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,
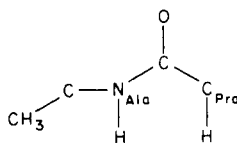
while the Phe N-H group is flanked by H_{Ala}^α and the Phe carbonyl oxygen
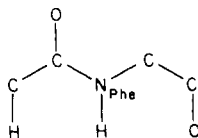
(24) Zhu, P.-P.; Go, A.; Kopple, K. D., unpublished.

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.

(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$\cdot$ 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$\cdot$ 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$\cdot$ 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 com-

pounds 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-CH₃, 5 = H, 5 = p-CN) 1,4-dihydronicotinamides, N-benzyl-3-carbamoyl-1,4-dihydroquinoline (7), and tritiated N-methylacridan (8) have been chosen
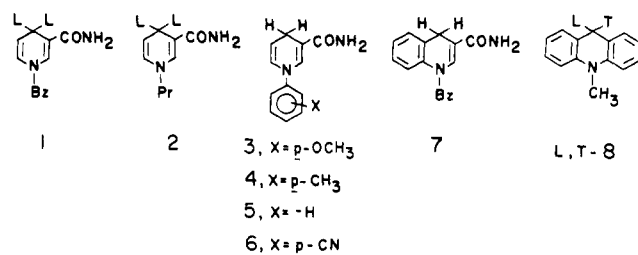
(where L,L = H,H or D,D; L,T = H,T or D,T)

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.

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%)

(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.
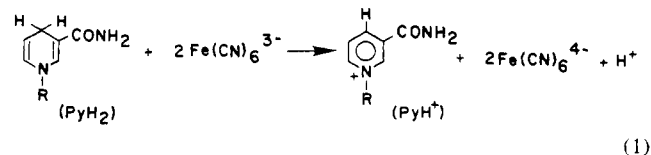
analogues for **1** and **2** (dideuterated at the 4-position) were available from an earlier study.[2] Tritium-labeled [9-³H]-N-methylacridan (H,T-8) and [9-²H,³H]-N-methylacridan (D,T-8) were prepared by NaBH₃T reduction of the 9-H- and 9-D-labeled methylacridinium ions, respectively. Solvent was prepared 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 $[Fe(CN)_6^{3-}]_0 = 0.02$ 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 $(2 \times 10^{-4})-(1 \times 10^{-3})$ 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" ($[Fe(CN)_6^{3-}]_0/[Fe(CN)_6^{4-}]_0$). 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

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} $[H,T-8]_o \simeq 10^{-12}$ M, $[H,H-8]_o \simeq 10^{-7}$ M.
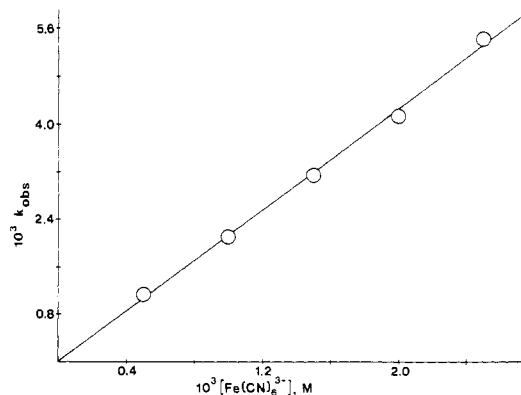
Figure 1. Dependence of k_{obsd} for reaction of **1** on the concentration of
$Fe(CN)_6^{3-}$ with constant $[Fe(CN)_6^{4-}]$ (1×10^{-4} M) and $[K^+]$ (6×10^{-2}
M) at 30 °C in 20% CH_3CN-H_2O containing 10^{-3} M $[CO_3^{2-}]$.

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).
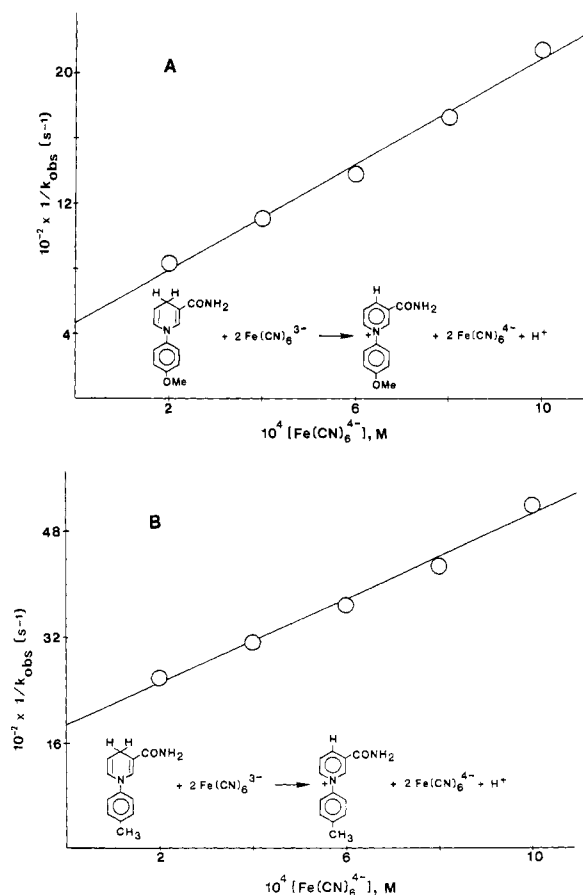
The dependence of the rate constants for reaction of **1-8** with
a constant excess of $Fe(CN)_6^{3-}$ and varying $[Fe(CN)_6^{4-}]$ in the
absence and presence of O_2 is shown in the linear plots of $1/k_{obsd}$
vs. $[Fe(CN)_6^{4-}]$ (Figures 2 and 3). Inspection of Figures 2 and
3 shows that the reactions 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)
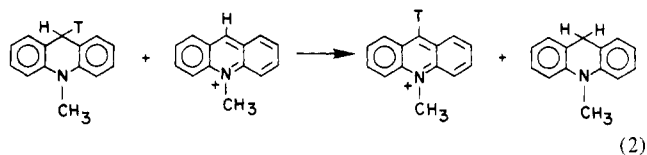
studied by tritium isotope exchange. This redox reaction has
previously been shown to involve H^- transfer.[2] In Table I there
is summarized the values of k_{obsd}. The high reliability of rate
constants measured by ^3H exchange permits an accurate as-
sessment as to the effect of $Fe(CN)_6^{4-}$ on this reaction. Exam-
ination 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])
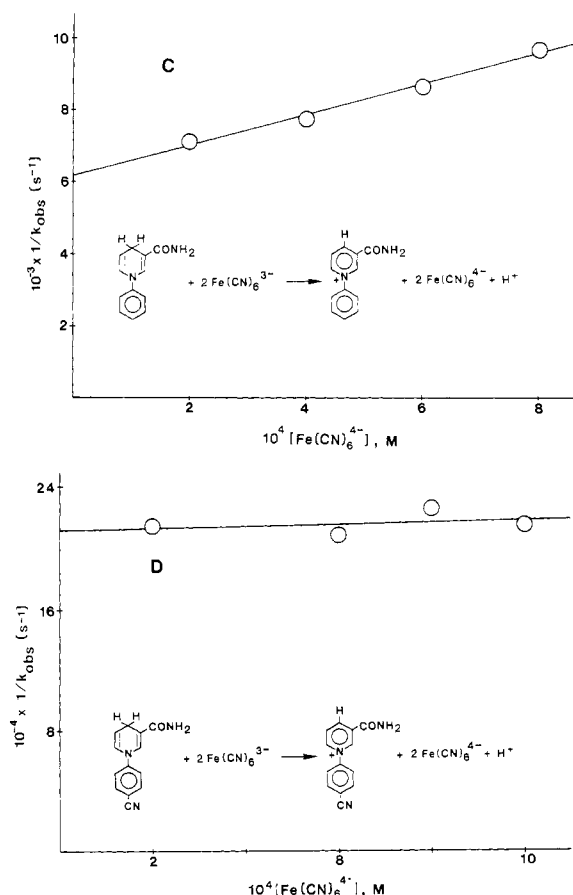
Figure 2. Dependence of $1/k_{obsd}$ on the concentration of $Fe(CN)_6^{4-}$ for reaction of N-phenyl-substituted dihydronicotinamides with $Fe(CN)_6^{3-}$ (2×10^{-3} M) at 30 °C in 20% CH_3CN-H_2O: (A) N-(p-methoxyphenyl)-1,4-dihydronicotinamide (**3**), (B) N-(p-methylphenyl)-1,4-dihydronicotinamide (**4**), (C) N-phenyl-1,4-dihydronicotinamide (**5**), (D) N-(p-cyanophenyl)-1,4-dihydronicotinamide (**6**).
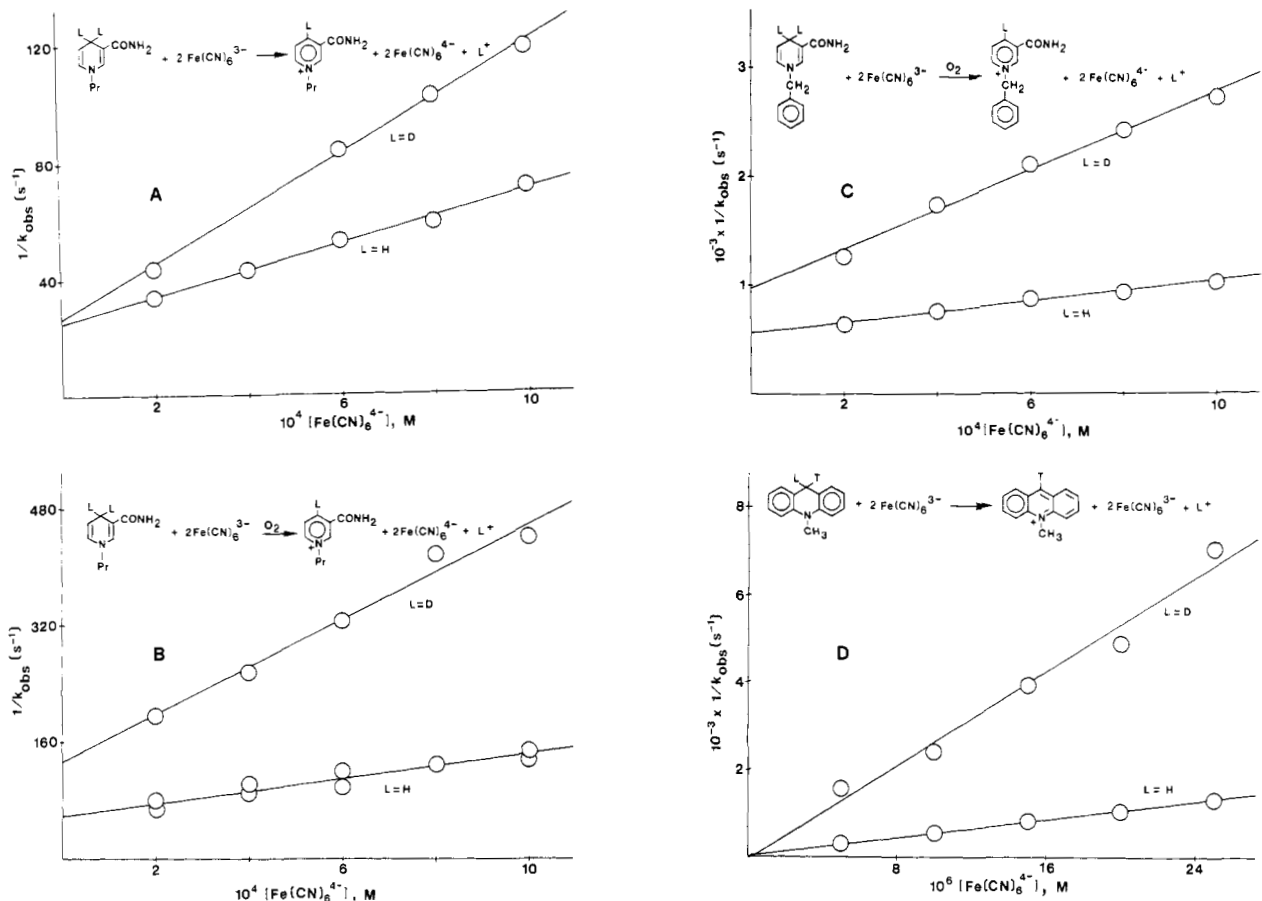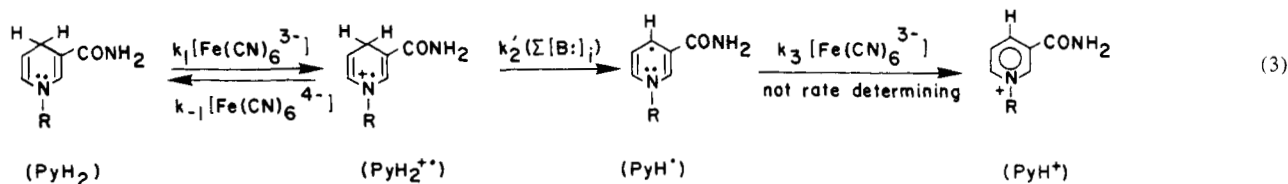
Figure 3. Dependence of $1/k_{obsd}$ on the concentration of $Fe(CN)_6^{4-}$ for reaction of NADH model compounds with $Fe(CN)_6^{3-}$ (see Experimental Section) at 30 °C in 20% CH_3CN–H_2O: (A) reaction of **2** under anaerobic conditions, (B) reaction of **2** with O_2 present, (C) reaction of **1** with O_2 present, (D) reaction of **8** under anerobic conditions.

Scheme I

$$\tag{3}$$

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 $[Fe(CN)_6^{3-}] \gg [PyH_2] < [Fe(CN)_6^{4-}]$. 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

$$k_{obsd} = \frac{k_1 k_2'[Fe(CN)_6^{3-}](\sum[B:]_i)}{k_{-1}[Fe(CN)_6^{4-}] + k_2'(\sum[B:]_i)} \tag{4}$$

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

$$\frac{1}{k_{obsd}} = \frac{k_{-1}[Fe(CN)_6^{4-}]}{k_1 k_2'[Fe(CN)_6^{3-}](\sum[B:]_i)} + \frac{1}{k_1[Fe(CN)_6^{3-}]} \tag{5}$$

vs. $[Fe(CN)_6^{4-}]$ should be linear and of slope $k_{-1}/\{k_1 k_2'[Fe(CN)_6^{3-}](\sum[B:]_i)\}$ 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

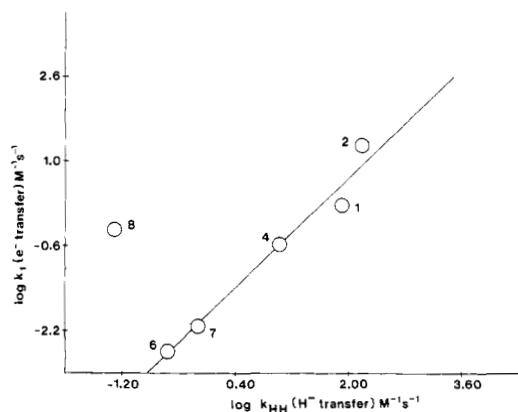course of the reaction. The derived values of k_1 and $k_{-1}/\{k_2'(\sum[B:]_i)\}$ are given in Table II.

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

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]

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

(6)

8
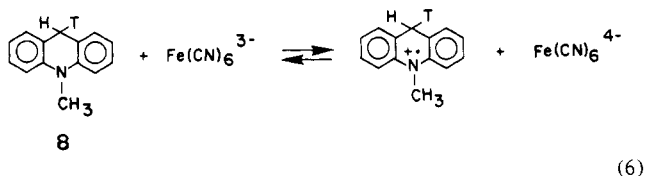
of magnitude greater than expected. The positive deviation of **8** from the plot of Figure 4 is even greater when the determined rate constant is corrected for the secondary tritium isotope effect (k_H^T vs. k_H^H) and when a statistical correction of 2 is applied (only one H is being transferred). 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)
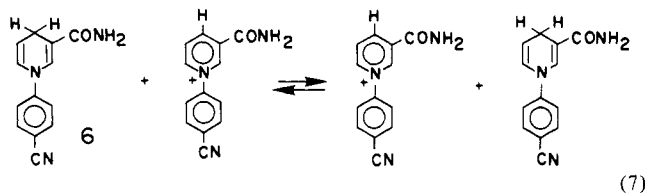
a "hydride-like" H-transfer mechanism due to the unstable nature of the radical cation formed. The definition of hydride-like follows.

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

(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.
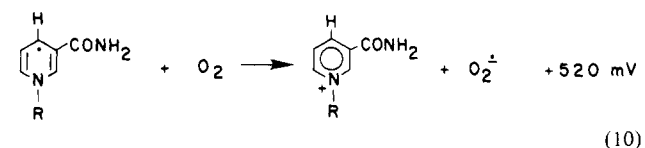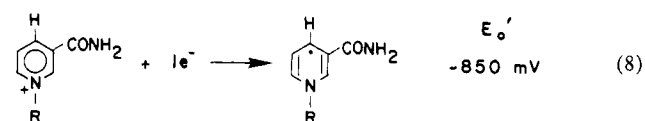
The kinetic isotope effects for e^- transfer (k_1) in the anaerobic ferricyanide oxidation of PyH_2 are close to unity as expected for a "pure" electron-transfer process, whereas k_H/k_D for proton abstraction (k_2') from the radical cation of **2** is 2.09 ± 0.14 and of **8** is 5.18 ± 0.49. This variation 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 $k_{-1}/\{k_2'(\sum[B:]_i)\}$ 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.
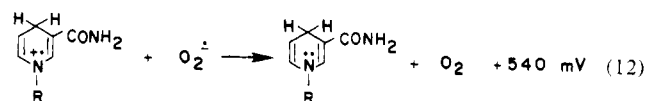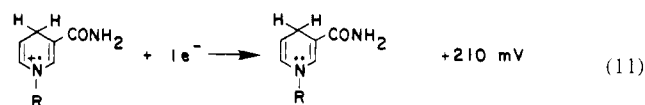
Ferricyanide oxidation of **2**, studied under anearobic conditions, gives an isotope effect on k_1 of unity (as shown by the common intercept of the plots of Figure 3A). However, in the reaction of **1** or **2** with $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ under aerobic conditions, a difference in intercepts 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 ap-
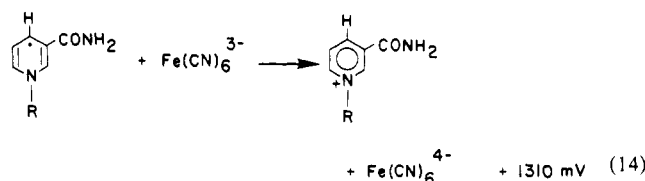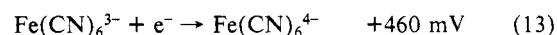
proximate potential of eq 8 (see Table III of ref 2) and the

$$\text{... } + \text{1e}^- \longrightarrow \text{... } \quad E_o' \quad -850 \text{ mV} \quad (8)$$

$$O_2 + 1e^- \longrightarrow O_2^{-\cdot} \quad -330 \text{ mV} \quad (9)$$

$$\text{... } + O_2 \longrightarrow \text{... } + O_2^{-\cdot} \quad +520 \text{ mV} \quad (10)$$

potential of eq 9[12] (pH 10.8) the O_2 oxidation of $PyH\cdot$ (eq 10) is associated with a standard free energy (ΔG°) of -50 kJ mol^{-1}. The reaction of O_2 with $NAD\cdot$ has been reported as being very rapid.[13] From the potentials of eq 9 and 11, the reaction of eq

$$\text{... } + \text{1e}^- \longrightarrow \text{... } \quad +210 \text{ mV} \quad (11)$$

$$\text{... } + O_2^{-\cdot} \longrightarrow \text{... } + O_2 \quad +540 \text{ mV} \quad (12)$$

12 is exothermic by $\Delta G^\circ = -52$ 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 ΔG_o

$$Fe(CN)_6^{3-} + e^- \longrightarrow Fe(CN)_6^{4-} \quad +460 \text{ mV} \quad (13)$$

$$\text{... } + Fe(CN)_6^{3-} \longrightarrow \text{... }$$
$$+ Fe(CN)_6^{4-} \quad + 1310 \text{ mV} \quad (14)$$

$= -126.3$ kJ mol^{-1}. 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]

(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
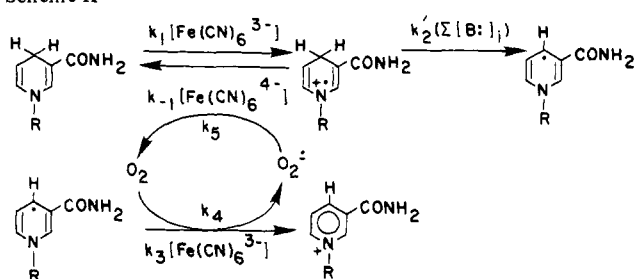
Conclusions: Alternative Mechanisms

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 $PyH^+ \cdot$ (i.e., $k_2' \sum [B:]_i$) and the elucidation of its character. The suggested[16] lifetime (1 μs) for "spontaneous" decomposition (i.e., $5t_{1/2}$) of $NADH^+ \cdot$ is akin to the assumption of a second-order rate constant equaling $\sim 6 \times 10^4 \ M^{-1} \ s^{-1}$ 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 $PyH_2^+ \cdot$

(16) Grodkowski, J.; Neta, P.; Carlson, B. W.; Miller, L. *J. Phys. Chem.* **1983**, *87*, 3135–3138.
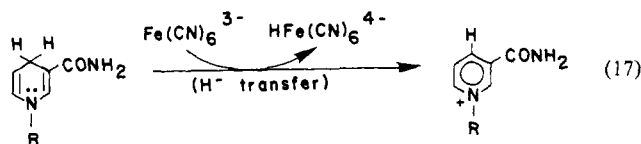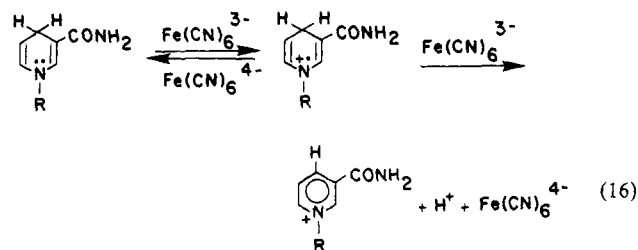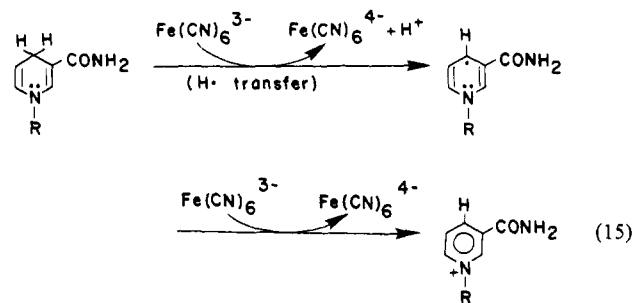
by $Fe(CN_6)^{4-}$ to yield PyH_2 and $Fe(CN)_6^{3-}$. As an example, for **2** the second-order rate constant for reaction of $Fe(CN_6)^{4-}$ with a radical cation would equal $\sim 5 \times 10^9 \ M^{-1} \ s^{-1}$. For a "back-of-an envelope" calculation this is reasonable.

(15)

(16)

(17)