11 the spectrum again exhibited a peak at 340 mμ.

The reversibility studies for the suggested 1,2- and 1,6-cyanide adducts were unfortunately not quite as clear-cut. The reaction solution containing the N-benzyl compound and 0.20 *M* cyanide, pH 11.3, was allowed to stand for 1 day at room temperature at which time the absorption spectrum showed peaks near 325 and 410 mμ and exhibited an increased absorption near 260 mμ. The pH was lowered to 2.0 and after 1 day the spectrum looked very much like that of the starting material with the same λ_{max} (265 mμ) and nearly the same peak height taking into account dilution of the solution, but the peak tailed off slightly and was not as sharp as that for the starting material itself. Thus, formation of those species absorbing maximally near 325 and 410 mμ seems to be substantially, but not completely, reversible. Base was added to bring the reaction solution to pH 11, and the spectrum taken 5 min later showed an increase in the absorption near 340 mμ and a decrease in the band at 265 mμ. This spectral change became even more pronounced shortly thereafter upon the addition of 1 *M* KCN (to replace CN^- lost as HCN at pH 2), as is to be expected if the cyanide ion has again added to the pyridinium ring of the N-benzyl compound at position 4. On further standing, the material showed evidence of decomposition.

The effect of ionic strength on the rate and equilibrium constants for addition of cyanide to N-methyl-3-carbamoylpyridinium ion was investigated in solutions of sodium perchlorate; the results are collected in Table I. The pseudo-first-order rate constants (k_{obsd}) were obtained as usual but only the relative equilibrium and second-order rate constants could be evaluated from the plots of product optical density against cyanide concentration. Increased ionic strength decreased the extent of cyanide addition to a point at which insufficient cyanide adduct existed at equilibrium to permit evaluation of the actual equilibrium constants. Values of K_{diss} increase and those for k_2 decrease with increasing ionic strength and do so to about equal extents.

Discussion

Ultraviolet, proton magnetic resonance, and mass spectra all suggest that the initial site of addition of cyanide to the N-substituted carbamoylpyridinium ions in water occurs at position 4, in agreement with the conclusions of a number of previous studies on related reactions.[7-13] In contrast, the 4 adducts seem not to be the stable species in aqueous solution. The spectrophotometric properties of products formed subsequent to formation of the 4 adducts (Figures 2 and 3) are related to those of isolated 1,2- and 1,6-dihydro species[32,39-41] suggesting formation of the corresponding cyanide adducts in this system. This conclusion must be considered very tentative owing to inability to isolate these products and to the existence of reasonable alternative explanations for the spectral changes. At any event, the 4 adducts are the kinetically stable, but not the thermodynamically stable, species in water in contrast to the observation of Lyle and Gauthier for related reactions in organic media.[19] The thermodynamic stability of the 4 adducts in organic solvents accounts for the difficulties in attempted isolation of the thermodynamically stable species in water (Experimental Section). It should be noted that no evidence for rearrangement of the 4-cyanide adducts of the pyridine nucleotides was obtained in this study. The apparent solvent dependence of the positional specificity

(39) K. Schenker and J. Druey, *Helv. Chim. Acta*, 42, 2571 (1959).
(40) R. E. Lyle and P. S. Anderson, *Advan. Heterocyclic Chem.*, 6, 45 (1966).
(41) R. E. Lyle, D. A. Nelson, and P. S. Anderson. *Tetrahedron Letters*, 553 (1962).

for addition of cyanide to N-alkylpyridinium ions has some parallels in the corresponding reactions of 1-alkoxypyridinium ions. For the latter reactions, addition of cyanide is followed by explusion of the alkoxy function with aromatization yielding stable substituted pyridines which may be isolated and characterized.[42-46] Decreasing the polarity of the solvent for addition of cyanide to 1-methoxypyridinium ion increased the fraction of 2-substituted product relative to that for the 4-substituted isomer; in contrast, if the corresponding 3-carboethoxy derivative is employed, decreasing polarity seems to favor the 4 isomer.[46] It is clear, at any event, that the nature of the solvent system employed may have a profound effect on the relative kinetic and thermodynamic stabilities of the possible positional isomers in nucleophilic reactions with pyridinium ion systems.

Inductive effects of polar substituents at the ring nitrogen of the 3-carbamoylpyridinium ions are marked for both the reactivity and affinity of these species toward cyanide ion; $\rho^* = 2.2$ and -3.7, respectively. While these values are quite large, they are not unexpected since the substrates possess a full positive charge on nitrogen that is partially lost in the transition states and completely lost in the products. The sensitivity of the equilibrium constants to polar effects is in accord with the findings of Wallenfels and Diekmann;[18] rate constants for these reactions seem not to have been measured previously. The sensitivity of the rate constants for cyanide addition to polar effects strongly suggests that the transition state resembles product more strongly than substrate. That is, the charge on nitrogen is probably largely destroyed and the carbon–carbon bond probably largely formed in the transition state. This conclusion is strengthened by the results relevant to changes in the relative rate and equilibrium constants for these reactions as a function of ionic strength (Table I). As expected, increasing ionic strength decreases both the reactivity and affinity of the pyridinium ions toward cyanide ion, presumably the result of salting in of the ionic reactants relative to the partially ionic transition state and nonionic product. The fact that the effects of changing ionic strength are nearly as large on the rate constants as they are on the equilibrium constants suggests that the transition state and product state resemble each other.

The increased affinity of nicotinamide ribosidyl derivatives compared to the remaining pyridiniums ions (Table II) is in accord with qualitative observations of Kaplan and co-workers[9] and may simply reflect the polar characteristics of the sugar moiety. The abnormal affinity of both isomers of NAD^+ and nicotinamide mononucleotide toward cyanide relative to their reactivity toward this nucleophile (Table II) is not completely understood. Nicotinamide mononucleotide, for which special intramolecular interactions ought not to occur, has about the same reactivity but ten times the affinity toward cyanide as N-(2,6-dichlorobenzyl)-3-carbamoylpyridinium ion. Results for nicotinamide mononucleotide are correlated well with those for the β isomer of NAD^+, the latter compound exhibiting both somewhat more reactivity and affinity for cyanide. On the other hand, the α isomer of NAD^+ is about as reactive as the mononucleotide but has only one-quarter of its affinity for cyanide and only 10% of that of the β isomer of NAD^+. This observation accords with the observation that α-NAD^+ is a weaker oxidizing agent than β-NAD^+;[47] *i.e.*, it has a lower affinity for the hydride ion. This distinction between the two isomeric forms of NAD^+ suggests that interactions between the pyridinium and adenine ring systems may be of some importance in determining reactivities and affinities toward nucleophilic reagents. Fluorescence and proton magnetic resonance studies have indicated that the β isomers of NAD^+ and NADH exist in a folded conformation in solution with the two ring systems existing in a stacked configuration.[48-50] Fluorescence studies suggest that α-NADH does not exist in such a configuration[51] while proton magnetic resonance studies suggest that α-NAD^+ probably does.[50] At any event, it is not unlikely that differences in the relative orientation of the two ring systems does in fact occur in the two isomers and that these differences account, in part at least, for the behavioral patterns observed.

The fact that the reactivities and affinities for those pyridinium ions substituted on nitrogen with the imidazolylethyl and indolylethyl groups, which exist as internal charge-transfer complexes,[23] can be reasonably well accounted for in terms of polar effects alone is somewhat surprising. In view of the established sensitivity of these reactions to polar effects, one might well have expected that electron donation to the pyridinium ion, which should largely disappear in the transition state and product, would markedly decrease the reactivity and affinity of these species for cyanide. The failure to obtain behavior of this type is additional evidence for the suggestion that little charge is transferred in charge-transfer complexes.[52]

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(42) W. E. Feely and E. M. Beavers, *J. Amer. Chem. Soc.*, **81**, 4004 (1959).
(43) S. Takahashi and H. Kanō, *Tetrahedron Letters*, 3789 (1965).
(44) T. Okamato and H. Tani, *Chem. Pharm. Bull.* (Tokyo), 7, 130 (1965).
(45) T. Okamato and H. Tani, *ibid.*, 7, 925 (1965).
(46) R. A. Abramovitch and J. G. Saha, *Advan. Heterocyclic Chem.*, **6**, 229 (1966).
(47) N. O. Kaplan, M. M. Ciotti, F. E. Stolzenbach, and N. R. Bachur, *J. Amer. Chem. Soc.*, 77, 815 (1955).
(48) G. Weber, *Nature*, 180, 1409 (1957).
(49) S. Shifrin and N. O. Kaplan, *ibid.*, **183**, 1529 (1959).
(50) O. Jardetzky and N. G. Wade-Jardetzky, *J. Biol. Chem.*, **241**, 85 (1966).
(51) S. Shifrin and N. O. Kaplan, "Light and Life," W. D. McElroy and B. Glass, Ed., Johns Hopkins University Press, Baltimore, Md., 1961, pp 144–156.
(52) E. M. Kosower, *Progr. Phys. Org. Chem.*, **3**, 82 (1965).