Time-Resolved Resonance Raman Spectra, Structure, and Proton Reactivity of 4-Carboxypyridinyl Radicals[†]

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Abstract: This paper presents the first molecular spectroscopic study of the structure, bonding, and vibrational modes of a short-lived pyridinyl radical, an important intermediate in a variety of chemical and biochemical reactions, using time-resolved resonance Raman spectroscopy and *ab initio* SCF molecular orbital calculations. A structural explanation has been provided for the proton reactivity, which plays a fundamental role in the aqueous chemistry of the electron adduct states of nitrogen-heterocyclic aromatic molecules. Three protonation forms of the radical derived from 4-pyridinecarboxylic (isonicotinic) acid have been examined as model systems. Theoretical calculations performed on the electron adduct of the isonicotinate anion show that most of the added charge goes to the ring nitrogen, which explains the rapid protonation of the species at this site in aqueous solution. The resulting pyridinyl radicals have very nearly a quinoid ring structure, as manifest in the unusually high Raman frequency for the ring stretching Wilson mode 8a. In the neutral 4-carboxypyridinyl radical, the frequency of the vibrational mode containing the C=O stretch is ~100 cm^{-1} lower than in isonicotinic acid, indicating some formal negative charge on the carboxylic group. This partially ionic structure explains the radical protonation at the carbonyl oxygen at moderately low pH ($H_0 \sim 0$), a chemical behavior which contrasts with that of the aromatic carboxylic acids. It also accounts for a significant increase in the $-CO_2H$ proton dissociation constant (pK_a 6.3) in the radical with respect to that in isonicotinic acid. This study illustrates the relationship between the vibrational structures and the acid−base properties of reactive intermediates, which are often quite different from those of their stable precursors.

Introduction

Pyridinyl radicals, because of their central role as redox intermediates in the biochemical reactions of enzyme-coupled nicotinamide adenine dinucleotide and its phosphate, are of considerable importance in biology.[1] They are formed by one-electron reduction of pyridine derivatives which include compounds of the vitamin B family, such as nicotinic acid and nicotinamide. The chemical properties and modes of reaction of these radicals have been studied extensively over the years.[1−10] It has been demonstrated previously by ESR studies that the electron addition to pyridines in aqueous solution is followed by fast protonation at the ring nitrogen position,[10] and subsequent reactions generally involve protonated pyridinyl radicals. This extreme proton reactivity is a fairly common property of the electron adduct species of nitrogen-heterocyclic aromatic systems. Heretofore, no molecular spectroscopic study of the vibrational modes and frequencies, bonding and structure of a model pyridinyl radical in solution has been reported. Structural information of this kind is essential for providing a molecular interpretation of the reactive properties of these important radical intermediates and for probing the site and strength of the chemical interactions in various chemical and biochemical environments.[11−13]

The reduced radical states of prototype pyridines are often highly reactive in aqueous media. Their spectroscopic observation requires time-resolved techniques applicable to dilute solutions, which have become available only in recent years.[14] However, their stable precursors, particularly the pyridine carboxylic acids and amides, have been the subjects of numerous vibrational spectroscopic studies, as model biochemical compounds, for several decades.[15−19] The relationships between

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(1) Land, E. J.; Swallow, A. J. *Biochim. Biophys. Acta* **1968**, *162*, 327. Carlson, B. W.; Miller, L. L. *J. Am. Chem. Soc.* **1983**, *105*, 7553 and references cited therein.

(2) Kosower, E. M.; Tuerstein, A.; Swallow, A. J. *J. Am. Chem. Soc.* **1973**, *95*, 6127. Kosower, E. M.; Land, E. J.; Swallow, A. J. *J. Am. Chem. Soc.* **1972**, *94*, 986. Kosower, E. M.; Poziomek, E. *J. Am. Chem. Soc.* **1964**, *86*, 5514. Schwarz, W. M.; Kosower, E. M.; Shain, I. *J. Am. Chem. Soc.* **1961**, *83*, 2013.

(3) Wang, G. W.; Hu, Z. B. *Electrochim. Acta* **1985**, *30*, 779. This paper presents a brief historical survey of various studies on electrochemical reduction of aqueous isonicotinic acid since 1940.

(4) Bruhlman, U.; Hayon, E. *J. Am. Chem. Soc.* **1974**, *96*, 6169.

(5) Shimura, M.; Espenson, J. H. *Inorg. Chem.* **1983**, *22*, 234.

(6) Ward, R. L. *J. Am. Chem. Soc.* **1961**, *83*, 3623.

(7) Powell, M. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1982**, *104*, 5834. Ohno, A.; Shio, T.; Yamamoto, H.; Oka, S. *J. Am. Chem. Soc.* **1981**, *103*, 2045.

(8) Grodkowski, J.; Neta, P.; Carlson B. W.; Miller, L. L. *J. Phys. Chem.* **1983**, *87*, 3135. Simic, M.; Ebert, E. *Int. J. Radiat. Phys. Chem.* **1971**, *3*, 259. Solar, S.; Solar, W.; Getoff, N.; Holcman, J.; Sehested, K. *Int. J. Radiat. Phys. Chem.* **1988**, *32*, 585.

(9) Solar, S.; Getoff, N.; Sehested, K.; Holcman, J. *Int. J. Radiat. Phys. Chem.* **1991**, *38*, 323. Neta, P.; Patterson, L. K. *J. Phys. Chem.* **1974**, *78*, 2211.

(10) Neta, P. *Radiat. Res.* **1972**, *52*, 471.

(11) *Biological Applications of Raman Spectroscopy*; Spiro, T. G., Ed.; John Wiley and Sons: New York, 1987, 1988; Vols. I, II, and III.

(12) *Spectroscopy of Biological Systems: Advances in Spectroscopy;* Clark, R. J. H., Hester, R. E., Eds.; John Wiley & Sons: New York, 1986; Vol. 13.

(13) Su, Y.; Tripathi, G. N. R. *J. Am. Chem. Soc.* **1994**, *116*, 4405.

(14) Tripathi, G. N. R.; In *Advances in Spectroscopy Vol. 18, Time-resolved Spectroscopy;* Clark, R. J. H, Hester, R. E., Eds.; John Wiley & Sons: New York, 1989; pp 157−218.

(15) Dollish, F. R.; Fateley, W. G.; Bentley, F. F. *Characteristic Raman Frequencies of Organic Compounds;* John Wiley and Sons: New York, 1974. References to 47 papers published between 1944 and 1970 on pyridine and its derivatives are given.

(16) Foglizzo, R.; Novak, A. *J. Chim. Phys.* **1969**, *66*, 1539. Spiner, E. *J. Chem. Soc.* **1960**, 1226; **1963**, 3870; *Aust. J. Chem.* **1967**, *20*, 1805.

(17) Patrick, D. N., II; Wilson, J. E.; Leroi, G. E. *Biochemistry* **1974**, *13*, 2813.

0002-7863/95/1517-5540$09.00/0

the molecular structures and vibrational energy states established by these studies are useful in visualizing the bond properties of their reaction intermediates from the observed vibrational features. Pyridine and its derivatives also exhibit tremendous enhancement of Raman scattering when adsorbed on metal surfaces. This enhancement, combined with the vibrational frequency shifts due to bonding with the metal, makes it possible to detect and distinguish the surface molecules from the bulk, providing a valuable spectroscopic tool for electrochemical investigation of surface catalysis. In several recent studies, 4-pyridinecarboxylic (isonicotinic) acid adsorbed on silver and copper films and electrodes has been probed by Raman spectroscopy, in order to provide a fundamental understanding of the surface-enhanced Raman scattering (SERS).[20,21] The SERS investigation of the redox chemistry of this molecule and other pyridines on metal surfaces is, therefore, potentially feasible, but it requires a prior knowledge of the vibrational frequencies of intermediates in solution, to be used as reference to ascertain the redox state,[22] its binding site and structure,[13] and their implications for the chemistry.

We present, in this paper, a time-resolved resonance Raman study of the pyridinyl radical produced on electron addition to isonicotinic acid in aqueous solution. Three protonation forms of the radical have been observed (see Figure 5 for chemical structures). *Ab initio* SCF molecular orbital calculations have been performed on the neutral, anion, and cation forms of the radical as an aid in vibrational assignments and for providing a detailed description of the molecular geometry, and charge and spin distributions. The acid–base properties of the carboxylic group and the ring N–H protons in pyridinyl radicals, which bear little correspondence with those in parent pyridinecarboxylic acids, have been explained in terms of their vibrational and electronic structures. The extreme reactivity of the electron adducts of the nitrogen-containing heterocyclic systems toward protons in water is one of the fundamental aspects of their chemistry, and the unprotonated intermediates have rarely been observed. This work provides a molecular understanding of this important chemical property, using isonicotinic acid as a model system.

Experimental and Theoretical Procedures

The one-electron reduction of isonicotinic acid (pK_a 1.75 and 4.86) in aqueous solution was achieved by pulse radiolysis.[8,9,23] Electron irradiation of oxygen-free water produces e_{aq}^- (2.6), hydroxyl radical (2.7), and H atom (0.6) (numbers in parentheses are *G* values, i.e., yields of radicals per 100 eV of energy absorbed)[24] on the ~100 ns time scale. In neutral and basic solutions, the $^{\bullet}OH$ radical was scavenged by tertiary butyl alcohol (*t*-BuOH), in order to produce free radicals by reaction of e_{aq}^- with isonicotinate anion (reactions 1 and 2). In acidic solutions, however, the e_{aq}^- reacts with H^+ ($k \sim 2.3 \times 10^{10}$ M^{-1} s^{-1}), to form H atoms.[24] Therefore, at low pH, the pyridinyl radicals were produced by electron transfer from acetone ketyl radical, $(CH_3)_2C^{\bullet}OH$, to isonicotinic acid (reactions 5–7) . The acetone ketyl radical was prepared by reaction of $^{\bullet}OH/H^{\bullet}$ radicals with isopropyl alcohol (*i*-PrOH) (reactions 3 and 4) in solutions saturated with N_2O, which converts e_{aq}^- into $^{\bullet}OH$ radical within a few nanoseconds. About 85–90% of the $^{\bullet}OH$ and $H^{\bullet}$ radicals produce acetone ketyl radical by reaction with *i*-PrOH.[24] Electron transfer from $(CH_3)_2C^{\bullet}OH$ to isonicotinic acid occurs only when the ring nitrogen is protonated (pK_a 4.86).[8,9]

(basic solutions)

$$4\,CO_2^-C_5H_4N + e_{aq}^- \rightarrow 4\,CO_2^-C_5H_4N^{\bullet -} \qquad (1)$$

$$4\,CO_2^-C_5H_4N^{\bullet -} + H_2O \rightarrow 4\,CO_2^-C_5H_4NH^{\bullet} + OH^- \qquad (2)$$

(acidic solutions)

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

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

$$4\,CO_2^-C_5H_4N^+H + (CH_3)_2C^{\bullet}OH \rightarrow 4\,CO_2HC_5H_4NH^{\bullet} + CH_3COCH_3 \qquad (5)$$

$$4\,CO_2HC_5H_4N^+H + (CH_3)_2C^{\bullet}OH \rightarrow 4\,CO_2HC_5H_4NH^{\bullet} + CH_3COCH_3 + H^+ \qquad (6)$$

$$4\,CO_2HC_5H_4NH^{\bullet} + H^+ \rightarrow 4\,C(OH)_2^+C_5H_4NH^{\bullet}\ (pH < 0) \qquad (7)$$

The optical absorption[25] and resonance Raman methods[14,26] applied here to study transient chemical species produced by pulse radiolysis in solution have been described in detail in several previous publications from this laboratory. Radiolysis by ~8 MeV, ~5 ns electron pulses from a linear accelerator, which produced a radical concentration of ~3 × 10^{-6} M per pulse, was used for optical absorption measurements. In Raman experiments, ~2 MeV, ~100 ns electron pulses, delivered by a van de Graaff accelerator, at a dose rate sufficient to produce ~3 × 10^{-4} M radical concentration per pulse, were applied. The Raman scattering was probed by an excimer laser (~100 mJ) pumped dye laser (~10 ns), tuned in resonance with the optical absorption of the radicals. The spectra were recorded by using an optical multichannel analyzer (OMA) accompanied with an intensified gated (~20 ns) diode array detector, with the gate pulse synchronized with the Raman signal pulse. Extensive signal averaging was performed to improve S/N ratio in the Raman spectra, with the accelerator and laser operated at a repetition rate of 7.5 Hz. In both experiments, a flow system was used to refresh solution between consecutive electron pulses. 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.

The *ab initio* molecular orbital calculations were carried out with the GAUSSIAN 92 computer program.[27] Electronic wave functions were first computed by the unrestricted Hartree–Fock (UHF) method using the standard split-valence 4-31G basis set, but this procedure displayed serious spin contamination and was abandoned in favor of

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(18) Spiner, E. *J. Phys. Chem.* **1988**, *92*, 3379. Prasad, R.; Dube, N. *Indian J. Pure Appl Phys.* **1987**, *25*, 178.

(19) Albert, N.; Badger, R. M. *J. Chem. Phys.* **1958**, *5*, 21. Shindo, H. *Chem. Farm. Bull. (Tokyo)* **1957**, *5*, 472. Yoshida, S.; Asai, M. *Chem. Farm. Bull. (Tokyo)* **1959**, *7*, 162. Yoshida, S. *Chem. Farm. Bull. (Tokyo)* **1963**, *11*, 628. Azizov, M. A.; Rashkes, Ya. V.; Kats, A. L. *Z. Struk. Khim.* **1967**, *8*, 262. Afifi, M. S.; Sabana, A. A. *Analusis* **1982**, *10*, 239. Osborne, B. G. *Analyst* **1987**, *112*, 313.

(20) Chen, C. Y.; Davoli, I.; Ritchie, S.; Burnstein, E. *Surf. Sci.* **1980**, *101*, 363. Dornhaus, R.; Benner, R. E.; Chang, R. K.; Chabay, H. *Surf. Sci.* **1980**, *101*, 367.

(21) Noda, L. K.; Sala, O. *J. Mol. Struct.* **1987**, *162*, 11.

(22) See, for example: Xu, J.; Birke, R. L.; Lombardi, J. R. *J. Am. Chem. Soc.* **1987**, *109*, 5645.

(23) Anbar, M.; Hart, E. J. *J. Am. Chem. Soc.* **1964**, *86*, 5633.

(24) Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513.

(25) Patterson, L. K.; Lilie, J. *Int. J. Radiat. Phys. Chem.* **1974**, *6*, 129. Janata, E.; Schuler, R. H. *J. Phys. Chem.* **1982**, *86*, 2078.

(26) Tripathi, G. N. R. In *Multichannel Image Detectors II*; Talmi, Y., Ed.; ACS Symposium Series 236; (American Chemical Society: Washington, DC, 1983; p 171.

(27) Frisch, M. J.; Trucks, G. W.; Head-Gordon, M.; Gill, P. M. W.; Wong, M. W.; Foresman, J. B.; Johnson, B. G.; Schlegel, H. B.; Robb, M. A.; Replogle, E. S.; Gomperts, R.; Andres, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. *GAUSSIAN 92* Revision C; (Gaussian, Inc. Pittsburgh, 1992).

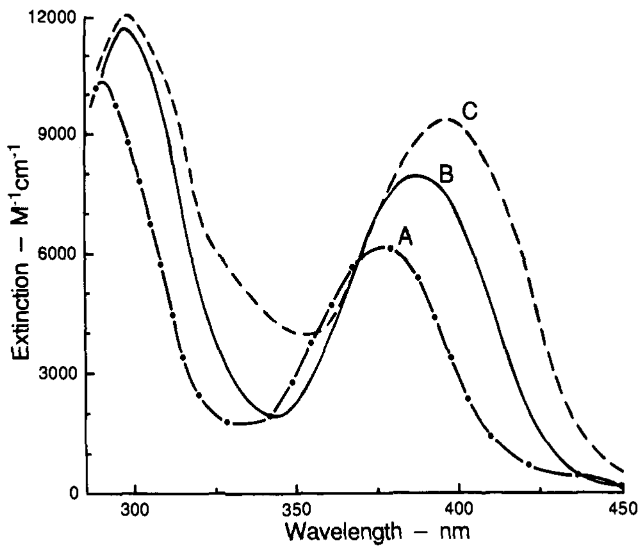

Figure 1. Absorption spectra observed 1–5 μs after electron pulse irradiation of oxygen-free aqueous solutions containing 0.5 M 2-propanol and 0.5–1.0 mM isonicotinic acid: (A) N_2-saturated, pH ~11; (B) N_2O-saturated, pH ~3.2; (C) N_2O-saturated, 2.5 M H_2SO_4 in solution ($H_0 \sim -1$). These spectra are attributed to 4-carboxypyridinyl anion (A), neutral (B), and cation (C) radicals.

the spin-restricted open shell Hartree–Fock method (ROHF).[27–29] The equilibrium geometries and harmonic force fields were obtained with methods based on analytical evaluation of the gradients of the total electronic energy. The combined errors from basis set incompleteness, neglect of electron correlation, and vibrational anharmonicity lead to a slight overestimation of the vibrational frequencies. Therefore, the *ab initio* frequencies computed for the isolated molecules were multiplied by an empirical scaling factor, which is a common practice,[28–30] before being compared with the experimental Raman frequencies in aqueous solution.

Transient Absorption

Figure 1A shows the optical absorption spectrum measured ~5 μs after electron pulse irradiation of a N_2-saturated aqueous solution containing 0.5 mM isonicotinic acid and 0.5 M *i*-PrOH at pH 11. When the solution was saturated with N_2O, this absorption disappeared, showing that the transient is formed by reaction of e_{aq}^- (reactions 1 and 2), and not of $(CH_3)_2C^{\bullet}OH$. An identical spectrum was obtained on replacing *i*-PrOH in solution by *t*-BuOH. The rate constant for the e_{aq}^- reaction with isonicotinate anion (reaction 1) was measured as 1.5×10^{10} M^{-1} s^{-1}, by monitoring the decay of the e_{aq}^- absorption at ~700 nm. The absorption spectrum displayed in Figure 1A, which has been scaled to account for the radiation yield (*G* value of e_{aq}^-), is similar to the spectrum of the species reported recently,[9] with an absorption maximum (λ_{max}) at 375–380 nm and extinction coefficient (ϵ_{max}) 6.1×10^3 M^{-1} cm^{-1}. The spectrum obtained in mildly acidic solution (pH ~3), with the solution saturated with N_2O, is given in Figure 1B. This spectrum is shifted to the red by ~10 nm with respect to the spectrum in Figure 1A, as also noted in earlier studies. Here, the transient is formed by electron transfer from $(CH_3)_2C^{\bullet}OH$ to N-protonated isonicotinate (pK_a 4.86). The reaction rate constant was measured as 9×10^8 M^{-1} s^{-1}. The ϵ_{max} at ~390 nm in Figure 1B, 7.8×10^3 M^{-1} cm^{-1}, is ~15% higher than its recent literature value. This discrepancy is due to the use of different radiation yields. Under the experimental conditions used, only ~85% of the primary radicals produced on radiolysis of water convert to $(CH_3)_2C^{\bullet}OH$,[24] which was not taken into consideration in earlier estimations. The minor radicals (14.5%) produced on reaction of $H^{\bullet}$ and $^{\bullet}OH$ with *i*-PrOH do not transfer electrons to isonicotinic acid, as is evident from a comparison of the optical absorption measured in the reaction of e_{aq}^- with isonicotinic acid at pH ~3.2, using *t*-BuOH to scavenge $^{\bullet}OH$ radicals. The e_{aq}^- reacts with isonicotinic acid at this pH at a rate constant of 1.6×10^{10} M^{-1} s^{-1}, which is similar to the rate constant in basic solutions. The spectrum seen in highly acidic solutions, on radiolysis of a N_2O-saturated solution containing 1 mM isonicotinic acid, 0.5 M *i*-PrOH, and 2.5 M H_2SO_4 ($H_0 \sim -1$),[31] is shown in Figure 1C. In this highly acidic solution, a small fraction of $HSO_4^{\bullet}$ radical is also produced by the direct action of radiation, at the expense of the $^{\bullet}OH$ radical,[32] and abstracts hydrogen from *i*-PrOH to form $(CH_3)_2C^{\bullet}OH$. The e_{aq}^- converts to $H^{\bullet}$ radical, within the duration of the electron pulse, by the reaction with H^+. The yield of the $(CH_3)_2C^{\bullet}OH$ radical in 2.5 M H_2SO_4 solution was taken to be similar to that at pH 3.2. The rate constant for the reaction of $(CH_3)_2C^{\bullet}OH$ radical with isonicotinic acid at pH < 0 was found to be 2.8×10^9 M^{-1} s^{-1}. The transients formed in the acidic solutions (Figure 1B,C) are very short-lived, as compared to the transient in basic solution (Figure 1A), and disappear by second-order reactions, at the rate constants (2*k*) of $(1.5 \pm 0.5) \times 10^9$ M^{-1} s^{-1}. The proton dissociation constant (pK_a) for the transient characterized by the absorption in Figure 1C has been determined previously[9] as −0.17 (H_0 scale) and that in Figure 1B as 6.3. The absorption in basic solution (Figure 1A) remained unchanged as the pH of the solution was raised to 14.

Resonance Raman Spectra

Raman scattering from chemical transients formed on pulse radiolytic reduction of isonicotinic acid in different chemical conditions was probed at several wavelengths between 375 and 400 nm to establish identification of the species and to confirm the chemistry. The initial radical concentration in Raman experiments being ~100 times higher than in optical absorption studies, it was often necessary to use a substrate concentration of 2–5 mM in solution to ensure that the reducing species, such as e_{aq}^- and acetone ketyl radicals, mainly react with isonicotinic acid and not with themselves. The chemical conditions were otherwise similar to those used in optical absorption studies. Initially, Raman spectra were recorded in the 1300–1800 cm^{-1} region, at several time intervals between 100 ns and 10 ms after electron pulse irradiation of solution and also without irradiation. The Raman signals which appeared in the 100 ns spectrum and decayed at later times were attributed to the transients formed on radiolytic reduction of aqueous isonicotinic acid. Using solvent bands as reference, spectra taken in the presence of the electron pulse under a particular set of chemical conditions were appropriately scaled to account for the attenuation of the probe laser beam and Raman signals by transient absorption. From these spectra the 10 ms spectrum or the spectrum of unirradiated solution was digitally subtracted, to obtain the transient Raman spectra. With the excitation wavelength fixed at 390 nm, the

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(28) Binkley, J. S.; Pople, J. A.; Hehre, W. J. *J. Am. Chem. Soc.* **1980**, *102*, 939. Gordon, M. S.; Binkley, J. S.; Pople, J. A.; Pietro, W. J.; Hehre, W. J. *J. Am. Chem. Soc.* **1982**, *104*, 2797. Pietro, W. J.; Francl, M. M.; Hehre, W. J; Defrees, D. J.; Pople, J. A.; Binkley, J. S. *J. Am. Chem. Soc.* **1982**, *104*, 5039. Pople, J. A.; Scott, A. P.; Wong, M. W.; Radom, L. *Isr. J. Chem.* **1993**, *33*, 345–350.

(29) Pople, J. A. Schlegel, H. B.; Krishnan, R.; Defrees, D. J.; Binkley, J. S.; Frisch, M. J.; Whiteside, R. A.; Hout, R. F.; Hehre,W. J. *Int. J. Quantum. Chem* **1981**, *S15*, 269.

(30) Pulay, P.; Fogarasi, G.; Pongor, G.; Boggs, J. E.; Vargha, A. *J. Am. Chem. Soc.* **1983**, *105*, 7037–7047.

(31) Lesigne, B.; Ferradini, C.; Pucheautt, J. *J Phys. Chem.* **1973**, *77*, 2156.

(32) Rochester, C. H. *Acidity Functions*, Academic Press: New York, 1970.

transient spectrum observed 1 μs after electron irradiation of N_2-saturated 2 mM aqueous solution of isonicotinic acid at pH ~ 13.5 was similar in intensity to the spectrum taken after adding 1 M *t*-BtOH in solution to scavenge OH radicals, showing that the OH adducts of isonicotinic acid do not react with the electron adducts at a high rate. When this solution was saturated with N_2O to convert e_{aq}^- into OH, the signal intensity was drastically reduced, confirming that the transient signals are due to a species formed by electron addition to isonicotinic acid in its ionized form (pK_a 4.86). When 0.5 M *i*-PrOH, instead of *t*-BuOH, was added to the N_2O-saturated solution, the signal intensity almost doubled, indicating that electron transfer from the acetone ketyl radical to isonicotinic acid was taking place. When the pH of the latter solution was decreased to ~11, the signal intensity fell drastically, showing that the acetone ketyl radical, in its un-ionized form (pK_a ~13.2), does not reduce isonicotinate anion. On further reduction of pH below 4, stronger Raman signals at shifted frequencies appeared, as protonation of isonicotinate at its nitrogen site made reduction possible. The spectral pattern changed gradually with addition of H_2SO_4 to solution at 1 M and higher concentrations. With 2.5 M H_2SO_4 in solution, the spectrum obtained was completely distinct from that in mildly acidic solutions.

Examination of these exploratory spectra did not reveal any signals attributable to transient or stable products evolving at microsecond or longer time scales. While the transient Raman spectra observed in basic solutions persisted even on the millisecond time scale, the spectra in acidic solutions completely disappeared in 100 μs. In basic solutions, maximum enhancement in Raman scattering was seen when excitation approached 375 nm, and in acidic solutions between 390 and 395 nm. The excitation wavelengths, however, have only a minor effect on the intensity profiles in the spectra. The chemical compositions of the irradiated solutions, decay kinetics of the signals, and excitation-dependence of the spectral intensities clearly indicate the Raman observation of three protonation forms of the reduced radical state of isonicotinic acid, associated with optical absorption spectra in Figure 1. The ESR spectra recorded previously on electron irradiation of similar solutions[10] identify the species with N-protonated pyridinyl radicals.

Figure 2 depicts the 1 μs Raman spectra (500–2300 cm^{-1}) of the 4-carboxypyridinyl anion, neutral, and cation radicals in H_2O solutions, using 380, 390, and 395 nm excitations, respectively. These difference spectra were obtained by averaging short- and long-time spectral data, each for ~9600 pulses, in four overlapping spectral segments. The 1700–2300 cm^{-1} region shows combination frequencies, none of which involve fundamental vibrations below 700 cm^{-1}. Figures 3 and 4 compare the Raman spectra of the neutral and cation radicals in H_2O and D_2O. The D_2O spectra were averaged over only ~2400 pulses. The shifts in the vibrational frequencies in D_2O indicate the presence of exchangeable protons in the radicals.

The fundamental frequencies of the anion radical are given in Table 1, and those of the neutral and cation radicals in Table 2.

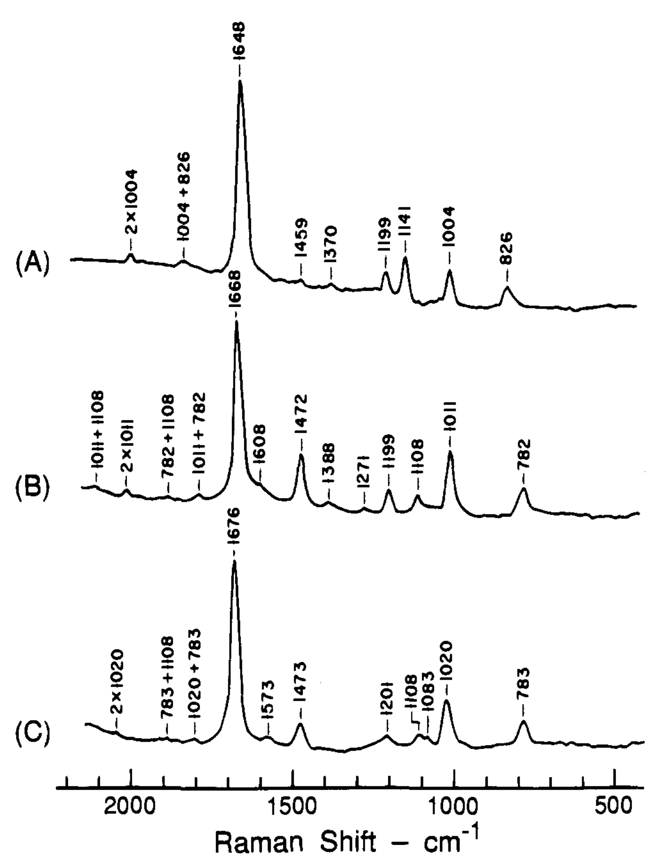

Figure 2. Resonance Raman spectra of 4-carboxypyridinyl radicals in aqueous solution. (A) anion radical, 380 nm excitation; (B) neutral radical, 390 nm excitation; (C) cation radical, 395 nm excitation (see text).

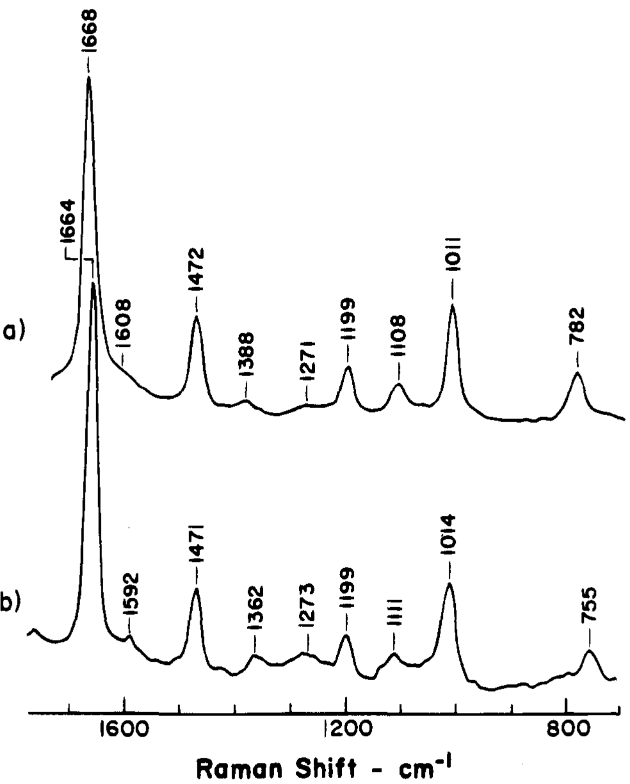

Figure 3. Resonance Raman spectra of neutral 4-carboxypyridinyl radical in (a) H_2O and (b) D_2O. Excitation at 390 nm.

Vibrational Structures

Unlike stable molecules, for which bond distances and bond angles are often known from X-ray, neutron, or electron diffraction studies and are used to ascertain the nature of the vibrational energy states, the main objective of the vibrational spectroscopy of reactive intermediates in solution is to provide an insight into their bond properties and chemical behavior. Therefore, a reliable interpretation of the Raman spectrum becomes extremely important. In principle, one can obtain molecular geometry by theoretical structure calculations, at a level of sophistication which reproduces observed frequencies. However, in a large-size radical with a number of vibrational

Table 1. Experimental and Calculated Fundamental Frequencies (cm^{-1}) of 4-Carboxypyridinyl Anion Radical

isonicotinic acid				4-carboxypyridinyl anion radical			
					calculated		
a	*b*	*c*	*d*	experimental *e*	*f*	*g*	assignment *h*
1691	1385	1320	1395	1370 vw	1377	1318	CO_2 symm stretch
1616	1645	1679	1610	1648 vs	1649	1578	8a (ring stretch)
1499	*i*	1536	*i*	1459 vw	1467	1404	19a (CH bend+ ring stretch)
1212	1205	1230	1220	1199 m-w	1232	1179	9a (CH bend)
1141	*i*	1171	*i*	1141 m	1163	1113	7a(C−CO_2 stretch + ring distort)
1027	1010	1024	1005	1004 m	990	947	1 (ring breathe)
823	850	805	850	826 m-w	801	766	CO_2 scissor + ring distort.

[a] Solid state frequencies of isonicotinic acid, ref 18. [b] Aqueous state frequencies of N-protonated isonicotinate anion, ref 21. [c] 0.93 x *ab initio* frequencies of N-protonated isonicotinate anion. [d] Aqueous state frequencies of N-deprotonated isonicotinate anion, ref 21. [e] This work. v = very, = strong, m = medium, w = weak. [f] *Ab initio* frequencies multiplied by scaling factor of 0.93. [g] *Ab initio* frequencies multiplied by scaling factor of 0.89. [h] Wilson notation for ring modes. [i] Not observed.

Table 2. Experimental and Calculated Fundamental Frequencies (cm^{-1}) of 4-Carboxypyridinyl Cation and Neutral Radicals

isonicotinic acid[a]	neutral radical[b]			cation radical[b]			assignment[e]
	exp(H_2O)	exp(D_2O)	calculated[c]	exp(H_2O)	exp(D_2O)	calculated[d]	
1730	1608 vw,sh	1592 vw	1630 1622				C═O stretch
1645	1668 vs	1664 vs	1663 1578	1676 vs	1669 vs	1669 1663	8a
(1499)	1472 s	1471 s	1452 1459	1573 w	1552 m	1570 1554	7a + 19a
	1388 vw	1362 vw	1355 1331	1473 m	1471w	1460 1448	19a + 7a
(1317)	1271 vw	1272 vw	1261 1258	*f*	*f*	1288 1237	14
1205	1199 m	1199 m	1202 1199	1201 w	1200 w	1217 1217	9a
(1058)	1108 w	1111 w	1064 1084	1108 w	1121 w	1002 1016	18a/12 + C−O stretch
1010	1011 s	1014 s	976 974	1020 s	1022 s	946 950	1
810	782 w	755 w	730 704	783 w	762 w	743 729	CO_2 scissor

[a] Aqueous state frequencies of N-protonated acid, ref 21. Solid state frequencies of N-deprotonated acid in parentheses, ref 18. [b] This work. v = very, s = strong, m = medium, w = weak. [c] *Ab initio* frequencies of 4-$CO_2HC_5H_4NH$ (column 4) and 4-$CO_2DC_5H_4ND$ (column 5), multiplied by 0.89. [d] *Ab initio* frequencies of 4-$C(OH)_2^+C_5H_4NH$ (column 8) and 4-$C(OD)_2^+C_5H_4ND$ (column 9) multiplied by 0.89. [e] Wilson notation for ring modes. [f] Not observed.

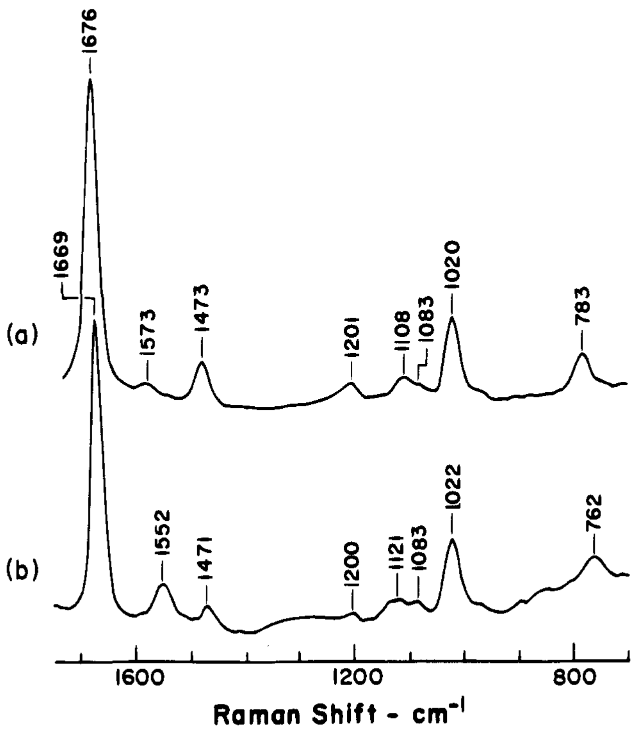

Figure 4. Resonance Raman spectra of neutral 4-carboxypyridinyl cation radical in (a) H_2O and (b) D_2O. Excitation at 395 nm.

modes, only a few vibrations are observably resonance-enhanced in Raman. It is often possible to find a calculated frequency in close proximity to the experimental frequency, irrespective of the radical structure used in computation.[33] Therefore, a more dependable approach is to use, first, the experimental arguments to assign the observed vibrational frequencies to approximate modes and to develop a qualitative picture of the radical structure on that basis. The theoretical results, if consistent with the experimental conclusions, can then be accepted for a quantitative description of the molecular structure.

The molecular point group symmetry and the nature of the resonant electronic transition allow us to sort out the vibrational modes which are likely to be prominent in a resonance Raman spectrum. Assuming a planar geometry with two equivalent CO bonds, the 4-carboxypyridinyl anion radical can be ascribed to the C_{2v} point group. The strongly allowed electronic transitions in the visible region in aromatic radicals invariably involve excitation of pπ electrons, which precludes enhancement of the vibrational modes confined to σ bonds only. With these considerations, vibrations which are likely to be prominent in the resonance Raman spectrum of the 4-carboxypyridinyl anion radical should relate to the pyridine modes 8a (CC stretch), 19a (CC stretch + CH bend), 7a (C−CO_2 stretch), 9a (CH bend), 18a (CH bend + ring distortion), 1 (ring breathe), 12 (trigonal bend), and 6a (ring distortion), using Wilson notation, and to carboxyl modes CO_2 symmetric stretch and scissor (bend).[15−18] These are Franck−Condon-allowed totally symmetric (a_1) vibrations. Pyridine and carboxylic group vibrations will generally couple, the coupling being more pronounced when the frequencies are comparable. This coupling should lead to shared intensities and some shifts from the zero-order frequencies. A slight departure from the C_{2v} symmetry may enhance additional bands in the spectrum. However, such bands are expected to be weak. The C_{2v} symmetry approximation can also be applied to interpret the resonance Raman spectrum of the 4-carboxypyridinyl cation radical. The neutral radical, however, must belong to C_s or a lower symmetry, and, in this case, careful correlation with the resonance Raman spectra of

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

the anion and cation forms of the radical is the only practical way for making vibrational assignments. Comparison of the Raman spectra of 4-carboxypyridinyl cation and neutral radicals, recorded in H_2O and D_2O solutions (Figures 3 and 4), is particularly useful in identifying the vibrational modes with significant contributions from the carboxylic group vibrations. Some modes, which acquire totally symmetric character due to the loss of the C_{2v} symmetry and involve N−H bending motion, may also show frequency shifts in D_2O. However, such modes should be considerably weaker in the cation radical, where structural distortion of the ring is expected to be much less pronounced than in the neutral radical.

The most prominent band in the resonance Raman spectra of 4-carboxypyridinyl radicals (Figures 2−4) appears in the frequency range 1648−1676 cm^{-1}. In the anion and cation forms of the radical the Wilson 8a mode, which involves a major contribution from the stretching motion of the central CC bonds, is the only vibration of a_1 symmetry which can occur in this frequency range and also be strongly resonance-enhanced. In the neutral radical, however, the carbonyl C=O stretch can also have a comparable frequency, and the two vibrations may couple. If this coupling is strong, two bands in the ~1600 cm^{-1} region, with shared intensities, should be enhanced. However, we observe one very intense band at 1668 cm^{-1} and a very weak shoulder band at ~1608 cm^{-1} in the 1600−1700 cm^{-1} region. In D_2O solution, the 1668 cm^{-1} band shifts downward by 4 cm^{-1}, while the ~1608 cm^{-1} band shifts by ~16 cm^{-1} (Figure 3). It is clear that coupling between the 8a and C=O stretching motions is small, and the 1668 cm^{-1} band in the neutral radical can be mainly attributed to the 8a vibration. In isonicotinic acid, the 8a frequency is reported at ~1620 cm^{-1}.[18] The unusually high frequency of the 8a vibration in the 4-carboxypyridinyl radicals, which is not interpretable in terms of the shifts due to couplings with the carboxylic group vibrations, must be attributed to a considerable increase in the central CC bond strengths. In the pyridine molecule, where the C(2)C(3) and C(5)C(6) bond lengths (~1.4 Å)[34] are slightly longer than in isonicotinic acid (~1.38 Å),[35] the 8a vibration is reported at 1583 cm^{-1}.[15] The Raman frequencies of the 8a vibration in the three 4-carboxypyridinyl radicals, observed in the 1648−1676 cm^{-1} region, indicate that the ring structures are nearly quinoid, with the central CC bonds considerably shorter than in isonicotinic acid, and probably very close in length to a double bond. The weakly enhanced ~1608 cm^{-1} band in the neutral radical very probably represents the vibrational mode containing the carbonyl C=O stretch. However, the Wilson mode 8b (CC/CN stretch + N−H bend),[15,16,18] which is Franck−Condon allowed in the C_s molecular symmetry but not in C_{2v}, can also have a frequency in this region, and a clear distinction cannot be made between the two possibilities with the experimental data in hand.

Identification of the vibrational modes involving CO_2 bending/scissor motions in 4-carboxypyridinyl radicals is straightforward. In isonicotinic acid, the most recent study reports this mode at 823 cm^{-1}.[18] There, it is believed to be mixed with the 6a ring distortion (CCC bend). The medium-weak intensity bands at 826 cm^{-1} in the anion radical, and at 782 and 783 cm^{-1} in the neutral and cation radicals, can be unambiguously assigned to this mode. In D_2O solution, the latter two frequencies shift downward to 755 and 762 cm^{-1}, respectively, which is the expected behavior. It is evident from the resonance Raman spectra in Figures 2−4 that the carboxyl scissor in these radicals does not derive its intensity by coupling with 6a, as the latter vibration, expected in the 400−700 cm^{-1} region, is not enhanced. From intensity considerations, the coupling partner is identified here as the ring vibration in the 1000−1020 cm^{-1} region.

The 1388 cm^{-1} mode of the neutral 4-carboxypyridinyl radical, which does not appear in the cation radical spectrum, also shifts downward to 1362 cm^{-1} in D_2O, suggesting an O−H or N−H bending component mixed with ring vibration. In contrast, the 1108 cm^{-1} band, observed in the neutral as well as in the cation radical, shifts upward by 3 and 13 cm^{-1}, respectively. The latter behavior is typical of the modes containing C−OH stretching and O−H bending coordinates.[15,18] The anomalous upward shifts in frequencies suggest unenhanced O−H bending modes in the ~1150 cm^{-1} region (the calculation discussed later predicts this mode in the 1170−1190 cm^{-1} range). The 1388 cm^{-1} mode in the neutral radical is not correlatable with the 1350 cm^{-1} CCO asymmetric stretching mode in isonicotinic acid, as the isotope shifts are in opposite directions,[18] and also not with the Wilson mode 14 (CC stretch), which is more appropriately assigned to a very weak band at 1271 cm^{-1}. Of the two other possibilities, i.e., 19a and 19b, the former is more likely to be observed if the ring is not too distorted from the C_{2v} symmetry. In several benzene-related radicals, we have observed a weakly enhanced 19a mode in the ~1400 cm^{-1} region, accompanied by a strongly enhanced 7a mode in the ~1500 cm^{-1} region.[14,36−38]

In isonicotinic acid, the 9a CH bending mode has been observed at ~1215 cm^{-1} (Tables 1 and 2), and there is no reason for the frequency of this mode to be drastically different in its radical states. We observe a weak band at ~1200 cm^{-1} in all three protonation forms of the 4-carboxypyridinyl radical which is attributable to this mode. The vibrational frequencies of the ring distortion modes below 1100 cm^{-1}, which seldom involve a predominant contribution from a particular bond, should also be comparable in the parent molecule and its radical state. The ring breathing/distortion mode 1 generally appears at ~1000 cm^{-1} in pyridine derivatives.[15,16,18] The prominent bands at 1004, 1011, and 1020 cm^{-1} in the anion, neutral, and cation radical spectra are assignable to this mode. This mode does not seem to couple effectively with the carboxylic group vibrations, as the frequencies remain virtually unchanged in D_2O.

The vibrational assignment of the remaining bands in the 1100−1600 cm^{-1} region is not so straightforward. This is the region where the 19a, 7a, and symmetric CO_2 stretching modes, which are helpful in visualizing some important aspects of the radical structures, are expected. In isonicotinic acid the frequency of the 19a vibration is reported at ~1500 cm^{-1}.[18] The symmetric CO_2 stretch in isonicotinate anion is observed at ~1390 cm^{-1},[21] and the C=O stretch in isonicotinic acid at ~1700 cm^{-1}.[18] The 7a vibration which represents the stretching motion of the ring−CO_2H bond appears at ~1140 cm^{-1}. However, these frequencies and mode compositions may change drastically in the radical state, and one-to-one correspondence with the parent molecule or among the three protonation forms of the radical may not exist. This point is clearly evident from the relative intensities of the bands in the spectral region in question (Figure 2). While a medium intensity band appears in the ~1470 cm^{-1} region in the cation and neutral radical spectra, no such band of comparable intensity can be found in the anion spectrum. On the other hand, a band at 1141 cm^{-1} is very prominent in the anion radical spectrum, but the Raman

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

(35) Takusagawa, F.; Shimada, A. *Acta Crystallogr.* **B1976**, *32*, 1925.

(36) Tripathi, G. N. R.; Schuler, R. H. *J. Chem. Phys.* **1984**, *81*, 113.

(37) Tripathi, G. N. R.; Schuler, R. H. *J. Phys. Chem.* **1988**, *92*, 5129.

(38) Chipman, D. M.; Liu, R.; Zhou, X.; Pulay, P. *J. Chem. Phys.* **1994**, *100*, 5023.

bands of comparable frequencies in the cation and neutral radical are very much weaker.

The symmetric CO_2 stretch and 7a mode (ring$-CO_2$ stretch) are two possible candidates for the assignment of the anion radical band at 1141 cm^{-1}, as the frequency is too low to be assigned to 19a. The symmetric CO_2 stretch can acquire a frequency as low as 1141 cm^{-1} only when the two CO bonds are very close to a single bond, i.e. when the added electronic charge gets confined on the oxygen atoms. This possibility can be readily discounted, as proton addition to $-CO_2^{2-}$ should have only minor effects on the bonds and a vibrational mode with the CO stretching character in the $\sim$1600 cm^{-1} region in the neutral radical will be hard to explain. The 1141 cm^{-1} band of the anion radical is, therefore, assignable to a mode containing ring$-CO_2$ stretch, as the 7a mode. The remaining very weak bands at 1459 and 1370 cm^{-1} can be asssigned to the 19a and CO_2 stretching modes, respectively. In the cation radical, the 1473 cm^{-1} band is too strong to be assigned to a predominantly 19a mode, as this vibration involves a large CH bending component, along with the ring stretch, and cannot be strongly resonance-enhanced by a $\pi\pi$ electronic transition. Its assignment to a mode with mixed 7a (ring$-C(OH)_2^+$) and 19a characters is more likely. It should be noted that the 1473 and 1573 cm^{-1} modes in the cation radical (Figure 4) represent two strongly coupled vibrations which share spectral intensity. This coupling increases as the frequency difference between the two modes decreases in D_2O, and a reversal of the relative intensities occurs (Figure 4). It is clear that the 1573 cm^{-1} mode also involves a ring$-C(OH)_2^+$ stretching coordinate, and this component becomes predominant on downward shift of frequency on deuteration. The frequency of the uncoupled stretching vibration is probably in the range of $\sim$1500 cm^{-1}, and the ring $-C(OH)_2^+$ bond is closer to a double bond. In the neutral radical, using similar arguments, the 1472 cm^{-1} mode can be associated with the stretching motion of the ring$-CO_2H$ bond (7a), with some 19a character. The other mode which results from the mixing of the 19a and 7a vibrations in the neutral radical is attributed to the 1388 cm^{-1} band.

In summary, the vibrational spectroscopic analysis of the resonance Raman spectra of the 4-carboxypyridinyl radicals indicates the central ring CC bonds to be very close to double bonds. In the anion form of the radical the resonance Raman frequencies are almost identical to the frequencies of the N-protonated isonicotinate anion (Table 1), which shows that the $\pi\pi$ bondings that undergo a major change on electronic excitation are very little affected in the radical, and the unpaired electron mostly remains on the ring. In N-protonated isonicotinic acid, however, a significant fraction of the added electron also goes to the antibonding $\pi\pi$ orbitals of the C=O bond, resulting in a reorganization of the bond structure. In particular, the ring$-CO_2H$ bond acquires a partial double-bond character, and the central ring CC bonds are strengthened, as reflected in a significant increase in the 7a and 8a frequencies of the neutral radical (Table 2). On protonation of the oxygen atom in the cation radical, the pπ electron on the CO carbon becomes free to compete for bonding with the adjacent ring pπ electron, thus greatly enhancing the double-bond character of the $C-C(OH)_2^+$ bond.

The effect of the proton on the vibrational modes and frequencies, Raman intensity profile, and structure in 4-carboxypyridinyl radicals provides standards by which chemical interactions in nonaqueous environments, such as proteins or metal surfaces, can be examined.[11,12] These interactions generally amount to partial removal or addition of protons to the radical system.

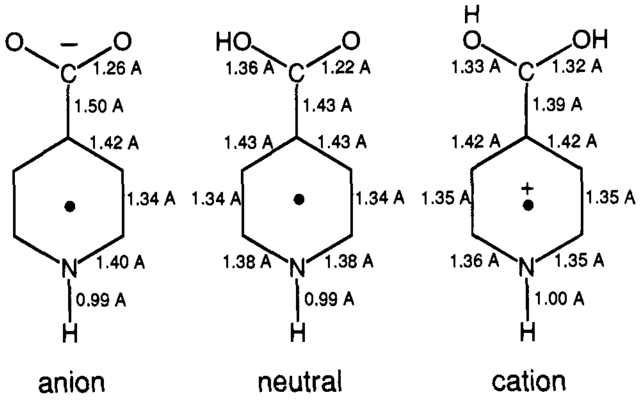

Figure 5. Calculated bond lengths in 4-carboxypyridinyl anion, neutral, and cation radicals.

Equilibrium Molecular Geometry

The calculated molecular geometries of the 4-carboxypyridinyl radicals were found to be planar. A variety of conformers, including twisting and tilting at the nitrogen and carboxylic hydrogens were used as starting points for the ground state geometry optimization, but these all led back to the same planar structure. The anion radical, observed experimentally in neutral and basic aqueous solutions, was found to have C_{2v} symmetry. The cation form of the radical, seen in strongly acidic solutions, shows only a slight asymmetry about the C_2 axis, which provides justification for the vibrational analysis discussed above using the C_{2v} point group approximation. The asymmetry becomes more pronounced in the case of the neutral radical, and here the molecular point group is undoubtedly C_s. The optimized ground state geometrical parameters for the 4-carboxypyridinyl anion, neutral, and cation radicals are shown in Figure 5.

Let us examine the theoretical structures in view of the experimental results. The calculated C(2)C(3) and C(5)C(6) bond lengths of $\sim$1.34 Å in the anion and neutral radicals and $\sim$1.35 Å in the cation radical are significantly shorter than 1.40 Å in pyridine and very close to the typical C=C bond lengths of $\sim$1.33 Å.[34] In this respect, the ring structures are nearly quinoid, as concluded previously from the Raman frequencies of the 8a modes. The CO bond lengths of 1.26 Å in the anion radical are comparable to those in the carboxylate anions,[34] as also indicated by the experimental CO_2 symmetric stretching frequency. In the neutral radical, the C=O bond (1.22 Å) is $\sim$0.01 Å longer than that in isonicotinic acid, which implies a relatively lower frequency of the stretching mode in the radical, as observed.

The theoretical calculations show that the nature of the CC bond which links the aromatic ring with the carboxyl group is markedly dependent on the protonation state of the radical. The bond length increases in the order cation (1.39 Å) < neutral (1.43 Å) < anion (1.50 Å) radical. In the anion radical, this CC bond is a single bond, comparable in strength to the corresponding bond in the isonicotinic acid. In the neutral radical, this bond acquires a significant double-bond character, and in the cation radical the bond length approaches that of a double bond. As noted earlier, the vibrational frequency of the 7a mode, which involves stretching motion of the carboxylic group with respect to the ring, occurs at 1141 cm^{-1} in the anion radical and in the 1470$-$1570 cm^{-1} region in the neutral and cation radicals, consistent with the calculated structures. The unusual effect of the substituent's protonation on its bonding with the ring is unique to the free radical state, and an analogue is difficult to find in closed shell systems.

It is clear from the above analysis that the theoretical structures furnished in Figure 5 provide fairly good descriptions

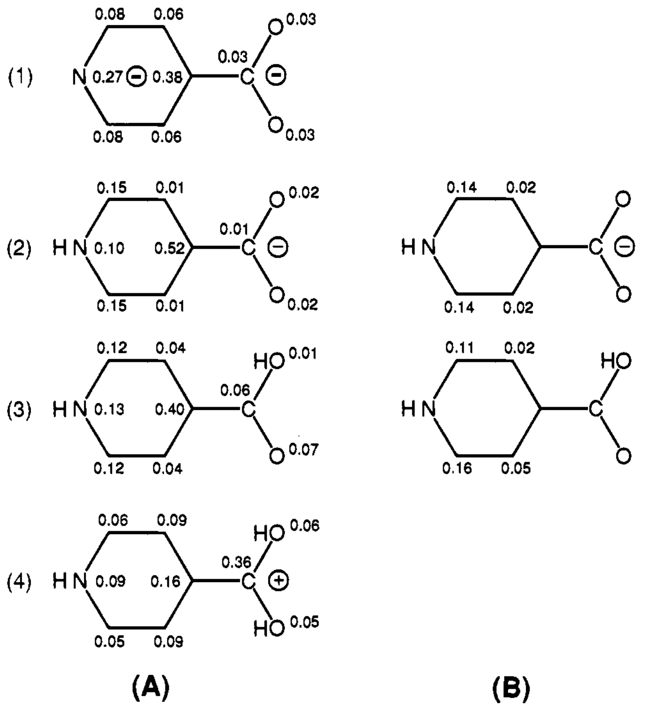

Figure 6. Unpaired electron distribution in 4-carboxypyridinyl radicals. (A) theoretical; (B) ESR-derived. (1) Electron adduct of isonicotinate anion. (2) 4-Carboxypyridinyl anion radical. (3) Neutral 4-carboxypyridinyl radical. (4) 4-Carboxypyridinyl cation radical.

of the bond properties of the 4-carboxypyridinyl radicals, as manifest in their resonance Raman spectra.

The ESR hyperfine constants provide a set of experimental data by which the theoretical prediction of the unpaired π electron distribution in a radical can be examined. Unfortunately, these data are available only for the anion and neutral forms of the 4-carboxypyridinyl radical and are not very extensive. Here, we use the McConnell relationship[39] to estimate the spin population on the ring atoms with the help of the experimental hyperfine constants of the adjacent hydrogens. The *experimental* spin distribution obtained this way can then be compared with the calculated unpaired π electron distribution using the ROHF procedure.[40] This is an indirect procedure, and it would be preferable to use the wave function to compute spin densities at the various nuclei and compare these directly to measured ESR coupling constants. However, such a calculation, to have predictive value, would require a larger basis set and a more complete wave function including electron correlation.[41]

According to the McConnell relationship, the spin population on the ring atom when multiplied by an appropriate constant gives the proton hyperfine constant on the hydrogen atom bonded to it. This constant was taken as $-27G$ for the C−H bond, as recommended in many ESR studies.[40] Figure 6 presents a comparison of the theoretical and *experimental* spin distributions in 4-carboxypyridinyl anion and neutral radicals. The ESR data were taken from ref 10, with the assumption that the reported proton hyperfine constants are all negative, as usually is the case in the related radicals.[40] It can be readily seen that the agreement between the spin populations derived from experimental results and those predicted by *ab initio* calculations is reasonable. Calculations predict significant spin population on the ring C(2) and C(6) sites, and very little on C(3) and C(5) sites, which finds experimental confirmation. It is interesting to note that the calculated spin distribution in the cation radical is quite distinct from that in the anion and neutral radicals. Unfortunately, there are no ESR data with which the theoretical predictions can be verified in this case. The consistency between the calculated unpaired electron distributions and the ESR parameters in 4-carboxypyridinyl anion and neutral radicals provides further indication of the adequacy of the theoretical approach adapted here.

Calculated Frequencies

Comparison between the observed and calculated vibrational frequencies is generally used for experimental verification of the equilibrium molecular geometry of the radical, although disagreements may frequently relate to the force field approximations. If agreement is good, a complete picture of the normal modes emerges which is not obtainable from the experimental data alone. Unfortunately, various approximations involved in frequency calculation by *ab initio* methods lead to an overestimate, and the calculated frequencies have to be scaled down before comparison is made with experimental frequencies. This is done either by applying a number of scale factors directly to the force constants, borrowed from a stable molecule with very similar bond properties,[29,38] or by simply multiplying the *ab initio* frequencies by a common scaling factor.[28,29,42,43] The chemical bonds in a free radical are often unusual, and stable molecules with similar bonds are difficult to find. Therefore, we prefer here the latter procedure. It should be emphasized, however, that the *uniform* scaling factor is essentially an adjustable empirical parameter which is used to bring *ab initio* frequencies closer to the observed frequencies. Therefore, it is the consistency between the theoretical and experimental sets of frequencies, and not the numerical agreement, that provides support for the theoretical structures and force fields. From the studies of a number of molecules, mostly closed shell systems, a scaling factor of 0.89 has been recommended for the *ab initio* method applied here.[28,29] We find this scaling factor quite reasonable for the neutral and cation forms of the 4-carboxypyridinyl radical, which will be discussed first. Tables 1 and 2 correlate the fundamental frequencies observed in resonance Raman with the calculated frequencies (0.89 × *ab initio* frequencies) for which the theoretical mode descriptions are consistent with the experimental mode assignments discussed earlier. The calculated frequencies given in Tables 1 and 2 are not necessarily the ones which are numerically closest to the observed frequencies.

In the 4-carboxypyridinyl cation radical ($-h_7$), the experimental frequencies, 1676, 1573, 1473, and 1201 cm^{-1}, in the 1200−1700 cm^{-1} region show excellent agreement with the calculated frequencies, 1669, 1570, 1460, and 1217 cm^{-1}, when a scaling factor of 0.89 is applied. The observed shifts in these frequencies in D_2O (7, 21, 2, and 0 cm^{-1}) also compare well with the calculated shifts (6, 16, 12 and 0 cm^{-1}, respectively) in the 4-carboxypyridinyl cation radical ($-h_4D_3$). The calculated frequencies of the lower frequency modes are, however, somewhat underestimated. It should be noted that the experimental ring breathing (Wilson mode 1) frequency 1020 cm^{-1} correlates with the calculated frequency 946 cm^{-1}, both showing a small upward shift in the deuterated radical, and not with the

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(39) McConnel, H. M. *J. Chem. Phys.* **1956**, *24*, 764. McConnel, 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) Chipman, D. M. *Theor. Chim. Acta* **1992**, *82*, 93.

(42) Chipman, D. M.; Prebenda, M. F. *J. Phys. Chem.* **1986**, *90*, 5557. Raymond, K. S.; Wheeler, R. A. *J. Chem. Soc., Faraday Trans.* **1993**, *89*, 665−670.

(43) Tripathi, G. N. R.; Sun, Q.; Armstrong, D. A.; Chipman, D. A.; Schuler, R. H. *J. Phys. Chem.* **1992**, *96*, 5344. Chipman, D. M.; Sun, Q.; Tripathi, G. N. R. *J. Chem. Phys.* **1992**, *97*, 8073.

calculated modes 1002 cm^{-1} (CH bend, 18a) or 1035 cm^{-1} (ring distortion, 12), which are closer in frequency. The upward shift of 13 cm^{-1} for the experimental 1108 cm^{-1} mode in D_2O is similar to the predicted isotopic shift of 12 cm^{-1} for the 18a mode, but much smaller than the calculated shift of 58 cm^{-1} for the trigonal mode 12, which appears not to be enhanced. The carboxyl scissor mode 783 cm^{-1}, which shifts downward by 21 cm^{-1} in D_2O solution, correlates well with the calculated mode 743 cm^{-1} with a predicted isotope shift of 14 cm^{-1}.

In the neutral 4-carboxypyridinyl radical, which is structurally more asymmetric than the cation radical, the general agreement between the experimental and calculated frequencies, isotope shifts, and mode descriptions is as good as in the cation radical, and the comparison given in Table 2 is self-explanatory. The calculation supports the experimental assignment of the weak shoulder band at ~1608 cm^{-1} to a mode constituting C=O stretch, calculated at 1630 cm^{-1}. The calculated 8b (ring stretch) mode at 1565 cm^{-1} does not relate to the experimental mode ~1608 cm^{-1}, as the calculated isotope shift of 60 cm^{-1} in the 4-carboxypyridinyl-h_5D_2 radical is too large, as compared to the observed shift of 21 cm^{-1} for the 1608 cm^{-1} mode. However, the calculated 1630 cm^{-1} C=O mode slightly overemphasizes the contribution of the ring stretching component, which is inconsistent with the very weak enhancement of this mode as compared to the 1668 cm^{-1} (predominantly 8a) mode in the resonance Raman spectrum. This argument is further supported by the experimental isotope shift, which is larger for the ~1608 cm^{-1} mode (16 cm^{-1}) than for the 8a mode (4 cm^{-1}). Since the theoretical modes involve nearly equal contributions, the predicted isotope shifts for the mixed 8a and C=O modes are also equal (8 cm^{-1}). It is likely that the C=O bond in the neutral radical is slightly (~0.01 Å) longer than the calculated bond length of 1.22 Å.

The frequencies calculated for the 4-carboxypyridinyl anion radical by multiplying the *ab initio* frequencies by a scaling factor of 0.89 are uniformly lower than the experimental frequencies, but a good numerical agreement is obtained by using a scaling factor of 0.93 (see Table 1). The frequencies of the totally symmetric modes are predicted in the order 8a > 19a > CO_2 stretch > 9a > 7a(+12) > 1 > CO_2 scissor, as observed. The calculation also suggests the C−CO_2 stretching coordinate to be distributed over several modes in the 950–1450 cm^{-1} region. Experimental isotope shifts, essential for making reliable correlation between the calculated and observed frequencies, are not available in this case, but this disadvantage is largely offset by the higher symmetry of the radical structure.

The comparisons made in Tables 1 and 2 show good agreement between the experimental and scaled *ab initio* frequencies of the 4-carboxypyridinyl radicals. It is clear that at the level of theory used in this work, the electronic and vibrational structures of the pyridinyl radicals can be satisfactorily described.

Chemical Implications of the Radical Structure

Proton Reactivity of the Electron Adducts. On addition of an electron, the nitrogen-heterocyclic aromatic molecules become extremely reactive toward water, and their initial chemical reaction usually involves proton addition on the ring nitrogen. It is the protonated species that participates in subsequent reactions in normal chemical conditions. The relationship between the vibrational structure and this fundamental chemical property will be discussed here by the example of the 4-carboxypyridinyl radicals.

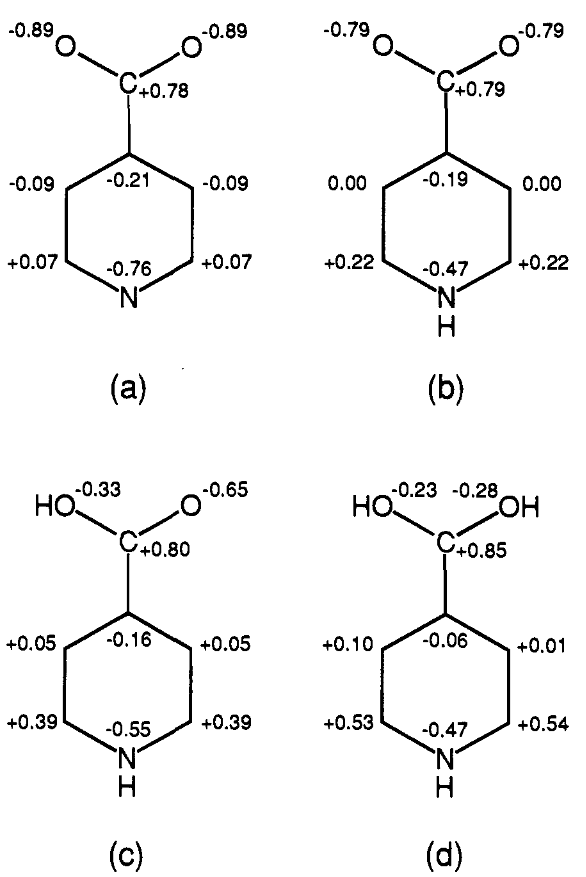

Figure 7. Theoretical charge distribution in (a) electron adduct of isonicotinate anion and (b) 4-carboxypyridinyl anion, (c) neutral, and (d) cation radicals. The charge on hydrogen atoms has been absorbed in the adjacent heavy atoms.

Raman observation of the electron adduct intermediate of isonicotinic acid, prior to its protonation, could not be made even at pH ~14, showing that the dissociation constant of the NH proton in the radical is at least 10 units higher than in the parent molecule. In kinetic terms, the electron adduct state of isonicotinate should react with water (see reaction 2) at subnanosecond or shorter time scale ($k > 10^{10}$ s^{-1}), assuming that the OH$^-$ reaction with 4-carboxypyridinyl anion radical occurs at a diffusion-controlled rate. Thus, the extreme ionic reactivity of a chemical species toward water is manifest in its high pK_a value. As shown in Table 1, the vibrational frequency of the CO_2 symmetric stretching mode in 4-carboxypyridinyl anion radical is only slightly (~20 cm^{-1}) lower than in N-protonated isonicotinate anion. This implies that the resonance structures corresponding to 4-$CO_2^-C_5H_4$−NH (structures A) are principal contributors to the molecular structure, and ionic resonance structures, 4-$CO_2^{2-}C_5H_4N^+H$ (structures B), are less important. The pK_a of the N−H proton (e.g., H_2N−H) is generally estimated to be ~25 units higher than that of the N$^+$−H proton (e.g., H_3N^+−H),[44] which suggests a pK_a close to 30 (using isonicotinic acid as reference) for structures A. The pK_a of structures B should be comparable to that in isonicotinate anion (i.e. ~5). The predominance of the resonance structures A, as reflected in the CO frequency, accounts for pK_a > 14 in 4-carboxypyridinyl anion radical and, in turn, explains the extreme reactivity of the electron adduct toward water.

A parallel argument can be made from the theoretical calculation of the charge distribution (Mulliken population analysis)[45] resulting from electron addition to isonicotinate anion, as shown in Figure 7. It can be seen that the total electronic charge on the carboxylate group is close to unity.

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

(45) Mulliken, R. S. *J. Chem. Phys.* **1955**, *23*, 1833.

Most of the added negative charge goes to the ring, a major fraction (~76%) of which is confined on the nitrogen atom, producing a high negative electrostatic field in its vicinity. Taking NH_3 as reference (protonation pK_a ~10, deprotonation pK_a ~35),[44] with total charge on N, the isonicotinate electron adduct, with ~76% of the charge, should be able to abstract H^+ from water even in most basic aqueous solutions. The charge distribution in the 4-carboxypyridinyl anion radical (Figure 7) conforms with the experimental conclusion that the resonance structures B are not important.

Acid–Base Properties of the Carboxyl Group. The charge distribution in 4-carboxypyridinyl radicals, as manifest in the vibrational frequencies of the carboxyl group, can also account for its acid–base properties which are quite distinct from those of isonicotinic acid. The frequency of the C=O stretch (1608 cm^{-1}) in neutral 4-carboxypyridinyl radical is significantly lower than in N-protonated isonicotinic acid (1730 cm^{-1}), suggesting substantial negative charge on the carboxyl group of the radical. For example, the CO frequency in *p*-benzoquinone at 1665 cm^{-1} [46] drops to 1435 cm^{-1} in *p*-benzosemiquinone anion radical,[47,48] with equal distribution of single electronic charge on two oxygen atoms,[34] and to ~1250 cm^{-1} in hydroquinone.[33,49] The downward shift in the CO frequency of the neutral 4-carboxypyridinyl radical, with respect to that of a typical C=O bond, is about 15–20% of the shift expected when bonding changes from C=O to $C-O^-$. Theoretical calculation estimates the excess electronic charge on the carboxyl group of the 4-carboxypyridinyl radical to be 18%. The pK_as of water and aliphatic alcohols, where almost all electronic charge resides on oxygen after deprotonation, are in the range 14–15 (OH proton is far more acidic than the NH proton).[44] In acetate and formate anions, where it is equally distributed between two oxygens, the pK_a drops to ~5.[34,44] Considering an electrostatic model for H^+ interaction with anions in acid–base equilibria,[44] a difference of 0.5 electronic charge on oxygen should amount to a change of about 10 units in pK_a. Therefore, the 18% of the excess charge on oxygen is roughly equivalent to an increase of ~4 units in the pK_a at which protonation should occur. Without attaching quantitative significance to these numerical values, used mainly for illustration, it can be safely stated that the experimental CO frequency and theoretical charge distribution in the neutral 4-carboxypyridinyl radical indicate that the radical should protonate in relatively milder acidic conditions than those required for stable carboxylic acids. Thus, the vibrational structure of the radical explains the experimental observation of the cation form of the 4-carboxypyridinyl radical below $H_0 \sim 0$. This, of course, is an interesting chemical property of the carboxyl group peculiar to the free radical state, as isonicotinic and many other carboxylic acids do not protonate at this site even in much stronger acids. The excess negative charge on the carboxylic group should also enhance the effective electrostatic attraction of the carboxyl proton in the neutral radical, which should result in an increase of its dissociation constant,[34,44] as observed.[9] A similar conclusion can be drawn from the symmetric CO_2 stretching frequency of the anion radical. The carboxyl proton in the 4-carboxypyridinyl radical dissociates at a pK_a of 6.3.[9] It should be noted that the C=O stretching frequency in the parent N-protonated isonicotinic acid is about 50–70 cm^{-1} higher than the typical C=O frequency in an aliphatic carboxylic acid.[15] This higher frequency correlates well with its relatively lower proton dissociation constant (pK_a 1.75).

Conclusion

This work illustrates the relationship between the vibrational spectra, structure, and proton reactivity of redox intermediates, with the example of 4-carboxypyridinyl radicals. Time-resolved resonance Raman spectroscopy, complemented by *ab initio* molecular orbital calculations, has led to a detailed description of the electronic structure and bond properties of these intermediates. It has been shown that a major fraction of the electronic charge added to the isonicotinic acid on its reduction goes to the ring nitrogen, and only a small fraction to the carboxylic group. As a result, the proton dissociation constants (pK_as) of the carboxylic group in the redox intermediate increase only by a few units, but the N–H dissociation constant goes up by more than 10 units.

The 4-carboxypyridinyl radicals serve as spectroscopic models for interpreting the resonance Raman spectra and structures of their relatively less symmetric isomers, such as 3-carboxypyridinyl radicals, related amides, and many other pyridine-derived radicals. Several of these radicals appear as redox intermediates in biochemical reactions and are ideal candidates for examining surface-catalyzed reactions by SERS spectroscopy. Measurements of the vibrational frequencies of the different protonation forms of the radical intermediates in solution and their reliable interpretation, as presented in this work, are essential for probing the site and strength of the chemical interactions in other environments, using Raman spectroscopy. In neutral 4-carboxypyridinyl radical, the binding of the carboxylic proton with proteins or metal surfaces should reflect in the shifts of the vibrational frequencies toward those of the cation radical, and binding at the oxygen site should show the shifts toward the frequencies of the anion radical. The NH–protein/metal binding, on the other hand, is more likely to manifest in the ring frequencies than the carboxyl group frequencies. This work thus lays the groundwork for the Raman investigation of the redox intermediates of pyridine derivatives in a variety of chemical and biochemical environments.

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(46) Becker, E. D. *J. Phys. Chem.* **1991,** *95,* 2818.

(47) Tripathi, G. N. R. *J. Chem. Phys.* **1981,** *74,* 6044. Tripathi, G. N. R.; Schuler, R. H. *J. Chem. Phys.* **1982,** *76,* 3139. Tripathi, G. N. R.; Sun, Q.; Schuler, R. H. *Chem. Phys. Lett.* **1989,** *156,* 51.

(48) Hester, R. E.; Williams, K. P. J. *J. Chem. Soc., Faraday Trans.* **1982,** *78,* 573. Beck, S. M.; Brus, L. E. *J. Am. Chem. Soc.* **1982,** *104,* 4789.

(49) Hidalgo, A.; Otero, C. *Spectrochim. Acta* **1960,** *16,* 528.