Time-Resolved Resonance Raman, Electron Spin Resonance, and *ab Initio* Molecular Orbital Study of the Structure and Proton Reactivity of 4-Carbamylpyridinyl Radicals†

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Abstract: Short-lived pyridinyl radicals (4-carbamylpyridinyl; neutral and cation forms) produced on one-electron reduction of isonicotinamide by e_{aq}^-, H• or $(CH_3)_2C^{\bullet}OH$ in aqueous solution have been probed by resonance Raman and ESR spectroscopies. It has been shown that the radical cation formation by direct addition of H• atom to N-protonated isonicotinamide is less efficient (29% yield) than the sequential additions of e_{aq}^- and H^+. The neutral carbamylpyridinyl radical (1-hydro-4-pyridine amide) protonates in mildly acidic solutions ($pK_a \sim 2$), in contrast to closed-shell amides which protonate only in highly concentrated aqueous acid solutions. This anomalous property of the amide group conjugated with semireduced pyridine has been explained in terms of *ab initio* molecular structures which are consistent with resonance Raman frequencies of the radicals and their deuterated isotopomers, and with ESR hyperfine constants. As with stable amides, the proton binds with the oxygen, and not the nitrogen atom, in the radical structure. However, the CO bond is ∼0.02 Å longer in the neutral radical than in isonicotinamide, and there is substantial negative charge on the oxygen atom, which makes this species very reactive toward the proton, as compared to the regular amides.

Introduction

The chemical properties and modes of reaction of pyridine and its derivatives, which are used for modeling some biochemical processes of common occurrence in nature, have been an area of active research for many years.[1−20] Reaction intermediates prepared by chemical, electrochemical, photochemical, and radiation-chemical methods in aqueous and nonaqueous environments have been characterized extensively by optical absorption[2,3] and ESR methods,[4−7] and their bimolecular and electron transfer reactions have been examined. These studies have addressed a number of questions of chemical importance in relation to the redox interconversion of coenzyme couples NADH/NAD^+ and NADPH/$NADP^+$, and the control mechanism of certain water-soluble vitamins (e.g., vitamin B_5).[3] Thus far, there has been little information on the electronic structure and bond properties of these important radical intermediates which could provide an understanding of their chemical behavior from a molecular perspective. The diffraction methods, widely used in chemistry and biology to directly measure the atomic separations in complex molecular systems, are not

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applicable to short-lived intermediates prepared in solution at low concentrations. Therefore, indirect procedures are the only alternatives. A promising approach, which is currently evolving, is to combine vibrational spectroscopy of the solvated reactive intermediate with appropriate theory to develop a model for the molecular structure. This structural model, if realistic, should provide an adequate explanation for the chemical properties of the intermediate. In a recent publication,[20] we have applied this approach to investigate the structure−reactivity relationship in 4-carboxypyridinyl radicals. Here, we examine the pyridinyl radicals derived from 4-pyridine amide (isonicotinamide).

Earlier studies of the redox reactions of nicotinamide and isonicotinamide have shown that some of the chemical properties, such as proton dissociation constants and reduction potentials, of pyridinyl radicals obtained from the two isomers are not too different.[3,21] However, isonicotinamide is a cleaner chemical system for resonance Raman studies, as other reaction products do not absorb in the same spectral region as the pyridinyl radicals.[3] Also, electronic transitions in the radical intermediates of this isomer in the visible region are stronger than those of nicotinamide which enhances the detection sensitivity in resonance Raman experiments. On the other hand, the structure−reactivity relationship discussed here for the 4-carbamylpyridinyl radicals ($H_2NCOC_5H_4NH^{\bullet}$) is of fairly general nature and can be readily extended to the other isomers. The crystal and molecular structures of several inorganic complexes of isonicotinamide have been studied recently because of their pharmaceutical importance.[12] Information on the structure and bonding in the reduced radical state of isonicotinamide is required for an understanding of the mode of action of these compounds. Vibrational frequencies of the 4-carbamylpyridinyl radicals in aqueous solution provide a valuable reference against which the redox reactions of isonicotinamide in nonaqueous environments, such as metal and semiconducting surfaces, can be examined.

One of the most striking chemical properties of the carbamylpyridinyl radicals is their protonation in mildly acidic solutions. The pK_a of the radical cation, $(H_2NCOH)^+C_5H_4NH^{\bullet}$, in isonicotinamide has been measured as 1.9, and that in nicotinamide as ∼1.4.[3] There is hardly any example of a stable amide or carboxylic acid , to our knowledge, which protonates at a pH this high. This chemical behavior also contrasts with that of the carboxypyridinyl radicals[3,20] where protonation occurs in relatively stronger acidic solutions ($H_0 \sim 0$). In neutral radicals formed from acids, the −CO oxygen is the only site available in the carboxylic group for the proton attachment. In the corresponding amide radicals, however, proton addition at the nitrogen position is also possible. Whether the proton binds with the amide oxygen or nitrogen atom in the radical structure, and why it displays an unusually high dissociation constant (pK_a) relative to closed-shell amides, are some of the chemical questions we have examined in this work with the help of transient Raman, ESR, and theoretical calculations. Protonation sites in stable amides in concentrated aqueous acid solutions have been the subject of a number of investigations in the past.[22] Thus far there has been no investigation on the protonation behavior of the amide group in a short-lived redox intermediate, to our knowledge.

In addition to the structural studies, resonance Raman spectroscopy has been applied here as a diagnostic tool to explore some interesting aspects of the chemistry. Since pyridinyl radical formation essentially involves addition of a hydrogen atom to the parent molecule at a nitrogen atom site, it would be interesting to know if the same species is also produced on direct reduction by the hydrogen atom. Pulse radiolysis of acidic aqueous solutions is a convenient method for production of hydrogen atoms in sufficient concentration to study their chemistry.[23] One can, in principle, examine the question posed here even by the optical absorption method which is relatively less time consuming. However, absorption spectra in solution are not very structure specific, and distinction between the pyridinyl and the other radicals formed by $H^{\bullet}$ addition at different sites of the molecule can be difficult.

The e_{aq}^- reduction of isonicotinamide was studied in highly basic solutions (0.1 to 1.5 M KOH) to observe the deprotonated radical which would allow us to determine whether the ring or amide N−H site was involved. The transient Raman spectrum recorded on the μs time scale at pH ∼14 differs from the one obtained at pH ∼11. However, the species observed is the anion form of the 4-pyridine carboxylic acid radical,[20] and not of the amide radical.

Experimental and Theoretical Procedures

For transient absorption and Raman studies of reaction intermediates, pulse radiolysis was used to induce one-electron reduction of isonicotinamide (pK_a of conjugate acid 3.6) in aqueous solution.[3,20,21,23−25] Radiolysis of oxygen-free water produces e_{aq}^- (2.6), $^{\bullet}OH$ (2.7), and $H^{\bullet}$ (0.6) radicals (numbers in parentheses are G values, i.e., yields of radicals per 100 eV of energy absorbed)[23] on the ∼100 ns time scale. In basic solutions, the $^{\bullet}OH$ radical is scavenged by an excess of *tert*-butyl alcohol (*t*-BuOH), so that reduction occurs mainly by reaction of e_{aq}^- (reactions 1 and 2). In acidic solutions, where the e_{aq}^- reacts with H^+ ($k \sim 2.3 \times 10^{10}$ M^{-1} s^{-1}) to form $H^{\bullet}$ atoms,[23] isonicotinamide

(basic solutions)

$$4\text{-}H_2NCOC_5H_4N + e_{aq}^- \rightarrow 4\text{-}H_2NCOC_5H_4N^{\bullet -} \quad (1)$$

$$4\text{-}H_2NCOC_5H_4N^{\bullet -} + H_2O \rightarrow 4\text{-}H_2NCOC_5H_4NH^{\bullet} + OH^- \quad (2)$$

was reduced by acetone ketyl radical, $(CH_3)_2C^{\bullet}OH$ (reactions 5 and 6). The latter radical was prepared by reaction of $^{\bullet}OH/H^{\bullet}$ radicals with isopropyl alcohol (*i*-PrOH) (reactions 3 and 4) in solutions saturated

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with N_2O which converts e_{aq}^- into •OH radical. About 85 to 90% of the •OH and H• radicals produce acetone ketyl radical by reaction with *i*-PrOH.[23] Electron transfer from $(CH_3)_2C^{\bullet}OH$ to isonicotinamide occurs only when the ring nitrogen is protonated (pK_a 3.6).[3] The various chemical steps involved in the pyridinyl radical formation are given below:

(acidic solutions)

$$^{\bullet}OH + (CH_3)_2CHOH \rightarrow (CH_3)_2C^{\bullet}OH + H_2O \quad (3)$$

$$^{\bullet}H + (CH_3)_2CHOH \rightarrow (CH_3)_2C^{\bullet}OH + H_2 \quad (4)$$

$$4\text{-}H_2NCOC_5H_4N^+H + (CH_3)_2C^{\bullet}OH \rightarrow 4\text{-}H_2NCOC_5H_4NH^{\bullet} + CH_3COCH_3 + H^+ \quad (5)$$

$$4\text{-}H_2NCOC_5H_4NH^{\bullet} + H^+ \rightarrow 4\text{-}(H_2NCOH)^+C_5H_4NH^{\bullet}\ (pH < 3) \quad (6)$$

The experimental procedure used in this Laboratory for time-resolved resonance Raman studies of short-lived chemical intermediates produced in solution has been described previously.[26,27] A Van de Graaff accelerator is used to generate ~2 MeV, ~100 ns electron pulses, at a radiation dose sufficient to produce $\sim 3 \times 10^{-4}$ M radical concentration per pulse. A tunable excimer laser pumped dye laser (~10 ns) system is used to probe Raman scattering, with an optical multichannel analyzer (OMA) accompanied by an intensified gated (~20 ns) diode array detector for detection. The accelerator and laser were operated at a repetition rate of 7.5 Hz which allowed efficient signal averaging. Raman band positions were measured with reference to the known Raman bands of common solvents, such as ethanol and carbon tetrachloride, and are accurate to within 2 cm^{-1} for sharp bands and 5 cm^{-1} for broad and shoulder bands.

Radiolysis by ~8 MeV, ~10 ns electron pulses from a linear accelerator, which produced a radical concentration of $\sim 3 \times 10^{-6}$ M per pulse, was used for spectrophotometric measurements of the absorption spectra, by methods described in earlier papers from this Laboratory.[28] In optical as well as Raman experiments a flow system was used to refresh solution between consecutive electron pulses to avoid product accumulation in the cell.

Photolysis of aqueous solutions containing acetone and *i*-PrOH was used to prepare acetone ketyl radical, $(CH_3)_2C^{\bullet}OH$, to reduce isonicotinamide for ESR experiments. The excited triplet state of acetone abstracts a hydrogen atom from *i*-PrOH to produce two molecules of ketyl radical (reaction 7). During the course of these studies it was

$$^1(CH_3)_2CO \xrightarrow{h\nu} {}^3(CH_3)_2CO \xrightarrow{+(CH_3)_2CHOH} 2(CH_3)_2C^{\bullet}OH \quad (7)$$

discovered that the photoexcited isonicotinamide also abstracts a hydrogen atom from *i*-PrOH, to produce 4-carbamylpyridinyl radical (reaction 8). Although it is not clear at this point if the excited state

$$4\text{-}H_2NCOC_5H_4N \xrightarrow{h\nu} [4\text{-}H_2NCOC_5H_4N]^* \xrightarrow{+(CH_3)_2CHOH} 4\text{-}H_2NCOC_5H_4NH^{\bullet} \quad (8)$$

involved is a singlet or a triplet, this finding would be an interesting topic for further investigation. The reaction allowed us to prepare neutral and cation forms of carbamylpyridinyl radicals simply by changing the pH of the solution. The steady-state photolysis ESR experiments were carried out in a manner similar to that of previous work, but with a Bruker ESP-300E spectrometer with an ESP 380-1010 microwave bridge.[29] The light source was a Hg−Xe high-pressure lamp with mirror and a filter solution to restrict the wavelength range to 250 to 300 nm. The sample flowed through a 0.4 mm thick silica flat cell at about 5 cm^3 min^{-1}.

The *ab initio* molecular orbital calculations were carried out with the GAUSSIAN 92 computer program.[30] Electronic wave functions were computed by the restricted open shell Hartree−Fock (ROHF) method using the standard split-valence 4-31G basis set. The unrestricted Hartree−Fock (UHF) procedure displays serious spin contamination, and is not suitable for the pyridinyl radicals.[20] The equilibrium geometries and harmonic force fields were obtained with methods based on analytical evaluation of the gradients of the total electronic energy. The various approximations involved in this theoretical procedure are known to result in a slight overestimation of the vibrational frequencies. Therefore, the calculated frequencies were multiplied by an empirical scaling factor, which is a common practice,[31−33] before being compared with the experimental Raman frequencies. It should be noted that the frequencies are calculated for the isolated radical, but the Raman frequencies have been observed in aqueous solution. The solute−solvent interaction shifts the vacuum frequencies, but these shifts for the ring vibrations are generally small in comparison to the prediction accuracy of the theoretical method applied here. Large frequency shifts in aqueous medium are expected only for the vibrations involving large-amplitude motions of hydrogen atoms bonded to oxygen and nitrogens. This can result in poor agreement between the predicted and observed isotope shifts for the ring modes strongly coupled with N−H/N−D bending motions.

Transient Absorption

Absorption spectra of the 4-carbamylpyridinyl radicals, required as reference for resonance Raman measurements, were obtained by using chemical conditions similar to those in earlier studies.[3] Figure 1a represents the absorption spectrum of the neutral 4-carbamylpyridinyl radical observed ~4 μs after electron pulse irradiation of a N_2-saturated aqueous solution containing 1 mM isonicotinamide and 0.5 M *t*-BuOH at pH ~11. Here, the transient is formed by reaction of e_{aq}^- with isonicotinamide (reactions 1 and 2). The reaction rate constant, measured by monitoring the decay of the e_{aq}^- absorption at ~700 nm, was determined as 2.7×10^{10} M^{-1} s^{-1}. The absorption spectrum of the conjugate acid of the 4-carbamylpyridinyl radical (referred to as the radical cation throughout the paper) was obtained on a similar time scale on radiolysis of a N_2O-saturated solution containing 0.5 M isonicotinamide and 2 M *i*-PrOH at pH ~0.5, and is given in Figure 1b. In this case, isonicotinamide is reduced by $(CH_3)_2C^{\bullet}OH$ (reactions 3−6). This reduction occurs at a rate constant of 1.9×10^9 M^{-1} s^{-1}. The spectra and rate constants measured here are in agreement with the corresponding data in the literature.[3]

ESR Spectra

While a number of ESR studies on the reduced radical states of pyridine derivatives have been reported in the past,[4−7] to date,

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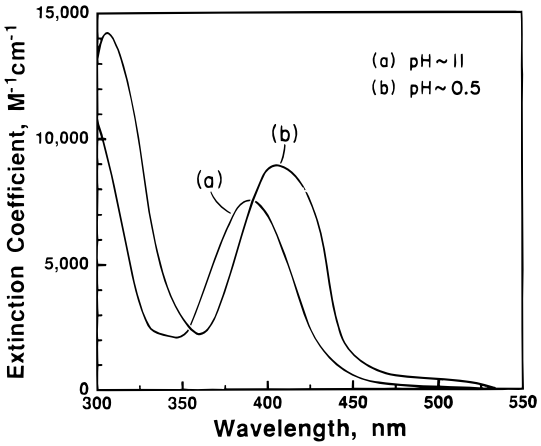

Figure 1. Absorption spectra of 4-carbamylpyridinyl radicals observed on pulse radiolysis of (a) 1 mM isonicotinamide and 0.5 M *tert*-butyl alcohol in N_2-saturated solution at pH 11, 4 μs after pulse, and (b) 0.5 mM isonicotinamide and 2 M isopropyl alcohol in N_2O-saturated aqueous solution at pH 0.5, 6 μs after pulse. The spectra are scaled for the radiation yields (*G* values) of the radicals.

the ESR observation of the cation form of a pyridinyl radical has not been made, to our knowledge. In a recent investigation of the 4-carboxypyridinyl radicals,[20] the spin populations in the neutral and anionic forms of the radical, predicted by the *ab initio* method used in this work, were found to be consistent with the ESR hyperfine constants. The prediction that the spin population in the cation form of the radical is drastically different from that in the neutral and anion forms could not be verified due to the non-availability of the experimental data. This ESR study of the 4-carbamylpyridinyl radicals was undertaken with two objectives: (1) to check if the ring proton hyperfine constants in the cation form of the radical are indeed of comparable magnitude, as suggested by the calculations on isoelectronic 4-carboxypyridinyl radicals, and (2) to complement the vibrational spectroscopic arguments to determine the protonation site in the amide group. The ESR spectrum of the neutral form of the radical was also obtained for comparison, as it has not been previously investigated.

The reduced form of isonicotinamide was prepared by photolysis, as described in the previous section. A number of different conditions were tried at different pH values. A strong spectrum of the neutral form of the radical was produced using 10% acetone and 10% *i*-PrOH in water with no added acid or base. Similar spectra were obtained at pH 5.6 with added phosphate buffer. This spectrum was analyzed by standard means by comparing synthesized spectra with the experimental spectrum. Preliminary values for the hyperfine constants of ring hydrogens and nitrogen were obtained from a study on the pyridinecarboxylic acids.[6] After adjustment of the values an excellent fit of both line positions and intensities was obtained as shown in Figure 2. The spectrum taken with low resolution (Figure 2a, large field modulation amplitude) establishes the larger splittings and the spectrum obtained with a lower field modulation (Figure 2b) clearly shows the sizes of the five smaller splittings. With such a complex spectrum it is difficult to be certain that the analysis is unique. However, the excellent fit and similarity of the values to those of the corresponding radical from the carboxylic acid give a high degree of confidence that the analysis is correct. The hyperfine constants are shown in Table 1.

When KH_2PO_4 was added to give pH 3, marked changes in the spectrum were observed. The overall pattern of intensities changed and the lines became wider. Spectra were taken with up to 5 M H_2SO_4 present, but all of these spectra were too weak

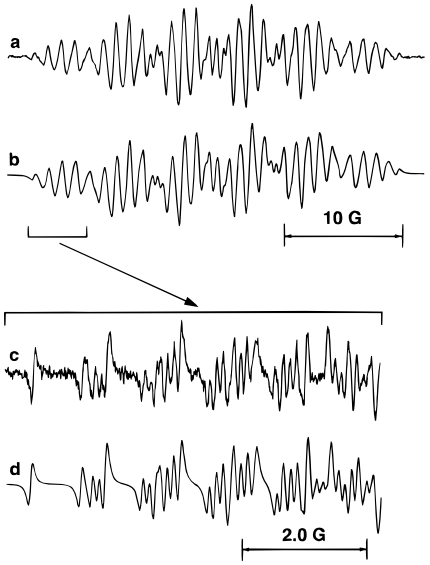

Figure 2. First-derivative ESR spectrum taken during photolysis of 20 mM isonicotinamide in water with 10% acetone and 10% isopropyl alcohol. Field increases to the right. Spectrum (a) was obtained with a large field modulation and (b) is the corresponding synthesized spectrum with splittings as given in Table 1. Partial spectrum (c) was obtained with a lower modulation amplitude and (d) is the simulation. The bracket at the lower left below (b) represents the portion expanded in (c). The five smallest splittings (two are equal) are clearly evident in the second line group from the left.

Table 1. Hyperfine Constants (G)[a] of Reduced Isonicotinamide

form	H_1	N_1	$H_{2,6}$[b]	$H_{3,5}$[b]	$H(NH_2)$[b]	$N(NH_2)$
neutral	5.13	5.13	3.25	1.05	0.88	1.08
			3.55	0.97	0.72	
acid	5.30	4.87	2.0	2.30		2.15
			2.0	2.30		

[a] The accuracy of the smaller values is about 0.01 G but the larger values could have two or three times larger error. [b] The assignment of proton hyperfine constants to the 2,6 and 3,5 and NH_2 positions for the neutral form is arbitrary as is the assignment to the 2, 3, 5, and 6 positions in the acid form.

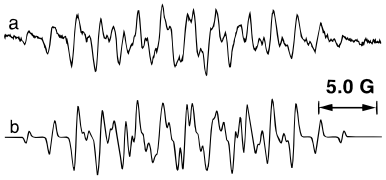

Figure 3. First-derivative ESR spectrum taken during photolysis of 40 mM isonicotinamide in a solution of 40% acetone, 50% isopropyl alcohol, and 10% water with 1 M H_2SO_4 (a) and the simulation (b).

for analysis. To give better spectra, solutions with less water and more acetone and *i*-PrOH were tried. A spectrum with a solution containing 40% acetone, 50% *i*-PrOH, and 10% water and 1 M H_2SO_4 was of sufficient intensity to analyze as shown in Figure 3. Again, the fit is excellent. A spectrum taken with 15% acetone and 10% *i*-PrOH with 1 M H_2SO_4 was weaker but seemed to be the same.

The hyperfine constants for the neutral radical are similar to those for a number of other pyridine derivatives such as the neutral form of the corresponding acid.[4−6] A particular feature is the larger values assigned to the protons *ortho* to the ring nitrogen relative to those for the *meta* hydrogens. The hyperfine constants of the form of the radical found in acid are quite distinct, the values for the ortho and meta ring protons being all near 2 G rather than having larger values for the ortho protons.

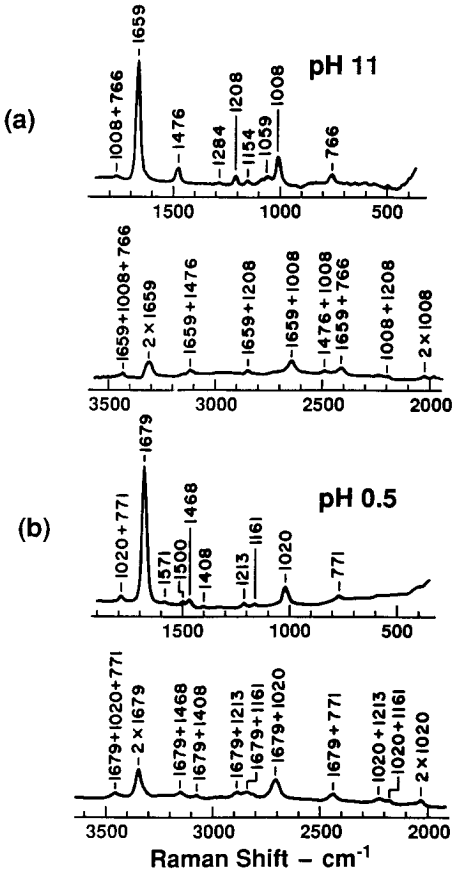

Figure 4. Resonance Raman spectra (1 μs) of 4-carbamylpyridinyl radicals in aqueous solution in the 500−3500-cm^{-1} region: (a) neutral radical, 409-nm excitation; and (b) radical cation, 409-nm excitation.

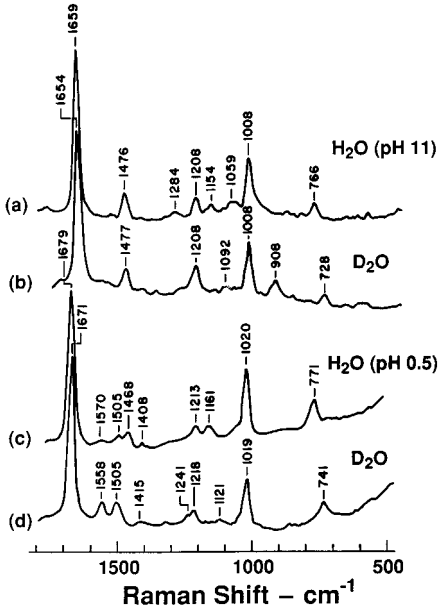

Figure 5. Resonance Raman spectra of 4-carbamylpyridinyl radicals in the fundamental vibration region: neutral radical in (a) H_2O and (b) D_2O, excitation at 395 nm; radical cation in (c) H_2O and (d) D_2O, excitation at 409 nm.

Resonance Raman Studies

Transient Raman Spectra. Raman experiments were performed at radiation dose rates ∼100 times higher than in optical absorption measurements. Therefore, it was often necessary to use substrate concentrations of 5 to 10 mM in solution to ensure that the reducing species, such as e_{aq}^- and acetone ketyl radicals, mainly react with isonicotinamide and not with themselves. The chemical conditions were otherwise similar. Raman spectra were recorded in the 500−3500-cm^{-1} region in seven overlapping segments, 1 μs and 10 ms after the electron pulse, by averaging data from ∼6000 pulses for each measurement. The difference spectra obtained by using solvent bands as reference were attributed to the pyridinyl radicals.

Figure 4a represents the 1-μs spectrum of the neutral 4-carbamylpyridinyl radical probed at 395 nm, on electron pulse irradiation of a N_2-saturated aqueous solution containing 6 mM isonicotinamide and 0.5 M *t*-BuOH at pH ∼11. The 500−1700-cm^{-1} region (Figures 4a and 5a) contains Raman bands representing fundamental vibrations. The spectrum of the radical in this region was also examined in D_2O solution, and is given in Figure 5b for comparison. A highly resonance-enhanced band at 1659 cm^{-1}, which shifts to 1654 cm^{-1} in D_2O, dominates the spectrum. Combination and overtone frequencies in the 1700−3500-cm^{-1} region (Figure 4a) mostly involve the 1659-cm^{-1} fundamental, and provide clear evidence that only one species is responsible for the entire spectrum.

Raman spectra of the 4-carbamylpyridinyl radical cation, depicted in Figures 4b and 5c, were obtained by 409-nm excitation, 1 μs after pulse irradiation of a N_2O-saturated aqueous solution containing 6 mM isonicotinamide and 2 M *i*-PrOH at pH 0.5. The prominent bands in the spectra are generally shifted to higher frequencies with respect to the corresponding bands in the neutral radical. The most noticeable difference in the two spectra occurs in the 1450−1600-cm^{-1} region where instead of one medium intensity band at 1476 cm^{-1} in the neutral radical three weaker bands at 1570, 1500, and 1468 cm^{-1} appear in the radical cation (Figures 4 and 5). The spectrum recorded in D_2O solution (Figure 5d) shows only two radical cation bands in this region. The combined intensities of the 1570- and 1500-cm^{-1} bands in H_2O are approximately equal to the intensity of the 1558-cm^{-1} band in D_2O, which indicates that one of the two strongly coupled vibrations in this region undergoes a large-frequency shift on radical deuteration. These isotopic shifts are extremely important in mode assignments. Combination bands in the 1700−3500-cm^{-1} region (Figure 4b) conclusively rule out the presence of more than one isomer of the radical cation in the spectrum, as fundamentals of two chemically distinct species do not combine.

The fundamental frequencies of the 4-carbamylpyridinyl radicals, obtained in this work, are compared with those of the isoelectronic 4-carboxypyridinyl radicals in Table 2, and their mode assignments are given in Tables 3 and 4.

Diagnostic Applications. (1) Proton Dissociation Constant. Acid−base equilibrium between the neutral and cation forms of the 4-carbamylpyridinyl radical has been studied previously by transient optical absorption, but accuracy of the reported pK_a value has been questioned.[3] The measurement error generally relates to the variation in the radical yields in the pH region 2 to 5. Radiolysis of water in acidic solutions produces e_{aq}^- and H$^\bullet$ in varying concentrations, as e_{aq}^- reacts with proton ($k \sim 2 \times 10^{10}$ M^{-1} s^{-1}) to form H$^\bullet$ atoms. Therefore, reduction by e_{aq}^- is not appropriate for the pK_a measurement of a reduced radical state in mildly acidic solutions. The pK_a of the N-protonated isonicotinamide is 3.6, and the unprotonated molecule is not reduced by $(CH_3)_2C^\bullet OH$. Therefore, as the pH of the solution is increased above 3.6, the fraction of the ketyl radical transferring an electron to isonicotinamide decreases, and the total concentration of the pyridinyl radicals may not remain constant. However, one does not anticipate a significant error if the pK_a of the radical cation is much lower than that of the parent molecule, as appears to be the case. Since the

Table 2. Comparison of the Fundamental Frequencies (cm^{-1}) and Band Intensities[a] in the Resonance Raman Spectra of 4-Carboxy- and 4-Carbamylpyridinyl Radicals

neutral radicals				radical cations			
b	*c*	*d*	*e*	*f*	*g*	*h*	*i*
1668 vs	1664 vs	1659 vs	1654 vs	1676 vs	1669 vs	1679 vs	1671 vs
1608 vw	1592 vw			1573 w	1552 m	1570 vw	1558 m
1472 s	1471 s	1476 m-s	1477 m-s	1473 m	1471 w	1500 w	1505 m
						1468 w	
1388 vw	1362 vw					1408 vw	1415 vw
1271 vw	1272 vw	1284 vw					
							1241 vw
1199 m	1199 m	1208 m	1208 m	1201 w	1200 w	1213 w	1218 w
		1154 vw	1092 vw			1161 w	1121 vw
1108 w	1111 w			1108 w	1121 w		
		1059 vw	908 m				
1011 s	1014 s	1008 s	1008 s	1020 s	1022 s	1020 s	1019 s
782 w	755 w	766 w	728 vw	783 w	762 w	771 m	741 w

[a] v = very, s = strong, m = medium, w = weak, sh = shoulder. [b] H_2O solution frequencies of neutral 4-carboxypyridinyl radical. [c] D_2O solution frequencies of neutral 4-carboxypyridinyl radical. [d] H_2O solution frequencies of neutral 4-carbamylpyridinyl radical. [e] D_2O solution frequencies of the 4-carbamylpyridinyl radical. [f] H_2O solution frequencies of the 4-carboxypyridinyl cation radical. [g] D_2O solution frequencies of the 4-carboxypyridinyl cation radical. [h] H_2O solution frequencies of the 4-carbamylpyridinyl cation radical. [i] D_2O solution frequencies of the 4-carbamylpyridinyl cation radical.

Table 3. Experimental and Calculated Frequencies (cm^{-1}) of 4-Pyridine Amide (Isonicotinamide) and the Neutral 4-carbamylpyridinyl Radical

isonicotinamide		4-carbamylpyridinyl radical				
		exptl		calc[b]		
exp[a]	calc[b]	H_2O[c]	D_2O[d]	H_2O[c]	D_2O[d]	assignment[e]
1593	1603	1659	1654	1641	1633	8a (ring stretch)
1621	1626			1635	1218	NH_2 scissor/bend (amide I)
1684	1673			1590	1591	C═O stretch (amide II)
1490	1492[f]	1476	1477	1447	1448	7a (C−$CONH_2$) + 19a (CH bend and ring stretch)
1130	1130[g]					
1372[h]	1350	1284		1251	1245	3/14 (CH bend + rind distortion)
1225	1212	1208	1208	1202	1206	9a (CH bend)
1149	1139	1154	1092	1133	1078	18a/12 (trigonal stretch + CH bend) + NH_2 rocking
1064	1068	1059	908	1034	893[i]	18b (CH bend) + NCO bend + CNH bend
1003	983	1008	1008	966	959	1/12 (ring distortion)
781	746	766	728	717	689[j]	Amide NCO bend + 6a (ring distortion)

[a] Infrared frequencies in Nujol (ref 17). [b] *Ab initio* frequencies multiplied by 0.89. [c] 4 -$H_2NCOC_5H_4NH^{\bullet}$. [d] 4-$D_2NCOC_5H_4ND^{\bullet}$. [e] Approximate description in terms of amide modes and Wilson modes of pyridine. [f] Wilson mode 19a (C−H bend + ring stretch). [g] Wilson mode 7a (C−$CONH_2$ stretch). [h] Frequency in solution (ref 17). In the deuterated isomer mode (i) has more NCO bending character than mode (j).

Table 4. Experimental and Calculated Fundamental frequencies (cm^{-1}) of the Cation Form of the 4-Carbamylpyridinyl Radical

exptl		calc[a]				
H_2O	D_2O	isomer I[b]		isomer II[c]		assignment[d]
1679	1671	1686	1676	1671	1667	8a (ring stretch)
1570	1558	1565	1546	1548[e]	1541[e]	7a (C − $COHNH_2$)+19a (CH bend + ring stretch)
1500	1505	1455	1443	1471	1469	19a + 7a;
1468		1589	1498	1591	1503	19b/8b (ring stretch + ring N−H bend)
1408	1415	1401	1400	1443	1442	amide CO/CN stretch + 8b
	1241	1663	1222	1646	1193	amide NH_2/ND_2 scissor/bend
1213	1218	1218	1217	1203	1203	9a (CH bend)
1161	1121	1185	1101	1015	1014	18a/12 (trigonal ring distortion + CH bend)
1020	1019	1019	1009	962	945	1/12 (ring distortion)
		943	950			
771	741	742	722	815[f]	741[f]	amide NCO bend

[a] *Ab initio* frequencis multiplied by 0.89. [b] First column: 4-$(H_2NCOH)^+$ $C_5H_4NH^{\bullet}$. Second column: 4-$(D_2NCOD)^+$ $C_5H_4ND^{\bullet}$. [c] First column: 4-H_3N^+CO $C_5H_4NH^{\bullet}$. Second column 4-D_3N^+CO $C_5H_4ND^{\bullet}$. [d] Approximate mode description for isomer I frequencies in terms of amide modes and Wilson modes of pyridine. [e] CO stretch. [f] This mode contains the C−N stretch, but not the NCO bend.

reported pK_a of the 4-carbamylpyridinyl radical cation is quite unusual, and a structural explanation for this chemical behavior is intended in this work, we have used resonance Raman spectroscopy to re-determine this constant. Raman studies clearly identify the transient species present in the system on the microsecond time scale as the radical cation at pH <1, and the neutral radical at pH >3. In the intermediate pH range, Raman signals due to both species are observed. At pH ∼2, the relative intensities of the signals do not change in the spectra taken at 100 ns or longer times, indicating a shorter equilibration time.

Initially, Raman spectra of the radical obtained in strongly acidic solution (pH ∼0.5) by reduction of isonicotinamide with $(CH_3)_2C^{\bullet}OH$ ($G = 5.01$), and in basic solutions (pH 11) by e_{aq}^- ($G = 2.6$), were properly scaled to represent equal contribution of the two species, and then combined (Figure 6). The Raman spectra obtained by $(CH_3)_2C^{\bullet}OH$ reduction of isonicotinamide at different pH were then compared with the composite

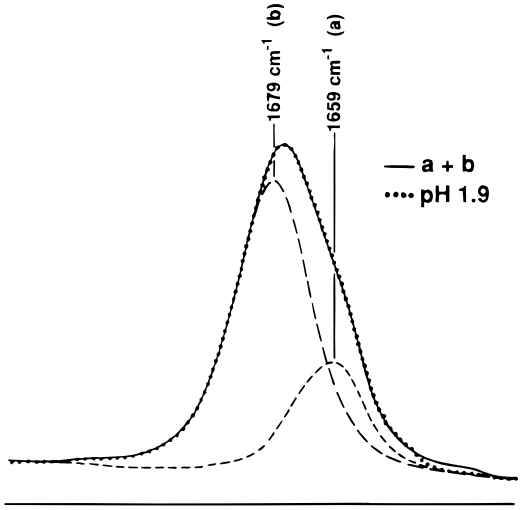

Figure 6. Raman estimation of the acid dissociation constant of 4-carbamylpyridinyl radical. Excitation at 420 nm, 1 μs after electron pulse. The spectrum (− −) represents the strongest Raman band of the radical cation at pH 0.5 and (- - -) of neutral radical at pH 11. Both signals are scaled for the radiation yield, to represent equal concentration of the two species, and combined to give a composite spectrum (—). Raman spectrum at pH 1.9 (···) matches well with the composite spectrum.

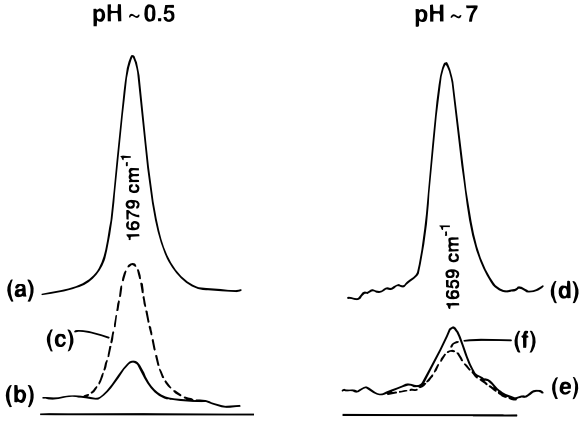

Figure 7. Comparison of the relative yields of 4-carbamylpyridinyl radicals by electron addition to isonicotinamide followed by protonation, and by H radical reaction. Raman signal (420 nm, 1 μs) of radical cation (pH ~0.5) produced by (a) electron transfer from acetone ketyl radical and (b) H reaction. (c) Expected Raman signal on electron transfer, if the radiation yield of acetone ketyl radical were equal to that of H atom. Raman signal (400 nm, 1 μs) of neutral radical (pH ~7) produced by (d) reaction of hydrated electron and (e) by H radical. (f) Expected Raman signal on electron addition, if the radiation yield of solvated electron were equal to that of H yield (see text).

spectrum, and pH at which they matched was taken as the pK_a of the cation form of the 4-carbamylpyridinyl radical. The spectrum drawn by a solid line in Figure 6 represents the composite spectrum. The Raman spectrum obtained at pH 1.9, shown in Figure 6 by a dotted line, is identical to this spectrum. The pK_a of the radical cation determined by the Raman method is not different from the reported value.[3]

(2) Reduction by H• Atom. Reactions 1 and 2 or 5 and 6 taken together show that electron transfer to isonicotinamide is followed by rapid protonation and the net result is hydrogen atom addition. Then the question arises if sequential e_{aq}^- and H^+ reactions are equivalent to direct reaction of hydrogen atom in this system. Since e_{aq}^- reacts with protons at a very high rate to convert into H• atoms, formation of the pyridinyl radicals by the reaction of H• with isonicotinamide can be accurately determined in moderately strong acidic solutions by Raman spectroscopy. The Raman spectrum of the radical cation was monitored at 420 nm, slightly off the absorption peak, to minimize attenuation of the probe laser and the scattered radiation by transient absorption. The pH of solution was kept at ~0.5. When 1 M *i*-PrOH was added to the N_2O-saturated solution containing 6 mM isonicotinamide to scavenge H• and •OH radicals (reactions 3 and 4), the 1679-cm^{-1} band of the radical cation, observed at 1 μs, appeared very prominently (Figure 7a). In this case reduction is by $(CH_3)_2C^{\bullet}OH$ radical (G = 5.1, 85% of the •OH and H• yields), and is believed to be quantitative. This signal is compared in Figure 7b with the signal observed on irradiation of the solution in the absence of *i*-PrOH, keeping other experimental conditions identical. In the latter case reduction is by H• atoms (G = 3.2), as the signal intensity does not change on adding *t*-BuOH in solution to remove •OH radical. The signal intensity expected if the $(CH_3)_2C^{\bullet}OH$ radical yield were equal to that of H• atoms is shown by a dotted line in Figure 7c. By comparison of the peak intensities in Figures 7b and 7c, it can be readily determined that the radical cation yield in H• reaction is only 29% of the total yield of the H• atoms. We conclude that about 29% of the H• atoms add on the oxygen position in N-protonated isonicotinamide to produce the 4-carbamylpyridinyl radical cation, and the remaining ~71% add to various ring carbon sites. The latter species very likely absorb below 350 nm and do not contribute to the resonance Raman scattering at the excitation wavelengths used in the experiment.

Reduction of isonicotinamide by e_{aq}^- and H• in neutral and mildly basic solutions was monitored at 400 nm. In this chemical condition an accurate determination of the pyridinyl radical yield by the reaction of H• atom is difficult for several reasons. The radiation yield of H• in water is only ~10% (G = 0.6) of the total radical yield. Its relatively lower reaction rate constant (6×10^8 M^{-1} s^{-1}) requires a high concentration of isonicotinamide in solution to ensure that the desired reaction occurs. On the other hand, the isonicotinamide concentration should be low for complete conversion of e_{aq}^- into •OH (scavenged by *t*-BuOH), by the chemical procedure of saturating solution with N_2O (reaction rate ~2.5×10^8 s^{-1}). Otherwise, a fraction of e_{aq}^- will react with isonicotinamide also, and the pyridinyl radical formed cannot be attributed to the H• reaction only. The 1659-cm^{-1} Raman signal of the neutral 4-carbamylpyridinyl radical, observed 2 μs after pulse irradiation of a N_2-saturated solution containing 1 mM isonicotinamide and 0.5 M *t*-BuOH at pH ~7 (buffered), is shown in Figure 7d. Here, reduction is by e_{aq}^- (G = 2.6) and H• (G = 0.6). The signal observed when the same solution was saturated with N_2O is given in Figure 7e. Figure 7f represents the signal expected by H• reduction, if all of H• coverted into pyridinyl radical (0.19 × signal intensity in Figure 7d). It can be seen that the 1659-cm^{-1} signal intensity in Figure 7e is 27% of the signal intensity in Figure 7d. Even if reduction by H• produced only pyridinyl radical, the signal intensity should not be more than 19% (Figure 7f). The additional signal is obviously due to a small fraction of unscavenged e_{aq}^- reacting with isonicotinamide. This fraction can be readily estimated to be ~10% from the rates of reaction of e_{aq}^- with ~ 26 mM N_2O (rate constant ~ 10^{10} M^{-1} s^{-1}) and 1 mM isonicotinamide (rate constant ~ 2.7×10^{10} M^{-1} s^{-1}) in solution. Thus, the pyridinyl radical yield by H• (G = 0.6) reduction is ~17% of the yield by $e_{aq}^- + H^{\bullet}$ (G = 2.6 + 0.6 = 3.2) reduction. This indicates that ~90% of the H• atoms add at the nitrogen site of the ring to produce neutral 4-carbamylpyridinyl radical. In view of the various uncertainties, we do not assign quantitative significance to this estimation. However, it is certain that a high percentage of H• atoms add

to the ring-N site in the reduction of isonicotinamide in neutral and basic solutions.

(3) Search for the Anion Form of the 4-Carbamylpyridinyl Radical. Transient Raman spectroscopy, in principle, can be applied in a straightforward manner to investigate if the 4-carbamylpyridinyl radical deprotonates in highly basic solution, and if it does, whether it is the amide or the ring N−H proton that is lost. A 380-nm Raman spectrum of the transient formed at 1 μs by e_{aq}^- reaction with 1 mM isonicotinamide and 1 M KOH in N_2O-saturated solution was obtained. The frequencies and relative intensities of the bands observed at 1648 (vs), 1198 (w), 1142 (m), 1004 (m), and 827 (m) match, within experimental error, with the bands at 1648 (vs), 1199 (w), 1141 (m), 1004 (m), and 826 (w) of the anion form of the 4-carboxypyridinyl radical.[20] A close similarity between the resonance Raman spectra of the pyridinyl radicals formed by loss of proton from the amide and carboxylic groups is expected, and one may interpret this resemblance to the observation of the anion form of the 4-carbamylpyridinyl radical deprotonated at the amide site. However, it is very unlikely that the frequencies and relative intensities will be the same for all the observed bands. At this point we believe that at pH >13 isonicotinamide is hydrolyzed to produce isonicotinic acid which is responsible for the observed transient Raman spectrum. The rate constant for the e_{aq}^- reaction with isonicotinamide at pH ∼14 was found to be 1.7×10^{10} M^{-1} s^{-1}, which is lower than the rate constant measured in mildly basic solutions (2.7×10^{10} M^{-1} s^{-1}). It is comparable, however, with the rate constant measured for the reaction of e_{aq}^- with isonicotinic acid in basic solutions (1.6×10^{10} M^{-1} s^{-1}),[20] which supports the vibrational spectroscopic conclusion.

Radical Structure and Proton Reactivity

(1) Theoretical Models for the Radical Structures. For a molecular description of the reactive properties of a chemical intermediate, it is essential to develop an appropriate model for its electronic structure. Experimental data can be used to construct resonance structures which provide considerable physical insight into the nature of the bonds and their implications to the chemistry.[34] For a numerical description of the bond properties, the commonly used quantum mechanical calculations for medium-size closed-shell molecules can be applied to the free radical state.[35] However, the theoretical model of the radical structure must conform with the experimental conclusions, in order to represent an acceptable approximation of the actual molecular geometry.[36] This requires a structural interpretation of the experimental results independent of the theory. In a recent study, using purely spectroscopic arguments to relate the resonance Raman frequencies with the bond properties, and then by their comparison with the theoretical results, it was established that the simple *ab initio* procedures used here give a reasonably good description of the structure, bonding, and vibrational modes of pyridinyl radicals.[20] Therefore, consistency with the experimental Raman frequencies and ESR proton hyperfine constants should be sufficient to justify the theoretical structural models for the 4-carbamylpyridinyl radicals.

Calculations were also performed on the parent isonicotinamide. A number of papers on X-ray diffraction studies of the crystal and molecular structures of isonicotinamide derivatives and its isomers,[8−12] e.g., picolinamide (2-pyridine amide) and nicotinamide (3-pyridine amide),[8,9] are reported in the literaure, but information on the unsubstituted compound could not be found. Since the bond properties of the pyridine amides show very little variation in the two isomers, we briefly compare the calculated bond lengths of isonicotinamide with the experimental bond lengths in its isomers. The calculated geometry of isonicotinamide is nonplanar, the amide plane making an angle of 22° with the pyridine plane. In picolinamide this angle is 19°, and in nicotinamide it is 25°. The calculated ring−amide CC bond length of 1.49 Å in isonicotinamide is equal to this bond length measured in its isomers. The N_1C_2 and N_1C_6 (1.33 Å), C_2C_3 and C_5C_6 (1.38 Å), and C_3C_4 and C_4C_5 (1.385 Å) bonds in isonicotinamide are all typical of the pyridine amides.[8−11] The frequencies of the molecule, calculated by multiplying the *ab initio* frequencies by an empirical constant of 0.89, are compared with the experimental frequencies[17] in Table 3, and the agreement is fairly good. This, of course, does not ensure that this theoretical procedure should work equally well for the open-shell pyridinyl radicals.

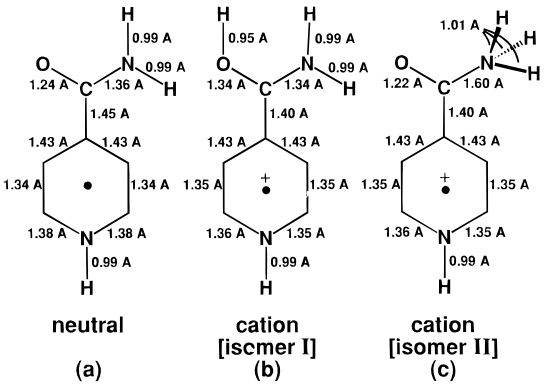

Figure 8. Calculated bond lengths in 4-carbamylpyridinyl radicals: (a) neutral radical, (b) radical cation, amide O-protonated; (c) radical cation, amide N-protonated.

The calculated equilibrium molecular geometries of the 4-carbamylpyridinyl radicals are given in Figure 8. Calculations were performed on two forms of the radical cation, one with the proton attached to the amide oxygen (structure **I**) and the other with the proton attached to the amide nitrogen (structure **II**). The molecular geometries are generally planar, with the exception of the isomer **II** of the radical cation where nonplanarity is indicated in the N^+H_3 group, as expected. The calculated C(2)C(3) and C(5)C(6) bond lengths of ∼1.34 Å in the neutral radicals and ∼1.35 Å in both forms of the radical cation are significantly shorter than the 1.40 Å in pyridine and the 1.38 Å in pyridine amides, and very close to the typical C═C bond lengths of ∼1.33 Å.[34] The CO bond length of 1.235 Å in the neutral radical is intermediate between that in carboxylic acids (∼1.21 Å) and the carboxylate anions (∼1.26 Å).[34] The CC bonding between the pyridinyl ring and the amide group has significant double bond character in the neutral radical. In both isomers of the radical cation, this bond is relatively shorter than in the neutral radical species.

The theoretical ring structures in isomers **I** and **II** of the radical cation are almost indistinguishable. A significant difference occurs only in the amide bonds. In isomer **I**, the CO bond is significantly longer (by ∼0.1 Å) than in the neutral radical, but in isomer **II** it is slightly shorter (by ∼0.02 Å). The ring−amide CC bonds in both cation forms have considerable double bond character. The calculation shows that isomer **I** should be more stable (by ∼0.9 eV) than isomer **II** in the gas phase. However, this energy difference cannot be taken too

(34) Pauling, L. *The Nature of the Chemical Bond*; Cornell University Press: Ithaca, NY, 1960.

(35) See, for example: Chipman, D. M.; Prebenda, M. F. *J. Phys. Chem.* **1987**, *90*, 5557. Schuler, R. H.; Tripathi, G. N. R.; Prebenda, M. F.; Chipman, D. M. *J. Phys. Chem.* **1983**, *87*, 3101. Tripathi, G. N. R.; Chipman, D. M.; Miderski, C. H.; Davis, H. F.; Fessenden, R. W.; Schuler, R. H. *J. Phys.Chem.* **1986**, *90*, 3968. Tripathi, G. N. R.; Sun, Q.; Armstrong, D. A.; Chipman, D. A.; Schuler, R. H. *J. Phys. Chem.* **1992**, *96*, 5344.

(36) Tripathi, G. N. R.; Schuler, R. H. *J. Chem. Soc., Faraday Trans.* **1993**, *89*, 4177−4180.

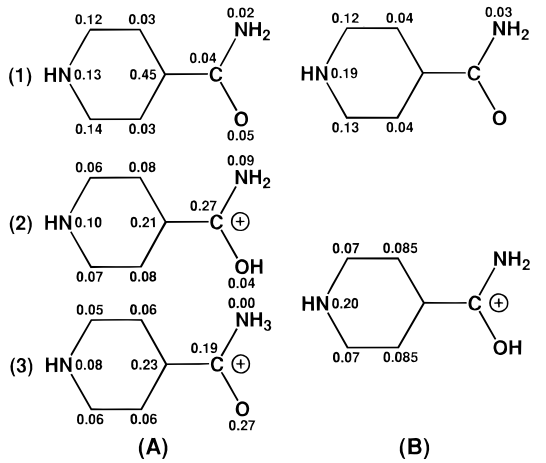

Figure 9. Unpaired π-electron distribution in 4-carbamylpyridinyl radicals: (A) theoretical and (B) ESR-derived; (1) neutral radical, (2) radical cation, amide O-protonated, and (3) radical cation, amide N-protonated.

seriously to predict the relative abundance of the two isomers at room temperature in aqueous solution.

It would be pertinent at this point to give the rationale behind the two possible structural forms for the radical cation. The $-NH_2$ group is intrinsically far more basic than $-O$, and if the two are isolated, it is the NH_2 group that will protonate first. The various amino acids are typical examples where zwitterion structures, such as $H_3N^+-R-COO^-$, are more stable than the low polarity structures, $H_2N-R-COOH$, in aqueous solution.[34,37,38] In amides, the resonance structures, $^-O-C=N^+H_2$, as manifest in the vibrational spectra and crystal structures, put significant amount of formal negative charge on oxygen and positive charge on nitrogen. Therefore, in 100% sulfuric acid solutions proton attachment occurs mostly on oxygen.[22] A minute fraction of protonation on nitrogen has also been detected in some systems.[22] This suggests that the free energy difference between the two isomers in solution is probably small (e.g., $\sim$0.2 eV for the population ratio 100:1). Interestingly, the nitrogen-protonated isomer is held responsible for the slow hydroxylation of the amides in concentrated aqueous acid solutions.[22] In the radical state, where charge distribution in the amide group is likely to be different, one cannot assume, without experimental evidence, that the relative abundance of the two isomers in aqueous solution should be similar to that in the precursor amides. The combination and overtone bands in the resonance Raman spectra of the 4-carbamylpyridinyl radical cation provide conclusive evidence of the predominance of only one species which can be either isomer **I** or **II**.

Figure 9 presents the calculated spin populations in the radicals. The calculated charge distribution in the radicals is given in Figure 10. Here, charge on each hydrogen atom has been absorbed on the adjacent heavy atom. A serious drawback of the Mulliken population analysis, used in these calculations, is the prediction of the excess charge on hydrogen atoms due to the delocalization of pπ electrons. Therefore, the numerical values lack absolute quantitative significance.

(2) Spectroscopic Justification for the Model Structures. Qualitative models for the radical structures can be conceived

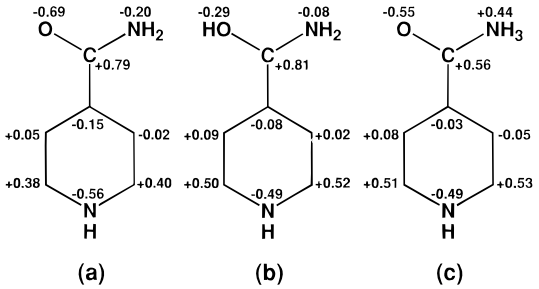

Figure 10. Theoretical charge distribution in 4-carbamylpyridinyl radicals: (a) neutral radical, (b) radical cation, amide O-protonated, and (c) radical cation, amide N-protonated. The charge on hydrogen atoms has been absorbed in the adjacent heavy atoms.

from the vibrational frequencies and intensity profiles in the resonance Raman spectra. It will be shown here that the theoretical models discussed above for the 4-carbamylpyridinyl radicals are consistent with the structural conclusions one can draw from the Raman spectroscopic data. The isoelectronic 4-carboxypyridinyl radicals, for which the resonance Raman spectra have been previously interpreted using standard spectroscopic arguments, provide valuable reference systems (see Table 2). The most intense band in the Raman spectra of the 4-carbamylpyridinyl radicals in the 1660$-$1680-cm^{-1} region represents the Wilson mode 8a which involves in-phase stretching motions of the C(2)C(3) and C(5)C(6) bonds, coupled with other stretching and bending motions. The frequency of this mode is unusually high. A large upward shift in the 8a frequency is possible due to strong coupling with a vibration of comparable frequency, but the two coupled modes should be enhanced together in the Raman spectrum. There is no evidence of any such vibration from the Raman spectra in Figures 4 and 5. Therefore, a high frequency for the 8a mode must be attributed mainly to the bond strengths. The uncoupled C(2)C(3) and C(5)C(6) stretching frequencies are probably in the range of $\sim$1650 cm^{-1}, representing bond lengths nearly equal to that of a double bond ($\sim$1.33 Å). The theoretical structures given in Figure 8 are consistent with this spectroscopic interpretation for the nature of the central CC bonds of the pyridinyl rings.

The C$=$O stretch at 1608 cm^{-1} is one of the vibrations which weakly couple with the 8a vibration in 4-carboxypyridinyl radical. In the corresponding amide radical, the C$=$O mode is not observed probably due to a much lower frequency which should reduce coupling with 8a. Consistent with this explanation, the 8a frequency is 9 cm^{-1} lower in the neutral amide radical (1659 cm^{-1}) than in the acid radical (1668 cm^{-1}). On the other hand, the 8a frequency, 1679 cm^{-1}, is slightly ($\sim$3 cm^{-1}) higher in the cation form of the amide radical than in the corresponding acid radical, as coupling with the CO stretch becomes less significant in both systems. The lack of enhancement of the CO stretching mode in 4-carbamylpyridinyl radical indicates, although indirectly, that the bond is much weaker than in the acid radical, and the theoretically estimated bond length of 1.235 Å appears reasonable. In the neutral 4-carboxypyridinyl radical this bond length is estimated as 1.22 Å.[20]

Comparison of the resonance Raman spectra of the 4-carbamylpyridinyl radicals with those of carboxylic acid radicals leads to the assignment of the medium intensity band at 1476 cm^{-1} in the neutral state to a mode containing ring-CONH$_2$ stretch (Wilson mode 7a) mixed with ring (CC/CN) stretch and CH bend (Wilson mode 19a). This frequency is intermediate between that of a typical double (bond length 1.33 Å) and single (bond-length $\sim$1.5A) CC bond, indicating a partial double bond character for the bond, as represented by the calculated bond

(37) Dake, Y.; Rauk, A.; Armstrong, D. A. *J. Am. Chem. Soc.* **1995**, *117*, 1789.

(38) Corey, R. B.; Donohue, J. *J. Am. Chem. Soc.* **1950**, *72*, 2899. Corey, R. B.; Pauling, L. *Proc. R. Soc. London* **1953**, *B141*, 10. Garfinkel, D.; Edsall, J. T. *J. Am. Chem. Soc.* **1958**, *80*, 3807, 3818, 3823. Takeda, M.; Iavazzo, R. E. S.; Garfinkel, D.; Scheinberg, I. H.; Edsall, J. T. *J. Am. Chem. Soc.* **1958**, *80*, 2813. Ghazanfar, S. A. S.; Myers, D. V.; Edsall, J. T. *J. Am. Chem. Soc.* **1964**, *86*, 3439 and references cited therein.

length of 1.45 Å. The comparison also places the 7a and 19a frequencies in the radical cation in the 1500−1580-cm^{-1} range, which suggests that the ring−amide CC bond is significantly shorter in the radical cation than in the neutral radical, as in the model structures. Because of the similar ring stuctures in isomers **I** and **II** of the radical cation, one does not expect a drastic difference in the frequencies of the vibrational modes mainly confined to the ring, which are generally enhanced in the resonance Raman spectrum. An insight into the species observed can be gained only by a careful comparison of the resonance Raman spectra of the isoelectronic carbamyl- and carboxypyridinyl radicals.

The *experimental* spin population for the neutral 4-carbamylpyridinyl radical and its caton, evaluated from the ESR proton hyperfine constants measured in this work (Table 1) using the McConnell relationship,[39,40] as described in a previous paper,[20] are compared in Figure 9 with the predicted spin populations. It can be seen that the agreement is reasonable, particularly when the solvent effects have not been taken into consideration in the calculation. The calculation predicts the spin population on the C(2) and C(6) ring sites in the neutral radical to be small as compared to the spin population on the C(3) and C(5) sites, but of comparable magnitude in both isomers of the radical cation. These predictions are readily verified by the experimental hyperfine constants. It is clear that the various approximations involved in the theoretical procedure are fairly reasonable for these pyridinyl radicals, and the calculated structures can be taken as good representations of the molecular geometries.

(3) Protonation Site in the Cation Radical. Vibrational frequencies and intensity profiles in the resonance Raman spectra of the neutral 4-carbamyl- and 4-carboxypyridinyl radicals are qualitatively similar in H_2O and D_2O (Table 2). This suggests that the composition of the resonance enhanced modes in the radicals and their deuterated isotopomers is not drastically affected by replacing the NH_2 group by OH. Protonation on NH_2 should have only a minor effect on the overall vibrational features in the resonance Raman spectrum, as the positive charge will reside on the amide nitrogen. Replacing two stronger σ N−H bonds in the neutral radical with three weaker σ N−H bonds in the radical cation will result in a minor increase in the CO strength and a decrease in the CN strength (loss of resonance structure $^{-}O-C{=}N^{+}H_2$), with ring bonds remaining largely unchanged. This simple argument is readily supported by the theoretical structures in Figure 8. Therefore, the spectrum of the radical cation, with the proton attached to the nitrogen atom, should show a greater resemblance with the spectrum of the neutral 4-carboxypyridinyl radical than with its protonated state, which is not the case. On the other hand, proton addition on oxygen will drastically change the nature of the CO bond, as a σ O−H bond is formed at the expense of $\pi\pi$ bonding, leading to reorganization of the π electron distribution and making the spectrum quite distinct from that of the neutral radical. There is one-to-one correspondence between most of the Raman bands, in frequency as well as relative intensity, of the 4-carbamylpyridinyl and 4-carboxypyridinyl radical cations. The similarity is more evident in the D_2O solution spectra as the modes with N−D or O−D bending components do not effectively couple with the strongly enhanced higher frequency modes. The experimental Raman data thus suggest that the radical cation observed is the one with a proton on the oxygen atom (isomer **I**).

It is difficult to determine a structure for the protonated form of the 4-carbamylpyridinyl radical using the ESR parameters given in Table 1. There are no experimental data available on analogous radical cations to relate the proton hyperfine constants with the protonation site. However, the following arguments can be considered. Protonation at the amide nitrogen produces a saturated group, NH_3^+. One may be inclined to think that the ring hyperfine constants should not be too different from the neutral radical for such a form. We note, however, that the ring proton splittings in the reduced form of 4-acetylpyridine[7] (NH_3^+ in carbamylpyridinyl replaced by CH_3) are all of comparable size, about 2 G. The form protonated at oxygen can, however, be considered as a substituted benzyl radical.[4] Such a structure might have the ordering of the ring proton splittings reversed with the larger values for the protons adjacent (*ortho*) to the carbon substituent rather than for those *ortho* to the ring nitrogen. Calculations presented in Figure 9 give spin populations on the various atoms for the two protonated forms. The conclusion from these is that either of the two forms will have very similar ring proton splittings. The only other splitting of use is that of the amide nitrogen. This value is dependent on the spin populations on both the nitrogen and the adjacent carbon. The radical form with NH_3^+ has no significant spin population on the nitrogen and so the splitting is derived from that on the carbon. A radical with a similar situation is the glycine radical $^{+}H_3NC^{\bullet}HCO_2^{-}$ in crystals of glycine. The isotropic nitrogen splitting is 3.2 G for this radical with essentially unit spin population on the carbon.[41] The measured value is 2.15 G for the acid form of the 4-carbamylpyridinyl radical, which would suggest ∼67% of the spin population to reside on the amide carbon atom. This value is too large in comparison to the ∼19% spin population predicted by the calculation. The ESR-derived spin populations on the C(2), C(3), C(5), and C(6) atoms add up to ∼31% (Figure 9) which would leave negligible spin on the other atoms, including ring nitrogen. However, the large ring nitrogen (4.87 G) and adjacent proton (5.3 G) splittings indicate the spin population on the ring nitrogen to be quite significant. More importantly, a radical structure with the spin population mostly on the amide group is inconsistent with the substantial double bond character of the ring−amide bond indicated by the resonance Raman frequencies. The radical form protonated on oxygen has spin population on the nitrogen and can have a larger splitting. Thus, the ESR hyperfine constants are consistent with protonation on the oxygen, but not on the amide nitrogen atom. We conclude that the theoretical prediction of the amide N-protonated radical cation to be a higher energy isomer of the O-protonated radical cation is also valid in aqueous solution.

(4) Experimental and Calculated Modes and Frequencies. Table 3 compares the fundamental frequencies observed in resonance Raman with the calculated frequencies of the neutral 4-carbamylpyridinyl radical. Table 4 presents a comparison of the frequencies calculated for isomers **I** and **II** of the radical cation and its isotopomers and the experimental frequencies. The spectroscopic arguments for the assignment of the vibrational modes to the resonance Raman frequencies are similar to those discussed in the paper on isoelectronic 4-carboxypyridinyl radicals[20] and will not be repeated. Here, we will briefly discuss some minor differences in the two spectra in the low-frequency region. In the neutral amide radical, the weak band at 1108 cm^{-1} in the acid radical is replaced by two weak bands at 1049 and 1064 cm^{-1}. The isotope shifts are also very different. For example, the 1108-cm^{-1} (ring distortion + C−OH stretch) band in the acid radical shifts upward in D_2O,

(39) McConnell, H. M. *J. Chem. Phys*. **1956**, *24*, 764. McConnell, H. M.; Chesnut, D. B. *J. Chem. Phys*. **1958**, *28*, 107.

(40) Wertz, J. E.; Bolton, J. R. *Electron Spin Resonance: Elementary Theory and Practical Applications*; McGraw-Hill Inc.: New York, 1972.

(41) Collins, M. A.; Whiffen, D. H. *Mol. Phys.* **1966**, *10*, 317.

while the 1149- and 1064-cm^{-1} bands shift downward by large wavenumbers. This different behavior arises because of the mixing of the ring distortion with the NH_2 rocking motion, as suggested by the calculations, and the isotope shifs of 62 and 151 cm^{-1} agree well with the calculated shifts of 55 and 141 cm^{-1}, respectively. The 1064-cm^{-1} mode in the radical also has some NCO bending component which increases in the deuterated isotopomers, explaining the relative enhancement of the mode at 908 cm^{-1} which derives its intensity probably due to mixing with the ring mode 1008 cm^{-1}. It should be noted that the analogous COC bending vibration in the acid radical is also enhanced due to mixing with the prominent ring vibration in the 1010-cm^{-1} region. The NCO bending mode at 766 cm^{-1}, on the other hand, becomes more of a ring-bending mode on deuteration of the radical, making the corresponding band in D_2O relatively weaker.

In the radical cation spectrum, identified with isomer **I**, there are three bands in the 1450−1600-cm^{-1} region instead of the two in the acid radical. However, in D_2O solution one of these bands disappears, suggesting that it represents a mode involving either amide or the ring N−H bending mode. The frequency is too low to be assigned to the NH_2 scissor mode,[42] calculated at 1645 cm^{-1}. Therefore, its assignment to a mode containing the ring N−H bend is more likely. The ring modes 8b and 19b, expected in this region, involve a N−H bending component. Calculation predicts the 19b mode at 1589 cm^{-1} with a large N−H planar bending amplitude. Since the experimental mode falls in close proximity of the 7a and 19a modes, it borrows their character and gets enhanced. The experimental 1161-cm^{-1} mode corresponds to the 1154-cm^{-1} mode of the neutral radical, involving NH_2 rocking motion. However, the 1059-cm^{-1} mode of the neutral radical has no counterpart in the cation spectrum, as it is predicted to have litttle NCO bending character in the latter, and is not enhanced.

Because of the low symmetry of the radical structures, the number of Franck−Condon allowed Raman transitions is too large in comparison to the resonance enhanced modes in the spectra. Therefore, it is impossible to associate the observed cation species with the O- or N-protonated radical, based on comparison between the calculated and experimental frequencies. It can be seen (Table 4) that the experimental frequencies show almost the same degree of agreement with the calculated frequencies of the N-protonated species (isomer **II**), only the mode assignments are different. This is an illustration of a fairly common situation in which it is possible to find a calculated frequency in close proximity of the resonance Raman frequency, irrespective of the molecular structure used in computation. Therefore, independent spectroscopic interpretation of the resonance Raman spectrum, based on comparison with structurally and isotopically related species, is often essential, in order to examine the validity of a model structure.

(5) Acid−Base Properties of the Amide Group. The anomalous acid−base properties of the amide group bonded to the semireduced state of pyridine ring can be satisfactorily explained by structural models discussed above. The carbamylpyridinyl radicals protonate in mildly acidic solutions, in contrast to closed-shell amides in which protonation is observed in extremely acidic aqueous solutions.[22] The pK_as of the conjugate acids of these radicals are in the same range as those for some aliphatic and aromatic carboxylic acids. What is peculiar about the carbamylpyridinyl radicals is that they are neutral. The aliphatic or aromatic carboxalate anions, on the other hand, are endowed with negative charge and their capability to bind with the proton can be readily understood. It is clear that the carbamylpyridinyl radicals have highly polar structures, with significant negative charge on the amide group and an equal amount of positive charge on the ring.

Let us examine the relationship between the amount of charge on the oxygen atom in a molecule and its effects on the acid−base equilibria.[43] In a previous study, by comparison of the pK_as of aliphatic alcohols and caboxylic acids, it was argued that a difference of unit electronic charge on oxygen should roughly amount to a change of 20 units in the pK_a.[20] For this qualitative discussion, using numerical values only as an illustration, the statistical factor of 2 in the equilibrium constant due to two oxygen positions available for protonation in carboxalate anions can be ignored. The pK_a (1.9) of the conjugate acid of the 4-carbamylpyridinyl radical suggests about 35% of the electronic charge on the oxygen atom, and that of the 3-carbamylpyridinyl radical (p$K_a \sim 1$) about ∼30%. There is a close relationship between the fraction of the electronic charge on the oxygen atom and the nature of the CO bond in a molecule. For example, the C═O bonds in carboxylic acids (∼1.21 Å) become significantly longer in carboxylate anions (∼1.26 Å). In aliphatic alcohols, the C−OH bond is often close to 1.41 Å in length.[34] The CO bond in the structural model for the neutral 4-carbamylpyridinyl radical has a length of l.235 Å, which is longer than that in a typical carboxylic acid or amide indicating a significant amount of negative charge on the amide oxygen and, therefore, protonation at much higher pH. On the other hand, this bond is shorter than that in a typical carboxylate anion, suggesting that pK_a of the conjugate acid of the amide radical should be lower than the pK_a of acetic acid, as observed. The calculated charge distribution in 4-carbamylpyridinyl radical presented in Figure 10 also supports a polar structure with excess negative charge on the amide group, but these estimations are of little quantitative significance, and a discussion in terms of the bond distances is more appropriate. We believe, the CO bond lengths in the actual molecular geometries of the hydrated carbamylpyridinyl radicals are probably closer to 1.26 Å (between 1.245 to 1.25 Å), to which the calculated bond length of 1.235 Å is a reasonable approximation.

The carbamylpyridinyl radicals protonate in milder acidic solutions than the carboxypyridinyl radicals. This can be readily explained by comparison of the CO bond lengths in the model structures of the para isomers of the two radical systems. The C═O bonds are generally slightly shorter in carboxylic acids than in corresponding amides.[34] This is because of the resonance ionic structures, mentioned earlier, being more important in amides than in acids. The difference in the bond lengths is generally of the order of ∼0.01 Å or less. The C═O bond length in neutral 4-carbamylpyridinyl radical, l.235 Å, is longer than that in 4-carboxypyridinyl radical, 1.22 Å, calculated by the same *ab initio* procedure.[20] From the arguments given in the preceding paragraph, the neutral amide radicals can be predicted to exist in the protonated form at much higher pH than the acid radicals, which is supported by the observations. The hydropyridine carboxylic acid radicals protonate at $H_0 \sim 0$, and the amide radicals at pH between 1 and 2.

(6) Acid−Base Properties of the Ring N−H Proton. Structural explanation for the extreme reactivity of the electron adducts of nitrogen−heterocyclic aromatic molecules toward water, discussed previously in the case of isonicotinic acid, can be readily extended to the pyridine amides. Here, we point out some important differences in the two radical systems based

(42) Tripathi, G. N. R. *J. Chem. Phys.* **1980**, *81*, 113. Tripathi, G. N. R.; Schuler, R. H. *J. Phys. Chem.* **1984**, *88*, 1706. Sun, Q.; Tripathi, G. N. R.; Schuler, R. H. *J. Phys. Chem.* **1990**, *94*, 6273. Chipman, D. M.; Sun, Q.; Tripathi, G. N. R. *J. Chem. Phys.* **1992**, *97*, 8073.

(43) Bell, R. P. *The Proton in Chemistry*; Cornell University Press: Ithaca, NY, 1959.

on the structural models for their reduced radical states. From the preceding discussion, a longer CO bond in amide radicals implies greater positive charge on the ring in comparison to the acid radicals. This positive charge differential should further increase with deprotonation of the carboxylic proton in acid radicals at pH >6. Since most of this charge is expected on the nitrogen atom, even if only qualitative significance is assigned to the charge distribution in Figure 10, the ring N−H proton should depart more readily than the amide proton. Also, the pK_a should be relatively lower than in acid radicals. Therefore, observation of the deprotonated form of pyridinyl radical in highly basic solutions is more likely in amide systems than in acid systems. Unfortunately, hydrolysis of the parent compound prevents an experimental verification of this prediction in 4-carbamylpyridinyl radical. However, an earlier report[3] of deprotonation of 3-carbamylpyridinyl radical at pH >13 seems to support this point, although the species formed remains to be identified by a structure-sensitive method.

Conclusion

Physicochemical properties of the redox intermediates of isonicotinamide, prepared in aqueous solution by pulse radiolysis and laser photolysis, have been examined by time-resolved resonance Raman and ESR spectroscopies. The effect of protonation of the substituent group on the ESR parameters, studied in a pyridinyl radical for the first time, shows significantly enhanced spin population on the *meta* carbon positions to the ring nitrogen, and reduced spin population on the *ortho* carbon positions, with respect to those in the neutral radical. Vibrational frequencies and proton hyperfine constants of the intermediates provide experimental verification for their molecular stuctures obtained by simple *ab initio* calculations. These structures explain the experimentally observed exceptionally high proton reactivity of the amide group on reduction of the pyridine moiety bonded to it. Conclusive evidence for pyridinyl radical formation by H$^\bullet$ atom addition to the ring nitrogen in isonicotinamide, and to the amide oxygen in N-protonated isonicotinamide, has been presented. The yields of the pyridinyl radicals by direct H$^\bullet$ reaction were found to be lower than the yields observed on sequential additions of solvated electron and proton. A previous estimation of the proton dissociation constant for the conjugate acid of the 4-carbamylpyridinyl radical has been confirmed by Raman measurements. It has been concluded that the proton binds with the amide oxygen in carbamylpyridinyl radicals, as in closed-shell amides, and not with the nitrogen atom.

A prediction has been made that the carbamylpyridinyl radicals should deprotonate at the ring nitrogen and not the amide nitrogen, and deprotonation should occur at a pH lower than that for the corresponding hydropyridine carboxylic acid radicals. The reported observation of the absorption spectrum of the anion form of the pyridinyl radical derived from nicotinamide, but not from nicotinic acid, in strongly basic solutions conforms with this prediction. The instability of isonicotinamide at pH >13 precluded a study of the deprotonation of its pyridinyl radical.

Experimental and theoretical studies of reactive intermediates of chemical and biochemical interest are currently an important topic in chemistry. Major disagreements between the structural conclusions, whether reached on the basis of purely spectroscopic arguments or by calculations, are not uncommon. The problem lies with the interpretation of the spectral data which are often very limited. Therefore, it is important to establish the proposed structure by relating it to the chemistry. We prescribe the acid−base properties as a simple chemical test for the structural models of the short-lived radicals, as illustrated in this work. These properties can be measured, in most situations, in straightforward experiments using the photochemical, radiation-chemical, or electrochemical method of preparation of the radical in aqueous solution.

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