Isotope Effects on Hydride Transfer between NAD^+ Analogues[1a,b]

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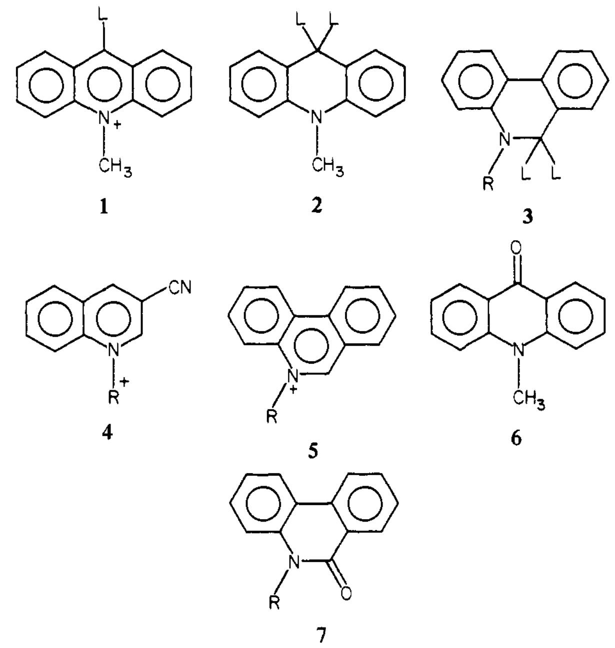
Abstract: Primary kinetic hydrogen isotope effects (KIE's) for hydride transfer between NAD^+ analogues are between 6 and 4, for reactions with equlibrium constants ranging from 0.7 to 10^{16}. There is no sign of the sharp change in KIE which would signal the change of a rate-limiting step in a multistep mechanism, and other evidence also indicates a one-step mechanism.[2] The secondary KIE produced by changing the hydrogen already attached at the acceptor site from H to D is about 10% smaller when the *transferred* atom is D than when it is H. These results, combined with the values of the primary KIE's, strongly suggest some nuclear tunneling in the reaction coordinate.

In a recent paper[2] we have shown that rate constants, k, for hydride transfer between NAD^+ analogues could be quite successfully predicted by Marcus' theory of atom transfer over a wide range of structures and equlibrium constants, K. This result indicates that the reaction involves no high-energy intermediates, because over the wide range of K values covered (~25 powers of 10) the bottle-neck step would change if a multistep mechanism were used, and the observed rate constant would be smaller than the predicted value. Another consequence of the multistep mechanism would be that the primary hydrogen isotope effect would fall off sharply with increasing or decreasing K, at the point where the rate-limiting step changed. Such an effect has been reported,[3] although its experimental foundations have been challenged.[4,5]

In the present paper we report primary hydrogen isotope effects for hydride transfer from **3a–c** to **1** (the product is **2** in each case) and from **2** to **4a,c**. These substances were chosen because the dideuterio variants of **2** and **3a–c** could be prepared by the unambiguous reduction of the corresponding carbonyl compounds, **6** and **7** with $LiAlD_4$. This avoids the problem about the location of the deuterium and also the estimation of a large primary isotope effect from the small effect of monodeuteration, which have undermined the earlier work. The corresponding K values vary from 0.7 to 2×10^6. Rates were measured at 25 °C in a 4:1 mixture of 2-propanol and water. The isotope effects all lie between 4.4 and 6.0. For a related reaction Powell and Bruice[6] have measured an isotope effect of 4.06 and estimated a K value of 10^{16}. Thus there is not sign of a break signifying a change in rate-limiting step.

In the course of this work it has also been possible to measure secondary isotope effects by changing L from H to D in **1**. This has been done with **3a** and **3c** as reducing agents, with both H and D as the atom transfered. For both **3a** and **3c** the secondary effect, $(k_H/k_D)_{II}$, is larger by 10% *when the transfering atom is H*. These effects are reminiscent of observations on related, enzyme-catalyzed reactions.[7] Combined with the observed primary effects, they are quantitatively consistent with the involvement of nuclear tunneling in the reaction coordinate. They are not readily explained in any other way.

Chart I



In all cases L can be either H or D: a, R = CH_3; b, R = $CH_2C_6H_5$; c, R = $CH_2C_6H_4$-4-CF_3.

Experimental

Materials. **1** (L = H), **4a**, and **5a** are well-known substances which were prepared by methylation of the tertiary bases and identified by their physical and spectroscopic properties.[2,5] They are prepared and used as the iodides.

4c and **5b,c** were prepared by treating the free bases with the corresponding benzyl bromide in ethanol.[2] **5b** is a known compound.[8] Its melting point 247–252 °C dec, was similar to that previously reported, 248–250 °C dec.[8] **4c** and **5c** have not been previously reported. Anal.[9] Calcd for $C_{18}H_{12}BrF_3N_2$: C, 54.98; H, 3.08; Br, 20.32; F, 14.50; N, 7.12. Found: C, 54.75; H, 3.25; Br, 20.19; F, 14.61, N, 7.17. **4c** has mp 192 °C dec. Anal. Calcd for $C_{21}H_{15}BrF_3N$: C, 60.30; H, 3.61; N, 3.35. Found: C, 60.47; H, 3.54; N, 3.44. **5c** has mp 240–244 °C dec, and the long-wavelength band in its electronic spectrum has λ_{max} at 363 nm, with long ϵ_{max} = 3.61.

2 (L = H) and all the variants of **3** (L = H) were prepared by reduction of the corresponding salts with $NaBH_4$. **2**, **3a**, and **3b** are pre-

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(2) Roberts, R. M. G.; Ostovič, D.; Kreevoy, M. M. *Faraday Discuss. Chem. Soc.*, in press.

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(4) Powell, M. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1982**, *104*, 5834.

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(7) Cleland, W. W., private communication.

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(9) Analyses were performed by the M-H-W Laboratories, Phoenix, AZ.

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Table I. Primary KIE's

oxidant	reductant	k_H, M^{-1} s^{-1}	$K^{a,b}$	$k_H/k_D{}^c$
4a	**2**	1.48×10^{-2}	0.70	5.71
4c	**2**	6.88×10^{-2}	2.8×10	5.45
1 (L = H)	**5c**	1.68	1.6×10^4	5.15
1 (L = H)	**5b**	5.22	6.9×10^4	4.42
1 (L = H)	**5a**	11.24	2×10^6	5.95

[a] All determined in the same solvent at 25 °C. [b] These values are somewhat different from those reported in ref 2 and are believed to be improved, but neither the conclusion of this paper nor those of the earlier one would change if the values were interchanged. [c] Uncorrected for isotopic contamination. A discussion of the scatter and possible systematic error is given in the text.

viously described compounds;[2] **3c** is not. Anal.[9] Calcd for $C_{21}H_{16}F_3N$: C, 74.32;, 4.75; N, 4.13. Found: C, 74.25; H, 4.71; N, 4.18. **3c** had mp 89–90 °C.

6 was purchased from Aldrich Chemical Co. **7a–c** were prepared by air oxidation of **3a–c**. The reactant was spread out on a watch glass and heated in an oven at 105 °C, in ordinary air, for ~10 h. The products were purified by thin-layer chromatography and, in the cases of **7a** and **7c**, with recrystallization. Yields were 30–40%. **7a** has been previously reported to have mp 108 °C.[10] We found mp 109–112 °C. **7c** had mp 145–147 °C. Anal.[9] Calcd for $C_{21}H_{14}F_3NO$: C, 71.38; H, 3.99; N, 3.96. Found: C, 71.18; H, 4.07; N, 3.95. **7b** could not be completely purified; however, it had appropriate ^1H NMR and IR spectra, and its could be reduced to **3b** which was identical with that prepared by $NaBH_4$ reduction of **5b**.

2 (L = D) and **3a–c** (L = D) were prepared by $LiAlD_4$ (sold as 98 atom % D by Aldrich Chemical Co.) reduction of **6** and **7a–c**, as described by Karrer and co-workers.[10] They had melting point identical with their undeuterated analogues and showed no signals in their ^1H NMR spectra at the frequencies identified with the replaced hydrogens. Their mass spectra indicated that they were at least 94% dideuteriated.[11]

1 (L = D) was prepared by methylation of acridine-*9-d*. The latter was obtained by oxidizing acridan-*9,9-d*$_2$.[12] Acridan-*9,9-d*$_2$ was parepared by reducing acridone (purchased from Aldrich Chemical Co.) with $LiAlD_4$.[10] The acridan-*9,9-d*$_2$ was 97% isotopically pure by mass spectroscopic analysis, and neither it nor **1** (L = D) showed a signal in their ^1H NMR spectra for the hydrogens replaced with deuterium.

The preparation of 3-cyano-1,4-dihydro-1-methylquinoline from **4a** has been previously described.[5]

Distilled water was used in this work and was redistilled from a small amount of H_2SO_4. 2-Propanol was purchased from several suppliers, "suitable for ultraviolet spectrophotometry", and was also redistilled from a small amount of H_2SO_4. Other reagents were purchased from a variety of sources. They were of the best available quality (generally Analytical Reagent Grade[13]) and were used without further purification.

Procedures. Rate constants, *k*, were all measured spectrophotometrically. It was independently verified that the Beer–Lambert law was obeyed under the conditions of our reactions. For slower reactions (half-lives of several minutes or more) a conventional spectrophotometer with a water jacketed cell compartment was used. The temperature in the cells was measured and found to be 25.0 ± 0.2 °C. The cell compartment of the spectrophotometer accomodates four cells, and isotope effects were calculated from pairs of experiments carried out at the same time.

For faster reactions (half-lives 0.2–200 s) *k* values were measured with a remote probe, light guide colorimeter in a reaction vessel which was immersed in a large thermostat and efficiently stirred to provide fast mixing and temperatures of 25.00 ± 0.02 °C. Changes in absorbance were monitored continuously and recorded during and after addition of the limiting reagent, and stirring was also maintained throughout this period. The rise time of this apparatus was ~0.1 s. Secondary isotope effects measured by adding the isotopic variants of **1** sequentially to solutions containing a large excess of reducing agent, so that *all* conditions were the same except the isotopic substituent.

Table II. Secondary KIE's for Reduction of **1**

reductant	k_H/k_D
a	1.06
5c (L = H)	1.11
5c (L = D)	0.98
5a (L = H)	1.05
5a (L = D)	0.96

[a] 3-Cyano-1,4-dihydro-1-methylquinoline.

All kinetic experiments were carried out with at least a 15-fold excess of the spectroscopically inactive constituent. In all cases reactions went to at least 99% completion. The rate law for pseudo-first-order kinetics was used[14] and successfully described and growth or decay of absorbance for at least 3 half-lives—usually for 4–5 half-lives. Infinite time points were stable for many half-lives and were in good agreement with calculated values.

For all kinetic measurements solution pH values were adjusted to ~5, by addition of very small amounts of $HClO_4$, to avoid pseudo-base formation by the cations. The reducing agents used in this study are not hydrated at any appreciable rate at that pH.

Results

Rate constants for undeuterated species and primary KIE's are given in Table I; secondary KIE's are given in Table II. Rate constants were replicated 4–16 times, giving average deviations from the mean of 2–9% and probable errors of 0.5–4% for the mean value. Most of this scatter is probably due to variation in temperature and solvent composition. Care was taken to obtain isotope effects from pairs of experiments carried out under identical conditions, as described in the Experimental, so that the probable error in the values of k_H/k_D is about 2%, rather than the 5% which would have resulted from measuring k_H and k_D in an uncorrelated fashion. The primary KIE's, however, are also quite sensitive to the isotopic purity of the reductants. This is a particular problem in the reactions of **3a–c** with **1**, where the isotopically substituted species were in excess. In those cases, for example, 10% contamination with the mono-deuterio analogue would give a rate constant 20% too high[15] and reduce the apparent isotope effect similarly. The worst problem was the reaction of **3b** (L = D) with **1**. In that case mass spectroscopic analysis showed up to 6% of mono-deuterio compound, which should give a value of *k*, 13% too high. However, when this system was studied with **1** in excess, the same value of k_D was obtained. With **1** in excess the precision is reduced because we are then studying a small change in a large absorbance, but there should be little or no systematic error due to isotopic contamination. Because of this, because the correction is hard to make accurately, and because the mass spectroscopic peak at M + 1 could have origins other than isotopic contamination, we have not attempted to correct for this error. We note, however, that the reaction of 3b with 1 gives the lowest value of k_H/k_D that we have observed. In part this may be due to isotopic contamination.

The secondary isotope effects do not suffer from this problem. They are so small that isotopic contamination at levels below 10% will hardly affect the values. In these cases we believe that the statistical probable errors in k_H/k_D, which are ~2%, are an accurate measure of the accuracy of the results. If this is so, then each of the secondary KIE's for transferring H is really larger than that for transferring D with confidence limits well over 99%.[16] The ratio of the KIE for transferring H to that for transferring D is not determined with great reliability, but it appears to be ~1.10, and it may well be the same in the two examples studied. The secondary KIE for transferring H may also be the same in

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(10) Karrer, P.; Szabo, L.; Krishna, H. J. V.; Schwyzer, R. *Helv. Chim. Acta* **1950**, *33*, 294.

(11) Determined from $(M + 2)/[(M + 2) + (M + 1)]$ after correction for ^{13}C. There should be not significant amount of undeuterated material, but fragmentation by loss of mass 2 keeps this from being determined. The M + 1 peak gives only an upper limit for the isotopic contamination, as it can have other origins.

(12) Clark, J.; Bakovoli, M. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1968.

(13) American Chemical Society "Reagent Chemicals"; Washington: D.C., 1974.

(14) Frost, A. A.; Pearson, R. G. "Kinetics and Mechanism"; 2nd ed.; Wiley: New York, 1962; pp 12 and 29.

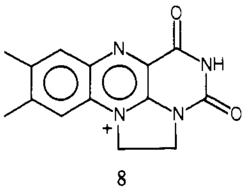
(15) An upper limit on the error was calculated from $k_{\text{obsd}} = \chi_{DD}k_{DD} + \chi_{HD}k_{HD}$ (χ's are mole fractions) with the assumption that $k_{HD} = 3k_{DD}$. This value of k_{HD} would be consistant with $k_{HH} = 5k_{DD}$.

(16) Dixon, W. J.; Massey, F. J., Jr. "Introduction to Statistical Analysis"; McGraw-Hill Book Co.: New York, 1957; pp 119–124.

the three examples studied, $\sim$1.07.

Discussion

The foregoing results show no sign of the change in primary KIE which would suggest a multistep mechanism.[3] The observed primary KIE's are not far below the value, 6.9, given by the Eyring and Cagle approximation[17] for the conversion of a 2800-cm^{-1} frequency of the reactant to the reaction coordinate. An even wider range of equilibrium constants can be obtained by considering the reaction of the substituted flavin, **8**, with 1-benzyl-1,4-dihydronicotinamide (**9**), for which Powell and Bruice have very recently reported a primary KIE of 4.06, measured in acetonitrile.[7] (The structure of **8** has been drawn in a somewhat unorthodox manner, to emphasize its similarity to the other oxidizing agents of our series.) From the redox potentials given by Powell and Bruice a K of 10^{16} can be estimated for this reaction. For a one-step reaction with λ of 72 kcal mol^{-1},[2] Marcus' theory of atom transfer would suggest that a primary KIE of 5.40 for a K of 1.0 would fall to 5.28 for a K of 2×10^6 and 4.7 for a K of 10^{16}.[18] Considering the scatter, the uncertainty in the equilibrium constant for the reaction of **8** and **9**, and the possible solvent effect on the KIE, these predictions are in reasonable accord with the observations. All these primary KIE values are, therefore, entirely consistent with the one-step model of the hydride transfer.[2]



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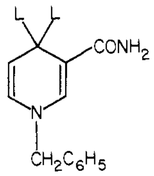

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It is somewhat harder to fit the secondary KIE's into this picture. The conversion of an sp^2-hybridized C–H bond to sp^3 hybridization is expected to produce a small *inverse* KIE. The equlibrium isotope effect (EIE) on the reduction of NAD$^+$ to NADH (isotopic substitution of H at the 4-position) is expected to be 0.89.[19] The transition states for the present reactions appear to be close to symmetrical, both structurally and energetically, so that in the absence of dynamic coupling, a secondary KIE of $\sim$0.95 might have been expected. This is approached for deuterium transfer, but for hydrogen transfer k_H/k_D is larger than 0.95 by 5–8 times the uncertainty of the experimental result. Results presenting similar difficulties have previously been found by Kurz and Frieden for the non-enzymic reduction of 4-cyano-2,6-dinitrobenzene sulfonate by NADH[20] and by Cleland and co-workers[21] for a number of enzymic reductions by NADH. The secondary KIE values can be accommodated by assuming strong coupling between the in-place and the in-flight hydrogens. However, since this involves several atomic motions in the reaction coordinate, it leads to calculated primary KIE values much below those observed. The dilemma can be resolved by allowing nuclear tunneling in the reaction coordinate. In that case less coupling is required, because some of the secondary KIE is provided by increasing the mass in the reaction coordinate when the in-place hydrogen is isotopically substituted. Also, the primary KIE is amplified by the tunneling. As a bonus, the diminuation of the secondary KIE when deuterium is in-flight is explained, because in that case, tunneling will be much less important. All of this has been quantified, and our results have been substantially rationalized.[22] In a model calculation intended to mimic the reaction of NADH with acetaldehyde, but including motion of the in-place hydrogen in the reaction coordinate and substantial tunneling, Huskey and Schowen obtained a primary KIE of 5.5, a secondary KIE of 1.17 with H in flight, and a secondary KIE of 1.06 with D in flight. These results depend strongly on the inclusion of tunneling. We therefore conclude that the model is correct.

Registry No. **1**·I, 948-43-6; **2**, 4217-54-3; **3a**, 30319-92-7; **3b**, 87861-94-7; **3c**, 87861-95-8; **4a**·I, 50741-48-5; **4c**·Br, 87861-96-9; **5a**·I, 5412-06-6; **5c**·Br, 87861-99-2; **6**, 719-54-0; **7a**, 4594-73-4; **7b**, 87861-97-0; **7c**, 87861-98-1; 3-cyanoquinoline, 34846-64-5; phenanthridine, 229-87-8; 3-cyano-1,4-dihydro-1-methylquinoline, 72594-76-4; deuterium, 7782-39-0; 4CF$_3$C$_6$H$_4$CH$_2$Br, 402-49-3; PhCH$_2$Br, 100-39-0.

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(18) Kreevoy, M. M. In "Isotopes in Organic Chemistry"; Buncel, E., Lee, C. C., Eds.; Elsevier Scientific Publishing Co.: Amsterdam, 1976; Vol. 2, p 26.

(19) Cleland, W. W. *Methods Enzymol.* **1980**, *64*, 101.

(20) Kurz, L. C.; Frieden, C. *J. Am. Chem. Soc.* **1980**, *102*, 4198.

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(22) Huskey, W. P.; Schowen, R. L. *J. Am. Chem. Soc.*, submitted for publication.

Energetics of Twisting in the Lowest Stilbene Triplet State[1]

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Abstract: The temperature dependence of the azulene effect on the benzophenone-sensitized photoisomerization of the stilbenes is reported in toluene, benzene, *tert*-butyl alcohol, and acetonitrile. The data are consistent with formation of identical stilbene triplets from the two stilbene isomers. Predictions concerning the shape of the potential energy curve for twisting about the central bond depend on the detailed mechanism assumed for the quenching interaction. A lower enthalpy for the twisted triplets is predicted, $\Delta H_{tp} = -2.1$ and -1.6 kcal/mol in toluene and *tert*-butyl alcohol, respectively, if it is assumed that the equlibrium population of transoid triplet geometries, $^3t^*$, is negligible and that quenching follows encounters of twisted stilbene triplets, $^3p^*$, with azulene when they achieve $^3t^*$ geometries in the encounter cage. On the other hand, if quenching is assumed to occur only by direct interaction between $^3t^*$ and azulene, equilibrium constants for the $^3t^* \rightleftarrows {}^3p^*$ process are obtained which predict a lower enthalpy content for transoid triplets: $\Delta H_{tp} = 0.5$ and 2.9 kcal/mol for toluene and *tert*-butyl alcohol, respectively. In the latter case entropy is predicted to favor twisted geometries so that for both mechanisms the free energy favors $^3p^*$ at the temperatures normally employed for photochemical studies. Reasons for favoring the $^3p^*$ quenching mechanism are presented.

Structural preferences of 1,2-diarylethene triplets in solution have been inferred from consequences of their interactions with quenchers. Encounter of olefin triplets in planar (or nearly so) conformations with molecules having lower lying triplet states

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