An Old but Simple and Efficient Method to Elucidate the Oxidation Mechanism of NAD(P)H Model 1-Aryl-1,4-dihydronicotinamides by Cations 2-Methyl-5-nitroisoquinolium, Tropylium, and Xanthylium in Aqueous Solution

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Cations 2-methyl-5-nitroisoquinolinium (IQ^+), tropylium (T^+), and xanthylium (Xn^+) were treated by an NAD(P)H model 1-(p-substituted phenyl)-1.4-dihydronicotinamide series (**1**) in buffered aqueous solution to give the corresponding reduced products by accepting hydride. Effects of the 4-substituents of **1** on the reaction rates were investigated. Hammett's linear free energy relationship analysis on the three reactions of **1** provides the reaction constants of −0.48, −2.2, and −1.4 with IQ^+, T^+, and Xn^+ as the hydride acceptors, respectively. Comparison of the present reactions with the reaction examples whose mechanisms are well-known, such as the reaction of **1** with a one-electron oxidant $Fe(CN)_6^{-3}$, shows that the active site of **1** in the oxidation with IQ^+ is at the 4-position on the dihydropyridine ring but that the active site of **1** in the oxidations with T^+ and Xn^+ is at the 1-position, which is in agreement with the results from the Brønsted-type linear analysis and the relation studies of the logarithm of the second-order rate constants with the oxidation potentials of the hydride donors. According to the dependence of the reaction mechanism on the active site of **1**, a conclusion can be made that the reaction of **1** with IQ^+ proceeds by direct one-step hydride transfer mechanism, but the reactions of **1** with T^+ and Xn^+ would take place via multistep hydride transfer mechanism initiated by one-electron transfer.

Introduction

The reduced form of the nicotinamide adenine dinucleotide coenzyme (NAD(P)H), with the biologically important 1,4-dihydropyridine partial structures,[1] plays an important role in many bioreductions by transferring a hydride ion or an electron to the surrounding substrates.[2] The mechanism of the hydride transfer has been a very interesting subject and has been drawing much more of the attention of many researchers in the world. 1-Benzyl-1,4-dihydronicotinamide (BNAH), Hantzsch 1,4-dihydropyridine (HEH), 10-methyl-9,10-dihydroacridine ($AcrH_2$), and many other 1,4-dihydropyridine derivatives have been used as NAD(P)H models to probe the mechanistic details of the hydride transfer.[3−8] However, many experimental results collected are not in line to support each other: some evidence supports that the hydride transfer occurs by a direct one-step mechanism,[9] but others support that the hydride transfer takes place via multistep sequence of e-H^+-e or e-$H^•$.[10] So, the mechanism of the hydride transfer is still obscure. Systematic examination of past publications on this subject shows that various research methods such as kinetic isotope effect,[11] isotope trace,[12] thermodynamics,[4] photochemistry,[13] electrochemistry,[14] computer-chemistry,[15] reaction intermediate trapping,[16] analogue simulator,[17] and ster-

(1) Murakami, Y.; Kikuchi, J.; Hisaeda, Y.; Hayashida, O. *Chem. Rev.* **1996**, *96*, 721−758.

(2) (a) Brewster, M. E.; Simay, A.; Czako, K.; Winwood, D.; Farag, H.; Bodor, N. *J. Org. Chem.* **1989**, *54*, 3721−3726. (b) Friedlos, F.; Knox, R. *J. Biochem. Pharmacol.* **1992**, *44*, 631−635.

(3) (a) Fukuzumi, S.; Tanaka, T. In *Photoinduced Electron Transfer*; Fox, M. A., Chanon, M., Eds.; Elsevier: Amsterdam, 1988; Part C, p 578. (b) Fukuzumi, S.; Suenbu, T.; Patz, M.; Hirasaka, T.; Itoh, S.; Fujitsuka, M.; Ito, O. *J. Am. Chem. Soc.* **1998**, *120*, 8060.

(4) (a) Anne, A.; Fraoua, S.; Grass, V.; Moiroux, J.; Saveant, J.-M. *J. Am. Chem. Soc.* **1998**, *120* 2951. (b) Anne, A.; Moiroux, J.; Saveant, J.-M. *J. Am. Chem. Soc.* **1993**, *115*, 10224. (c) Anne, A.; Hapiot, P.; Moiroux, J.; Neta, P.; Saveant, J.-M. *J. Am. Chem. Soc.* **1992**, *114*, 4694.

(5) (a) Miller, L. L.; Valentine, J. R. *J. Am. Chem. Soc.* **1988**, *110*, 3982. (b) Carlson, B. W.; Miller, L. L. *J. Am. Chem. Soc.* **1980**, *102*, 6533.

(6) Almarsson, O.; Sinha, A.; Gopinath, E.; Bruice, T. C. *J. Am. Chem. Soc.* **1993**, *115*, 7093.

(7) Marcinek, A.; Adamus, J.; Huben, K.; Gebicki, J.; Bartczak, T.; Bednarek, P.; Bally, T. *J. Am. Chem. Soc.* **2000**, *122*, 437−443.

(8) (a) Zhu, X.-Q.; Liu, Y.-C. *J. Org. Chem.* **1998**, *63*, 2786. (b) Zhu, X.-Q.; Liu, Y.-C.; Wang, H.-Y.; Wang W. *J. Org. Chem.* **1999**, *64*, 8983. (c) Zhu, X.-Q.; Zou, H.-L.; Yuan, P.-W.; Liu, Y.; Cao, L.; Cheng, J.-P. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1857. (d) Zhu, X.-Q.; Liu, Y.-C.; Li, J.; Wang, H.-Y. *J. Chem. Soc., Perkin Trans. 2* **1997**, 2191. (e) Zhu, X.-Q.; Wang, H.-Y.; Wang, J.-S.; Liu, Y.-C. *J. Org. Chem.* **2000**, in press (JO001434F). (f) Zhao, B.-J.; Zhu, X.-Q.; He, J.-Q.; Xia, Z.-Z.; Cheng, J.-P. *Tetrahedron Lett.* **2000**, *41*, 257. (g) Liu, Y.-C.; Li, B.; Guo, Q.-X. *Tetrahedron* **1995**, *51*, 9671.

(9) (a) Powell, M. F.; Wu, S. C.; Bruice, T. C. *J. Am. Chem. Soc.* **1984**, *106*, 3850. (b) Carlson, B. W.; Miller, L. L. *J. Am. Chem. Soc.* **1983**, *105*, 7453. (c) Fukuzumi, S.; Koumitsu, S.; Hironaka, K.; Tanaka, T. *J Am. Chem. Soc.* **1987**, *109*, 305. (d) Sinha, A.; Bruice, T. C. *J. Am. Chem. Soc.* **1984**, *106*, 7291. (e) Lai, C. C.; Colter, A. K. *J. Chem. Soc., Chem. Commun.* **1980**, 1115. (f) Fukuzumi, S.; Kondo, Y.; Tanaka, T. *J. Chem. Soc., Perkin Trans. 2* **1984**, 673. (g) Fukuzumi, S.; Ishikawa, M.; Tanaka, T. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1811.

(10) (a) Powell, M. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1982**, *104*, 5834. (b) Powell, M. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 1014. (c) Verhoeven, J. W.; van Gerresheim, W.; Martiens, F. M.; van der Kerk, S. M. *Tetrahedron* **1986**, *42*, 975. (d) Carlson, B. W.; Miller, L. L. *J. Am. Chem. Soc.* **1985**, *107*, 479. (e) Ostovic, D.; Roberts, R. M. G.; Kreevoy, M. M. *J. Am. Chem. Soc.* **1983**, *105*, 7629. (f) Ostovic, D.; Lee, I. S.; Roberts, R. M. G.; Kreevoy, M. M. *J. Org. Chem.* **1985**, *50*, 4206.

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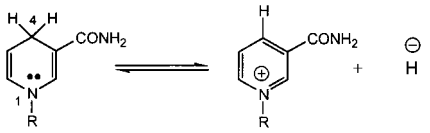
Scheme 1



eochemistry,[18] etc. are widely used. But, to our best knowledge, very little attention has been paid to the dependence of the active sites of 1,4-dihydropyridine on the mechanisms of the hydride transfer. As is well-known, there are two active sites on the 1,4-dihydropyridine ring responsible for the NAD(P)H reductions, one of which is at the 1- or N-position on the ring, the other is at the 4-position (see Scheme 1). It is conceivable that if the hydride transfer takes place by a direct one-step mechanism, the active site of 1,4-dihydropyridine would be at the 4-position due to the absence of hydrogen at the N-position. If the hydride transfer is initiated by one-electron transfer, the active site would be at the N-position rather than the 4-position, since it is only the nitrogen atom at 1-position that can lose an electron in the initial reaction step. So it is a facile and effective method to elucidate the oxidation mechanisms of NAD(P)H model reactions by determining the active center position of 1,4-dihydropyridine during the reaction coordination. This idea induced us to design and synthesize a series of 1-(p-substituted phenyl)-1.4-dihydronicotinamides (**1**) as NAD(P)H models and choose 2-methyl-5-nitroisoquinolinium (**2**), tropylium (**3**), and xanthylium (**4**) cations as $NAD(P)^+$ models (Scheme 2) to determine the reaction center of **1** in the oxidations with **2**, **3**, and **4** by using Hammett's linear free energy correlation method.

Results

Reduction of 5-Nitroisoquinolinium Cation (2) by 1 in Aqueous Solution. Treatment of *N*-methyl-5-nitroisoquinolinium cation by **1** in 30% acetonitrile/70% H_2O gave 1,2-dihydro-5-nitroisoquinoline (eq 1) which can be identified by UV−vis, MS, and ^{1}H NMR spectra analysis (see Experimental Section). Since 1,2-dihydro-5-nitroisoquinoline is the only species in eq 1 which absorbs at 450 nm, the kinetics of this reaction may be conveniently monitored by following the time dependent absorbance at this wavelength. The increase in absorbance at 450 nm was kinetically first order in dihydronicotinamide under conditions in which the hydride acceptor was in 164- to 336-fold excess (Figure 1a). Pseudo-first-order rate constants, k_{obs}, for five X are tabulated in Table 1 (Supporting Information). The second-order rate constants (k_2) obtained from the k_{obs} as a function of the concentration of the hydride acceptor are summarized in Table 2.

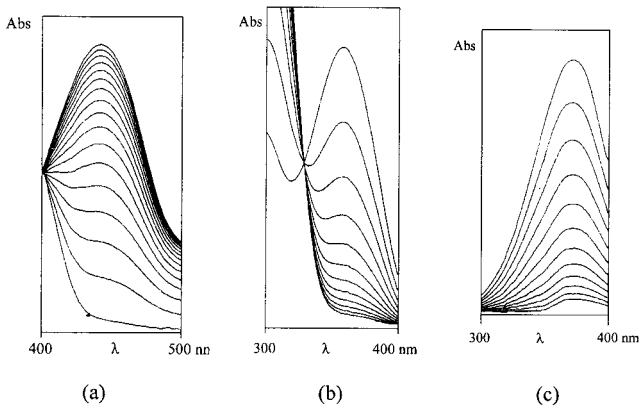
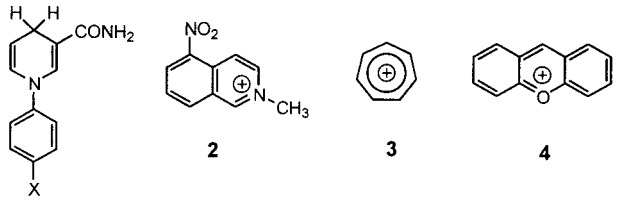
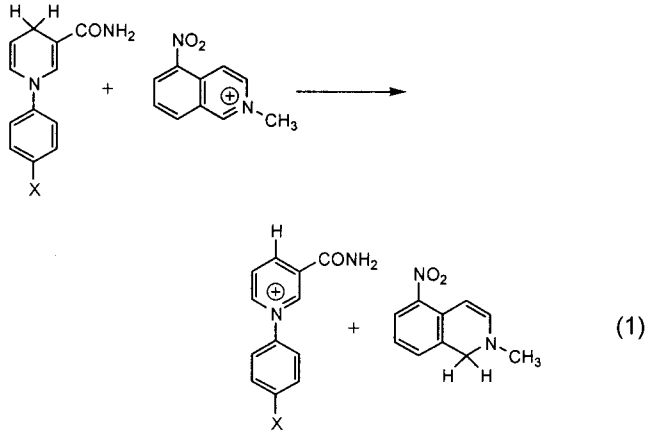


Figure 1. Time dependence of the electronic absorption spectra of the following reactions at room temperature: (a) $IQ^+ + \mathbf{1}$ (X = H), $[IQ^+] = 12.64 \times 10^{-3}$ M, $\Delta t = 1$ min; (b) $T^+ + \mathbf{1}$ (X = CH_3), $[T^+] = 3 \times 10^{-3}$ M, $\Delta t = 1$ min; (c) $Xn^+ + \mathbf{1}$ (X = H), $[Xn^+] = 2.2 \times 10^{-3}$ M, $\Delta t = 1$ min.

Scheme 2



1 X = OCH_3 (**a**), CH_3 (**b**), H (**c**), Cl (**d**), Br (**e**)



Reduction of Tropylium (3) and Xanthylium (4) Cations by 1 in Aqueous Solutions. Tropylium and

(11) (a) Ohno, A.; Yamamoto, H.; Oka, S. *J. Am. Chem. Soc.* **1981**, *103*, 2041−2045. (b) Ohno, A.; Shio, T.; Yamamoto, H.; Oka, S. *J. Am. Chem. Soc.* **1981**, *103*, 2045−2048. (c) Creighton, D. J.; Hajdu, J.; Sigman, D. S. *J. Am. Chem. Soc.* **1976**, *98*, 4619−4625. (d) Hermes, J. D.; Roeske, C. A.; Oleay, M. H.; Cleland, W. *Biochemistry* **1982**, *21*, 5106. (e) Belasco, J. G.; Albery, W. J.; Knowles, J. R. *J. Am. Chem. Soc.* **1983**, *105*, 2475.

(12) (a) Kalyanasundaram, K.; Colassis, T.; Humphry-Baker, R.; Savarino, P.; Barni, E.; Pelizzetti, E.; Graetzel, M. *J. Am. Chem. Soc.* **1989**, *111*, 3300−3311. (b) Fukuzumi, S.; Ishikawa, W.; Tanaka, T. *J. Chem. Soc., Perkin Trans 2* **1989**, *8*, 1037−1045. (c) Martens, F. M.; Verhoeven, J. W.; Gase, R. A.; Pandit, U. K.; de Boer, Th. *Tetrahedron* **1978**, *34*, 843−846. (d) Hitoshi, I.; Koji, T.; Toshio, T. *Chem. Lett.* **1988**, *2*, 339−342. (e) Yamashita, K.; Ishida, H.; Ohkubo, K. *Chem. Lett.* **1991**, *9*, 1637−1640.

(13) (a) Nesterenko, A. M.; Buryak, N. I.; Polumbrik, O. M.; Yasnikov, A. A.; Dopov Akad. Nauk *Ukr. RSR. Ser. B: Geol. Khim. Biol. Naoki* **1986**, *11*, 36−40. (b) Kreavoy, M. M.; Ostovie, D.; Lee, I. S. H.; Binder, D. A.; King, G. W. *J. Am. Chem. Soc.* **1988**, *110*, 524−530. (c) Brewster, M. E.; Kaminski, J. J.; Huang, M. J.; Bodor, N. *J. Org. Chem.* **1990**, *55*, 2361−2366.

(14) Gelvin, R. M.; Rodriquez, M. J. M. *J. Electroanal. Chem. Interfacial Electrochem.* **1989**, *265*, 195−2029.

(15) (a) Wu, Y.-D.; Hook, K. N. *J. Am. Chem. Soc.* **1987**, *109*, 2226. (b) Wu, Y.-D.; Hook, K. N. *J. Am. Chem. Soc.* **1991**, *113*, 2353. (c) Wu, Y.-D.; Lai, D. K. W. *J. Am. Chem. Soc.* **1995**, *117*, 4100. (d) Nonkebel, S. T.; Orszulid, S. T.; Suckling, C. T. *J. Chem. Soc., Chem. Commun.* **1982**, 146. (e) Lauries, D.; Lucas, E.; Nonkebel, D. C.; Suckling, C. T. *Tetrahedron* **1986**, *42*, 1035. (f) Merjer, L. H. P.; Van Niel, J. C. G.; Pandit, U. K. *Tetrahedron* **1984**, *40*, 5185.

(16) (a) Li, B.; Liu, Y.-C.; Guo, Q.-X. *J. Photochem. Photobiol. A: Chemistry* **1997**, *103*, 101−104. (b) Deng, G.; Xu, H. J.; Chen, D. W. *J. Chem. Soc., Perkin Trans. 2*, **1990**, *7*, 1133.

(17) Meijer, L. H. P.; Pandit, U. K. *Tetrahedron* **1985**, *41*, 467.

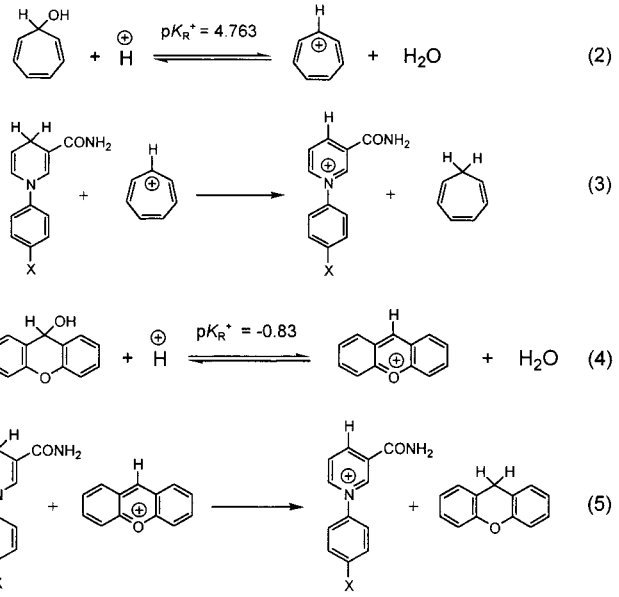
(18) Kill, K. J.; Widdowson, D. A. *J. Chem. Soc., Chem. Commun.* **1976**, 755.

Table 2. The Second-Order Rate Constants, Free Energy Changes of One-Electron Transfer from the NAD(P)H Models to the Cations, and Relative Redox Potentials of the NAD(P)H Models

	k_2 ($M^{-1}\cdot s^{-1}$)				
S	**a**	**b**	**c**	**d**	**e**
IQ^+ [a]	9.86×10^{-2}	7.18×10^{-2}	5.80×10^{-2}	4.93×10^{-2}	5.01×10^{-2}
T^+ [b]	3.79×10^{3}	1.35×10^{3}	8.01×10^{2}	2.76×10^{2}	2.52×10^{2}
Xn^+ [c]	2.22×10^{7}	1.98×10^{7}	1.06×10^{7}	0.49×10^{7}	0.48×10^{7}
	oxidation potentials of the NAD(P)H models				
E_{ox}	0.281	0.313	0.363	0.395	0.400
	free energy changes of one-electron transfer from the NAD(P)H models to the cations ($\Delta G(eT)$) [d]				
IQ^+ [e]	27.8	28.6	29.7	30.5	30.6
T^+ [f]	19.2	20.0	21.1	21.9	22.0
Xn^+ [g]	11.2	11.7	12.4	13.4	13.3

[a] Standard deviation $< \pm 0.24 \times 10^{-2}$. [b] Standard deviation $< \pm 0.31 \times 10^{3}$. [c] Standard deviation $< \pm 0.32 \times 10^{7}$. [d] Units: kcal/mol; $E_{red}(IQ^+) = -0.926$ (V vs Fc^+/Fc in acetonitrile), $E_{red}(T^+) = -0.553$ (V vs Fc^+/Fc in acetonitrile), $E_{red}(Xn^+) = -0.293$ (V vs Fc^+/Fc in acetonitrile). [e] Standard deviation $< \pm 1.5$. [f] Standard deviation $< \pm 1.5$. [g] Standard deviation $< \pm 2.0$.

xanthylium cations are very active species and easily hydrolyze in aqueous solutions to give their corresponding hydroxide adducts. In acidic aqueous solutions, the tropylium and xanthylium cations exist in equilibrium with their corresponding hydroxide adducts and the equilibrium constant was measured to be 4.786[19] and −0.83,[20] respectively (see eqs 2 and 4). Obviously, the



concentrations of cations **3** and **4** in acidic aqueous solutions can be calculated from the concentration of the corresponding alcohol and the pH value of the buffered aqueous solutions, respectively.

The reductions of **3** and **4** by **1** in aqueous solutions gave the corresponding reduced products by hydride transfer from **1** to **3** and **4** (eqs 3 and 5, respectively), which were verified by checking their spectral properties against those of the authentic samples. The kinetics studies of the reactions were carried out in a buffered aqueous solution containing 30% acetonitrile under pseudo-first-order conditions in 20- to 70- or 20- to 43.4-fold excess of the hydride acceptor at constant pH by measuring the maximum absorption of **1** (see Figure 1b and c). The pseudo-first-order rate constants (k_{obs}) were evaluated at six concentrations of the cations from the

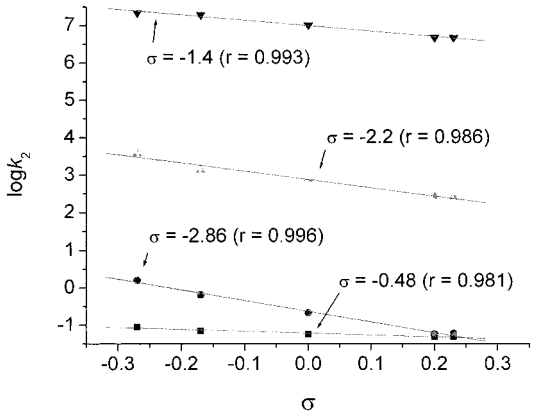


Figure 2. Hammett $\sigma-\rho$ analysis for a series of 1-(4-substituted phenyl)-1,4-dihydronicotinamides and their rates of (■) IQ^+-, (●) $Fe(CN)_6^{-3}$-, (▲) T^+-, and (▼) Xn^+-mediated oxidations.

plots of $\ln(A_t - A_{t+\Delta})$ vs t (Table 1, Supporting Information). The apparent second-order rate constants (k_2^{app}) were evaluated from the slopes of k_{obs} vs ($[R^+] + [ROH]$). The pH-dependent second-order rate constants were evaluated from eq 6.

$$k_2^{app} = k_2/(1 + K_R + /[H^+]) \qquad (6)$$

If $K_R^+ \gg [H^+]$, eq 6 simplifies to $k_2^{app} = k_2[H^+]/K_R^+$, i.e.,[21]

$$\log k_2^{app} = \log k_2 + pK_R^+ - pH \qquad (7)$$

The second-order rate constants obtained from eq 7 are summarized in Table 2.

Discussion

The reduction rates of cations **2**, **3**, and **4** by **1** collected in Tables 1 and 2 show that all the three reactions obey second-order kinetics with first-order dependence on each reactant concentration. A Hammett-type free energy analysis on the three reactions provides three excellent lines of log k_2 against the σ constant of the substituents X with reaction constant ρ values of -0.48 ± 0.07, -2.2 ± 0.2, and -1.4 ± 0.1 for IQ^+, T^+, and Xn^+ as the hydride acceptors, respectively (Figure 2). The negative ρ values indicate the developing positive charge on the 1,4-dihydropyridine ring in the rate-limiting step. The mag-

(19) Ritchie, C. D.; Fleischhauer, H. *J. Am. Chem. Soc.* **1972**, *94*, 3481.

(20) Martin, J. C.; Smith, R. G. *J. Am. Chem. Soc.* **1964**, *86*, 2252.

(21) Bunting, J. W.; Conn, M. M. *Can. J. Chem.* **1990**, *68*, 537−54.

nitude of the ρ values is a measure of the sensitivity of the three reactions to the effects of the substituent X changes.[22]

Comparing the magnitude of the second-order reaction rates among the three reactions, it is found that the magnitude order of the rate constants is k_2 (IQ^+) $\ll$ k_2 (T^+) $\ll$ k_2 (Xn^+) at the same temperature. This result indicates that if the three reactions proceeded by an identical or similar pathway, the absolute value magnitude of the reaction constants ρ would decrease in the following order: $|\rho(IQ^+)| > |\rho(T^+)| > |\rho(Xn^+)|$ on the basis of the reactivity−selectivity principle (RSP).[23] However, the fact is that the absolute value of ρ in the case of IQ^+ as the hydride acceptor ($\rho = -0.48$) is much smaller than those in the cases of T^+ and Xn^+ as the hydride acceptor ($\rho = -2.2$ and -1.4, respectively), which means that the three reactions took place via different mechanisms. Obviously, the active site of **1** in the former reaction is farther away from the source of the substituent X perturbation than those in the latter two reactions. This result allows us to make a proposal that, for the reaction with IQ^+, the active site of **1** would be at the 4-position on the 1,4-dihydronpyridine ring, whereas, for the reactions with T^+ and Xn^+, the active site of **1** would be at the 1- rather than the 4-position on the ring, which means that the reaction of IQ^+ by **1** proceeds by direct one step hydride transfer, but the other two reactions would be initiated by one-electron transfer.

To further confirm the different active sites of **1** in the three oxidations, it is necessary to compare the present reactions with the well-known reaction examples of the Hammett correlation. From Figure 2, the ρ-value of the oxidation of **1** by IQ^+ ($\rho = -0.48$) is close to that of the following known reaction: $ArCOCH_3 + Br_2 \rightarrow ArCOCH_2Br + HBr$ ($\rho = -0.46$).[24] This indicates that the active site of **1** in the oxidation could not be at the 1-position. However, the ρ-values of the oxidations of **1** by T^+ ($\rho = -2.2$) and Xn^+ ($\rho = -1.4$) are similar to those of the known reactions $X-ArNH_2 + ArCOCl \rightarrow X-ArNHCOAr$ ($\rho = -2.78$) and $ArNH_2 + HCO_2H \rightarrow ArNHCHO + H_2O$ ($\rho = -1.43$), respectively.[24] This implies that the active site of **1** in the two present oxidations would be at the heterocyclic nitrogen. It is more efficient to compare the oxidations of **1** by IQ^+, T^+, and Xn^+ with the oxidation of **1** by $Fe(CN)_6^{-3}$ in aqueous solutions.[25] Since the ferricyanide is a well-known one-electron oxidant, the ferricyanide-mediated oxidation of **1** must be initiated by one-electron transfer, and the active site of **1** in this reaction should be at the 1-(N)-position. Examination of the oxidation of **1** by IQ^+ and the oxidation of **1** by $Fe(CN)_6^{-3}$ shows that the rate constant of the former reaction is obviously smaller than that of the latter reaction, but the absolute value of the reaction constant ρ is quite larger than that of the latter. This result strongly supports that the active center of **1** in the oxidation with IQ^+ is at the 4- rather than the 1-position on the 1,4-dihydronicotinamide ring and unambiguously indicates that the reaction of **1** with IQ^+ proceeded by the direct hydride transfer mechanism. Similar analysis can also be done on the comparison of the oxidations of **1** by T^+ and Xn^+ with the ferricyanide-mediated oxidation. As shown in Figure 2, for the three reactions of **1** with T^+, Xn^+, and $Fe(CN)_6^{-3}$, the magnitude order of the rate constants is $k_2(Fe(CN)_6^{-3}) < k_2(T^+) < k_2(Xn^+)$, and in agreement with the absolute value order of the reaction constants $|\rho(Fe(CN)_6^{-3})| > |\rho(T^+)| > |\rho(Xn^+)|$ in terms of RSP,[23] if the three reactions take place by an identical or similar pathway. In other words, this result indicates that the oxidations of **1** by T^+ and Xn^+, as the ferricyanide-mediated oxidation, would be initiated by one-electron transfer from the nitrogen atom on the 1,4-dihydropyridine ring.

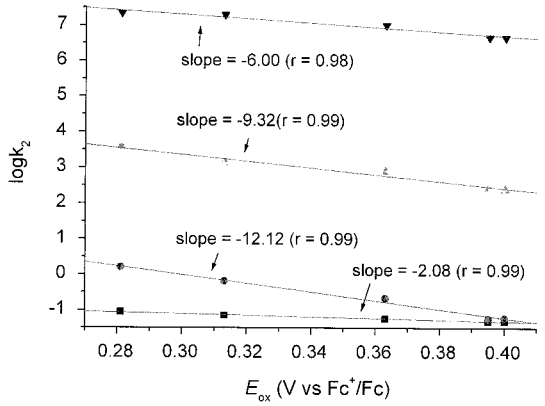


Figure 3. Plots of the logarithm of the second-order rate constants for the reductions of the cations (■) IQ^+, (●) $Fe(CN)_6^{-3}$,[25] (▲) T^+, and (▼) Xn^+ with 1-(p-substituted phenyl)-1,4-dihydronicotinamides against the oxidation potentials of **1**.

To support the above proposal, the dependence of the rate constants (k_2) of the oxidations of **1** by IQ^+, $Fe(CN)_6^{-3}$, T^+, and Xn^+ on the oxidation potentials (E_{ox}) of the NAD(P)H models **1** were examined. Figure 3 shows the plots of log k_2 against E_{ox} of **1** with the slopes of -2.08 ($r = 0.99$), -12.12 ($r = 0.99$), -9.32 ($r = 0.99$), and -6.00 ($r = 0.98$), respectively. Since the slope value represents only the pure electron loss component of the rate constants, the greater dependence of the rate on the E_{ox} of the NAD(P)H models indicates that the reaction is more favorable to take place by the initial one-electron-transfer mechanism. From Figure 3, even though the rate constants for the oxidation of **1** by T^+ and Xn^+ are much larger than that for the oxidation of **1** by IQ^+, the absolute values of the slope for the former two reactions are quite larger than that for the latter reaction. This result shows that the former two reactions are largely affected by the energy of the electron escape from the nitrogen atom, but a small tendency was observed for the latter reaction, which indicates that the former two reactions proceed by initial one-electron transfer, however the other reaction would take place via hydride one-step direct transfer.

A similar analysis can also be done on the dependence of the logarithms of the second-order rate constants on the pK_a's of the corresponding substituted anilines[26] for the four reactions of **1** with IQ^+, $Fe(CN)_6^{-3}$, T^+, and Xn^+.

(22) Isaacs, N. S. *Physical Organic Chemistry*, 1st ed.; John Wiley & Sons: New York, 1987; Chapter 7.

(23) Schmidt, M. W.; Baldrige, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. H.; Koseki, S.; Matsunnage, N.; Nguyen, K. A.; Su, S. J.; Windus, T. L.; Dupouis, M.; Montgomery, J. A. *J. Comput. Chem.* **1993**, *14*, 1347.

(24) Page, M.; Williams, A. *Organic and Bio-organic Mechanisms*, 1st ed.; Addison-Wesley Longman Ltd.: London, 1997; p 54.

(25) Second-order rate constants for the reduction of $Fe(CN)_6^{-3}$ by **1** are obtained from: Brewster, M. E.; Simay, A.; Czako, K.; Winwood, D.; Farag, H.; Bodor, N. *J. Org. Chem.* **1989**, *54*, 3721−3726.

(26) pK_a's of anilines are taken from: Kotake, M. *Constants of Organic Compounds*; Asakury Publishing Co., Ltd.: Tokyo, 1963; pp 593−595.

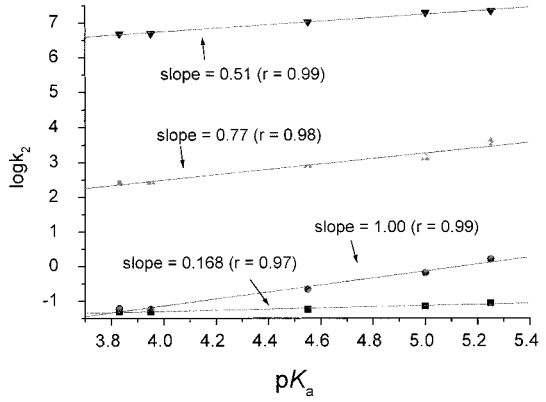


Figure 4. Plots of the logarithm of the second-order rate constants for the reductions of the cations (■) IQ^+, (●) $Fe(CN)_6^{-3}$,[24] (▲) T^+, and (▼) Xn^+ with 1-(p-substituted phenyl)-1,4-dihydronicotinamides against the pK_a of the corresponding arylamine.[26]

The Brønsted-type linear plots were shown in Figure 4 with the slopes of 0.168, 1.00, 0.77, and 0.51 for the oxidations by IQ^+, $Fe(CN)_6^{-3}$, T^+, and Xn^+, respectively. Reacting with the oxidants $Fe(CN)_6^{-3}$, T^+, and Xn^+, the reactivity of **1** is largely influenced by the lone-pair electron density on the ring nitrogen, but reacting with IQ^+, the reactivity is weakly affected, which also indicated that the active site of **1** in the oxidation with IQ^+ is at the 4-position of the 1,4-dihydropyridine, however the active site of **1** is at the 1-position in the oxidations with $Fe(CN)_6^{-3}$, T^+, and Xn^+.

On the other hand, distinction between the one-step and the multistep hydride transfer mechanisms may also be made on the basis of the thermodynamic point of view.[27] For this purpose, electrochemical measurement of the each reactant was performed and showed that the free energy changes of electron transfer from **1** to IQ^+, T^+, and Xn^+ is 27.8−30.6 kcal/mol, 19.2−22.0 kcal/mol, and 11.2−13.3 kcal/mol, respectively. According to the free energetic criteria proposed for differentiating the formal hydride transfer mechanism of the NAD(P)H model-mediated reactions,[27] if the free energy change of one-electron transfer is larger than 23.06 kcal/mol, the reaction is advantageous to taking place by one-step hydride transfer mechanism; if the case is reverse, the reaction proceeds in a direction favorable to one-electron transfer. Obviously, the reaction of **1** with IQ^+ is advantageous to carrying out by direct one-step hydride transfer, but the reactions of **1** with T^+ and Xn^+ proceed in a direction favorable to one-electron transfer.

Conclusions

In the present work, we elucidate the reaction mechanisms of NAD(P)H models 1-(4-substituted phenyl)-1,4-dihydronicotinamides with IQ^+, T^+ and Xn^+ by determining the active site of **1** in the oxidations using Hammett's linear relation analysis. On the basis of the reaction constant comparison of the present reactions with the well-known reaction examples of Hammett correlation, it is found that the active site of **1** in the oxidation with IQ^+ is at the 4-position on the dihydropyridine ring, but the active site of **1** in the oxidation with T^+ and Xn^+ is at the 1-position, from which a conclusion was naturally deduced that the reaction of **1** with IQ^+ proceeds by direct one-step hydride transfer, whereas the reactions of **1** with T^+ and Xn^+ take place via multistep hydride transfer initiated by one-electron transfer. This is an old but simple and efficient method to elucidate the mechanism of NAD(P)H mimic reactions, which, to our knowledge, appears to be the first work in the mechanism investigation of NAD(P)H mimic reactions.[28]

Experimental Section

Materials. 2-Methyl-5-nitroisoquinolinium iodide was prepared according to the literature method[29,30] and recrystallized from methanol/2-proanol before use. Tropylium tetrafluoroborate was purchased from Aldrich Chemical Co. and recrystallized from ethanol prior to use. 9-Hydroxylxanthene (9-xanthydrol) was synthesized from the corresponding xanthenone by reduction with $NaBH_4$ in water. 1-(4-Substituted phenyl)-1,4-dihydronicotinamides were prepared according to the following general method: 1 mmol of the appropriate aniline dissolved in dry methanol was added to a solution of 1 mmol of 1-(2,4-dinitrophenyl)nicotinomide chloride in 100 mL of methanol. The resulting red solution was then heated gently overnight or until the red color faded to yellow, indicating the formation of 2,4-dinitroaniline. The solution was cooled, and the precipitated side product was removed by filtration. The filtrate was then evaporated in a vacuum, and the residue was dissolved in 100 mL of H_2O. The aqueous phase was then exhaustively washed with ethyl ether. The water layer was then evaporated under reduced pressure to give a crude product, which was recrystallized from methanol−ether. Reduction of the pyridilium salt was performed in aqueous basic sodium dithionite to give the appropriate reduced pyridine derivatives.

Productions of the Reaction of 1 by IQ^+. To 4 mL of a 0.2 M solution of **1** (X = H) in acetonitrile was added 10 mL of a 0.08 M solution of IQ^+I^- in pH 7.0 phosphate buffer containing 2 M KCl and 6.0 mL of H_2O to make a total reaction volume of 20 mL. The mixture was stirred for 1 h, and the dark red product was extracted into dichloromethane. After removal of dichloromethane on a rotator, 1,2-dihydro-2-methyl-5-isoquinoline was obtained: UV−vis λ_{max} = 450 nm; MS *m*/*z* = 190; ^{1}H NMR (90 MHz, $CDCl_3$) δ = 2.76 (3H, s), 4.20 (2H, s), 5.93 (1H, d), 6.27 (1H, d), 6.77 (1H, m), 7.02 (1H, dd), 7.73 (1H, dd). The product in the aqueous layer was evaporated under vacuum to give the cation of **1**.

Products from the Oxidation of 1 by T^+ (or Xn^+). A mixture of 1 mmol **1** (X = H) and 1.5 mmol T^+ (or Xn^+) in 20 mL 30% acetonitrile/70% water was magnetically stirred at room temperature in the dark for 24 (or 16) hours. The organic layer was extracted with ether (3 × 20 mL), and the combined organic layers were washed with water and dried with $MgSO_4$ and concentrated. The crude material was chromatographed to give cycloheptatriene (or xanthene). The product in the aqueous layer was treated to give the cation of **1**.

Cycloheptatriene: ^{1}H NMR (90 MHz, $CDCl_3$) δ = 2.2−2.4 (2H, m), 5.15−5.6 (2H, m), 6.1−6.35 (2H, m), 6.45−6.62 (2H, m); MS *m*/*z* = 92 ($M^{+\bullet}$), 91 (100%). Xanthene: ^{1}H NMR (90 MHz, $CDCl_3$) δ = 4.0 (2H, s), 6.9−7.3(8H, m); MS *m*/*z* = 182 ($M^{+\bullet}$), 181 (100%).

Kinetic Measurements. All rate data were obtained in buffered aqueous solutions containing 20% or 30% acetonitrile (v/v) (see below) at 25 °C or 45 °C and ionic strength 1.0 or 0.5 (KCl). Stock solution of reagents for kinetic studies were

(27) Cheng, J.-P.; Lu, Y.; Zhu, X.-Q.; Mu, L. J. *J. Org. Chem.* **1998**, *63*, 6108.

(28) In the previous paper,[11b] a λ-shaped correlation of kinetic (or product) isotope effect (k_H/k_D or Y_H/Y_D) with σ was reported to support a stepwise hydride transfer mechanism. Since the correlation of isotope effect with σ is not due to Hammett's LFER, the slope of the lines is not reaction constant ρ, which is different from our method in nature.

(29) Bunting, J. W.; Norris, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 1189.

(30) Bunting, J. W.; Kabir, S. H. *J. Org. Chem.* **1978**, *43*, 3662.

kept in the dark when not in use and discarded after a maximum of 24 h. Care was taken to ensure that complete solubility of the reactants and products was maintained before and during all kinetic runs. Solubility limitation was the major consideration in defining the highest accessible concentration of the excess component in each reaction system. The reaction of Xn^+ and **1** proved to be incompatible with 20% acetonitrile containing phosphate buffer, although solubility was maintained under these conditions in 30% acetonitrile.

All reactions were followed on a U-3000 spectrophotometer with digitized absorbance data being recorded on Pentium-586 computer under pseudo-first-order condition with 20- to 336-fold excesses of the acceptor species over the hydride donor ([**1**] = 0.05 mM). Data were collected for at least 95% of each reaction. The pH was measured at the end of each kinetic run on a Beckman Φ 72 pH meter.

Pseudo-first-order rate constants were calculated by fitting the absorbance (A) at time (t) to the relationship $A = A_f + (A_i - A_f) \exp(-k_{obs}t)$ via the Marquardt algorithm, treating the initial absorbance (A_i), the final absorbance (A_f), and the pseudo-first-order rate constant as evaluated parameters.

Measurement of Redox Potentials. All electrochemical measurements were carried out in dry CH_3CN solution under an argon atmosphere as described previously.[31] n-Bu_4NPF_6 (0.1M) was employed as the supporting electrolyte. A standard three-electrode assembly consisting of a Pt disk as the working electrode, $AgNO_3$/Ag as reference, and a platinum wire as counter electrode was used in CV measurements. All sample solutions were 1.5 mM. The ferrocenium/ferrocene redox couple (Fc^+/Fc) was taken as an internal standard. Reproducibility is generally within 5 mV.

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Supporting Information Available: Pseudo-first-order rate constants for the reactions of IQ^+, T^+, and Xn^+ by the NAD(P)H models **1** [X = OCH_3 (a), CH_3 (b), H (c), Cl (d), and Br (e)] in buffered aqueous solutions (Table 1). This material is available free of charge via the Internet at http://pubs.acs.org.

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(31) Zhu, X.-Q.; Xian, M.; Wang, K.; Cheng, J.-P. *J. Org. Chem.* **1999**, *64*, 4187.