Bimolecular Reactions of Pyridinyl Radicals in Water and the Mechanism of NAD⁺-NADH Dehydrogenase Reactions

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Abstract: 1-Methyl-3-carbamido- (3) and 4-carbamidopyridinyl (4) radicals, generated through pulse radiolysis via 1e addition to the pyridinium ions, disappear by rather different pathways in water. 3-dimerizes in a pH-independent reaction, whereas 4 reacts by electron transfer with protonated 4 (4H⁺) and 4H⁺ reagents with other 4H⁺, leading to a pH-dependent reaction yielding pyridinium ion and a dihydropyridine. The latter reaction was defined with respect to both kinetics and products by a study of the disappearance of 1-ethyl- and 1-tert-butyl-4-carboxyhexapropypyrindinyl in aqueous solutions between pH 8 and 9. At higher pH, ester hydrolysis produces the highly reactive carboxylate-substituted radical. A log k−pH plot suggests pKₐ differences between ester and amide radicals. Dimerization and electron-transfer reactions of 2, 3, and 4- pyridinyl radicals generated by radiolytic techniques can be distinguished by careful measurement of pH changes of unbuffered solutions. Both the 3-dimer and the dihydropyridine from 4 react with water in pH-dependent reactions to yield derivatives absorbing at much shorter wave lengths. The pKₐ for 3 is 1.4, but the structurally similar radical from nicotinamide adenine dinucleotide (NAD⁻) is unprotonated at pH 0.3. The NAD⁻ dimer reacts with water at about 0.03 of the rate found for 3-dimer. Some properties of the product of combination of the hydroxy-tert butyl radical and 3 are compared to those of the 3-dimer. The suggested 1e, H⁺, pathway for NAD⁺-NADH enzyme-catalyzed reactions is made more attractive by our results.

Introduction

Two streams of thought underlie current interest in pyridinyl radicals (Py). The first began with the work of Gomberg on the triphenylmethyl radical and proceeded through dissociable

The authors dedicate this paper to the memory of Gabriel Stein, late Professor at the Hebrew University, Jerusalem, who contributed so significantly to the field of the present paper and to other areas of radiation chemistry, through both his own work and his enthusiastic encouragement of others.

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generation of the NAD$^-$ radical,13 and continues at present with suggestive evidence from many recent model experiments.13-16 The mechanistic question of whether or not NAD-catalyzed reactions proceed via a single two-electron ("hydride-transfer") step (eq 1) or a pair of one-electron steps accompanied by proton transfer (eq 2) is still open and requires a detailed understanding of the behavior of pyridinyl radicals in aqueous systems.

\[
\begin{align*}
\text{Py}^+ + RH & \rightarrow \text{PyH} + R^+ \quad (1) \\
\text{Py}^+ + RH & \rightarrow \text{Py} + RH^+ \quad (2a) \\
\text{Py} + RH^+ & \rightarrow \text{PyH}^+ + R \quad (2b) \\
\text{PyH}^+ + R & \rightarrow \text{PyH} + R^+ \quad (2c)
\end{align*}
\]

To simplify the discussion of the small number of pyridinyl radicals with which we shall be concerned, we adopt the following abbreviations: 3, 4, 4EE, 4EB, NAD$^{-17}$ and give some of the key formulas below.

![Diagram of pyridinyl radicals]

The first simple pyridinyl radicals observed in water solutions were 3- and NAD$_{-}^-$, generated through pulse radiolysis of the corresponding pyridinium ions. Both disappeared at high rates in bimolecular reactions with products presumed to be dimers.12 Later, Kosower, Teuerstein, and Swallow$^{18}$ discovered that the 4-radical could be generated in the same way and also disappeared in a bimolecular reaction. However, it was found that the reaction of the 4-radical was strongly influenced by pH, whereas that of 3-radical was essentially unaffected. Neta and Patterson$^{19}$ confirmed these findings for 3- and 4-radicals and about the mechanisms involved in the reaction of the radicals. Data reported by Brühlmann and Hayon$^{20}$ for 3-related radicals agree in many respects with the other results.

Many important points were left unclear or untouched by the previous work. In particular, the striking difference in the mechanism of reaction of 3- and 4-radicals should show up as a sharp difference in the nature of the products. Further, a clear dependence on (H$^+$), equivalent to defining the reaction, 4$-$ + 4H$^+$, was not established. In the present work, we present information on these points and, in addition, kinetic results and evidence on the products and their behavior.

**Results**

**Generation of Pyridinyl Radicals.** The 1-methylcarbami[doprydyl radicals, 3- and 4-, and NAD$^-$ were produced through 1e reaction of the corresponding pyridinium compounds, utilizing 1e-transfer agents formed by pulse radiolysis of appropriate solutions. Reductions proceeded according to eq 3a-c. The chemical basis for favoring high concentrations of particular species in the solutions being pulsed is given elsewhere.22

\[
\begin{align*}
\text{Py}^+ + e^- & \rightarrow \text{Py}^- \quad (3a) \\
\text{Py}^+ + \text{CO}_2^- & \rightarrow \text{Py}^- \quad (3b) \\
\text{Py}^+ + (\text{CH}_3)_2\text{COH} & \rightarrow \text{Py}^- \quad (3c)
\end{align*}
\]

The pyridinyl radicals are readily recognized by their characteristic absorption spectra (three bands, two strong and one weak, past 270 nm)$^7$ and their high rates of formation ($k_a > 10^{10}$ M$^{-1}$ s$^{-1}$). Maxima are cited in the Experimental Section. The UV spectrum of 4-, as generated by pulse radiolysis, is illustrated in Figure 1.

The 4-ester radicals (4EE- and 4EB-) are stable enough to be prepared by a chemical procedure, reduction with sodium amalgam in acetonitrile in the absence of oxygen, carried out on a vacuum line (eq 4).23,24 Absorption coefficients are established by titration. The spectroscopic data for 4EB- in acetonitrile are given elsewhere.24 For comparison with the other pyridinyl radicals, maxima for 4EE- are cited in the Experimental Section.

**pK$_a$ of Radicals.** Acid produces a shift of the uncorrected absorption maxima of 3- from 280 and 420 nm to 300 and 440 nm, attributed to protonation of the carbonyl oxygen atom.$^{19,20}$ Analysis by a graphical procedure for the effect of pH on the absorption at 470 nm led to a pK$_a$ value of 1.43 ($\pm 0.07$), in agreement with that found by others.19,20 The pK$_a$ of 4- was determined in the same way as 2.0 $\pm$ 0.1, in good agreement with the value of 2.1 reported for 1-hexyl-4-carbamidopyridyl radical.19 The 405-nm maximum of 4- shifts to 425 nm in the protonated form. The pK$_a$ of the 1-tert-butylcarbomethoxypyridyl radical (4EB) is estimated as ca. 0.6 from the log k vs. pH plot (Figure 2) (see below).

The 400-nm peak of the NAD- radical found at pH 7 is found in the same position in a pH 0.4 solution, implying that the pyridinyl radical is not protonated. However, the short wavelength absorption (containing contributions from both the pyridinyl radical and adenine) is shifted 10 nm to longer wavelengths. Further, in ca. 1.5 M HClO$_4$ the 400-nm band shifts to 430 (±5) nm, giving a lower limit to the pK$_a$ of the NAD- radical.

**Kinetics of Radical Disappearance.** As we reported in our preliminary communication,18 the most striking aspect of the behavior of the 3- and 4-radicals in water was that 3-radical disappeared in a virtually pH-independent reaction while the reaction of 4-radical with itself was strongly pH dependent. We then noted that 14EE- disappearance could be followed (with difficulty) at pH 8.0. In order to define the pH dependence for the reaction of 4-substituted radicals further, the disappearance of the 4EB-radical was examined between pH 8.0 and 11.0. A linear dependence of log k on [H$^+$] was found between pH 8.0 and ca. 9.2, the rate decreasing in the expected fashion. At higher pH values, the rate increased, the rate being first order
in the pyridinyl radical and log k linearly dependent upon the pH. Moderately rapid hydrolyses of ester, \( k_2 = 50 \text{ M}^{-1} \text{s}^{-1} \), accounted for the results (eq 5). The kinetic constants for pyridinyl radical reactions in aqueous systems are collected in Table 1, and the pH dependence is illustrated by the plots in Figure 2.

Initial Products of Radical Reactions. The radical 3- at pH 7.2 gives rise to a product with \( \lambda_{	ext{max}} = 355 (\pm 5) \text{ nm} \). This is very similar to the maximum obtained after \( \gamma \) radiolysis of a solution of 3+ with identical composition and like the absorption reported after \( X \) irradiation of oxygen-free aqueous solutions of 3+ containing 0.5 M ethanol.\(^{23}\) The instability of the product in acidic solutions will be described in the next section. The NAD-radical yielded a product with \( \lambda_{	ext{max}} = 355 (\pm 5) \text{ nm} \) which is also unstable in acidic solution (see below).

Using tert-butyl alcohol in place of isopropyl alcohol or formate as hydroxyl radical scavenger, products were also formed from the reaction of 3- or NAD- with hydroxy-tert-butyl radical (HtB*- formed in the scavenging reaction) at rates comparable to those of the 3+ or NAD+-NAD- reactions. Although the absorption maxima were not very different from those of products formed in the presence of isopropyl alcohol or formate, a clear distinction could be made on the basis of fluorescence studies. Following radiolysis of solutions of 3+ or NAD+ in the presence of tert-butyl alcohol, the products were found to possess broad, structureless fluorescence around 460 nm which were completely formed within less than 40 ms. However, while the emission maxima were in the same region as the NADH fluorescence, the product from NAD+ was found not to be NADH, when tested for coenzyme activity using lactic dehydrogenase. For 3+, the fluorescence intensities of the products of pyridinyl radical + HtB- reaction were at least five times greater than those of the product(s) when isopropyl alcohol was used as scavenger,\(^{27}\) and when formate was used no significant fluorescence was detected.

The behavior of the products is accounted for by radical combination products, i.e., dimers from reactions of the same radicals and combination products for cases in which the reacting radicals are different (eq 6 and 7). Assignment of structure is made on the basis of: (a) mechanism of formation, since spin densities of radicals are highest at 4-;\(^{7,19,28-30}\) (b) known formation of 4,4'-dimers (e.g., Paraquat); (c) NMR results for related cases;\(^{25a}\) (d) the stability of radicals substituted by alkyl groups at the 4 position.

The disappearance of 4-species does not lead to any products with absorption maxima at wavelengths longer than 300 nm. At pH values lower than 9, the ultraviolet absorption corresponds to a yield of 50% of the material in the form of the 4-carboxybenzoylpyridinium ion, suggesting the disproportionation reaction (to 4EE- and 4PMH) shown in eq 8.\(^{25a}\) At higher

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**Table 1. Kinetic Constants for the Disappearance of Pyridinyl Radicals in Water}\(^{a}\)**

<table>
<thead>
<tr>
<th>Radical</th>
<th>pH, Buffer</th>
<th>Method</th>
<th>log k</th>
<th>Ref</th>
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<tbody>
<tr>
<td>3-</td>
<td>0.46, H</td>
<td>p</td>
<td>9.06</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>7.20, P</td>
<td>p</td>
<td>9.03</td>
<td>e/f</td>
</tr>
<tr>
<td>NAD-</td>
<td>0.4, H</td>
<td>p</td>
<td>8.64</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>7.2, P</td>
<td>p</td>
<td>8.11</td>
<td>f</td>
</tr>
<tr>
<td>4-</td>
<td>1.44, H</td>
<td>p</td>
<td>8.66</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>1.89, H</td>
<td>p</td>
<td>8.61</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>2.35, H</td>
<td>p</td>
<td>8.61</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>2.84, H</td>
<td>p</td>
<td>8.33</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>3.31, H</td>
<td>p</td>
<td>8.01</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>3.43, F</td>
<td>p</td>
<td>8.12</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>4.07, F</td>
<td>p</td>
<td>7.72</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>5.62, P</td>
<td>p</td>
<td>6.43</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>6.63, P</td>
<td>p</td>
<td>5.26</td>
<td>e</td>
</tr>
<tr>
<td>4EE-</td>
<td>8.00 P</td>
<td>v</td>
<td>3.28</td>
<td>g</td>
</tr>
<tr>
<td>4EB-</td>
<td>8.00 P</td>
<td>v</td>
<td>2.71</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>8.25, B</td>
<td>v</td>
<td>2.27</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>8.68, B</td>
<td>v</td>
<td>2.08</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>9.86, C</td>
<td>v</td>
<td>1.76</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>9.67, C</td>
<td>v</td>
<td>2.79</td>
<td>h</td>
</tr>
<tr>
<td></td>
<td>9.91, C</td>
<td>v</td>
<td>2.17</td>
<td>h</td>
</tr>
<tr>
<td></td>
<td>10.34 C</td>
<td>v</td>
<td>-1.91</td>
<td>h</td>
</tr>
<tr>
<td></td>
<td>10.64 C</td>
<td>v</td>
<td>-1.84</td>
<td>h</td>
</tr>
<tr>
<td></td>
<td>10.85 C</td>
<td>v</td>
<td>-1.52</td>
<td>h</td>
</tr>
</tbody>
</table>

\( ^{a} \text{Defined by } d[\text{Py}]/dt = 2k[\text{Py}]^2. \) \( ^{b} \text{H} = \text{perchloric acid; F} = \text{isopropanol-formic acid; A} = \text{acetic acid-sodium acetate; P} = \text{phosphate; FP} = \text{sodium formate-phosphate; B} = \text{barbitol; C} = \text{carbonate.} \) \( ^{c} \text{p} = \text{pulse radiolysis; v = vacuum-line technique.} \) \( ^{d} \text{k in } \text{dm}^3 \text{ mol}^{-1} \text{s}^{-1}. \) \( ^{e} \text{Present work, with } e_00 = 8900 \text{ M}^{-1} \text{cm}^{-1}. \) \( ^{f} \text{The value given for the rate constant for the decay of 3-in ref 12a and 18 was calculated incorrectly and is now superseded.} \) \( ^{g} \text{The rate constant for the decay of NAD was correct. Comparisons of electrochemical and pulse-radiolysis data [C. O. Schmaken, K. S. V. Santhanam, and P. J. Elving, J. Am. Chem. Soc., 97, 5083 (1975)] now need to be revised.} \) \( ^{h} \text{Pseudo-first order reaction with OH- which supersedes the bimolecular reaction at the concentrations used for the pH values given.} \) \( ^{i} \text{in } \text{s}^{-1}. \)
In experiments the hydrolysis product, we think that the scheme shown in eq 1 is required to explain the “loss” of protons. Direct evidence for the formation of alkylamine was obtained from radiolysis experiments.

### pH Changes on Radiolysis

Irradiation of solutions of 1-methyl-3-carbamido- and 1-methyl-4-carbamidopyridinium perchlorates in water containing 0.5 M isopropyl alcohol but no buffer led to pH changes. Data for 1-methyl-2-carbamidopyridinium perchlorate solutions irradiated under the same conditions were also obtained, the radical 2+ being the expected intermediate as shown by pulse radiolysis experiments. Irradiation with 60Co γ rays of the solution of 3+ led to the greatest decrease in pH, with 2+ exhibiting substantially less pH change. The pH of the irradiated 4+ solution actually increased in pH by a small amount, a result which was traced to the formation of a small amount of methylamine, enough to convert the net change in pH into a small increase. Given the quantities of substances used and the radiation doses applied, the results for the three compounds may be summarized to a reasonable approximation by the following equations. The data are given in Table II, and the details of the 60Co irradiations are noted in the Experimental Section.

- (proton formation)

<table>
<thead>
<tr>
<th>compd</th>
<th>0</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+</td>
<td>7.00</td>
<td>7.84</td>
<td>8.24</td>
<td>8.47</td>
<td>8.49</td>
<td>8.40</td>
</tr>
<tr>
<td>3+</td>
<td>6.81</td>
<td>4.16</td>
<td>4.17</td>
<td>3.95</td>
<td>3.54</td>
<td>3.24</td>
</tr>
<tr>
<td>2+</td>
<td>6.71</td>
<td>5.12</td>
<td>4.72</td>
<td>4.55</td>
<td>4.46</td>
<td>4.33</td>
</tr>
</tbody>
</table>

a Fricke dosimetry indicated an approximate 7000 rad/min, but our experimental solutions were not prepared under absolutely controlled conditions and the expected G value was approached only after considerable irradiation. The trends, however, are clearly in agreement with eq 13-15. b Large-scale irradiation for product examination.

### Instability of Initial Products of Radical Reaction

Almost all of the initial products formed in the reactions of radicals are unstable under some or all of the conditions used. The dimers and combination products disappear in acid-catalyzed hydration reactions to form monounsaturated amides, \( \lambda_{\text{max}} \) 290 nm.26a (eq 10.) Observed rates of hydration could be followed at a number of pH values and are compatible with a process first order in hydrogen ion (Experimental Section). The NAD dimer reacts with protons and water at about 1% of the rate for the 4,4'-dimer of 3+, possibly due to a steric effect exerted by the group on nitrogen.
4-radicals react with one another so slowly under these conditions that no reaction should be observed.

Discussion

The most significant part of our current work is the sharp difference we have established between the reactions of 3- and 4-radicals. The 3-radicals react with one another to yield dimer, and the 4-radicals react with one another (primarily via a process which involves protonation of one 4-radical) to yield disproportionation products, formed by electron transfer between the reactant species. We can summarize these results in the form of two general equations, eq 16 and 17.

3Py4 + 3Py3 → 3Py3-3Py (probably 4,4')

4Py3 + 4Py3H+ → 4Py3H + 4Py4

In considering the reasons for this divergence of behavior, it became clear from the work of Mumm and Ludwig2 that steric hindrance at the 4 position promoted the formation of 3-radicals from their dimers. The importance of the 4 position is evident in the spin distribution for many pyridinyl radicals,28,29,30 and supported by the calculations of Neita and Patterson.19 If we were able to hinder the dimerization reaction, a 3-radical might then participate in 1e reactions. An enzyme which binds NAD might be regarded as a highly bulky, sterically hindering substituent on the pyridinium (or pyridyl) ring. In fact, Gunsalus and co-workers31 have produced NAD+ radicals in the presence of ferredoxin-NADP+ radicals react with one another so slowly under these conditions that no reaction should be observed.

Pulse Radiolysis Technique. Pulses of electrons (approximately 0.1-10 μs, 10 MeV) were delivered from a linear accelerator.21 Optical absorption of irradiated solutions was monitored at all necessary wavelengths and recorded as a function of time using Polaroid film. Treatment of data was carried out as in earlier work, thiocyanate being used for dosimetry.37

Irradiations were carried out on an argon-bubbled solutions containing either 0.1 M sodium formate/formic acid, or isopropyl alcohol-0.1 M sodium phosphate. pH was controlled by the addition of perchloric acid or, in certain cases, sulfuric acid.

Spectroscopic Data. λmax (nm) (εmax; ε): 3-370 (6500), 365 (3000), 900 (—); 2-380 (10000), 450 (4300), 1250 (—); 4- 305 (15700), 402 (8900), 650 (—80).

Radiolysis Technique. Aqueous solutions of 1-alkyl-4-carbomethoxydipyrindinyl perchlorates (5 × 10−3 M) containing 0.5 M isopropyl alcohol were made up with a glove bag under N2 using CO2-free solvents and then bubbled with pure N2 within the glove bag for 30 min. All pH measurements were made within the glove bag using an Orion digital pH meter.

Irradiations were made with the 60Co source at the Weizmann Institute, Rehovot. Radiation doses were measured with a Fricke dosimeter. However, the technique used for preparing the solutions does not rigorously exclude oxygen or other electron-consuming impurities, requiring doses greater than those which could accurately be measured with the dosimeter. It was possible to obtain an approximate idea of the effective dose by carrying out a large-scale irradiation and measuring the amount of pyridinium salt (absorption) remaining after 60 min of irradiation: 1-methyl-2-carbamido (80%), 1-methyl-3-carbamido (59%), and 1-methyl-4-carbamido (74%). Since disproportionation for the 3- and 4-radicals yields the starting pyridinium ion, these results imply amounts reacted of 40, 41, and 52%, respectively, for the 2-, 3-, and 4-ions. At the concentrations used (5 × 10−3 M), 2 × 10−3 M salt reacted. The dosimeter results (7000 rad/min) suggested that 2.5 × 10−3 M salt should have reacted [G = 6 (cf. eq 10)]. The agreement is satisfactory and compatible with the limitations of the techniques used. The pH of the 3-solution indicated 6 × 10−3 M H+ or only 30% of that expected. Either the 3-4 h delay between irradiation and measurement led to consumption of product (e.g., slow hydrolysis, protonization or disproportionation), or disproportionation occurred in another reaction such as hal-protonation of the dimer, which would lead to an ultraviolet spectrum similar to that of the unprotonated but diminished in intensity. In fact, the product has an estimated absorption coefficient at 295 nm of 10 000 in place of the 40 000 which might have been expected from the results of Kim and Chaykin.38 To simplify the discussion, the possible contribution of other dimers (e.g., 6,6′ or 4,6′, etc.) has been ignored.35

Methylamine formation was observed in the case of 4+ (ca. 4.5 × 10−6 M) and 2+ (ca. 1.1 × 10−6 M), using fluorescamine to generate a fluorescent product from methylamine and quantitative fluorimetry with a Perkin-Elmer MFP-4 spectrofluorimeter and comparison with known concentrations of methylamine-fluorescamine product.39 Thin-layer chromatography confirmed that the fluorescent product was indeed that derived from methylamine, readily distinguishable from the fluorescent product derived from 6-butyramine. The formation of methylamine in radiolysis confirms the conclusions reached on the basis of the NMR spectrum of the product of reaction of 4EE with itself in water at neutral pH (see following experiment).

Disproportionation of 1-Ethyl-4-carbomethoxydipyrindyl Radical in Water. Tetramethylammonium phosphate, pH 5.94, in D2O (2 cm3) was mixed with ca. 50 mg of 1-ethyl-4-carbomethoxydipyrindyl (CH3CN soln: solvent evapd, CD3CN introduced, solvent evapd, 0.4 cm3 CD3CN introduced) with stirring. Within a short time, the blue-green color of the solution had changed to pale orange. A portion (0.5 cm3) of the mixture was poured into an NMR tube, the tube was sealed off, and the NMR spectrum was immediately taken. The 1-ethyl-4-carbomethoxydipyrindyl ion was readily identified; δ 8.11, 8.41 (each doublet, ring H), 4.00 (CH3 of ester), 1.63 (CH3 of 1-ethyl). Quantitative analysis was not possible. The only other readily visible peaks were at 8.12 and 0.90. Each was a triplet. The ap
proximate ratio between the $\delta 1.63$ and the sum of the $\delta 1.24$ and 0.90 peaks was 1:1. No dihydropyridine ring protons could be seen, $\delta 4.5$ and 6.00 (see previous product study above). The dihydropyridine which must have formed as a result of disproportionation must have hydrolyzed, completely or partially after hydration, yielding ethylamine or a cyclic saturated amine in roughly equivalent amounts. The UV spectrum of the solutions after kinetic experiments shows that no UV-absorbing product except for the 1-ethyl-4-carboxylypyridinium ion is present (down to 200 nm).

Disappearance of 4EE or 4EB in Water. An amount of radical sufficient to give a suitable optical density for kinetic studies (between ca. 0.01 and 1.0) was introduced into an all-glass apparatus in an acetone/acetonitrile solution. Most of the acetone was removed, and a degassed buffer of the desired pH was added. The nominal pH of the buffer was that obtained before degassing (phosphate, pH 6–8; carbonate, pH 10–11; barbitral, pH 8–9; acetate, pH 5–5.5), but the pH was taken as that measured after the completion of the kinetic run, since small amounts of acetonitrile change the pH of an aqueous buffer. Although a number of successful experiments were carried through in which the buffer was introduced directly from the breakseal onto the radical sample located within the cell with the Cary spectrophotometer running, it was usually simpler to mix outside the spectrophotometer and then quickly insert the cell into the cell holder.

Plots of $\log$ optical density vs. time gave linear plots for experiments carried out below pH 9.5. At pH 9.7 or above, the log of OD vs. time was linear, indicating a pseudo-first-order reaction, since the constant varied with pH. The rate constant, defined as rate times the extinction coefficient, remained roughly constant down to 200 nm. The slope of the second-order plot times the extinction coefficient gave the bimolecular rate constants recorded in Table I. A UV spectrum taken after the completion of the kinetic runs below pH 9 revealed a spectrum corresponding to that of the 1-alkyl-4-carboxylypyridinium ion, $\lambda_{max}$ 275 nm ($\epsilon_{max}$ 4400) in almost exactly 50% yield. The spectrum changed at a moderate rate to that of the 1-alkyl-4-carboxylypyridinium ion, in exactly the way observed for an authentic solution of the 1-alkyl-4-carboxylypyridinium ion. The log of OD vs. time was linear, indicating a pseudo-first-order reaction with oxygen to form superoxide ion, $O_2^-$, and $H_2O$. These chemically reactive species apparently attack components of the membrane and eventually cause destruction of the plant. The kinetic constant of the radical reaction with oxygen is thus a subject of considerable economic as well as scientific importance. Compare J. A. Farrington, M. E. Baxendale, E. J. Ladd, and K. F. L. Boland, Biophys. Acta 1172 (1973), and A. K. Nightingale, E. J. Ladd, and N. H. Rees, J. Chem. Soc., Perkin Trans. 2, 855 (1976). The direct importance of superoxide ion has been questioned by B. Goldberg and A. Stahl, Mol. Pharmacol., 5, 253 (1969), G. Maitland, J. M. Morrison, and E. T. Seo, J. Am. Chem. Soc., 100, 627 (1978). See also J. A. Farrington, M. E. Baxendale, and E. J. Ladd, J. Chem. Soc. Faraday Trans. 1, 665 (1978).


(17) $pK_a = 1$-methyl-3-carboxylypyridinium ion; $pK_a = 1$-ethyl-4-carboxylypyridinium ion; $pK_a = 1$-tert-buty1-4-carboxylypyridinium ion; NAD$^+$ = pyridyl radical derived by 1 reduction of nicotinamide adenine dinucleotide. It should be pointed out that meta- $m$ and para $p$ are not correct as designators of substituent locations on the pyridine ring.


(a) U. Brunnmann and E. Heyon, J. Am. Chem. Soc., 96, 6168 (1974). (b) The protonation occurs on the amide group rather than on the ring carbon. This can be clearly seen by comparison of this spectrum with the spectrum of the product of hydrogen atom addition to the ring of the N-methyl nicotinamide ion. The adduct spectrum shows a fairly sharp maximum at 340 (8.5 nm); H. D. Burrows, A. J. Swallow, and E. M. Kosower, unpublished observations.


(27) In the scavenging of hydroxyl radicals by isopropyl alcohol, 13.3% of the reaction proceeds via formation of $\text{CH}_2\text{CH}($OH)$_2$ (K. O. Asmus, H. Mocckel, and A. Henglein, J. Phys. Chem., 77, 1218 (1973)). The small percentage of fluorescent product formed here may result from cross-reaction of these radicals with...