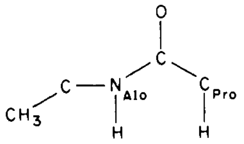
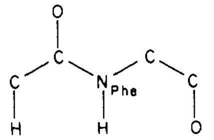
ppm/deg for H_{Phe}^{N}, a value considered to indicate solvent exposure, but only 0.0002 ppm/deg for H_{Ala}^{N}, a value usually taken to indicate shielding from the solvent. This distinction occurs in other peptides of the *cyclo*(Xxx-Pro-D-Ala)$_2$ series. The observed temperature coefficients (Xxx/D-Ala) for analogous peptides in which Phe is replaced by the indicated Xxx residue are as follows: Xxx = Ala, 0.003/0.006, Leu, 0.004/0.0027; Val, 0.005/0.0013 and Glu(O-*t*-Bu), 0.0046/0.0024. The distinction is absent, however, in methanol: Phe, 0.003/0.0029, and Leu, 0.0055/0.0065.[24] The temperature coefficients in dimethyl sulfoxide and the much larger upfield shift of the H_{Phe}^{N} resonance on shifting from dimethyl sulfoxide to sulfolane do suggest that H_{Phe}^{N} is more exposed to dimethyl sulfoxide than is H_{Ala}^{N}. A visible difference between them is that the Ala N–H group is flanked by H_{Pro}^{α} and the Ala β-CH_3 group,



while the Phe N–H group is flanked by H_{Ala}^{α} and the Phe carbonyl oxygen



Whether and how this difference explains the apparent solvent exposure is yet open to discussion.

One difference between crystal and solution conformations is apparent. Analysis of the H_{Phe}^{α}–H_{Phe}^{β} coupling constants[25] shows that one α–β rotamer with a trans pair of protons is dominant, about 0.65 mol fraction. The 0.3-ppm upfield shift of the Ala methyl protons from their normal value near 1.1 ppm (as in *cyclo*(Leu-Pro-D-Ala)$_2$, see Table V) suggests that the favored rotamer has $\chi_1 = -60°$. In the crystal $\chi_1 = 180°$; were this true in solution, large upfield shifts of some Pro protons would be expected. These were not found.

Acknowledgment. The synthesis and NMR work were supported by grants from the National Institute of General Medical Sciences, GM 26071 and GM 26071-2S1. The crystallography work was supported by National Institutes of Health Grant GM 22490 and the New York State Department of Health.

Registry No. *cyclo*(Phe-Pro-D-Ala)$_2$, 85761-33-7; H-Pro-D-Ala-OMe, 90107-57-6; *cyclo*(Leu-Pro-D-Ala)$_2$, 79546-58-0; benzyloxycarbonyl-L-phenylalanyl-L-prolyl-D-alanine methyl ester, 90107-58-7; benzyloxycarbonyl-L-phenylalanine-*N*-hydroxysuccinimide ester, 3397-32-8; benzyloxycarbonyl-L-phenylalanyl-L-prolyl-D-alanine hydrazide, 90107-59-8; L-phenylalanyl-L-prolyl-D-alanine methyl ester, 90107-60-1; benzyloxycarbonyl-L-phenylalanyl-L-prolyl-D-alanyl-L-phenylalanyl-L-prolyl-D-alanine methyl ester, 90107-61-2.

Supplementary Material Available: A listing of F_o and F_c values and hydrogen atom coordinates (41 pages). Ordering information is given on any current masthead page.

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[24] Zhu, P.-P.; Go, A.; Kopple, K. D., unpublished.

[25] Kopple, K. D.; Wiley, G. R.; Tauke, R. *Biopolymers* **1973**, *12*, 627–636.

Ferricyanide Oxidation of Dihydropyridines and Analogues

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Abstract: The reaction of the N^1-substituted dihydronicotinamides (**1**–**6**), *N*-benzyl-3-carbomyl-1,4-dihydropyridine (**7**), and tritiated *N*-methylacridan (**8**) with $Fe(CN)_6^{3-}$ is first order in $[Fe(CN)_6^{3-}]$ and [substrate]. The oxidations of **1**–**7** were followed spectrophotometrically (350 nm) while the oxidation of **8** was followed by radiometric assay. In the instances of **1**, **2**, and **8** kinetic deuterium isotope effects were determined by employing dideuterio analogues (of **1** and **2**) and deuterio–tritium substitution (for **8**). Under the conditions of $[Fe(CN)_6^{3-}] >> [PyL_2] < [Fe(CN)_6^{4-}]$, it is found that the reciprocal of the pseudo-first-order rate constant increases with increase in $[Fe(CN)_6^{4-}]$—at constant $[Fe(CN)_6^{3-}]$. This inhibition of the $Fe(CN)_6^{3-}$ oxidation of PyL_2 (L = H or D) compounds by $Fe(CN)_6^{4-}$ decreases with increase in the electronegativity of the pyridine nitrogens. This observation finds explanation in the 1e$^-$ oxidation of PyH_2 to $PyH_2^{+}\cdot$ by $Fe(CN)_6^{3-}$ and reduction of $PyH^{+}\cdot$ to PyH_2 by $Fe(CN)_6^{4-}$. A plot of the log of the rate constants (k_1) for the 1e$^-$ oxidations of a series of N^1-substituted dihydropyridines vs. the log of the rate constants (k_{HH}) for hydride transfer from N^1-substituted dihydropyridines to *N*-methylacridinium ion is linear (PyH_2 = **1**, **2**, **4**, **6**, **7**) with slope ~1. This finding shows that equal positive charges are generated on the pyridine nitrogen in the transition states for both the 1e$^-$ oxidation of and H$^-$ abstraction from any of the dihydropyridines. This result establishes that such linear free energy plots do not differentiate between the mechanism of formation of $PyH_2^{+}\cdot$ and PyH^+ by 1e$^-$ and H$^-$ oxidations. The point in the log k_1 vs. log k_{HH} plot for **8** exhibits a deviation of $>10^3$ commensurate with the greater stability of acridinyl radical cation as compared to radical cations generated from N^1-substituted dihydropyridines. The observation that the ferricyanide oxidation is first order in $Fe(CN)_6^{3-}$ and that both primary deuterium kinetic isotope effects (e.g., PyH_2 vs. PyD_2) and $Fe(CN)_6^{4-}$ inhibition are observed establishes that $PyH_2^{+}\cdot$ must exist as an intermediate. Either 1e$^-$ transfer from PyH_2 to $Fe(CN)_6^{3-}$ or H$^+$ transfer from $PyH_2^{+}\cdot$ to yield PyH· may be rate determining. The mechanism of Scheme I is favored in that it allows a rationalization of these observations. On increase in electron withdrawal, partitioning of $PyH_2^{+}\cdot$ species to PyH· by H$^+$ loss becomes favored over partitioning of $PyH_2^{+}\cdot$ to PyH_2 by reduction with $Fe(CN)_6^{4-}$. This is shown not only by the kinetically determined partition coefficient but also by the lack of inhibition of $Fe(CN)_6^{3-}$ oxidation of the electron deficient **6** by $Fe(CN)_6^{4-}$. Stabilization of the radical cation, as seen with **8**, is accompanied by marked $Fe(CN)_6^{4-}$ inhibition and a large kinetic deuterium isotope effect accompanying H$^+$ transfer from the radical cation. The inhibiting effect of O_2 is attributed (Scheme II) to the 1e$^-$ oxidation of PyH· to PyH^+ (not rate determining) by O_2 and reaction of the resultant $O_2^{-}\cdot$ with $PyH_2^{+}\cdot$ to regenerate O_2 and PyH_2.

Much of the controversy[1] regarding the detailed mechanism of NADH model compound (PyH,H) reduction of organic compounds that do not possess appreciable 1e$^-$ redox potentials has largely been resolved with the conclusion that H$^-$ transfer is

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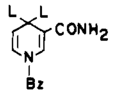
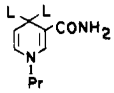
involved. The large discrepancies between kinetic and product isotope effects, which were offered in support of 1e$^-$, H$^+$, 1e$^-$ transfer, have been shown to be due to such anomalies as isotope scrambling due to the use of PyH,D rather than PyH,H/PyD,D in the determination of product and kinetic isotope effects.[2,3] For any given dihydropyridine analogue, however, increase in 1e$^-$ redox potential of the oxidant should result in H$^-$ transfer giving way to a 1e$^-$ transfer mechanism. Alternately a merging of 1e$^-$ and H$^-$ pathways for H$^-$ equivalent transfer should be detectable if the redox potentials for a homologous set of structurally similar dihydropyridines can be made to vary significantly.

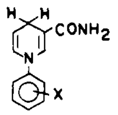
It has been reported that the primary kinetic isotope effect for reaction of *N*-propyl-1,4-dihydronicotinamide (**2**) with $Fe(CN)_6^{3-}$ is small[4] and that the reaction is retarded by $Fe(CN)_6^{4-}$ as expected for a 1e$^-$ transfer reaction involving a nicotinamide radical cation intermediate.[5] Other evidence offered in support of a 1e$^-$ transfer in this reaction is the rate-enhancing effect of added alkali salts.[6] It has been proposed that this effect of alkali metal ions involves the promotion of rate-determining e$^-$ transfer in a multistep mechanism by assistance to the e$^-$ transfer per se and by decreasing electrostatic repulsion between reactants. We have yet to investigate this aspect but it is clear that **2** reacts with the strong 1e$^-$ oxidant $Fe(CN)_6^{3-}$ by an electron-transfer mechanism. In this report there are provided results of an investigation of the $Fe(CN)_6^{3-}$ oxidation of dihydropyridines which include a kinetic analysis of the stepwise process, the influence of added ferrocyanide, and the effect of O_2 upon rate and deuterium kinetic isotope effects. It should be noted that oxygen has an inhibiting effect on the ferricyanide oxidations[7] and hence must be rigorously excluded from studies of the dependence of the oxidation rates upon $[Fe(CN)_6^{3-}]$ and $[Fe(CN)_6^{4-}]$. The model compounds **1**, **2**, *N*-aryl-substituted (**3** = *p*-MeO, **4** = *p*-CH$_3$, **5** = H, **5** = *p*-CN) 1,4-dihydronicotinamides, *N*-benzyl-3-carbamoyl-1,4-dihydroquinoline (**7**), and tritiated *N*-methylacridan (**8**) have been chosen since they exhibit a wide range of reactivity in H$^-$ transfer reactions in CH_3CN[2] and the rates of H$^-$ transfer from some of these compounds to acridinium ion in CH_3CN at 30 °C are available for comparison.



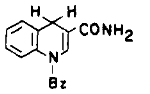
1



2



3, X = p-OCH$_3$
4, X = p-CH$_3$
5, X = -H
6, X = p-CN



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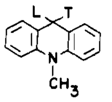


L,T-8

(where L,L = H,H or D,D; L,T = H,T or D,T)

Experimental Section

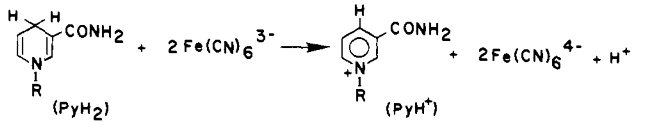
Materials. The NADH model compounds *N*-benzyl-1,4-dihydronicotinamide (**1**), *N*-propyl-1,4-dinicotinamide (**2**), *N*-(*p*-methoxyphenyl)-1,4-dihydronicotinamide (**3**), *N*-(*p*-methylphenyl)-1,4-dihydronicotinamide (**4**), *N*-phenyl-1,4-dihydronicotinamide (**5**), *N*-(*p*-cyanophenyl)-1,4-dihydronicotinamide (**6**), *N*-benzyl-3-carbamoyl-1,4-dihydroquinoline (**7**), *N*-methylacridan (**8**), and the deuterated (>98%) analogues for **1** and **2** (dideuterated at the 4-position) were available from an earlier study.[2] Tritium-labeled [9-^{3}H]-*N*-methylacridan (H,T-**8**) and [9-^{2}H,^{3}H]-*N*-methylacridan (D,T-**8**) were prepared by $NaBH_3T$ reduction of the 9-H- and 9-D-labeled methylacridinium ions, respectively. Solvent was prepared in large batches by adding 400 mL of deaerated spectroscopic grade CH_3CN to enough deaerated triply distilled deionized water at 22 ± 2 °C such that the final volume was 2 L. KCl, K_2CO_3, $K_3Fe(CN)_6$, and $K_4Fe(CN)_6$ were of the best commercially available grades. Prepared solutions were stored in the dark under N_2 to prevent any undesired side reactions that may occur with photosensitive solutions of this type.

Kinetic Studies. Rate constants for the oxidation of **1**–**6** were determined at 30 °C in 20% CH_3CN–H_2O with a Cary 118 spectrophotometer equipped with a temperature-equilibrated multicell sample changer. The anaerobic kinetic runs were carried out by using a thermostated Perkin-Elmer Model 559 spectrophotometer housed in a nitrogen glovebox. Potassium carbonate was added to all reaction solutions (except for **7** and **8** at $[Fe(CN)_6^{3-}]_o = 0.02$ M) such that the final $[CO_3^{2-}]$ was 10^{-2} M. The potassium ion concentration was held constant at 0.012 M by the addition of KCl. Control experiments demonstrated that **1** did not undergo hydration in 20% CH_3CN–H_2O with added KCl and K_2CO_3. Ferricyanide oxidation of **1** was also monitored at $\lambda = 290$ nm to verify that the hydration product was not formed during the course of reaction. Rate constants for reaction of **7** and **8** with $Fe(CN)_6^{3-}$ were also determined by using concentrations of $Fe(CN)_6^{3-}$ sufficiently great to prevent the monitoring of the course of the reaction by the change in UV absorption. Instead, a reactant isolation and quantitative analysis technique was used. For example, reaction of **7** with $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ was carried out as follows: Into 50 mL of temperature-equilibrated and deoxygenated reaction solution of 0.02 M $Fe(CN)_6^{3-}$ and 1×10^{-4} M $Fe(CN)_6^{4-}$ was added a small amount (~10 μL) of a stock solution of **7** in CH_3CN such that the final concentration of **7** was $\simeq 1 \times 10^{-5}$ M. The reaction solution was shaken vigorously for 30 s, and then sample aliquots were removed at known time intervals and extracted with 10 mL of toluene and a large excess of H_2O. The toluene layer was removed and added to a calibrated 1-cm UV cuvette. The UV spectra of 10 samples were recorded in this way, with special attention being paid to base-line calibration of the spectra at 400 nm. The pseudo-first-order rate of reaction was obtained from the decrease in absorbance at $\lambda = 350$ nm. Disappearance of radiolabeled **8** with time was followed by measuring the radioactivity in 5 mL of the toluene sample extract by liquid scintillation counting. The concentration of H,H-**8** in the radiotracer experiments was $\simeq 1 \times 10^{-7}$ M, whereas [H,T-**8**] $\simeq 10^{-12}$ M. Reaction of H,T-**8** with acridinium ion in the presence of varying concentrations of $Fe(CN)_6^{4-}$ was followed by tritium-exchange kinetics as described earlier.[2] In all experiments, the change in radioactivity (or absorbance) followed the first-order rate law for at least 4–6 half-lives. Rate constants for the reaction of **7** with $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ were calculated from data points taken during only the first half-life of reaction (although the run was followed to completion) in order to minimize any changes in rate with respect to time caused by the slight buildup of $Fe(CN)_6^{4-}$ during the course of the reaction. In all other kinetic runs for reaction of **1**–**6** and **8** with $Fe(CN)_6^{3-}$, rate constants were calculated from the total time course using ~10 half-lives of reaction.

First-order rates were calculated by a nonlinear least-squares analysis program used for earlier studies.[2] Linear least-squares analysis was used to obtain the slopes and intercepts of $1/k_{obsd}$ vs. $[Fe(CN)_6^{4-}]$ plots.

Results

Rates of reaction of the NADH model compounds **1**–**8** with ferricyanide were carried out in solutions containing both $Fe(CN)_6^{3-}$ (usually 2×10^{-3} M) and $Fe(CN)_6^{4-}$ (usually (2×10^{-4})–(1×10^{-3}) M) at 20% CH_3CN–H_2O under an O_2-free N_2 atmosphere (unless stated otherwise) at 30 °C. In most reactions $[Fe(CN)_6^{3-}]$ and $[Fe(CN)_6^{4-}]$ were much greater than the concentrations of **1**–**8** so that $Fe(CN)_6^{4-}$ produced during the reaction did not appreciably change the "redox buffer ratio" ($[Fe(CN)_6^{3-}]_o/[Fe(CN)_6^{4-}]_o$). Disappearance of PyH_2 analogues **1**–**7** was monitored at $\lambda = 350$ nm (eq 1). The change in concentration



(1)

of **8** with time was monitored by extraction of remaining reactant at given times and liquid scintillation counting of the organic

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(1) See introduction of: Powell, M. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 1014–1021.

(2) Powell, M. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 7139–7149.

(3) van Laar, A.; van Ramesdonk, H. J.; Verhoeven, J. W. *Recl. Trav. Chim. Pays-Bas*, in press.

(4) Okamoto, T.; Ohno, A.; Oka, S. *J. Chem. Soc., Chem. Commun.* **1977**, 181–182.

(5) Okamoto, T.; Ohno, A.; Oka, S. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 330–333.

(6) Okamoto, T.; Ohno, A.; Oka, S. *J. Chem. Soc., Chem. Commun.* **1977**, 784–786.

(7) Boiko, T. S.; Grishin, O. M.; Yashikov, A. A. *Ukr. Khim. Zh.* (*Ukr. Ed.*) **1982**, *48*, 390–395.

Table I. Summary of Rate Constants for Reaction of **8** and *N*-Methylacridinium Ion with added $Fe(CN)_6^{4-}$ at 30 °C in 20% CH_3CN-H_2O[a]

$[Fe(CN)_6^{4-}]$, M	k, M^{-1} s^{-1}	$[Fe(CN)_6^{4-}]$, M	k, M^{-1} s^{-1}
0	0.344 ± 0.007	6.0×10^{-4}	0.340 ± 0.007
2.0×10^{-5}	0.337 ± 0.007	7.4×10^{-3}	0.360 ± 0.021

[a] $[H,T\text{-}\mathbf{8}]_o \simeq 10^{-12}$ M, $[H,H\text{-}\mathbf{8}]_o \simeq 10^{-7}$ M.

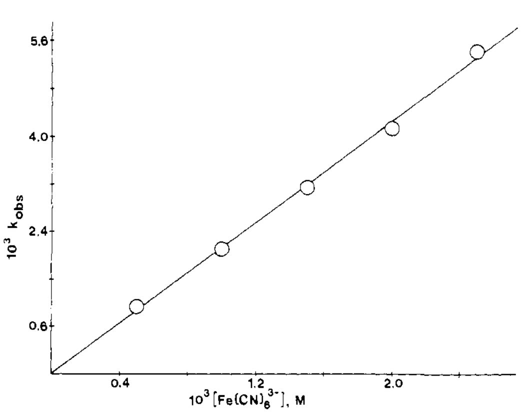
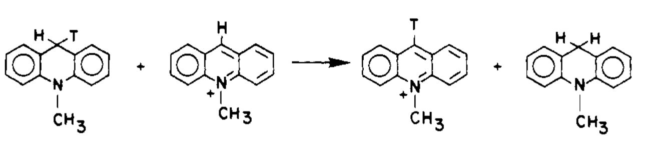


Figure 1. Dependence of k_{obsd} for reaction of **1** on the concentration of $Fe(CN)_6^{3-}$ with constant $[Fe(CN)_6^{4-}]$ (1×10^{-4} M) and $[K^+]$ (6×10^{-2} M) at 30 °C in 20% CH_3CN-H_2O containing 10^{-3} M $[CO_3^{2-}]$.

extracts. In all cases the disappearance of **1**–**8** obeyed the first-order rate law to completion of reaction. The pseudo-first-order rate constant (k_{obsd}) depended linearly on $[Fe(CN)_6^{3-}]$ when $[Fe(CN)_6^{4-}]$ and $[K^+]$ were held constant (Figure 1).

The dependence of the rate constants for reaction of **1**–**8** with a constant excess of $Fe(CN)_6^{3-}$ and varying $[Fe(CN)_6^{4-}]$ in the absence and presence of O_2 is shown in the linear plots of $1/k_{obsd}$ vs. $[Fe(CN)_6^{4-}]$ (Figures 2 and 3). Inspection of Figures 2 and 3 shows that the reactions of **1**–**8** with $Fe(CN)_6^{3-}$ are inhibited by added $Fe(CN)_6^{4-}$. The extent of inhibition is variable. For example, reaction of **8** with $Fe(CN)_6^{3-}$ is strongly inhibited by $Fe(CN)_6^{4-}$ whereas the rate of reaction of **6** with $Fe(CN)_6^{3-}$ is almost unaffected by change in $[Fe(CN)_6^{4-}]$ over the same range.

Reaction of H,T-**8** with *N*-methylacridinium ion (eq 2) in the presence of $Fe(CN)_6^{4-}$ (20% CH_3CN-H_2O at 30 °C) was also



(2)

studied by tritium isotope exchange. This redox reaction has previously been shown to involve H^- transfer.[2] In Table I there is summarized the values of k_{obsd}. The high reliability of rate constants measured by ^3H exchange permits an accurate assessment as to the effect of $Fe(CN)_6^{4-}$ on this reaction. Examination of Table I clearly shows that $Fe(CN)_6^{4-}$ does not inhibit the hydride-transfer reaction between **8** and methylacridinium ion, even at ferrocyanide concentrations up to 7×10^{-3} M.

Discussion

In this study we have examined the kinetics for the reaction of ferricyanide with NADH model compounds at constant $[K^+]$ and over a limited pH range. Our results adhere to the sequence of reactions shown in Scheme I. What follows is a justification for the proposal of this scheme. Although the promotional role of alkali metal ions (as was observed by Ohno and colleagues[6])

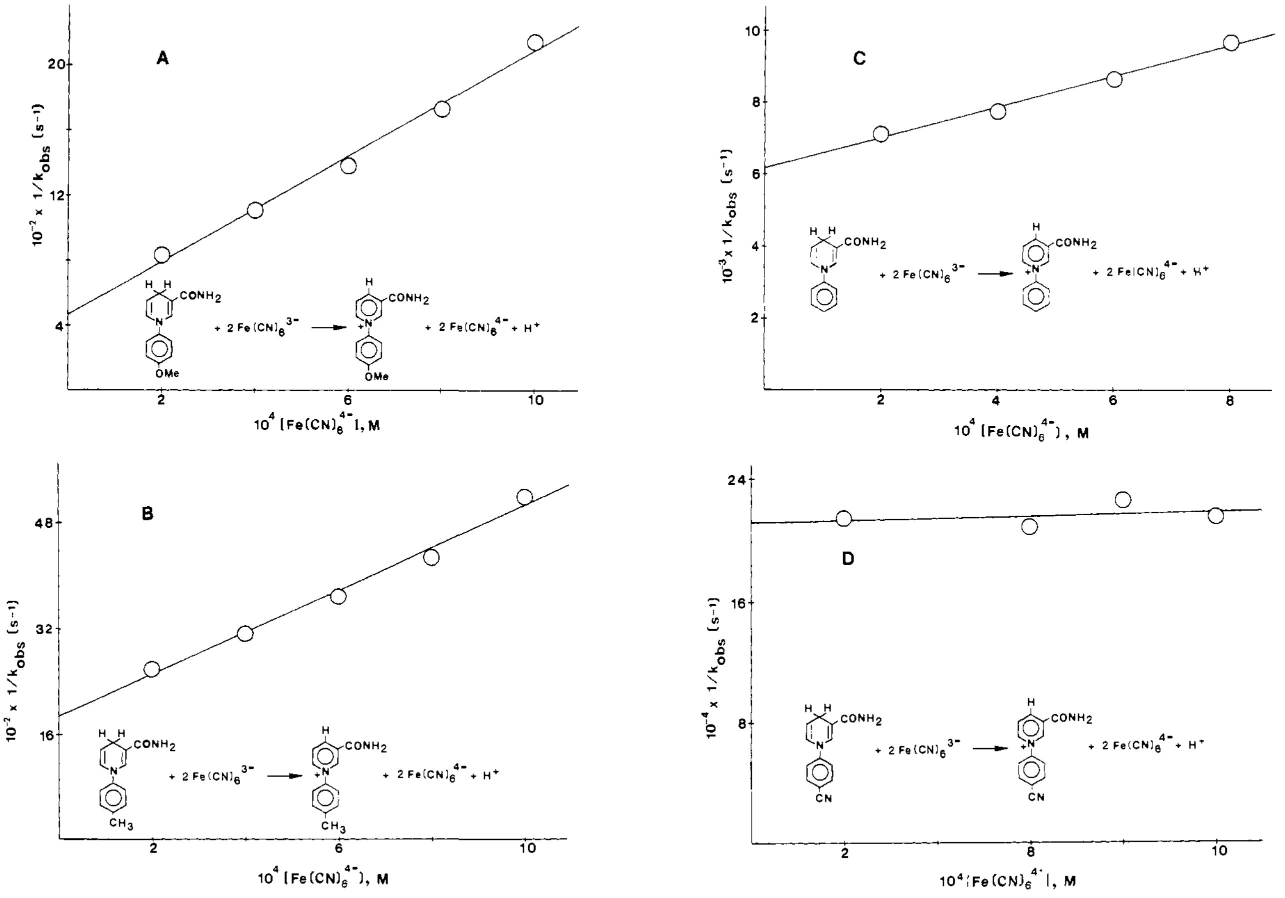


Figure 2. Dependence of $1/k_{obsd}$ on the concentration of $Fe(CN)_6^{4-}$ for reaction of *N*-phenyl-substituted dihydronicotinamides with $Fe(CN)_6^{3-}$ (2×10^{-3} M) at 30 °C in 20% CH_3CN-H_2O: (A) *N*-(*p*-methoxyphenyl)-1,4-dihydronicotinamide (**3**), (B) *N*-(*p*-methylphenyl)-1,4-dihydronicotinamide (**4**), (C) *N*-phenyl-1,4-dihydronicotinamide (**5**), (D) *N*-(*p*-cyanophenyl)-1,4-dihydronicotinamide (**6**).

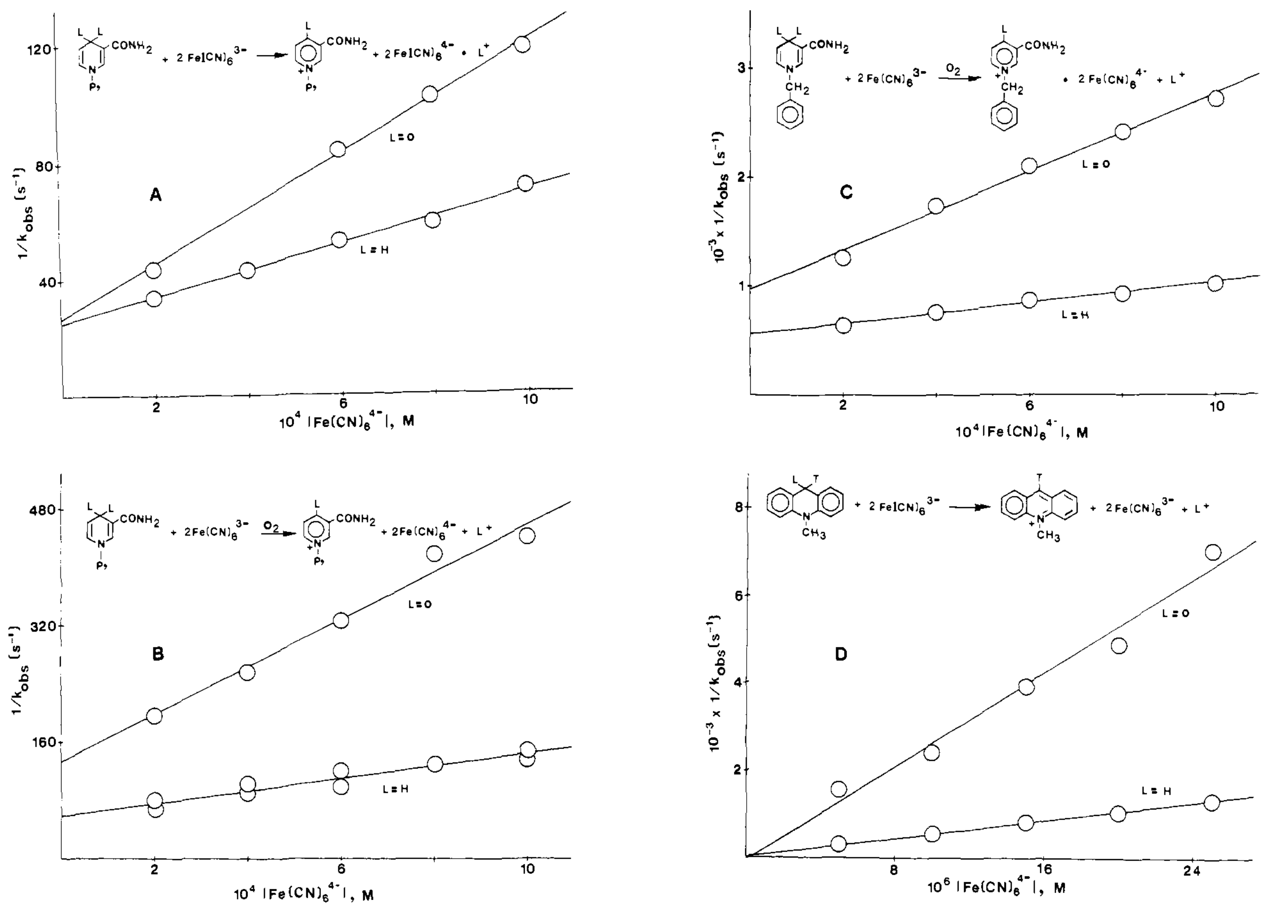
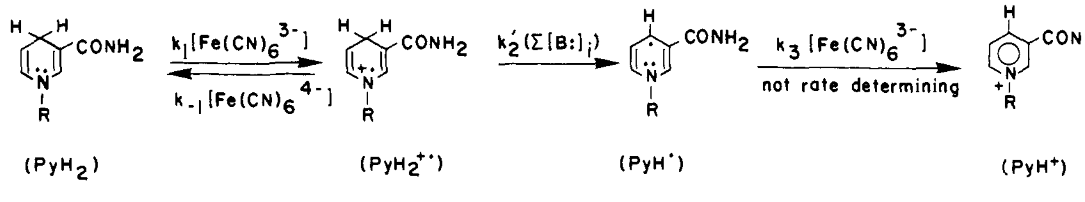


Figure 3. Dependence of $1/k_{obsd}$ on the concentration of $Fe(CN)_6^{4-}$ for reaction of NADH model compounds with $Fe(CN)_6^{3-}$ (see Experimental Section) at 30 °C in 20% CH_2CN-H_2O: (A) reaction of **2** under anaerobic conditions, (B) reaction of **2** with O_2 present, (C) reaction of **1** with O_2 present, (D) reaction of **8** under anerobic conditions.

Scheme I



(3)

and inhibition by O_2[7] are not taken into account in Scheme I, all experiments were carried out at constant [K^+] and under anaerobic conditions (unless otherwise specified). The O_2 effect will be discussed later.

Kinetic studies of the oxidation of PyH_2 compounds by ferricyanide were carried out under the pseudo-first-order conditions of constant "pH" values and $[Fe(CN)_6^{3-}] >> [PyH_2] < [Fe(CN)_6^{4-}]$. According to Scheme I, the pseudo-first-order rate constant (k_{obsd}) for disappearance of dihydronicotinamide (PyH_2) is described by eq 4 when $PyH_2^{+}\cdot$ is at steady state. Inversion

$$k_{obsd} = \frac{k_1k_2'[Fe(CN)_6^{3-}](\sum[B:]_i)}{k_{-1}[Fe(CN)_6^{4-}] + k_2'(\sum[B:]_i)} \quad (4)$$

of eq 4 provides eq 5. From eq 5 it follows that plots of $1/k_{obsd}$

$$\frac{1}{k_{obsd}} = \frac{k_{-1}[Fe(CN)_6^{4-}]}{k_1k_2'[Fe(CN)_6^{3-}](\sum[B:]_i)} + \frac{1}{k_1[Fe(CN)_6^{3-}]} \quad (5)$$

vs. $[Fe(CN)_6^{4-}]$ should be linear and of slope $k_{-1}/\{k_1k_2'[Fe(CN)_6^{3-}](\sum[B:]_i)\}$ and intercept $1/(k_1[Fe(CN)_6^{3-}])$ (Figures 2 and 3). Determination of k_1 and $k_{-1}/\{k_2'(\sum[B:]_i)\}$ from such plots is straightforward since the concentrations of $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ are known and are essentially invariant during the course of the reaction. The derived values of k_1 and $k_{-1}/\{k_2'(\sum[B:]_i)\}$ are given in Table II.

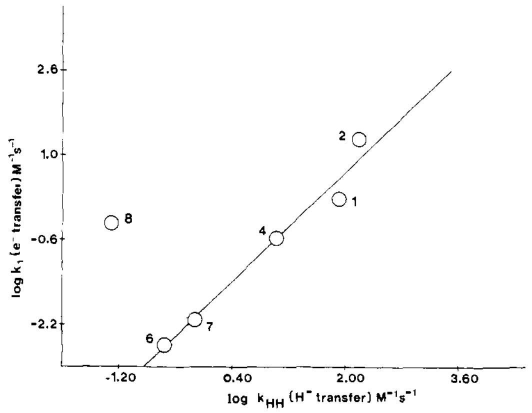


Figure 4. Correlation of k_1^H for electron transfer from NADH model compounds **1**, **2**, **4**, **6**, **7**, and **8** at 30 °C in 20% CH_3CN-H_2O with rates of hydride transfer to *N*-methylacridinium ion (k_{HH}) at 30 °C in CH_3CN reported earlier.[2]

The log of the rate constants for electron transfer (log k_i) from PyH_2 species to $Fe(CN)_6^{3-}$ vary proportionally with the log of the

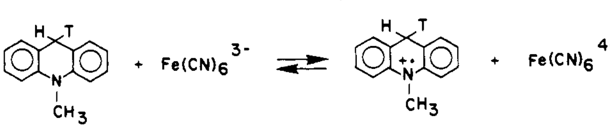
Table II. Summary of Rate Constants, Partition Ratios, and Isotope Effects for Reaction of NADH Model Compounds with $Fe(CN)_6^{3-}$ at 30 °C in 20% CH_3CN-H_2O Containing 10^{-2} M CO_3^{2-}

substrate	O_2 present	k_1, $M^{-1}\ s^{-1}$	k_1^H/k_1^D	$k_{-1}/\{k_2(\sum[B:]_i)\}$	k_2^H/k_2^D [a]
H,H-**1**	no	1.36 ± 0.13		$(1.29 \pm 0.18) \times 10^3$	
H,H-**1**	yes	$(8.93 \pm 0.36) \times 10^{-1}$		$(8.55 \pm 0.60) \times 10^2$	
D,D-**1**	yes	$(5.16 \pm 0.35) \times 10^{-1}$	1.73 ± 0.14	$(1.87 \pm 0.16) \times 10^3$	3.78 ± 0.46
H,H-**2**	no	$(2.01 \pm 0.13) \times 10^1$		$(1.80 \pm 0.15) \times 10^3$	
D,D-**2**	no	$(1.91 \pm 0.20) \times 10^1$	1.05 ± 0.13	$(3.59 \pm 0.40) \times 10^3$	2.09 ± 0.14
H,H-**2**	yes	8.62 ± 0.91		$(1.50 \pm 0.22) \times 10^3$	
D,D-**2**	yes	3.79 ± 0.54	2.27 ± 0.40	$(2.47 \pm 0.41) \times 10^3$	3.76 ± 0.51
H,H-**3**	no	1.08 ± 0.13		$(3.50 \pm 0.47) \times 10^3$	
H,H-**3**	yes	$(9.82 \pm 1.40) \times 10^{-1}$		$(3.73 \pm 0.57) \times 10^3$	
H,H-**4**	no	$(2.66 \pm 0.20) \times 10^{-1}$		$(1.68 \pm 0.17) \times 10^3$	
H,H-**4**	yes	$(1.94 \pm 0.11) \times 10^{-1}$		$(1.37 \pm 0.12) \times 10^3$	
H,H-**5**	no	$(8.17 \pm 0.21) \times 10^{-2}$		$(7.05 \pm 0.50) \times 10^2$	
H,H-**6**	no	$(2.59 \pm 0.27) \times 10^{-3}$		$(3.57 \pm 0.37) \times 10^1$	
H,H-**7**	no	$(3.23 \pm 0.17) \times 10^{-3}$		$(5.19 \pm 0.30) \times 10^2$	
H,H-**7**[b]	no	$(7.81 \pm 0.49) \times 10^{-3}$		$(4.46 \pm 0.97) \times 10^3$	
H,T-**8**[b]	no	$(5.18 \pm 3.28) \times 10^{-1}$		$(2.01 \pm 1.27) \times 10^6$	
D,T-**8**[b]	no	*c*	*c*	*c*	5.18 ± 0.49

[a] These isotope effects are calculated from the ratio of the slopes from $1/k_{obsd}$ vs. $[Fe(CN)_6^{4-}]$ plots for H,H and D,D substrate by assuming $k_1^H = k_1^D$ and $k_{-1}^H = k_{-1}^D$. [b] 0.02 M $Fe(CN)_6^{3-}$, no addition of CO_3^{2-}. [c] A zero intercept (within experimental error) prevented the calculation of these quantities.

rate constants (log k_{HH}) for hydride transfer from PyH_2 to *N*-methylacridinium ion[8,9] (Figure 4). For example, the smallest rate constants for both sets of reactions were observed for reaction of *N*-(*p*-cyanophenyl)-1,4-dihydronicotinamide (**6**), whereas the largest rate constants are associated, in both reactions, with *N*-propyl-1,4-dihydronicotinamide (**2**). This is not unexpected since electron-withdrawing groups destabilize the developing radical cation in electron transfer and the quaternary pyridinium ion in hydride transfer from PyH_2. The slope of the plot of log k_1 vs. log k_{HH} (Figure 4) is close to unity for the compounds **1**, **2**, **4**, **6**, and **7**, indicating that the free energies of H^- transfer to *N*-methylacridinium ion and e^- transfer to $Fe(CN)_6^{3-}$ exhibit the same substituent dependence. Rate constants for the reaction of **3** and **5** with *N*-methylacridinium ion at 30 °C are currently unavailable, but values measured at 50 °C[8] deviate only slightly from the plot of Figure 4. The solvent systems employed in determining k_1 and k_{HH} differ (20% CH_3CN-H_2O vs. neat CH_3CN). Transfer of the hydride reduction of acridinium ion by **1**, **2**, and **8** from 20% CH_3CN-H_2O to neat CH_3CN is accompanied by decreases in k_{HH} of 5-fold, 20-fold, and 14-fold, respectively. It would appear, therefore, that the free energies of activation for the two reactions possess nearly identical sensitivities to electronic effects of substituents. This interesting result suggests that differentiation between H^- and e^- transfer on the basis of electronic effects may not be possible.

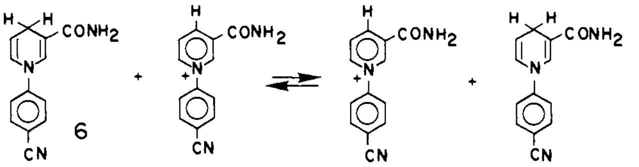
On the basis of the plot of Figure 4, the rate constant for e^- transfer from **8** to $Fe(CN)_6^{3-}$ (eq 6) is approximately 3 orders



8

(6)

of magnitude greater than expected. The positive deviation of **8** from the plot of Figure 4 is even greater when the determined rate constant is corrected for the secondary tritium isotope effect (k_H^T vs. k_H^H) and when a statistical correction of 2 is applied (only one H is being transferred). The much greater susceptibility of **8** to 1e^- oxidation relates to the stability of the acridinyl radical cation.[10] The reaction of **8** with *N*-methylacridinium ion (eq 2) in 20% CH_3CN-H_2O is not inhibited by the addition of ferrocyanide up to 7×10^{-3} M (Table I). These results are in accord with H^- transfer, a process that does not involve the formation of the acridinyl radical cation. This observation, coupled with the positive deviation of **8** in Figure 4, suggests that any reaction of **6** or **7** with their oxidized counterparts (i.e., eq 7) would yield



(7)

a "hydride-like" H-transfer mechanism due to the unstable nature of the radical cation formed. The definition of hydride-like follows.

Forward and Reverse Partitioning from the Radical Cation Intermediate. In terms of Scheme I, $Fe(CN)_6^{4-}$ inhibition indicates that bimolecular reaction of $Fe(CN)_6^{4-}$ with $PyH_2^{+}\cdot$ can compete with proton transfer from $PyH_2^{+}\cdot$, while lack of such inhibition indicates that it cannot. It should be noted that, in the reaction of **6** with $Fe(CN)_6^{3-}$, there cannot be detected any inhibition by $Fe(CN)_6^{4-}$ (Figure 2D). Hydride-like H-transfer implies that the unstable radical cation ($PyH_2^{+}\cdot$) favorably partitions via proton transfer (k_2) rather than e^- transfer ($k_{-1}[Fe(CN)_6^{4-}]$). The hydrogen transfer in reaction of **6** with $Fe(CN)_6^{3-}$ is best described as hydride-like (slow e^- transfer with rapid H^+ transfer). A pure hydride- (H^-) transfer mechanism implies that the electrons and proton are transferred in a single step to a common species. Hydride reduction of $Fe(CN)_6^{3-}$ would result in the formation of $HFe(CN)_6^{4-}$.[11] This species would then be required to rapidly disproportionate with $Fe(CN)_6^{3-}$ to fulfill the stoichiometry requirements of the reaction. The transfer of hydride to $Fe(CN)_6^{3-}$, as previously proposed for another system,[11] cannot apply to the oxidations of this study (see Conclusions).

Inspection of the partition coefficient $k_{-1}/\{k_2'(\sum[B:]_i)\} = \alpha$ for reaction of *N*-phenyl-substituted dihydronicotinamides with $Fe(CN)_6^{3-}$ (Table II) shows that α decreases smoothly as the substituent becomes increasingly electronegative. For example, α at constant $\sum[B:]_i$ for the *p*-methoxy-substituted derivative, **3** is $(3.50 \pm 0.47) \times 10^3$ whereas for the *p*-cyano-substituted analogue

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[8] Ohno, A.; Shio, T.; Yamamoto, H.; Oka, S. *J. Am. Chem. Soc.* **1981**, *103*, 2045–2048.

[9] Reaction of **2** with *N*-methylacridinium ion at 30 °C in neat CH_3CN gives $k_{HH} = (4.20 \pm 0.11) \times 10^2\ M^{-1}\ s^{-1}$ (Powell, M. F.; Bruice, T. C., unpublished results).

[10] Bunting, J. W.; Chew, V. S.; Chu, G.; Fitzgerald, N. P.; Gunasekara, A.; Oh, H. T. P. *Bioorg. Chem.*, submitted for publication.

[11] Bunting, J. W.; Lee-Young, P. A.; Norris, D. J. *J. Org. Chem.* **1978**, *43*, 1132–1140.

(**6**), α equals $(3.57 \pm 0.37) \times 10^1$. The ratio becomes smaller as the radical cation stability decreases. It is to be expected that both k_{-1} and k_2' would increase as $PyH_2^{+}\cdot$ becomes less stable. Thus, the free energy barrier for the forward rate (k_2') must fall off faster than the free energy barrier for the back rate (k_{-1}) as the substituent becomes electron withdrawing. This finding is in accord with lack of $Fe(CN)_6^{4-}$ inhibition of the oxidation of the electron-deficient **6** by $Fe(CN)_6^{3-}$.

The value of α is sensitive to the basicity of the reaction solution. For reaction of **7** with $Fe(CN)_6^{3-}$ at "pH" 7.6, $\alpha = (4.46 \pm 0.97) \times 10^3$, whereas in carbonate buffer (10^{-2} M) at "pH" 10.8, $\alpha = (5.19 \pm 0.30) \times 10^2$. Thus a change of H^+ activity of $\sim 10^3$ changes α by $\sim 10^1$. The rate of ferricyanide oxidation of **2** in 20% aqueous methanol[5] also shows a similar insensitivity of observed rate to $[HO^-]$. This less than first-order dependence upon $[HO^-]$ may be explained by a general base-catalyzed photon abstraction with a Brønsted $\beta \simeq 0.5$ if it is assumed that HO^- exhibits its usual negative deviation (10^2) and H_2O its usual positive deviation (10^1) from Brønsted plots. In such a situation the summed rate constants for $k_{H_2O}[H_2O] + k_{CO_3^{2-}}[CO_3^{2-}] + k_{HO^-}[HO^-]$ are approximately 10-fold greater at pH 10.8 with 0.01 M CO_3^{2-} than at pH 7.6. The observed pseudo-first-order rate constants for oxidation of **2** ($[Fe(CN)_6^{3-}] = [Fe(CN)_6^{4-}] = 1 \times 10^{-3}$ M) at "pH" 9.3 ($[KHCO_3^-] = 1 \times 10^{-2}$ M) and "pH" 11.3 ($[K_2CO_3] = 1 \times 10^{-2}$ M) at constant $[K^+] \sim 0.035$ also shows little variation (4.3-fold). The very much required further investigation of the influence of pH and buffer bases upon the kinetics of $Fe(CN)_6^{3-}$ oxidation of dihydropyridines is in progress.

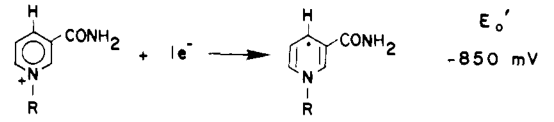
Kinetic Isotope Effects. For the reaction of PyH_2 and PyD_2 with $Fe(CN)_6^{3-}$, the reaction sequence of Scheme I predicts (eq 5) that the ratio of the slopes of plots of $1/k_{obsd}$ vs. $[Fe(CN)_6^{4-}]$ for PyH_2 and PyD_2 species equals $k_2'^{H}/k_2'^{D}$, provided that the secondary isotope effects on k_1 and k_{-1} are unity (i.e., $k_1^H = k_1^D$ and $k_{-1}^H = k_{-1}^D$). Isotope effects ($k_2'^{H}/k_2'^{D}$) derived in this manner range from 2 to 5 and thus indicate rate-determining C–H bond cleavage. Values of $k_2'^{H}/k_2'^{D}$ may also be calculated from the values of α (Table II) determined in the absence of O_2.

The kinetic isotope effects for e^- transfer (k_1) in the anaerobic ferricyanide oxidation of PyH_2 are close to unity as expected for a "pure" electron-transfer process, whereas k_H/k_D for proton abstraction (k_2') from the radical cation of **2** is 2.09 ± 0.14 and of **8** is 5.18 ± 0.49. This variation in primary isotope effect is not unexpected since the lessened stability of the radical cation of **2** results in a more exothermic proton transfer with a reactant-like transition state for k_2. Proton abstraction from the more stable acridinyl radical cation presumably proceeds through a more symmetric reaction coordinate for k_2 and thus shows larger kinetic isotope effects.

Oxygen Effect. The values of k_1 obtained from plots of Figure 2 and 3 were reduced somewhat when O_2 was not rigorously excluded from the reaction solutions. For example, in the presence of air k_1 decreases from 1.36 ± 0.13 to 0.89 ± 0.04 $M^{-1}\ s^{-1}$ for the ferricyanide oxidation of **1** and from 0.266 ± 0.020 to 0.194 ± 0.011 for the ferricyanide oxidation of **4**. Quenching of the radical cation by O_2 obviously compromises Scheme I and eq 4 so that true values of k_1 and $k_{-1}/\{k_2'(\sum[\mathrm{B}:]_i)\}$ cannot be determined in the presence of O_2. The rate constants, partition ratios (α), and kinetic isotope effects obtained with O_2 present should be used only to show that O_2 effects the reaction.

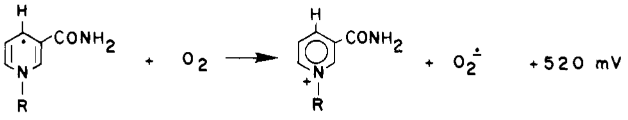
Ferricyanide oxidation of **2**, studied under anearobic conditions, gives an isotope effect on k_1 of unity (as shown by the common intercept of the plots of Figure 3A). However, in the reaction of **1** or **2** with $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ under aerobic conditions, a difference in intercepts for $1/k_{obsd}$ vs. $[Fe(CN)_6^{4-}]$ plots was observed (Figure 3B,C), yet the ratio of the slopes remained high ($k_2'^{H}/k_2'^{D} \sim 3$–4). A plausible explanation for the O_2 effect involves reaction of $PyH\cdot$, formed upon deprotonation of $PyH_2^{+}\cdot$, with O_2 to yield PyH^+ and $O_2^{-}\cdot$ and reaction of superoxide so generated with $PyH_2^{+}\cdot$ to give O_2 and dihydronicotinamide (Scheme II).

The reasonableness of Scheme II may also be judged on the basis of the following thermodynamic arguments. From the approximate potential of eq 8 (see Table III of ref 2) and the potential of eq 9[12] (pH 10.8) the O_2 oxidation of $PyH\cdot$ (eq 10) is associated with a standard free energy ($\Delta G°$) of −50 kJ mol^{-1}. The reaction of O_2 with NAD· has been reported as being very rapid.[13]



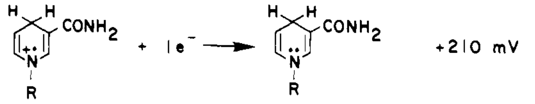
(8)

$$O_2 + 1e^- \longrightarrow O_2^{\dot{-}} \qquad -330\ \mathrm{mV} \tag{9}$$

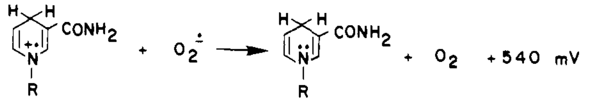


(10)

From the potentials of eq 9 and 11, the reaction of eq 12 is exothermic by $\Delta G° = -52$ kJ mol^{-1}. Thus oxygen will inhibit the ferricyanide oxidation of **1–8** providing O_2 can compete with $Fe(CN)_6^{3-}$ for $PyH\cdot$ Scheme II). Considering the half-cells of eq 8 and 13[14] provides eq 14, which allows the calculation of $\Delta G_0 = -126.3$ kJ mol^{-1}.

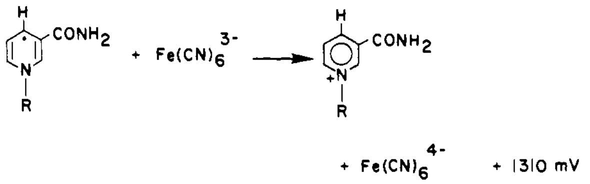


(11)



(12)

$$Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-} \qquad +460\ \mathrm{mV} \tag{13}$$



(14)

Reaction of $Fe(CN)_6^{3-}$ with $PyH\cdot$ is 76 kJ mol^{-1} more exergonic than reaction of $PyH\cdot$ with O_2. This should result in only small amounts of $O_2^{-}\cdot$ formation. However, this is compensated for by the expected facility of the reaction of $O_2^{-}\cdot$ with $PyH_2^{+}\cdot$. Thus, from the half-cells of eq 11 and 13, the reaction of $O_2^{-}\cdot$ with $PyH_2^{+}\cdot$ is calculated to be 65 kJ mol^{-1} more exergonic than is the reaction of ferrocyanide ion with $PyH_2^{+}\cdot$. The inhibition of the reaction of O_2 may then be due to the generation of $O_2^{-}\cdot$ in a non-rate-determining step and its consumption in the reversal of a rate-limiting step. Thus addition of O_2 to reaction solutions of **1–8** and $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ should slow the reaction based on the sequence of Scheme II.[15]

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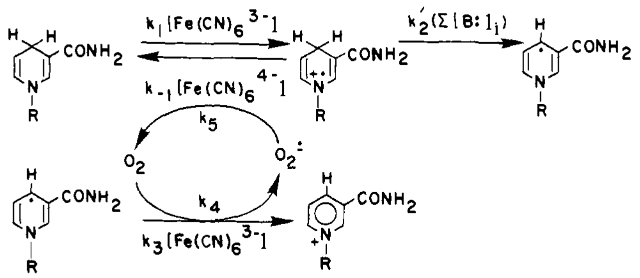
(12) Wilshire, J.; Sawyer, D. T. *Acc. Chem. Res.* **1979**, *12*, 105–110.

(13) Hore, P. J.; Volbeda, A.; Dijkstra, K.; Kaptein, R. *J. Am. Chem. Soc.* **1982**, *104*, 6262–6267.

(14) Clark, W. M. "Oxidation-Reduction Potentials of Organic Systems"; R. E. Krieger: Huntington, NY, 1972; p 132.

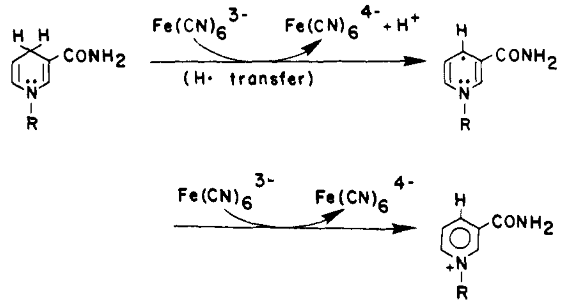
(15) Recent reports of the potential of eq 11 as ~1.0 V (based on kinetic data, cf.: Carlson, B. W.; Miller, L. L. *J. Am. Chem. Soc.* **1983**, *105*, 7453) and fluorescence quenching experiments (cf.: Fukuzumi, S.; Hironaka, K.; Nishizawa, N.; Tanaka, T. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2220) strengthen our argument for Scheme II as an explanation for the O_2 effect.

Scheme II



Conclusions: Alternative Mechanisms

Aside from the mechanism of Scheme I for the oxidation of 1,4-dihydropyridines, the mechanisms of eq 15–17 should be considered. The mechanism of eq 15 does not allow a logical explanation of the inhibition by $Fe(CN)_6^{4-}$. The mechanism of eq 16 would require the second step to be (at least partially) rate controlling in order to explain the observed deuterium isotope effects in reactions of PyH_2 and PyD_2. This feature would demand that the oxidation be second order in $[Fe(CN)_6^{3-}]$, which it is not. The mechanism of eq 17 does not allow an explanation of inhibition by $Fe(CN)_6^{4-}$ nor O_2. Further studies are in progress to test the competence of the presently favored mechanism of Scheme I. Of particular concern is the establishment of the presence (or absence) of general-base proton abstraction from $PyH^{+\bullet}$ (i.e., $k_2'\sum[\mathrm{B}:]_i$) and the elucidation of its character. The suggested[16] lifetime (1 μs) for "spontaneous" decomposition (i.e., $5t_{1/2}$) of $NADH^{+\bullet}$ is akin to the assumption of a second-order rate constant equaling ~6×10^4 M^{-1} s^{-1} for proton transfer to a water molecule. If this is so, then by use of the partition coefficients, α (Table II), one can calculate the rate of reduction of $PyH_2^{+\bullet}$ by $Fe(CN_6)^{4-}$ to yield PyH_2 and $Fe(CN)_6^{3-}$. As an example, for **2** the second-order rate constant for reaction of $Fe(CN_6)^{4-}$ with a radical cation would equal ~5×10^9 M^{-1} s^{-1}. For a "back-of-an envelope" calculation this is reasonable.



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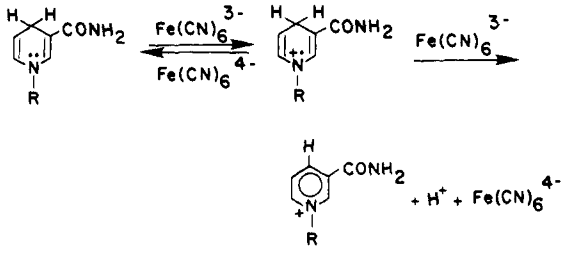

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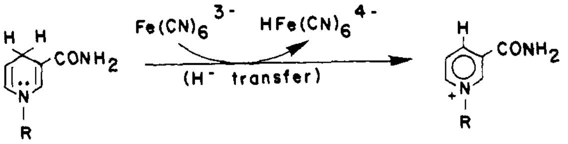

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(16) Grodkowski, J.; Neta, P.; Carlson, B. W.; Miller, L. *J. Phys. Chem.* **1983**, *87*, 3135–3138.