One-Electron Redox Reactions of Water-Soluble Vitamins. I. Nicotinamide (Vitamin B_5) and Related Compounds

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Abstract: The one-electron reduction of nicotinamide (N), N_1-methylnicotinamide (MN), isonicotinamide (*i*-N), and 3-acetylpyridine (3-AcPy) was studied in aqueous solution. Reduction was brought about by reaction with hydrated electrons or by electron transfer from acetone ketyl radicals $(CH_3)_2\dot{C}OH$. The intermediates produced were observed using the technique of pulse radiolysis and kinetic absorption spectrophotometry. Characteristic transient absorption spectra and extinction coefficients were observed. These spectra were found to be pH-dependent, and, from an examination of the "titration" curves of the free radicals produced, two ionization constants were obtained. For nicotinamide, the $\cdot NH_2^+$ radical has a $pK_a^1 = 1.1 \pm 0.2$, due to ionization of a proton from the $-\dot{C}(OH)NH_2$ group, and $pK_a^2 = 13.4 \pm 0.2$ due to ionization of the ring nitrogen proton $-\dot{N}H-$. For isonicotinamide, the corresponding pK_a values of the radicals are 1.9 ± 0.2 and >14.0. For N_1-methylnicotinamide, only one pK_a (radical) $= 1.3 \pm 0.2$ is observed. The efficiency and rate of reduction of N, MN, *i*-N, and 3-AcPy by electron transfer from $(CH_3)_2\dot{C}OH$ radicals (kinetic redox potential $E^{01} = -0.82$ V) were found to be dependent upon the redox potential of the acceptors. Similarly, from the kinetic redox potentials of the ·NH, ·MN and NAD· (produced from NAD^+) radicals, it is shown that the ·NH and ·MN radicals are stronger reducing agents than the NAD· radical. Rate constants for electron transfer from these radicals to a range of acceptors, *e.g.*, eosin Y ($E^{01} = -0.50$ V) and *p*-benzoquinone ($E^{01} = +0.293$ V), are close to diffusion-controlled $k \leq 6.0 \times 10^9$ M^{-1} sec^{-1}. In the absence of acceptors, these radicals decay by second-order kinetics to give permanent products with characteristic absorption spectra. These and other results are discussed.

The properties and modes of action of vitamins as controlling agents in metabolic processes are areas of continued active research (see, *e.g.*, ref 2 and 3). The functional and dynamic aspects of the chemistry and biochemistry of these vital compounds are of considerable importance, particularly the key role which vitamins play in the control mechanisms.

The oxidation and reduction properties and reactions which vitamins undergo are an area of great interest. One aspect of such studies deals with the observation of the intermediates produced from the one-electron redox reactions of vitamins, the factors which control the formation of these intermediates, and their subsequent reactions. A considerable amount of work has been carried out during the last few years on a few vitamins: riboflavin,[4] ascorbic acid,[5] and vitamin K.[6,7]

In part I of this series, the one-electron reduction of nicotinamide (vitamin B_5) and related compounds in water has been investigated. Emphasis will be given to the one-electron transfer reactions to nicotinamide (N) to produce the ·NH radical, as well as the one-electron transfer reactions from ·NH radical to acceptor compounds. The kinetic redox potential of these radicals is presented and discussed.

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Experimental Section

The one-electron reduction of nicotinamide (N) and related compounds in water was brought about by reaction of these systems with hydrated electrons, e_{aq}^-. The technique of pulse radiolysis and kinetic absorption spectrophotometry was used to generate e_{aq}^- and follow the formation and subsequent reactions of the short-lived intermediates produced. The experimental setup and conditions have been described elsewhere.[8,9]

The radiation chemistry of water produces e_{aq}^-, hydroxyl radicals, and H atoms

$$H_2O \xrightarrow{\sim\sim} e_{aq}^- (2.8), OH (2.8), \text{and } H (0.6)$$

where the numbers in parentheses are the *G* values (yields of radicals produced per 100 eV of energy absorbed). The free radicals formed by reaction of the substrates with e_{aq}^- were generated on pulse radiolysis of oxygen-free aqueous solutions in the presence of *tert*-butyl alcohol, in order to scavenge the OH radicals. The radical produced[8] from the reaction of OH with *t*-BuOH was found to be inert in this work and not to interfere with the experimental observations reported below.

In acidic solutions, there is a competition between reactions 1 and 2 and the ·NH radicals cannot be formed *via* reaction 1. The ·NH

$$e_{aq}^- + N \longrightarrow \cdot NH \qquad k = 2.4 \times 10^{10}\ M^{-1}\ \text{sec}^{-1} \qquad (1)$$

$$e_{aq}^- + H^+ \longrightarrow H \qquad k = 2.3 \times 10^{10}\ M^{-1}\ \text{sec}^{-1}\ (\text{ref } 10) \qquad (2)$$

radicals were therefore produced by electron transfer from the acetone ketyl radical $(CH_3)_2\dot{C}OH$ (eq 3). The ketyl radical was

$$(CH_3)_2\dot{C}OH + NH^+ \longrightarrow \cdot NH + CH_3COCH_3 + H^+ \qquad (3)$$

produced *via* reactions 4 and 5. Experiments were carried out in

$$OH + (CH_3)_2CHOH \longrightarrow (CH_3)_2\dot{C}OH + H_2O \qquad (4)$$

$$H + (CH_3)_2CHOH \longrightarrow (CH_3)_2\dot{C}OH + H_2 \qquad (5)$$

1-2 *M* isopropyl alcohol and the solution was saturated with N_2O ($\sim 2.2 \times 10^{-2}\ M$) in order to convert e_{aq}^- into OH radicals.

$$e_{aq}^- + N_2O \longrightarrow N_2 + OH + OH^-$$

(8) M. Simic, P. Neta, and E. Hayon, *J. Phys. Chem.*, **73**, 3794 (1969).

(9) J. P. Keene, E. D. Black, and E. Hayon, *Rev. Sci. Instrum.*, **40**, 1199 (1969).

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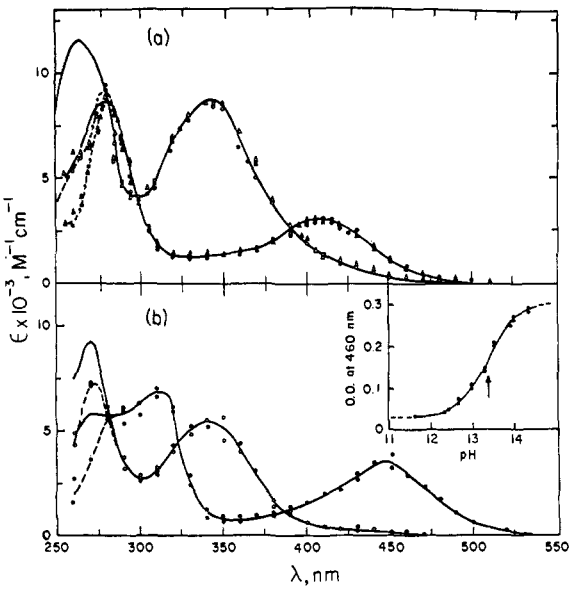

Figure 1. Absorption spectra of intermediates and final products produced from the one-electron reduction by e_{aq}^- of nicotinamide (1.0 m*M*, in the presence of 1.0 *M* *tert*-butyl alcohol, 1 atm of argon). Dark symbols denote intermediates and open symbols denote products measured ~15 sec after the pulse: (a) at pH 5.9 (●) and pH 11.1 (▲); (b) at pH 14.2 (●); and insert ΔOD *vs.* pH at 460 nm. In the wavelength region 290–700 nm, 13.1 krads/pulse was used; in the 250–330-nm region 1.5×10^{-4} *M* nicotinamide was used, 0.3 *M* *t*-BuOH, 5.1 krads/pulse. Dotted lines represent uncorrected data. Full lines represent data corrected for depletion of nicotinamide. See text for further details.

Since ~15% of the H and OH radicals produces a β-alcohol radical (reactions are not shown), the *G* value of 0.85 [G(H) + G(OH) + $G(e_{aq}^-)$] = 5.0 was used to calculate extinction coefficients.

All the chemicals used were the highest purity research grades available commercially and were obtained from Sigma, Calbiochem, Nutritional Biochemicals, Baker and Adamson, and Mallinckrodt. Solutions were buffered with perchloric acid, potassium hydroxide, and ~1 m*M* phosphate and tetraborate. Dosimetry was carried out using KCNS, as described.[8]

The stability in acid and basic solutions of the compounds studied here was checked by absorption spectrophotometry. Experiments were carried out at the extremes of the pH range only if the compounds were found to be completely stable for at least 2 hr (less than 1.5% change in OD_{max}).

The transient optical absorption spectra reported below were corrected for depletion of the substrates at the appropriate wavelengths. The correction was done assuming depletion of 1 mol of substrate per 1 mol of e_{aq}^- produced. The absorption spectra of the final products were measured several seconds after the pulse and are presented on an extinction coefficient basis assuming that 2 mol of pyridinyl radicals produce 1 mol of final product (radical dimerization).

$$NH\cdot + NH\cdot \longrightarrow (NH)_2$$

$$(NH)_2 \longrightarrow P \text{ in acid solutions}$$

Results

The reactivity of hydrated electrons with nicotinamide and related compounds was determined by following the decay kinetics of e_{aq}^- at 700 nm. From the pseudo-first-order decay, the reaction rate constants were obtained and are presented in Table I. Since the substrates undergo acid–base equilibria, the pH values of the experiments were chosen such that they are present in their basic form. The k_1 rates for nicotinamide (N) and *N*-methylnicotinamide (MN) were previously determined[11,12] and are in good agreement.

(11) M. Simic and M. Ebert, *Int. J. Radiat. Phys. Chem.*, **3**, 259 (1971).

Table I. Reaction Rate Constants of e_{aq}^- and $(CH_3)_2\dot{C}OH$ Radicals with Nicotinamide and Related Compounds in Aqueous Solutions

Compound	pK_a (solute)	$k(e_{aq}^- + S)$, M^{-1} sec^{-1} [a,b]	$k[(CH_3)_2\dot{C}OH + S]$, M^{-1} sec^{-1} [c]
Nicotinamide	3.4	2.4×10^{10} (7.5)	2.1×10^{8} (0.9)
N-Methylnicotinamide		4.1×10^{10} (9.0)	3.6×10^{8} (9.5)
Isonicotinamide	3.6	3.2×10^{10} (9.0)	3.1×10^{9} (0.7)
3-Acetylpyridine	4.9	2.4×10^{10} (9.6)	8.6×10^{9} (0.6)

[a] Value in parentheses is the pH at which the rate constant was determined. [b] Values to ±5%. [c] Values to ±20%.

Nicotinamide. The one-electron reduction of nicotinamide (N) by e_{aq}^- at pH 5.9 and 11.1 forms pyridinyl radicals with absorption maxima at 405 and ~280 nm and extinction coefficients 3.0×10^3 and $\sim 8.6 \times 10^3$ M^{-1} cm^{-1}, respectively [Figure 1(a) and Table II]. This intermediate is quite similar to that previously reported in the literature[11] at pH 7.0. In very alkaline solutions, pH 14.2, the transient absorption spectra of the pyridinyl radical are red shifted with maxima at 445 and ~310 nm, see Figure 1(b). This change in absorbance can be "titrated" at 460 nm, and a titration-type curve was obtained [insert, Figure 1(b)]. A pK_a ~ 13.4 ± 0.2 was derived. Nicotinamide was found to be absolutely stable in these relatively alkaline solutions under the experimental conditions used.

Nicotinamide has a pK_a = 3.4, and protonation occurs on the ring nitrogen to form NH^+. In acidic solutions, below pH ~3.0, reaction 1 cannot occur due to reaction 2. The H atoms produced in reaction 2 and the OH radicals produced from the radiolysis of water can react instead quantitatively with isopropyl alcohol, reactions 4 and 5. The pyridinyl radical is now produced by electron transfer from the $(CH_3)_2\dot{C}OH$ radical, reaction 3. The k_3 rate is 2.1×10^8 M^{-1} sec^{-1} for nicotinamide, determined at pH 0.9. In neutral solutions the $(CH_3)_2\dot{C}OH$ radical does not transfer an electron to nicotinamide, whereas it can transfer an electron to *N*-methylnicotinamide (Table I). This difference is due to the fact that the redox potential of nicotinamide is $E^{01} = -1.20$ V and the "kinetic" redox potential of the $(CH_3)_2\dot{C}OH$ radical[13] is $E^{01} = -0.82$ V. The redox potential of the acid form NH^+ of nicotinamide is not known but can be considered to be fairly close to that of *N*-methylnicotinamide, $E^{01} = -0.42$ V (see Table III).[14–16] Reaction 3 can then occur with NH^+, since the redox potentials are favorable. At pH 0.5 the $(CH_3)_2\dot{C}OH$ radical remains unprotonated and its potential value is unchanged.[13]

In 1.0 *M* perchloric acid, the transient spectrum produced immediately after the pulse has maxima at 445

(12) E. J. Land and A. J. Swallow, *Biochim. Biophys. Acta*, **162**, 327 (1968).

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(14) L. Meites, "Polarographic Techniques," Interscience, New York, N. Y., 1971; I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience, New York, N. Y., 1952; M. Brezina and P. Zuman, "Polarography," Interscience, New York, N. Y., 1958; "Handbook of Biochemistry," Chemical Rubber Publishing Co., Cleveland, Ohio, 1970, p J-33.

(15) P. S. Rao and E. Hayon, submitted for publication to *J. Amer. Chem. Soc.*

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Table II. Absorption Maxima, Extinction Coefficients, Decay Kinetics, and Ionization Constants of Intermediates and Products Produced from the One-Electron Reduction of Nicotinamide and Related Compounds in Water

Compound[a]	Intermediates: pH	λ_{max}, nm	ϵ_{max}, M^{-1} cm^{-1}	Decay kinetics,[c] $2k$, M^{-1} sec^{-1}	pK_a (radical)	Suggested radical	Products: pH	λ_{max}, nm	ϵ_{max}, M^{-1} cm^{-1}
Nicotinamide (3.4, N)	0	445	4.6×10^3	9.6×10^8		$\cdot NH_2^+$	0	P_1 350	8.6×10^3
		304	1.4×10^4		1.1 ± 0.2	⇅		P_2 ~300	9.0×10^3
	5.9, 11.1	410	3.0×10^3	1.7×10^9		$\cdot NH$	5.9, 11.1	345	8.7×10^3
		280[b]	8.6×10^3[b]			⇅		265[b]	1.2×10^4[b]
	14.2	445	3.6×10^3	2.0×10^8	13.4 ± 0.2	⇅	14.2	342	5.5×10^3
		~310[b]	~7.0×10^3[b]			$\cdot N^-$		270[b]	9.2×10^3
N_1-Methylnicotinamide (MN^+)	0.4	445	4.7×10^3	5.7×10^8		$\cdot MNH^+$	0.4	P_1 360	1.1×10^4
						⇅		300	2.4×10^4
		305	1.8×10^4		1.3 ± 0.2	⇅		P_2 305	1.6×10^4
	4.8, 9.5	420	3.3×10^3	1.2×10^9		$\cdot MN$	4.8, 9.5	360	1.0×10^4
		285	6.8×10^3					280[b]	1.1×10^4[b]
Isonicotinamide (3.6, *i*-N)	0.6	410	6.0×10^3	6.4×10^8		$\cdot i\text{-}NH_2^+$	0.6		
		310	9.7×10^3		1.9 ± 0.2	⇅			
	9.0	395	7.0×10^3	8.4×10^8		$\cdot i\text{-}NH$	9.0		
						⇅			
		292	1.3×10^4		>14.0	$\cdot i\text{-}N^-$			
3-Acetylpyridine (4.9, 3-AcPy)	1.9	430	2.8×10^3	2.5×10^8	~3.5–4.5	3-AcPyH$_2^+$	1.9	350	6.2×10^3
		290	1.8×10^4			⇅			
	9.4	460	4.6×10^3	3.7×10^9		3-AcPyH	9.4	355	3.0×10^3
		310	1.6×10^4		13.4 ± 0.2	⇅			
	14.0	465	4.2×10^3	7.1×10^8		3-AcPy$^-$	14.0	350	7.8×10^3
		310	6.8×10^3					280[b]	3.5×10^3[b]
		270	5.5×10^3[b]						

[a] Numbers given in parentheses are the pK_a values of the parent compounds. [b] Values obtained after experimental results were corrected for solute depletion. [c] Not corrected for effect of ionic strength on decay kinetics; rates may be high due to reaction with *t*-BuOH radical or $(CH_3)_2\dot{C}OH$ radical.

Table III. Redox Potentials of Nicotinamide and Related Compounds and Their Corresponding Pyridinyl Free Radicals in Aqueous Solution

Compound	E^{01}, V[a]	Free radical[b]	E^{01}, V[c] (radical)	Ref
Nicotinamide (N)	−1.20	·NH	−0.90	16
N-Methylnicotinamide (MN^+)	−0.42	·MN	−0.90	16
Isonicotinamide (*i*-N)	−0.78	·*i*-NH		
Nicotinic acid (NA)	−1.26	·NAH	−0.90	16
NAD^+	−0.34	NAD·	−0.75	16
3-Acetylpyridine (3-AcPy)	−0.90	3-AcPyH		

[a] Values at pH 7.0 and 25° from ref 14; no E^{01} values available for the acid form (protonation on ring nitrogen). [b] All free radicals produced at pH 7.0. [c] At pH 7.0 and 25° for the couples ·NH/N. These are "kinetic" potentials and not thermodynamic redox potentials (see ref 15 and 16).

and ~304 nm; see Figure 2 and Table II. On monitoring the change in absorbance at 460 nm with pH, a titration curve is obtained from which a p$K_a \sim 1.1 \pm 0.2$ can be derived.

Based on the results presented above and the decay kinetics of the pyridinyl radicals to be discussed below (see Table II), Scheme I is suggested. In support of these assignments, the pyridinyl radical present at pH 5.4 was found to be a neutral species since its second-order decay kinetics, $2k = 1.7 \times 10^9$ M^{-1} sec^{-1}, was independent of ionic strength up to $\mu = 0.6$ (K_2SO_4). The second-order decay kinetics of the $\cdot NH_2^+$ and $\cdot N^-$ radicals are lower (Table II), consistent with the charges carried by these species.

The decay of these radical intermediates gave rise to new absorption bands, formed by second-order kinetics (see Figures 1 and 2). In neutral and alkaline solutions,

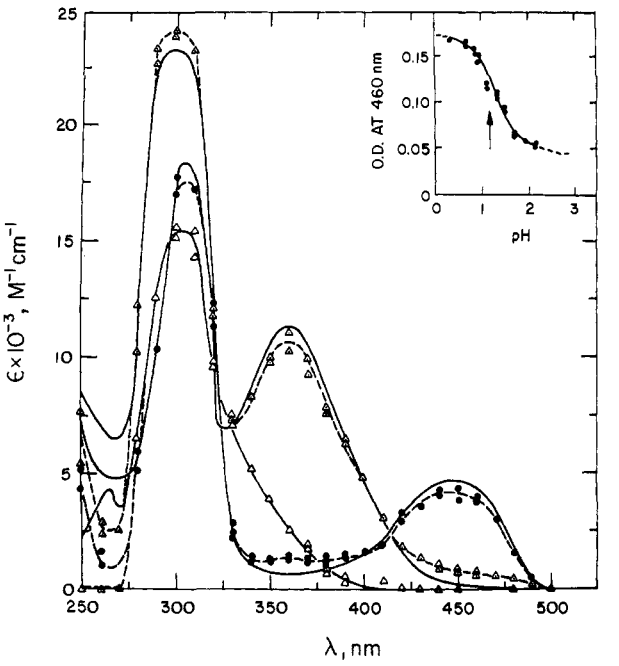


Figure 2. Absorption spectra of intermediates and final products produced from the one-electron reduction of nicotinamide by $(CH_3)_2\dot{C}OH$ radicals (1.0 m*M*, 2.0 *M* isopropyl alcohol, 1 atm of argon) at pH 0, 7.3 krads/pulse. Absorbance was read at 10 μsec (●), 440 μsec (△), and 5 sec (△) after the pulse. Insert: ΔOD at 460 nm *vs.* pH. Dotted lines represent experimental data as collected. Full lines represent data corrected for partial or incomplete decay of species measured. In the 260–380-nm region, 2×10^{-4} *M* nicotinamide and 1.0 *M* *i*-PrOH, 3.1 krads/pulse, pH 0, were used. The first spectrum was measured at 28 μsec after the pulse, and correction for solute depletion was made. The ε measured at 440 μsec was based on 2 mol of intermediate giving 1 mol of first product; the ε measured at 5 sec was based on 1 mol of first product giving 1 mol of final product.

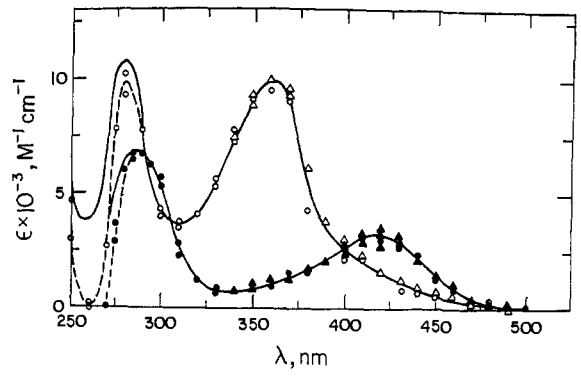


Figure 3. Absorption spectra of intermediates and final products produced from the reaction of e_{aq}^- with N_1-methylnicotinamide (1.0 mM, 1.0 M t-BuOH, 1 atm of argon) at pH 4.8 (▲) and pH 9.5 (●). Dark symbols denote intermediates (measured at OD_{max}), open symbols denote final products (measured 5–15 sec after the pulse), and 7.8 krads/pulse was used. In the 250–340-nm region, 1.5 × 10^{-4} M N_1-methylnicotinamide, 0.5 M t-BuOH, and 3.7 krads/pulse were used. Dotted lines represent uncorrected data; full lines represent data corrected for depletion of solute. The ε of product was based on 2 mol of intermediates giving 1 mol of product.

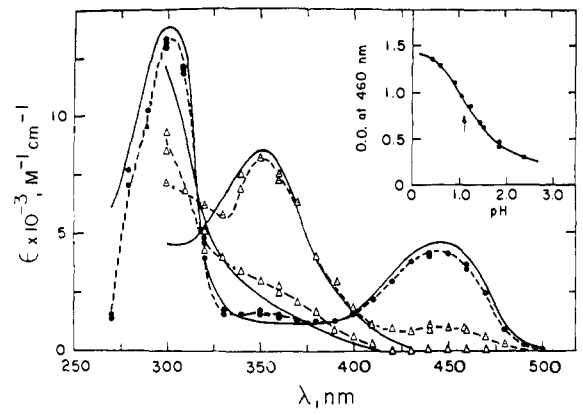
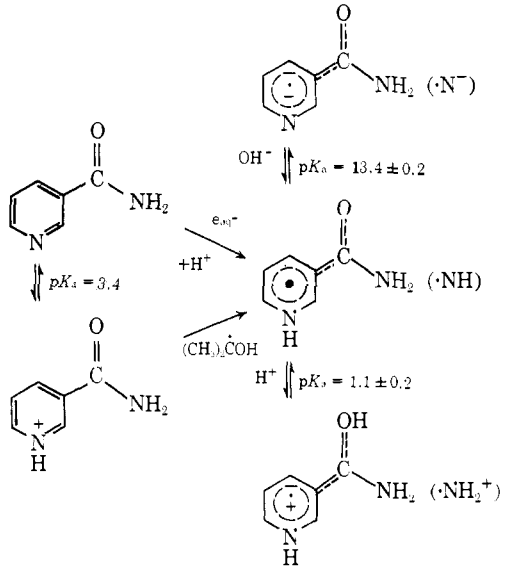


Figure 4. Absorption spectra of intermediates and final products produced from the one-electron reduction of N_1-methylnicotinamide by $(CH_3)_2\dot{C}OH$ radicals (5 × 10^{-4} M, 2.0 M i-PrOH, pH 0.4, 1 atm of argon, 7.5 krads/pulse). Insert: ΔOD at 460 nm *vs.* pH. In 300–700-nm region, absorbance was read at 6.2 μsec (●), 730 μsec, (△), and 5 sec (△) after the pulse. In 250–330-nm region (1.5 × 10^{-4} M solute, 1.5 M i-PrOH, pH 0.4, 2.2 krads/pulse, 1 atm of argon), corresponding absorbance was read at 17 μsec, 91 μsec, and 15 sec after the pulse, respectively. Dotted lines represent experimental data as collected; full lines represent data corrected for partial or incomplete decay of species measured. The ε measured at 730 μsec was based on 2 mol of intermediate giving 1 mol of product P_1; the ε measured at 5–15 sec after the pulse was based on 1 mol of P_1 → 1 mol of P_2.

Scheme I



these bands have maxima at ~345 and ~265 nm. These absorptions are stable even in the presence of air and appear to be permanent products. These products could be either dihydro derivatives of nicotinamide (produced by disproportionation) or dimers (produced by dimerization). The spectra of the dihydro compounds and the dimers (for the corresponding isomers) are fairly similar.[17–19] Based on the observed absorption spectra, however, it would seem that the final products are either the 6,6′ dimers or the 1,6-dihydro derivatives. The formation of dimers appears to be favored.[17–19]

In very acidic solutions, an intermediate is formed with λ_{max} ~350 nm and another band below 300 nm. This intermediate decays with τ ~2 sec to give rise to a permanent product having a maximum at <300 nm (possibly at 290 nm). Reduced (*i.e.*, dihydro) nicotinamides and NADH are known[18,20] to be unstable in acid solutions leading to an opening of the heterocyclic ring. The aldehyde suggested[20] to be formed absorbs at 290 nm.

***N*-Methylnicotinamide.** The one-electron reduction of N_1-methylnicotinamide (MN^+), a model compound for NAD^+, by e_{aq}^- at pH 4.8 and 9.5 forms a transient absorption spectrum which is red shifted, compared to that of nicotinamide at the same pH; see Figure 3 and Table II. These spectra are similar to those reported in the literature.[12,21]

Due to the instability of N_1-methylnicotinamide at pH >11, it was not possible to examine this system in very alkaline solutions. However, no changes were observed between pH 4.8 and 10.0 (Figure 3). In acidic solutions, N_1-methylnicotinamide reacted with $(CH_3)_2\dot{C}OH$, reaction 3, to produce a radical very similar to that formed from nicotinamide; see Figure 4 and Table II. At pH 0.4, the radical has absorption maxima at 445 and 305 nm, with extinction coefficients of 4.7 × 10^3 and 1.8 × 10^4 M^{-1} cm^{-1}, respectively. On monitoring the change in absorbance at 460 nm with pH, a titration curve is obtained (insert, Figure 4) from which a pK_a = 1.3 ± 0.2 can be derived.

Scheme II is suggested to account for the results obtained. The $(CH_3)_2\dot{C}OH$ radical can produce the ·MN radical in both neutral and acid solutions. N_1-Methylnicotinamide has no pK_a, and therefore the redox potential remains unchanged; E^{01} = −0.42 V. This is in contrast to nicotinamide, as discussed above. The neutral species ·MN produced at pH 4.8–11.0 is similar to the ·NH radical (see Scheme I). However, the pres-

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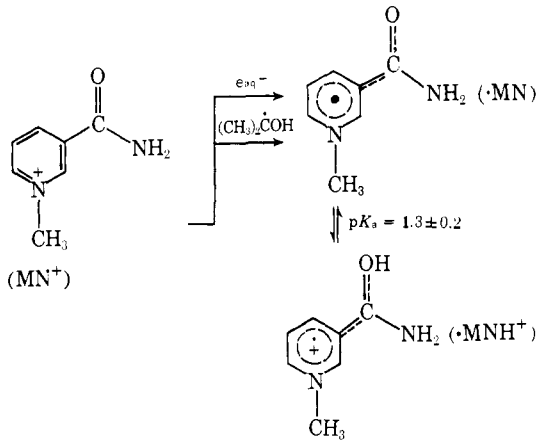
Scheme II



ence of a methyl group on the ring nitrogen results in red shifting of the transient absorption spectra. This observation is consistent with the strong interaction of the odd electron with the ring nitrogen. Furthermore, it leads to a slightly higher ionization constant for the $\cdot MNH^+$ radical compared to the $\cdot NH_2^+$ radical.

The $\cdot MN$ and $\cdot MNH^+$ radicals decay by second-order kinetics; see Table II. No ionic strength effect was observed for the decay kinetics of the $\cdot MN$ radical at pH 5.0.

In neutral solutions, a permanent product is produced from the decay of the $\cdot MN$ radicals (Figure 3 and Table II), with absorption maxima at 360 and 280 nm. These bands are also red shifted compared to the corresponding bands of the permanent product produced from the decay of $\cdot NH$ radicals. In very acidic solutions, the results observed are similar to those for nicotinamide. The second-order decay of the $\cdot MNH^+$ radicals produces an intermediate (product P_1) with λ_{max} at 360 and ~300 nm. This intermediate is unstable,[18,20] $\tau \sim 2$ sec, at low pH and presumably decomposes by opening of the ring to give a final product (P_2) which absorbs at ~305 nm. P_1 is suggested to be either the dihydro derivative or a dimer.

Isonicotinamide. Isonicotinamide (*i*-N) has a pK_a = 3.6 and its reactivity toward e_{aq}^- at pH 6–7 is slightly higher than that of nicotinamide (Table I) but somewhat lower than that of N_1-methylnicotinamide. This is understandable since the latter compound has a positive charge on the ring nitrogen—the most likely site of addition of e_{aq}^-.

The reaction of e_{aq}^- with *i*-N produced at pH 6–7 a radical with absorption maxima at 395 and 292 nm; see Figure 5 (a). These bands are close to those reported[22,23] for isonicotinamide produced electrochemically in organic solvents by metal reduction or by pulse radiolysis in water.[11] No change could be observed for this transient species up to pH ~13.0. Isonicotinamide becomes unstable at pH ≥14.0. It is therefore interesting to note that the radical produced does not ionize up to pH ~13.0. In acid solutions, electron transfer[24] occurs from the $(CH_3)_2\dot{C}OH$ radical

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(23) M. Itoh and S. Nagakura, *Tetrahedron Lett.*, **8**, 417 (1965); *Bull. Chem. Soc. Jap.*, **39**, 369 (1966).

(24) No electron transfer from the $(CH_3)_2\dot{C}OH$ radical ($E^{01} = -0.82$ V) to neutral solutions of isonicotinamide ($E^{01} = -0.78$) was observed even though these redox potential values indicate otherwise. It is suggested that the E^{01} literature value for isonicotinamide is too positive and may be closer to that for nicotinamide.

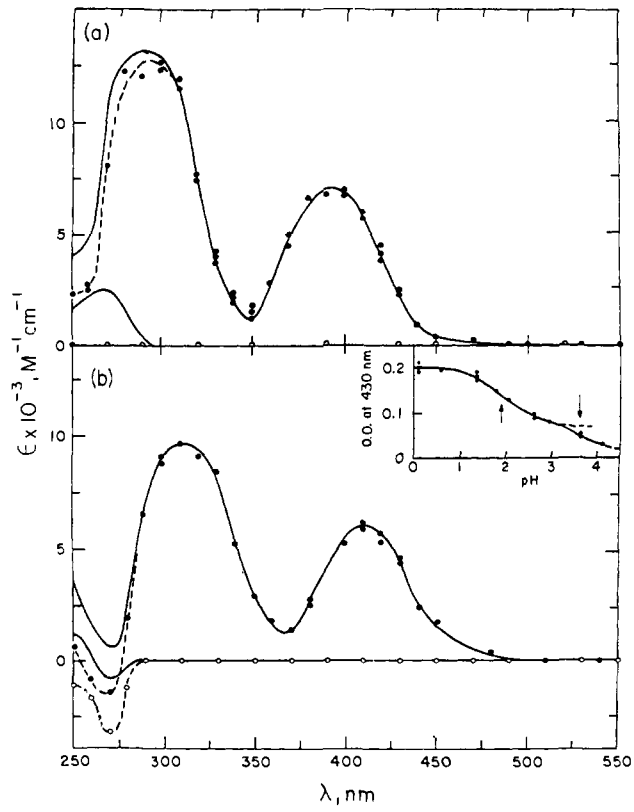


Figure 5. Absorption spectra of intermediates and final products produced from the reaction of (a) e_{aq}^- with isonicotinamide (1.0 m*M*, 1.0 *M* *t*-BuOH, 1 atm of argon, 12.2 krads/pulse) at pH 9.0. Absorbance was read at 0.2 μsec (●) and 15 sec (○) after the pulse. Full line represents data corrected for depletion of solute. The product peak at 265 nm comes about on correction for solute depletion (see text). In 250–350-nm region, 10^{-4} *M* isonicotinamide, 1.0 *M* *t*-BuOH, 2.5 krads/pulse, and pH 9.0 were used. (b) $(CH_3)_2\dot{C}OH$ radicals with isonicotinamide (1.0×10^{-4} *M*, 2.0 *M* *i*-PrOH, 2.5 krads/pulse, 1 atm of argon) at pH 0.6. Absorbances were measured at 17 μsec (●) and 5 sec (○) after the pulse. Full lines are data corrected for solute depletion. Insert: ΔOD at 430 nm *vs.* pH.

to produce a transient with λ_{max} at 410 and ~310 nm [Figure 5 (b)].

The radicals produced in neutral and acid solutions are in equilibrium [see insert, Figure 5 (b)], and a $pK_a \sim 1.9 \pm 0.2$ was found. This value may be slightly incorrect, since the change in absorbance at 430 nm with pH overlapped with the $pK_a = 3.6$ of isonicotinamide.

Scheme III presents the reactions suggested to take place. The assignment of the radicals follows similar trends as those given above for nicotinamide: electron addition in neutral solution is followed by rapid protonation to form the neutral radical $\cdot i\text{-NH}$; in acid solutions, a second proton adds, suggested to be on the carbonyl group, to form radical $\cdot i\text{-}NH_2^+$. The second-order decay kinetics of these radicals, $2k = 8.4 \times 10^8\ M^{-1}\ sec^{-1}$, and $2k = 6.4 \times 10^8\ M^{-1}\ sec^{-1}$, respectively, are in general agreement with these assignments.

No absorption due to the formation of permanent products was observed in the wavelength region ~250–700 nm, as a result of the decay of $\cdot i\text{-NH}$ and $\cdot i\text{-}NH_2^+$ radicals (Figure 5). This is in marked contrast to the results obtained from nicotinamide, N_1-methylnicotinamide, and 3-acetylpyridine (see below).

3-Acetylpyridine. The addition of e_{aq}^- to 3-acetyl-

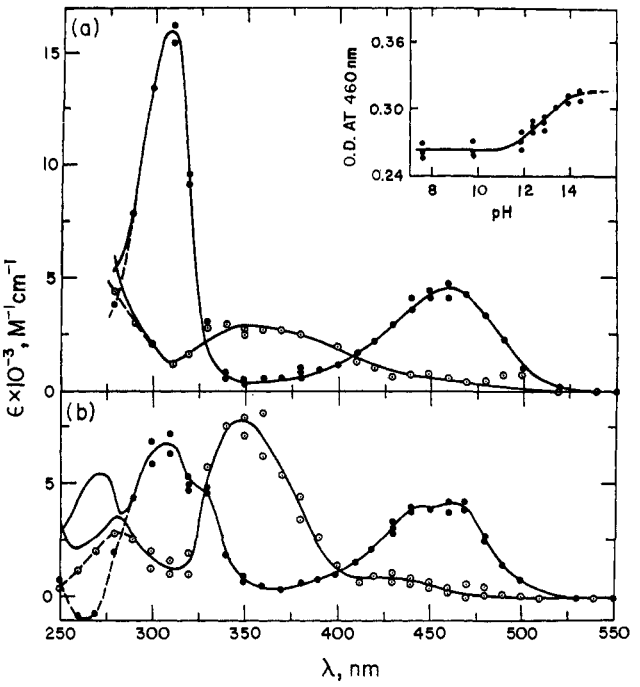


Figure 6. Absorption spectra of intermediates and final products produced from the reaction of e_{aq}^- with 3-acetylpyridine (1.0 m*M*, 1.5 *M* *t*-BuOH, 1 atm of argon, 9.5 krads/pulse) at (a) pH 9.4. Insert: ΔOD at 460 nm *vs.* pH. (b) At pH 14.0. Absorbances read at 0.2 μsec (●) and 5–15 sec (○) after the pulse. The ε of final products was based on 2 mol of intermediate giving 1 mol of product. Full lines represent spectra corrected for solute depletion. In 250–350-nm region, 2×10^{-4} *M* 3-acetylpyridine, 0.5 *M* *t*-BuOH, 4.0 krads/pulse were used.

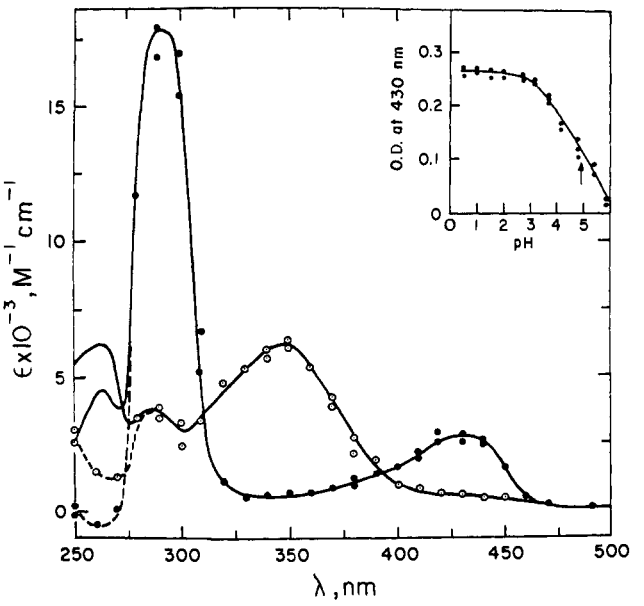
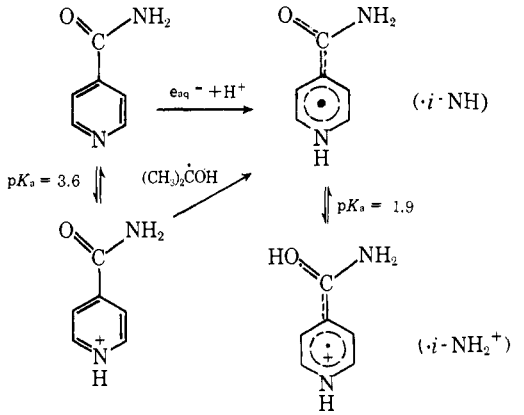


Figure 7. Absorption spectra of intermediates and final products produced from the one-electron reduction by $(CH_3)_2\dot{C}OH$ radicals of 3-acetylpyridine (5×10^{-4} *M*, 2.0 *M* *i*-PrOH, 5.0–10.0 krads/pulse) at pH 1.9. Absorbances were read 5 μsec (●) and 15 sec (⊙) after the pulse. The ε of the final product was based on 2 mol of intermediate giving 1 mol of product. Full lines represent spectra after correction for solute depletion. Insert: ΔOD at 430 nm *vs.* pH.

Scheme III

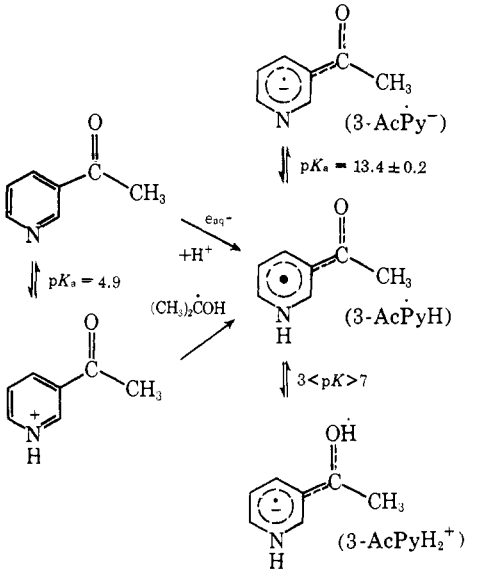


pyridine (3-AcPy) produces transient species at pH 7–10 whose absorption maxima are considerably red shifted compared to nicotinamide [see Figure 6 (a)], with λ_{max} at 460 and 310 nm. Small shifts are observed in alkaline solutions at pH ~14.0 [Figure 6 (b) and Table II]. From this change with pH, a $pK_a \sim 13.4 \pm 0.2$ can be derived [insert, Figure 6 (a)].

In neutral solutions, the $(CH_3)_2\dot{C}OH$ radical does not transfer to 3-acetylpyridine ($pK_a = 4.9$) since the $E^{01} = -0.90$ V; see Table III. In acid solutions, electron transfer is very efficient, $k = 8.6 \times 10^9\ M^{-1}\ sec^{-1}$ (Table I), indicating that the redox potential of the protonated 3-acetylpyridine is considerably more positive (no value is available). The transient species produced is shown in Figure 7. In this case, the bands are blue shifted compared to the species produced in neutral and alkaline solutions. This trend is the opposite to that found for nicotinamide, isonicotinamide, and N_1-methylnicotinamide. From the change in absorption with pH, only an approximate value could be derived for these species, $pK_a = 3.5$–4.5, since the change overlaps with the ionization constant of 3-AcPy itself.

Scheme IV is suggested. The radicals 3-AcPyH,

Scheme IV



3-AcPy⁻, and 3-AcPyH_2^+ all decay by second-order kinetics; see Table II. This decay gives rise to absorptions due to permanent products (see Figures 6 and 7 and Table II). It is interesting to note that the product formed at pH 1.9 is stable whereas the product from nicotinamide at pH 0 was short-lived and gave rise to another product which was permanent.

Discussion

The one-electron reduction [by e_{aq}^- or by electron transfer from the $(CH_3)_2\dot{C}OH$ radical] of nicotinamide leads, in neutral solution, to the reduction and *rapid* protonation of the ring nitrogen. The odd electron is in conjugation with the ring and the 3-$CONH_2$ group. Esr results[25] on the reduction of pyridine compounds are in agreement with this assignment. The reduction of N_1-methylnicotinamide and NAD^+ also probably takes place initially at the ring nitrogen. Ionization of the $-\dot{N}H-$ radical has been observed for the first time and occurs at very high pH values (see Table II). This high pK_a (radical) value for nicotinamide is consistent with the correlation[26] between the ionization constants of free radicals and the redox potentials of the parent compounds.

Another novel feature is the suggested protonation on oxygen in very acidic solutions; see Schemes I–IV. It is interesting to note that in acid solutions the one-electron reduction of benzoylpyridines[27] was suggested to form a radical with protonation of both the ring nitrogen and the carbonyl group. However, the first proton to ionize was suggested[27] to be the $-\dot{N}H-$ proton followed, in alkaline solutions, by the $-\dot{C}(OH)-$ proton. In view of the results obtained above with different nicotinamides, the assignment for the benzoylpyridines may be incorrect and should be reversed [*i.e.*, the first proton to ionize may be coming from the $-C(OH)-$ group].

The ·NH and ·MN radicals present in neutral solutions are relatively strong reducing agents and have kinetic redox potentials of ~ -0.90 V; see Table III. The NAD· radical with a $E^{01} = -0.75$ V is a weaker reducing agent. However, the three radicals can react with oxygen to regenerate the parent compound[16,28] (possibly *via* a peroxy radical as an intermediate)

$$\cdot NH + O_2 \longrightarrow N + \cdot O_2^- + H^+$$

This reaction is consistent with the determined[29,30] redox potential for the $O_2/\cdot O_2^-$ redox couple.

Electron transfer from ·NH, ·MN, ·*i*-NH, and NAD· radicals to a range of electron-acceptor compounds can be observed to take place; see Table IV.

Table IV. Reaction Rate Constants for Electron Transfer from Some Nicotinamide Free Radicals to Selected Acceptors in Aqueous Solution (at pH 7.0)

Donor radical	Acceptor compd[a] (V)	k, M^{-1} sec^{-1} [b]
Nicotinamide (·NH)	Eosin Y (−0.50)	2.5×10^9
	Menaquinone (+0.002)	5.1×10^9
	p-Benzoquinone (+0.293)	5.0×10^9
Nicotinic acid (·NAH)	Eosin Y (−0.50)	3.0×10^9
	Menaquinone (+0.002)	4.4×10^9
	p-Benzoquinone (+0.293)	5.2×10^9
N-Methylnicotinamide (·MN)	3-Benzoylpyridine (−0.75)	2.6×10^9
	Menaquinone (+0.002)	4.9×10^9
	p-Benzoquinone (+0.293)	5.2×10^9
NAD^+ (NAD·)	Riboflavin (−0.208)	1.0×10^9
	Oxygen	2.0×10^9
	Menaquinone (+0.002)	3.1×10^9
	p-Benzoquinone (+0.293)	4.4×10^9, 3.6×10^9

[a] Values in parentheses are the redox potentials E^{01} of the acceptors at pH 7.0. [b] From ref 16.

These rate constants reach near diffusion-controlled rates of $\leq 6.0 \times 10^9$ M^{-1} sec^{-1}. All these electron acceptors have redox potentials which are more positive than the kinetic potentials of the donor radials.

Thus, nicotinamide, nicotinic acid, and isonicotinamide are not as readily reduced by one-electron reducing agents, compared to N_1-methylnicotinamide and NAD^+. This follows from their redox potential values, Table III. In contrast, the radicals found from N, NA, *i*-N, and MN are strong reducing agents and somewhat stronger than the NAD· radical.

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Communications to the Editor

Relative Diylophylic Reactivities of Olefins toward a Trimethylenemethane[1]

Sir:

The cycloadditions of olefins with trimethylenemethane (**1**)[2] or the closely related substance 2-isopropylidenecyclopentane-1,3-diyl (**2**)[3–5] are among the few examples of intermolecular chemical interception of bidentate diradicals. An accompanying paper[6a] shows that it is possible to observe reactions of both a singlet (S) and a triplet (T) state of the diyl **2**.[6b] We report here a quantitative ranking of the rela-

(1) The support of this work by the National Institute of General Medical Sciences (Grant No. GM-16962), the National Science Foundation (Grant No. 33909X), the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the Hoffmann-LaRoche Fund is gratefully acknowledged.
(2) For lists of references to early studies in this field see (a) P. Dowd, *Accounts Chem. Res.*, 5, 242 (1972); (b) F. Weiss, *Quart. Rev., Chem. Soc.*, 24, 278 (1970); (c) ref 4.
(3) J. A. Berson, R. J. Bushby, J. M. McBride, and M. Tremelling, *J. Amer. Chem. Soc.*, 93, 1544 (1971).
(4) J. A. Berson, D. M. McDaniel, L. R. Corwin, and J. H. Davis, *J. Amer. Chem. Soc.*, 94, 5508 (1972).
(5) J. A. Berson, D. M. McDaniel, and L. R. Corwin, *J. Amer. Chem. Soc.*, 94, 5509 (1972).
(6) (a) J. A. Berson, L. R. Corwin, and J. H. Davis, *ibid.*, 96, 6175 (1974). (b) Although the properties of the singlet permit it to be the bicyclic hydrocarbon 5-isopropylidenebicyclo[2.1.0]pentane, the diyl S provides a simpler formulation at present.