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Hydride Reduction of NAD^+ Analogues by Isopropyl Alcohol: Kinetics, Deuterium Isotope Effects and Mechanism

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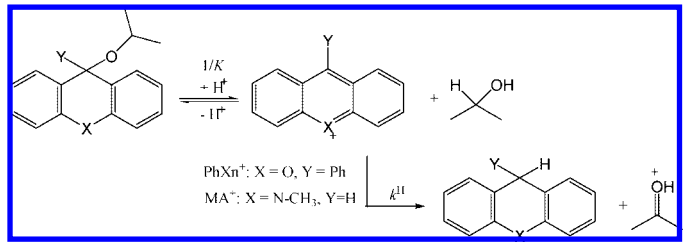

Observed pseudo-first-order rate constants (k^{obs}) of the hydride-transfer reactions from isopropyl alcohol (*i*-PrOH) to two NAD^+ analogues, 9-phenylxanthylium ion ($PhXn^+$) and 10-methylacridinium ion (MA^+), were determined at temperatures ranging from 49 to 82 °C in *i*-PrOH containing various amounts of AN or water. Formations of the alcohol−cation ether adducts (ROPr-*i*) were observed as side equilibria. The equilibrium constants for the conversion of $PhXn^+$ to PhXnOPr-*i* in *i*-PrOH/AN (v/v = 1) were determined, and the equilibrium isotope effect (EIE = K(*i*-PrOH)/K(*i*-PrOD)) at 62 °C was calculated to be 2.67. The k^H of the hydride-transfer step for both reactions were calculated on the basis of the k^{obs} and K. The corresponding deuterium kinetic isotope effects (e.g., $KIE_{OD}{}^H = k^H$(*i*-PrOH)/k^H(*i*-PrOD) and $KIE_{\beta\text{-}D6}{}^H = k^{obs}$(*i*-PrOH)/$k^{obs}$($(CD_3)_2CHOH$)), as well as the activation parameters, were derived. For the reaction of $PhXn^+$ (62 °C) and MA^+ (67 °C), primary $KIE_{\alpha\text{-}D}{}^H$ (4.4 and 2.1, respectively) as well as secondary $KIE_{OD}{}^H$ (1.07 and 1.18) and $KIE_{\beta\text{-}D6}{}^H$ (1.1 and 1.5) were observed. The observed EIE and $KIE_{OD}{}^H$ were explained in terms of the fractionation factors for deuterium between OH and $OH^+(OH^{\delta+})$ sites. The observed inverse kinetic solvent isotope effect for the reaction of $PhXn^+$ (k^{obs}(*i*-PrOH)/k^{obs}(*i*-PrOD) = 0.39) is consistent with the intermolecular hydride-transfer mechanism. The dramatic reduction of the reaction rate for MA^+, when the water or *i*-PrOH cosolvent was replaced by AN, suggests that the hydride-transfer T.S. is stabilized by H-bonding between O of the solvent OH and the substrate alcohol $OH^{\delta+}$. This result suggests an H-bonding stabilization effect on the T.S. of the alcohol dehydrogenase reactions.

Introduction

The chemistry of the metabolism of alcohols in living organisms involves the hydride transfer from the α-H of alcohols to the C-4 position of the pyridinium ring of the NAD^+ coenzyme (**1**, R^1 = adenine dinucleotide, R^2 = $CONH_2$). Alcohols are oxidized to ketones or aldehydes, and NAD^+ is reduced to NADH (**2**, R^1 and R^2 are same as in NAD^+). The reversible biochemical conversion can be mediated by alcohol dehydrogenases.[1–6] Searching for and understanding the hydride-transfer reaction from alcohols to NAD^+ models has thus

(1) (a) Nagel, Z. D.; Klinman, J. P. *Chem. Rev.* **2006**, *106*, 3095–3118. (b) Ramaswamy, S.; Eklund, H.; Plapp, B. V. *Biochemistry* **1994**, *33*, 5230–5237.
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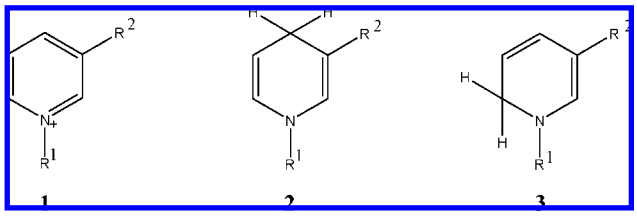
become a target of biomimetic research of the alcohol dehydrogenase reaction.[7]

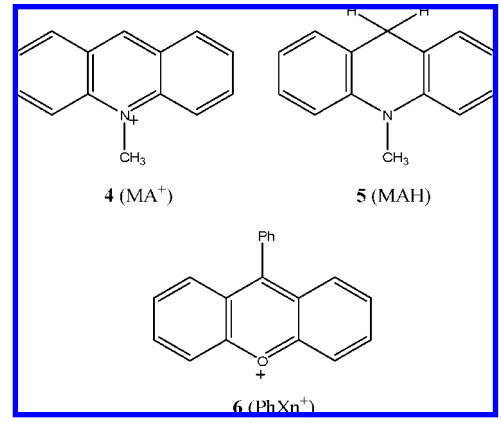


Thus far, biomimetic investigation of the enzymatic reaction has used *N*-alkyl-3-substituted pyridinium cations (**1**, R^2 = $CONH_2$ or $CONR_2$) as NAD^+ models. Since the hydride-transfer reactions from alcohols to these model compounds are thermodynamically unfavorable, reactive metal alcoholates containing lithium,[8,9] magnesium,[10] and zinc[11,12] have been used as hydride sources. These reactions give mainly 1,4-dihydropyridine products (**2**), but formation of the 1,6-dihydropyridine isomers (**3**) was often observed. The nonenzymatic reactions, some of which are less effective, are inconsistent with the regiospecific enzymatic reaction in which only the former is produced.

We recently reported an effective thermal oxidation of isopropyl alcohol (*i*-PrOH) via hydride transfer to another NAD^+ model, 10-methylacridinium ion (MA^+, **4**).[13] Since the central pyridinium ring of MA^+ is blocked by fused benzene rings, only one electrophilic center, i.e., the 4-position of the central ring, is available for the hydride ion donor to attack. Only the hydride-transfer product, 9,10-dihydroacridan (MAH, **5**), was isolated, and the kinetics of the reaction were determined. It is believed that this is the first kinetic determination of the hydride transfer from a neutral alcohol to an NAD^+ model. This reaction can serve as a useful model of the dehydrogenase reaction.

We report herein our detailed kinetic investigation of the hydride-transfer reaction from *i*-PrOH to MA^+ and to another more reactive analogue of MA^+, 9-phenylxanthylium ion (**6**, $PhXn^+$). Like MA^+, $PhXn^+$ has a central pyrylium ring fused with benzene rings on both sides. The effect of several variables on the rate of the hydride-transfer reduction of both $PhXn^+$ and MA^+ by isopropyl alcohol in either the parent alcohol solvent or a mixed solvent containing the parent alcohol were studied. These include the effects of the presence of mono- and perdeuterated isopropyl alcohol (*i*-PrOD, *i*-PrOH-*2-d*, and *i*-PrOD-d_7), protic acid, as well as that of varying the temperature. The results including kinetic solvent effects, kinetic temperature effects, and deuterium kinetic isotope effects at positions of α-C, β-C, and alcohol O on the hydride transfer from α-CH to both cations were used to elucidate the mechanism of the reactions and to provide insights into the mechanism of the alcohol dehydrogenase reactions.

At the same time, this work serves to provide insights into the general hydride-transfer reaction from alcohols to organic



cations, study of which has been ignored and undeveloped for half a century. This kind of reaction last appeared in the literature in the late 1950s when Bartlett[14] and Franzen[15] reported a number of examples of triphenylmethanol and derivatives (as their cations) abstracting hydride ion from aliphatic alcohols in 50% sulfuric acid aqueous solution. The function of the concentrated acid was to completely convert the aryl alcohols to their cationic forms so that they are activated toward hydride abstraction from the alcohols and a direct monitoring of the disappearance of the cation was possible for kinetic determinations. However, the acid also deactivates the alcohols by protonation, complicating the reaction system and thus constituting the major reason for the stagnant investigation of these important reactions.[16]

Results

Reduction of $PhXn^+$ by *i*-PrOH: Analogue to the MA^+ Reduction. $PhXn^+BF_4^-$ (0.096 mmol) in 2 mL of acetonitrile (AN) was added to 10 mL of *i*-PrOH at room temperature. The yellow color of the $PhXn^+$ disappeared immediately, implying that the $PhXn^+$ was consumed. The colorless product was isolated and characterized to be the corresponding isopropyl alcohol adduct, 9-isopropoxy-9-phenylxanthene (PhXnOPr-*i*, **7**). The same reaction solution was subjected to reflux conditions (82 °C), and the progress of the reaction was monitored by TLC. As the reaction time increased, PhXnOPr-*i* gradually disappeared and another product formed. After about 6 h, PhXnOPr-*i* was not detectable. The product was isolated and characterized to be the hydride-transfer product 9-phenylxanthene (PhXnH, **8**).

Reactions in which *i*-PrOH was replaced by either *i*-PrOD or perdeuterated isopropyl alcohol (*i*-PrOD-d_7) were carried out. Only the latter reaction led to the incorporation of the deuterium into the 9-position of the **4**, indicating that (1) the source of the transferring hydrogen was the α-H of the alcohol and (2) the hydrogen did not exchange with the aromatic hydrogens of the $PhXn^+$, and hence, PhXnH was the primary net hydride-transfer product. Our results indicate the involvement of an equilibrium between $PhXn^+$ and its isopropyl alcohol adduct (**7**) in the overall irreversible hydride-transfer reaction.

The reaction is analogous to the irreversible hydride reduction of MA^+ by *i*-PrOH[13] that we reported earlier. In the latter reaction, the isopropyl alcohol adduct of MA^+ (MAOPr, **9**) was

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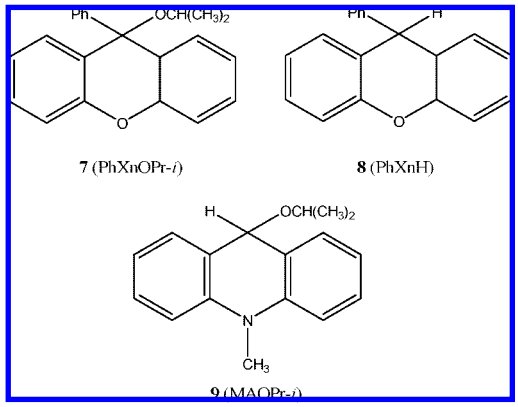
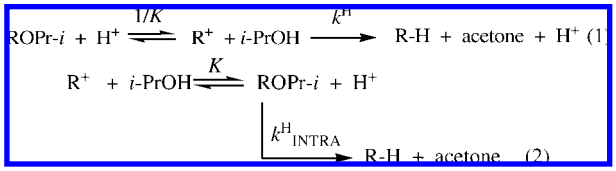
(15) Dr. rer. Nat. Franzen, V.; Katonah, Z. *Chem.—Ztg.* **1957**, *81*, 205–206.
(16) Deno, N. C.; Peterson, H. J.; Saines, G. S. *Chem. Rev.* **1960**, *60*, 7–14, and references cited therein.



also detected. That adduct is not as stable as PhXnOPr-*i* and was not isolatable. This is apparently due to the higher stability of its precursor cation MA$^+$ as compared to PhXn$^+$ with respect to the corresponding adducts. According to the hydride affinity values, MA$^+$ is 13 kcal/mol more stable than PhXn$^+$.[17] The formation of MAOPr-*i* was detected by its UV absorption (at 284 nm) and the NMR spectra in the mixture of MA$^+$ and *i*-PrOD-d_7.[13]

Two possible pathways for the involvement of the adducts in the corresponding irreversible hydride-transfer reactions are described in eqs 1 and 2 where R$^+$ represents the organic cations. Note that in the intramolecular hydride-transfer pathway (2), the adduct (ROPr-*i*) is an intermediate in the formation of the hydride-transfer product, while in the intermolecular hydride-transfer pathway (1) it is involved in a side equilibrium with its precursor compounds. Also note that, in pathway (2), the hydride-transfer step requires a four-membered ring transition state (T.S.).



It should be mentioned that searching for the evidence to discriminate between the two possible mechanisms for the general hydride-transfer reaction from alcohols to organic cations started as early as when these reactions were first discovered. However, an answer to this mechanistic question had never been found.[14,16] For example, although Bartlett and co-workers studied the kinetics of the hydride-transfer reactions from aliphatic alcohols to carbocations derived from the corresponding arylcarbinols by treating them as intermolecular reactions, they concluded that "*the kinetic data cannot exclude the possibility that the actual hydride transfer which is preferred in these systems is a intramolecular one between the two* α*-positions of the mixed ether ion.*"[14]

Determination of the Rates of the Hydride-Transfer Reaction from *i*-PrOH to PhXn$^+$ and to MA$^+$: Method, Kinetic Solvent Effect, Kinetic (Solvent) Isotope Effect, and *β*-Secondary Kinetic Isotope Effect. The UV−vis spectral kinetic scans were recorded for the reaction of PhXn$^+$ (0.001M) in *i*-PrOH/AN (v/v = 1) over a range of temperatures by

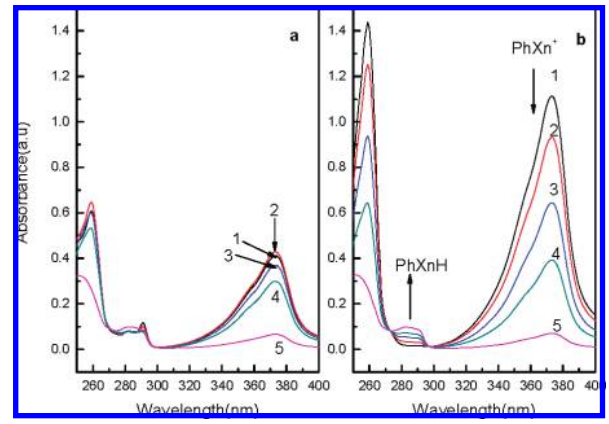


FIGURE 1. Kinetic scans determined by analysis of the reaction aliquots, taken from the reaction solution of [PhXn$^+$]$_0$ = 0.001 M in *i*-PrOH/AN (v/v = 1) at 62 °C, diluted 25 times with (a) pure AN and (b) AN containing $HClO_4$ (3 M). Reaction times for spectra 1− are 30, 120, 271, 506, and 1402 min, respectively.

determining the decay of absorbance due to the cation in aliquots at various time intervals. Spectral data showed that about 98% of the PhXn$^+$ was converted to the PhXnOPr-*i* adduct at 62 °C upon addition of the cation in *i*-PrOH/AN (v/v = 1). Spectra in Figure 1a correspond with the aliquots diluted with *pure* acetonitrile. Note that these spectra specially used for kinetic analysis are different from those of the real reaction system in that the PhXn$^+$/PhXnOPr-*i* equilibrium system is shifted to some extent to the side of the cation when the solution is diluted with acetonitrile. The characteristic absorption band with λ_{max} = 373 nm is due to PhXn$^+$ and that with λ_{max} = 283 nm is due to both the PhXnOPr-*i* adduct and the PhXnH product, which are known to absorb over the same wavelength range. The absorbance band at λ_{max} = 373 nm is unsuitable for monitoring the conversion of the cation to PhXnH product. This is due to the fact that the cation is in equilibrium with PhXnOPr-*i*. Furthermore, the absorbance band at λ_{max} = 283 nm is due to both the PhXnOPr-*i* adduct and the PhXnH product, which prohibits its use for monitoring the formation of PhXnH.

Since the equilibrium between the adduct and PhXn$^+$ favors the formation of the latter under sufficiently acidic conditions, spectral kinetic scans that reflect the genuine conversion from PhXn$^+$ to PhXnH were recorded for the reaction aliquots diluted with acetonitrile containing $HClO_4$ (Figure 1b). The [$HClO_4$] in AN was 3 M, sufficiently high that the concentration of adduct was negligible. The observed pseudo-first-order rate constant (k^{obs}) for the formation of PhXnH was thus computed according to the decay of PhXn$^+$ at 373nm. Here, $k^{obs} = -d[PhXn^+]_T/dt = d[PhXnH]/dt$ where the total [PhXn$^+$] measured, denoted as [PhXn$^+$]$_T$, is the sum of [PhXn$^+$] and [PhXnOPr-*i*] in the reaction solution.

As stated in the Introduction, previous kinetic studies of the hydride-transfer reactions from alcohols to carbocations were carried out in concentrated aqueous acid solutions.[14] Our method allows the determination of the rates of these reactions under neutral aprotic conditions, avoiding the complications due to the protonation of the alcohols by concentrated acids.

Data illustrating the effect of mono- and perdeuterated isopropyl alcohol (*i*-PrOD, *i*-PrOH-*2*-*d*, and *i*-PrOD-d_7) (entries 1-2 to 1-5), temperature (entries 1-1, 1-2, 1-6, and 1-7) and solvent (entries 1-8 and 1-9) on the k^{obs} of the reaction are summarized in Table 1. When AN solvent was replaced by *i*-PrOH or the nonpolar solvent cyclohexane, no hydride-transfer

(17) The hydride affinities ($-\Delta G_H$-(R$^+$)) of the two cations are 76 kcal/mol (MA$^+$) and 89 kcal/mol (PhXn$^+$), respectively, see: (a) Cheng, J. -P.; Lu, Y.; Zhu, X.; Mu, L. *J. Org. Chem.* **1998**, *63*, 6108–6114. (b) Cheng, J. -P.; Handoo, K. L.; Parker, V. D. *J. Am. Chem. Soc.* **1993**, *115*, 2655–2660.

TABLE 1. Effect of D-Substitution in Isopropyl Alcohol, the Temperature, and the Solvent on the Observed Pseudo-First-Order Rate Constants for the Hydride-Transfer Reaction from Isopropyl Alcohol to $PhXn^+$ and MA^+[a]

entry	T (°C)	reaction conditions	$k^{obs} \times 10^3$[b] (min^{-1})
		reduction of $PhXn^+$ (v/v = 1[c])	
1−1	69	*i*-PrOH/AN	4.49 ± 0.08
1−2	62	*i*-PrOH/AN	2.13 ± 0.09
1−3	62	*i*-PrOH-2-d/AN	0.480 ± 0.016
1−4	62	*i*-PrOD/AN	5.53 ± 0.14
1−5	62	*i*-PrOD-d_7/AN	1.11 ± 0.01
1−6	56	*i*-PrOH/AN	1.11 ± 0.02
1−7	48	*i*-PrOH/AN	0.755 ± 0.069
1−8	62	*i*-PrOH/cyclohexane	n.r.[d]
1−9	62	*i*-PrOH	n.r.[d]
		reduction of MA^+ (in alcohol containing 4.7% H_2O[e])	
1−10	67	*i*-PrOH	0.711 ± 0.013
1−11	67	*i*-PrOH-*2-d*	0.341 ± 0.063
1−12	67	*i*-PrOD	0.602 ± 0.025
1−13	67	*i*-PrOH/AN (v/v =1)	0.134 ± 0.018
1−14	82	*i*-PrOH/4.7% AN (v/v)	1.28 ± 0.21
1−15	82	*i*-PrOH	1.74 ± 0.17[f]

[a] $[PhXn^+]_0$ = 1 mM; $[MA^+]_0$ = 0.186 mM. [b] Based on three determinations. [c] For mixed solvents. [d] n.r. = no hydride-transfer reaction; only adduct production was observed. [e] v/v, unless otherwise indicated; Or D_2O for the use of *i*-PrOD. Note that the initial concentration of MA^+ (0.186 mM) is different from that (0.398 mM) used in our previous paper.[13] [f] Reference.[13]

reaction was observed. Spectral studies indicate that under these conditions $PhXn^+$ appears to be completely converted into the corresponding isopropyl alcohol adduct PhXnOPr-*i*.

Observed kinetic isotope effects (KIE^{obs}) at 62 °C were calculated and are listed in Table 2. The $KIE_{\alpha\text{-}D}{}^{obs}$ in first column reflects the kinetic isotope effect of the hydride-transfer process since the adduct formation equilibrium is not affected by the presence of D at the 2(or α)-position of the alcohol. The inverse KIE (row 1, column 2) represents the *solvent* KIE since *i*-PrOH acts as both reactant and cosolvent. The inverse $KIE_{OD}{}^{obs}$ is unlikely to originate from the facile O−H bond cleavage, with which the rate-determining C−H bond breaking cannot compete. The observed solvent $KIE_{OD}{}^{obs}$ should involve the isotope effect on the adduct formation equilibrium which involves O−H bond cleavage to form solvated H^+ (see the following paragraphs and the Discussion section). The last $KIE_{D8}{}^{obs}$ (row 1, column 3) is an overall value derived from the reaction of perdeuterated isopropyl alcohol. With the assumption that KIE is multiplicative, the β-secondary KIE observed upon deuteration of the two CH_3 positions of *i*-PrOH during the $PhXn^+$ reaction was calculated to be equal to 1.1 (row 1, column 4). Note that this KIE only applies to the hydride-transfer step since the presence of CD_3 groups would not be expected to affect the adduct formation equilibrium.

We have reported a deuterium $KIE_{D8}{}^{obs}$ of 3.8 for the hydride reduction of MA^+ at 67 °C in *i*-PrOH (or *i*-PrOD-d_7) containing 4.7% (v/v) H_2O (or D_2O).[13] We have now determined the rates of the reaction with *i*-PrOH-*2-d* and with *i*-PrOD in the same reaction medium at 67 °C (Table 1, entries 1-10−1-12). The $KIE_{\alpha\text{-}D}{}^{obs}$ = 2.1 and $KIE_{OD}{}^{obs}$ = 1.18 were derived, and the β-secondary kinetic isotope effect ($KIE_{\beta\text{-}D6}{}^{obs}$) is thus calculated to be 1.5 (Table 2, in row 2). Note that here the corresponding solvent KIE value ($KIE_{OD}{}^{obs}$ = 1.18) is normal rather than inverse (0.39) as was observed in the $PhXn^+$ reaction.

The rate constant of the reaction of *i*-PrOH with MA^+ in *i*-PrOH/AN (v/v = 1) at 67 °C was also determined (entry 1-13). The rate of the reaction is dramatically diminished (5.3-fold) compared to the reaction in *i*-PrOH containing 4.7% water (v/v) (entries 1-13 vs 1-10). In order to further understand the observed kinetic solvent effect, and because we have the rate data (entry 1-15) of the reaction in *i*-PrOH containing 4.7% water at 82 °C in hand for comparison,[13] the rate of the reaction in *i*-PrOH containing 4.7% AN at 82 °C was determined (entry 1-14). A reduction in rate (1.4-fold) resulted from the change of the cosolvent from water to AN was observed as well.

The method used to determine the rate constants of the MA^+ reaction was the same as that used in the kinetic determinations of the $PhXn^+$ reaction described above. The decay of absorbance with time at 358 nm due to MA^+ was recorded and used to determine the rate of the reaction.

The Effect of $[H^+]$ on the Kinetics of the Reactions of $PhXn^+$ and MA^+: An Opposite Effect. Since acid H^+ is involved in both of the assumed pathways (1) and (2), an examination of the hydride-transfer mechanism for the reactions of both $PhXn^+$ and MA^+ would need to take into account the effect of $[H^+]$ upon the kinetics of the reactions. Since acid favors the conversion of the adduct to the free cation, it is expected to facilitate pathway (1) but to inhibit pathway (2). This is apparent from the rate equations (3) and (4) for the formation of the hydride-transfer product (R−H) derived from pathways (1) and (2), respectively. Note that eqs 3 and 4 are only suitable when the protonation of *i*-PrOH to form *i*-$PrOH_2^+$, which does not undergo hydride-transfer reaction, is negligible. The rate of the reaction (1) increases with increasing $[H^+]$ until $[H^+] \gg K$ [*i*-PrOH] conditions under which the rate of the reaction no longer changes with $[H^+]$ and k^{obs} is equal to the rate constant of the hydride-transfer step (k^H), while that of the reaction (2) is expected to decrease with increasing $[H^+]$.

$$\begin{aligned} d[R-H]/dt &= -d[R^+]_T/dt \\ &= k^H[R^+]_T[i\text{-PrOH}][H^+]/([H^+] + K[i\text{-PrOH}]) \\ &= k^{obs}[R^+]_T[i\text{-PrOH}] \end{aligned} \quad (3)$$

$$\begin{aligned} d[R-H]/dt &= -d[R^+]_T/dt \\ &= k^H{}_{INTRA}[R^+]_T[i\text{-PrOH}]/([H^+] + K[i\text{-PrOH}]) \end{aligned} \quad (4)$$

The effects of added $[H^+]$ (= $[HBF_4]$) on the rate of the reaction of $PhXn^+$ in *i*-PrOH/AN (v/v = 1) and on that of MA^+ in *i*-PrOH containing 4.7% of water (v/v) were determined (Table 3, entries 3-1−3-2 for the $PhXn^+$ reaction, entries 3-6−3-8 for the MA^+ reaction). The effect of the $[H^+]$ on the rate of the $PhXn^+$ reaction in *i*-PrOH containing 2.5% water (v/v), whose rate was not measurable under neutral conditions, was also determined (entries 3-3−3-5). Since under the conditions studied the concentration range of the H^+ added is limited, a thorough investigation of the acid effect on the kinetics of the reaction is restricted. The current data, however, clearly show that an increase of $[H^+]$ accelerates the hydride-transfer reaction from *i*-PrOH to $PhXn^+$ but inhibits the rate of the reaction to MA^+.

Equilibrium Constant (*K*), Thermodynamic Parameters, and the Equilibrium Isotope Effect (EIE). In order to understand the effect of the adduct PhXnOPr-*i* formation equilibrium on the overall rate constant for the corresponding reaction (k^{obs}) and on the observed *inverse* $KIE_{OD}{}^{obs}$, the equilibrium constants (*K*) defined in eqs 1 and 2 at different temperatures in *i*-PrOH (or *i*-PrOD)/AN (v/v = 1) were determined using a spectral method based on the extinction

TABLE 2. Kinetic Isotope Effects on the Hydride-Transfer Reactions from *i*-PrOH to PhXn$^+$ and MA$^+$[a]

$KIE_{\alpha\text{-}D}^{obs}$[b] (*i*-PrOH vs *i*-PrOH-*2-d*)	KIE_{OD}^{obs}[b](*i*-PrOH vs *i*-PrOD)	KIE_{D8}^{obs}[b](*i*-PrOH vs *i*-PrOD-d_7)	$KIE_{\beta\text{-}D6}^{obs}$[b,c](*i*-PrOH vs $(CD_3)_2CHOH$)
	reduction of PhXn$^+$ in *i*-PrOH/AN (v/v = 1) at 62 °C		
4.4	0.39	1.9	1.1
	reduction of MA$^+$ in *i*-PrOH containing 4.7% water at 67 °C[d]		
2.1	1.18	3.8[e]	1.5

[a] $[PhXn^+]_0 = 1$ mM; $[MA^+]_0 = 0.186$ mM. [b] Definitions of KIE can be found in the context. [c] Equals $KIE_{D8}^{obs}/[(KIE_{\alpha\text{-}D}^{obs})(KIE_{OD}^{obs})]$ [d] Note that the initial concentration of the MA$^+$ (0.186 mM) is different from that (0.398 mM) used in our previous paper.[13] [e] From ref 13.

TABLE 3. Effect of Added [H$^+$] on the Observed Pseudo-First-Order Rate Constants for the Hydride-Transfer Reaction from *i*-PrOH to PhXn$^+$ and MA$^+$[a]

entry	reaction conditions	k^{obs} x10^3 (min^{-1})[b]
	reduction of PhXn$^+$ in *i*-PrOH/AN (v/v = 1) at 62 °C[c]	
3–1	$[H^+] = 0$ M	2.13 ± 0.09[d]
3–2	$[H^+] = 0.001$ M	7.13 ± 0.01
	in *i*-PrOH containing 2.5% water (v/v) at 62 °C[e]	
3–3	$[H^+] = 0.0098$ M	0.264 ± 0.001
3–4	$[H^+] = 0.098$ M	8.98 ± 0.38
3–5	$[H^+] = 0.196$ M	34.60 ± 0.30
	reduction of MA$^+$ in *i*-PrOH containing 4.7% water (v/v) at 82 °C[f]	
3–6	$[H^+] = 0$ M	1.74 ± 0.17[g]
3–7	$[H^+] = 0.050$ M	1.18 ± 0.14
3–8	$[H^+] = 0.196$ M	0.574 ± 0.006

[a] $[H^+] = [HBF_4]$; note that it is the concentration of the H$^+$ added rather than the real [H$^+$] in the reaction system. [b] Based on two determinations unless otherwise indicated. [c] $[PhXn^+]_0 = 1$ mM. [d] Same as entry 1-2. [e] $[PhXn^+]_0 = 0.398$ mM. [f] $[MA^+]_0 = 0.398$ mM. [g] Same as entry 1-15.

TABLE 4. Equilibrium Constants (*K*), Percentage Conversion, and Equilibrium Isotope Effect (EIE) for the Equilibration of PhXn$^+$ with PhXnOPr-*i* in *i*-PrOH (or *i*-PrOD)/AN (v/v = 1)

entry	*T* (°C)	*K*	% conversion	EIE[a]
4–1	69	0.00881	98	
4–2	62	0.0119	98	2.67
4–3	56	0.0108	99	
4–4	49	0.0123	99	

[a] EIE = *K*(*i*-PrOH)/*K*(*i*-PrOD).

coefficient of the PhXn$^+$ determined (Table 4). Interestingly, *K* does not change significantly within the temperature range from 49 to 69 °C. Using the van't Hoff equation, a small negative standard enthalpy change ($\Delta H° = -12.7$ kJ/mol) and a negative standard entropy change ($\Delta S° = -76$ J/mol *K*) were derived. The large negative $\Delta S°$ may be due to the formation of the adduct and the well-solvated proton in the equilibrium, which are expected to possess fewer degrees of freedom. Under the reaction conditions, the degree of conversion of the PhXn$^+$ to the adduct was calculated to be about 98%. The equilibrium isotope effect (EIE = *K*(*i*-PrOH)/*K*(*i*-PrOD)) was calculated to be 2.67 (Table 4), indicating that under these conditions the equilibrium concentration of the PhXn$^+$ is higher in *i*-PrOD than in *i*-PrOH.

The degree of conversion of MA$^+$ to MAOPr-*i* under the reaction conditions, i.e., in *i*-PrOH containing 4.7% of water, was too low to obtain an equilibrium constant. The equilibrium constant for the interconversion between 9-phenyl-10-methyl acridinium ion and its methanol adduct in methanol has been previously reported to be as small as 1.5×10^{-8} at 22 °C.[18] It would be reasonable to assume that the equilibrium between MA$^+$ and its isopropyl alcohol adduct would have an equilibrium constant (*K*) of a similar order of magnitude.

Intermolecular Mechanism (1) of the Hydride Reduction of PhXn$^+$. It appears clear that all of the results obtained from the reaction of PhXn$^+$ with *i*-PrOH are consistent with the intermolecular reaction pathway (1).

Hydride-transfer reaction was not observed in pure *i*-PrOH and in *i*-PrOH/cyclohexane (v/v=1) where all the PhXn$^+$ appeared to be converted to the adduct. The same result was observed when basic K_2CO_3 was added to the *i*-PrOH/AN system. The presence of the base facilitates the formation of the adduct. All these results suggest that the presence of the free cation is a prerequisite for the hydride-transfer reaction to occur. The function of the polar solvent, AN, may be to enhance ionization of the adduct to the PhXn$^+$ cation. This is also consistent with the observed catalytic effect of H$^+$ on the reaction. The greater than unity EIE value (2.67) and the inverse KIE_{OD}^{obs} (0.39) indicate that more free cation exists in the *i*-PrOD in which the reaction rate is higher, lending strong support for mechanism (1) (also see the Discussion section).

The $KIE_{\alpha\text{-}D}^{obs}$ (4.4) observed for the hydride-transfer process in the reduction of PhXn$^+$ at 62 °C is more likely consistent with the intermolecular mechanism that has a linear or slightly bent T.S. In general, KIE is lower in a hydrogen transfer reaction with a bent T.S. than that with a linear T.S.[19] The intramolecular mechanism requires a severely bent four-membered ring T.S., implying that a smaller KIE would be observed. The observed $KIE_{\alpha\text{-}D}^{obs}$ of 4.4 at 62 °C renders the latter mechanism less likely. It is of interest to compare the magnitude of the observed value with the KIE (4.1 at 52 °C) for the intermolecular hydride transfer from MAH (**5**) to 1-benzyl-3-cyanoquinolinium cation in acetonitrile recently reported by us.[20]

The observed normal β-secondary KIE ($KIE_{\beta\text{-}D6}^{obs} = 1.1$) is consistent with the intermolecular hydride-transfer reaction mechanism with a T.S. in which positive charge is developing on the α-C−OH group.[21] This type of KIE is believed to originate from the T.S. stabilization effect caused by the hyperconjugation between the β-C−H σ orbital and the p/or π orbital of the α-C$\cdots$OH group developing the positive charge.[21,22] Compared to the C-D bond, the more easily weakened C−H bond provides better orbital overlap for hyperconjugation, and promotes a normal KIE. [21,22] However

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TABLE 5. Pseudo-First-Order Rate Constants (k^H) for the Intermolecular Hydride-Transfer Step in the Reaction of $PhXn^+$ in *i*-PrOH(D)/AN (v/v = 1) as a Function of Temperature[a]

entry	Reaction conditions	$[H^+]_{eq}$ (mM)	k^H (min^{-1})	$KIE_{OD}{}^{H}$[b]
5–1	69 °C (*i*-PrOH)	0.982	0.250	
5–2	62 °C (*i*-PrOH)	0.979	0.196	
5–3	62 °C (*i*-PrOD)	0.968[c]	0.184	1.07
5–3	56 °C (*i*-PrOH)	0.987	0.0835	
5–4	49 °C (*i*-PrOH)	0.988	0.0595	

[a] $[PhXn^+]_0 = 1$ mM. [b] $KIE_{OD}{}^{H} = k^H(i\text{-PrOH})/k^H(i\text{-PrOD})$. [c] $[D^+]_{eq}$.

the KIE value cannot be used to distinguish between the two mechanisms (1) and (2) since hyperconjugation is also present involving the β−C-H σ orbital and the p/or π orbital of the developing polar α-C$^{\delta+}$---O$^{\delta-}$ group in the T.S. of the *concerted* intramolecular hydride-transfer process (2). This analysis also applies to the reaction of MA^+ in which a normal β-secondary KIE ($KIE_{\beta\text{-}D6}{}^{obs}$ =1.5) was observed as well.

Mechanism of the Hydride Reduction of MA^+ and the Relevant k^H and $KIE_{OD}{}^{H}$. It is reasonable to suggest that the hydride-transfer reduction of the more stable MA^+ follows the intermolecular mechanism (1) as well. An examination of the data in Table 3, however, shows that k^{obs} decreases as $[H^+]$ increases, opposite to the effect observed for the $PhXn^+$ reaction. This, at first sight, conflicts with mechanism (1). However, it is likely that the side equilibrium to form the unstable MAOPr-*i* adduct is insignificant in the overall reaction so that the introduction of acid lowers the rate of the reaction by H-bonding to and/or protonation of *i*-PrOH, lowering the hydride donation ability of *i*-PrOH.

The very small K value results in $K[i\text{-PrOH}] \ll [H^+]$ and rate eq 3 then reduces to; $d[MAH]/dt = -d[MA^+]/dt = k^H[MA^+][i\text{-PrOH}] \approx k^{obs}[MA^+][i\text{-PrOH}]$. This leads to the suggestion that in this case, k^{obs} can be considered to be the rate constant for the hydride-transfer process from *i*-PrOH to MA^+, i.e., $k^{obs} = k^H$. Therefore, the kinetic isotope effect for the hydride-transfer step accompanying deuteration at the OH group of the alcohol is $KIE_{OD}{}^{H} = k^H(i\text{-PrOH})/k^H(i\text{-PrOD}) = k^{obs}(i\text{-PrOH})/k^{obs}(i\text{-PrOD}) = KIE_{OD}{}^{obs} = 1.18$. The activation parameters for the hydride-transfer step have been reported for this reaction under similar conditions.[13]

Derivation of k^H, $KIE_{OD}{}^{H}$, and Activation Parameters for the $PhXn^+$ Reaction. The rate constants for the hydride-transfer step ($k^H(i\text{-PrOH})$ and $k^H(i\text{-PrOD})$) in the $PhXn^+$ reaction at different temperatures in *i*-PrOH(D)/AN (v/v = 1) were calculated by transforming the corresponding k^{obs}, K, [*i*-PrOH(D)], and $[H(D)^+]_{eq}$ into $k^{obs} = k^H[H^+]/([H^+] + K[i\text{-PrOH}])$ derived from the rate eq (3) (Table 5). The $[H(D)^+]_{eq}$ is expected to remain constant throughout most of the reaction according to the stoichiometric eq 1. Using the Arrhenius and Eyring equations, the activation parameters of the hydride-transfer process were derived; $E_a{}^H = 71.5$ kJ/mol, $\Delta H^{\ddagger H} = 68.6$ kJ/mol, $\Delta S^{\ddagger H} = -57.3$ J/mol·K. The corresponding $KIE_{OD}{}^{H} = k^H(i\text{-PrOH})/k^H(i\text{-PrOD})$ was calculated to be 1.07, a normal secondary KIE that reflects the O-D KIE on the hydride-transfer step. The KIE value is consistent with the secondary $KIE_{OD}{}^{H}$ of 1.18 for the hydride-transfer reaction of MA^+ (see the preceding section).

Discussion and Conclusions

Alcohols containing α-H act specifically as hydride donors when oxidized by NAD^+ coenzyme mediated by alcohol dehydrogenase. In addition to this reactivity, alcohols also function as nucleophiles when treated with NAD^+ analogues in less selective nonenzymatic reactions. The latter reactivity of the alcohol results in the possible formation of the alcohol adduct of the NAD^+ models. In this study, the adducts (PhXnOPr-*i* and MAOPr-*i*) were formed in side equilibria, complicating the study of the dehydrogenase model reactions between isopropyl alcohol and the two NAD^+ analogues $PhXn^+$ and MA^+. All of the results are consistent with the intermolecular hydride-transfer mechanism (1) (from *i*-PrOH to the cations). The alternative intramolecular hydride-transfer mechanism (2) requires a four-membered ring T.S., rendering the mechanism less likely. The adduct PhXnOPr-*i* is isolatable but readily decomposed into its precursor cation in polar solvents or under low acid concentration conditions. In contrast, the adduct MAOPr-*i* is extremely unstable such that the formation of this adduct is insignificant even under the conditions where *i*-PrOH is the major solvent component (*i*-PrOH containing 4.7% of water or AN). The observed primary $KIE_{\alpha\text{-}D}{}^{obs}$ (=$KIE_{\alpha\text{-}D}{}^{H}$) for both reactions indicates that the hydride-transfer step is rate-determining. The observed β-secondary kinetic isotope effect ($KIE_{\beta\text{-}D6}{}^{obs} = KIE_{\beta\text{-}D6}{}^{H}$) is consistent with a hydride-transfer T.S. in which positive charge has developed on the α-C−OH group of the alcohol moiety. The net hydride-transfer reaction of $PhXn^+$ (k^H) is faster than that of the reaction of MA^+ ($k^H = k^{obs}$) by ~3 orders of magnitude. For example, the former reaction in *i*-PrOH/AN (v/v = 1) at 69 °C is about 1800 times faster than that of the latter in the same medium at 67 °C (entry 5-1 vs entry 1-13).

Kinetic solvent isotope effects have played an important role in determining the acid-catalyzed reaction mechanisms.[23] Many studies of the kinetic effect brought about by a change of solvent from H_2O to D_2O have been reported.[24,25] Generally, reactions involving a pre-equilibrium formation of the conjugate acid (XH^+) of the substrate (X) (eq 5) were found to be faster in D_2O than in H_2O, resulting in inverse kinetic solvent isotope effects. This has been explained in terms of the known greater acid dissociation constants of weak acids in H_2O than in D_2O, resulting in higher equilibrium concentrations of the conjugate acid of the substrate in D_2O.[25,26] The observations are limited to the condition that the subsequent rate-determining step does not involve a proton or deuteron transfer, which accompanies a primary KIE that masks the equilibrium isotope effect contribution to the observed KIE.[27] In this study, a change of solvent/reactant from *i*-PrOH to *i*-PrOD resulted in a rate increase for the hydride-transfer reaction from isopropyl alcohol to $PhXn^+$. The latter is consistent with the kinetic effect for a change of solvent from H_2O to D_2O in the acid-catalyzed reactions (5), lending strong support for the intermolecular hydride-transfer mechanism (1). Our observation of the inverse $KIE_{OD}{}^{obs}$ (0.39) can be explained in terms of the greater equilibrium constant ($K(i\text{-PrOH})$) for formation of the adduct between $PhXn^+$ and *i*-PrOH as compared to that involving *i*-PrOD ($K(i\text{-PrOD})$) so that the cation necessary for the hydride-transfer reaction is present in higher concentration in the *i*-PrOD system. Although isotopic substitution of the alcohol O−H results in a normal isotope effect on the subsequent hydride-

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transfer step, the corresponding secondary KIE_{OD}^{H} (1.07) is masked by the EIE (2.67) of the side-equilibrium in the overall KIE of the reaction ($KIE_{OD}^{obs} = KIE_{OD}^{H}/EIE$).

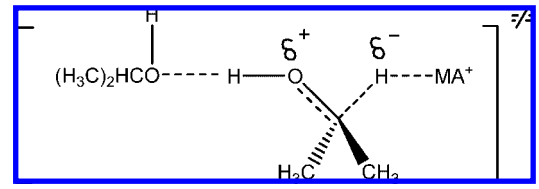
$$X + H^+ \overset{\text{rapid}}{\rightleftharpoons} XH^+ \xrightarrow{\text{slow}} \text{products} \qquad (5)$$

The EIE and KIE_{OD}^{H} can be explained in terms of fractionation factors (φ) for deuterium between ROH and ROH_2^+ sites and between ROH and its hydride-transfer T.S. R$\cdots$O$H^{\delta+}$ sites, respectively.[28] In these systems, φ can be defined to be the D/H ratio in positively charged OL^+ or $OL^{\delta+}$ bonds (L = H or D) divided by the D/H ratio in neutral OL bonds, i.e., $\varphi = (D/H)_{OL^+}/(D/H)_{OL}$.[28,29] A $\varphi = 0.69$, determined for the hydronium ion in aqueous solution, has been commonly assigned to all positively charged OL^+ bonds.[28,29] The less than unity φ value suggests a weaker O$-$L$^+$ bond than is an O$-$L bond. It also means that, in a deuterated solvent at room temperature, D has only 69% preference compared with H in the undeuterated solvent for placement in OL^+. In another sense, it also reflects that H_2O (or ROH) is a stronger base than is D_2O (or ROD). Thus, considering the equilibrium PhXn$^+$ + 2 *i*-PrOL $\leftrightarrow$ PhXnOPr-*i* + *i*-PrOL_2^+, and assuming that D and H have equal opportunity of being in *i*-PrOL, the EIE (K(*i*-PrOH)/K(*i*-PrOD)) of the equilibrium can be estimated to be $1^2/(0.69)^2 = 2.1$, not far from the value of 2.67 determined from this work. Note that in our equilibrium system L^+ may exist in a form other than *i*-PrOL_2^+, e.g. (*i*-PrOL)$_n$L$^+$, plus our experimental conditions (60 °C in *i*-PrOH/AN (v/v = 1)) are different from those used to obtain the 0.69 value, a quantitative comparison between the two EIE values (2.1 and 2.67) may thus be meaningless. Nevertheless, our larger than unity EIE and KIE_{OD}^{H} values correspond with the equilibrium process from OL to OL^+ and the activation process from OL to $[OL^{\delta+}]^{\ddagger}$, respectively. Also, that KIE_{OD}^{H} is masked by EIE in the overall KIE of the reaction may be relevant to the fact that the O-L bond in $[OL^{\delta+}]^{\ddagger}$ (carrying a partial positive charge) is stronger than that in OL^+ (bearing a full positive charge). Importantly, to the best of our knowledge, this is the first observation of KIE_{OD}^{H} in the hydride-transfer reaction involving alcohols as hydride donors. It is highly likely that the KIE_{OD} should also be observed in other types of reactions in which the T.S.'s contain a center of partial positive charge α to a hydroxyl group.

The observed $KIE_{\beta\text{-}D6}^{H}$ and KIE_{OD}^{H} are worthy of further comment. The results show that both β-C$-$D and O$-$D connected to the α-C of the alcohol exert isotope effects on the rate of the hydride transfer from α-C$-$H to the cations. The former is a result of a hyperconjugation effect involving the σ orbital of the β-C$-$H bond and the p/or π orbital of the α-C$\cdots$OH group where a partial positive charge is developing in the T.S., altering the β-C$-$H bond vibration frequency. The latter is a result of direct resonance effect involving a partial positive charge being delocalized onto the O$-$H group of the T.S. through the C$\cdots$O bond formation, affecting the vibration frequency of the O$-$H bond in it. Both effects are a consequence of the positive charge developing in the T.S. The O$-$D isotope effect (resulting from the direct conjugation effect) on the hydride-transfer process is expected to be greater than the β-C$-$D isotope effect (resulting from the hyperconjugation effect). This seems to be the case, at least in our systems. Note that the $KIE_{\beta\text{-}D6}^{H}$ values obtained in this work are a result of the cumulative effect of six β-C$-$D bonds.

One feature of interest is the rate reduction of the MA$^+$ reaction (~1.4-fold) when the cosolvent was changed from water (4.7%) to AN (entries 1$-$14 vs 1$-$15). When AN was increased to 50% (v/v), accompanied with a 2-fold decrease in the alcohol concentration, the rate of the reaction decreased by as large as 5.3-fold (entries 1-10 vs 1-13). Furthermore, in AN without *i*-PrOH as cosolvent, for example, [*i*-PrOH] = 0.04 M in AN, no reaction was observed over a period of days. This strongly suggests that the protic solvents, either *i*-PrOH or water, strongly stabilize the T.S. of the hydride-transfer process through H-bonding interactions with partial positively charged H of the alcohol moiety (Scheme 1). on the OH group of the alcohol moiety is highly developed in the T.S. Such a late T.S. is consistent with the observed small primary $KIE_{\alpha\text{-}D}^{H}$ (2.1) for the reaction of MA$^+$ and the relatively small normal secondary $KIE_{\beta\text{-}D6}^{H} = 1.5$ for the same reaction as well as $KIE_{\beta\text{-}D6}^{H} = 1.1$ for the reaction of PhXn$^+$. Note that a β-secondary KIE as large as 3.5 has been observed in the oxidation of isopropyl alcohol by a ruthenium(IV) complex.[21a]

SCHEME 1. Proposed Hydride Transfer T.S. Stabilized by *i*-PrOH through H-Bonding



This T.S. structural pattern can be used to understand the unexpected relatively small rate ratio for the hydride-transfer reaction of PhXn$^+$ and MA$^+$ (e.g., ~1.8×10^3 in *i*-PrOH/AN (v/v=1) at 69 °C), in spite of the large (~13 kcal/mol [30]) difference in thermodynamic stability of the two cations. Differences in the degree of H-bonding stabilization effect on the T.S.'s most likely diminish the difference in T.S. energies for the two hydride-transfer processes. This is interestingly quite consistent with the facile biological NAD$^+$ oxidation of alcohols where alcohol hydroxyl hydrogen apparently interacts favorably in the T.S. by H-bonding with the hydroxyl oxygen of an amino acid residue (e.g., serine) in the active site of alcohol dehydrogenases.[1b,2] Our results reflect the significance of the kind of base catalysis in lowering the T.S. energy of the biological hydride-transfer processes.

It should be mentioned that the hydride reduction of an NAD$^+$ model BNA$^+$ (**1**, R^1 = benzyl, R^2 = CONH$_2$) by alkyl alcohols including *i*-PrOH catalyzed by various Zn(II) complexes has been studied and found to be inefficient.[7,11,12] The addition of the Zn(II) complexes was meant to imitate the complexed zinc environment in the active site of the alcohol dehydrogenases.

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The Lewis acid Zn(II) complexes are believed to catalyze the hydride transfer from alcohol to NAD^+ by the formation of a reactive zinc alkoxide intermediate through Lewis acid catalyzed deprotonation of the alcohol.[1–6] Our observation of the hydride reduction of MA^+ by isopropyl alcohol, in which the adduct formation is insignificant, provides a model reaction for possible further study of the role of the Zn(II) complexes in the biological hydride-transfer reaction.

In conclusion, the kinetics, the deuterium isotope effects and the mechanism of the hydride-transfer reaction from isopropyl alcohol to two stable organic cations (NAD^+ analogues) were, for the first time, thoroughly studied in the parent alcohol medium containing acetonitrile or water. Both C−D and O−D connected to the α-C of the alcohol molecule exert isotope effects on the rates of the hydride transfers from the α-C−H to the cations. The observed *inverse* kinetic solvent isotope effect excludes the alternative intramolecular mechanism (2) for the hydride-transfer reaction. This model study also imitates the H-bonding stabilization effect on the T.S. of the alcohol dehydrogenase reaction. These reactions can be useful alcohol dehydrogenase model reactions, thus helping us understand this important biological hydride-transfer reaction and, further, the function of the Zn(II) ion in the active site of the alcohol dehydrogenases.

Experimental Section

General Procedures. 9-Phenylxanthylium ion ($PhXn^+BF_4^-$, **6**)[31] and 10-methylacridinium ion ($MA^+ClO_4^-$, **4**)[32] were synthesized according to the published procedures. The structures were characterized by comparing their ^{1}H NMR data and melting point values with those reported. Commercially available deuterated isopropyl alcohol (*i*-PrOD, *i*-PrOH-*2-d*, and *i*-PrOD-d_7) and the normal isopropyl alcohol were all purified before use by distillation over K_2CO_3. Acetonitrile was redistilled twice.

Formation of the Isopropyl Alcohol Adduct of the $PhXn^+$ (PhXnOPr-*i*, 7). $PhXn^+BF_4^-$ (0.02 g) was dissolved in 2 mL of AN followed by the addition of 10 mL of *i*-PrOH at room temperature. The yellow color of the reaction solution disappeared immediately. The solution was allowed to stay for another 10 min, and the isopropyl alcohol and acetontrile were evaporated off in a rotary evaporator. The addition of K_2CO_3 in the reaction hinders the decomposition of the adduct in the separation process. The PhXnOPr-*i* of quantitative yield was extracted with CH_2Cl_2: mp 153−155 °C; ^{1}HNMR (δ, CD_3Cl) 6.95−7.45 (m, 13H), 3.42−3.55 (septet, 1H), 0.73−0.82 (d, 6H).

Formation of the hydride-Transfer Product of $PhXn^+$ (PhXnH, 8). Instead of the separation of the adduct, the above reaction solution was subjected to reflux conditions (∼82 °C). After about 6 h, TLC results showed that the adduct PhXnOPr-*i* disappeared and the hydride-transfer product PhXnH was formed. The reaction solution was allowed to reflux for another 2 h, and the *i*-PrOH and AN were removed in a rotary evaporator. Extraction of the residue with CH_2Cl_2 gave PhXnH with a quantitative yield: mp 140−142 °C (lit.[33] mp 145 °C); ^{1}HNMR (δ, CD_3Cl) 6.94−7.32 (13H), 5.22−5.28 (s, 1H).

Determination of the Equilibrium Constant (*K*). The equilibrium constant (*K*) of the PhXnOPr-*i* formation equilibration was determined spectroscopically (UV−vis) by immediately recording the absorbance at 373 nm (ϵ = 30503) attributable to $PhXn^+$ upon adding various concentration of $PhXn^+$ to *i*-PrOH/AN (v/v = 1) at different temperatures. Two parallel determinations were performed.

General Kinetic Determination Procedure. A certain amount of the stock solution of $PhXn^+$ in AN and MA^+ in water or in AN was added to 8 mL *i*-PrOH containing AN or water in a well sealed 10 mL reaction vial thermostatted in a water bath of certain temperature (<70 °C). About 300 μL of the reaction aliquots were periodically taken into a sample vial precooled in an ice-bath. The samples were kept in a freezer (∼−20 °C) until six to eight reaction aliquots within 1−3 half-lives of the reaction were collected. The aliquots were analyzed by diluting certain amount of them in AN containing 3 M $HClO_4$ (for the reaction of $PhXn^+$) and in AN containing 30 mM of the Lewis acid $Zn(OTf)_2$ (for the reaction of MA^+) and determining the corresponding UV spectra. The absorbance (Abs) decays with time at 373 nm ($PhXn^+$) or at 358nm (MA^+) were recorded. The obtained Abs−*t* data were fitted into the first-order integrated rate equation, $-\ln(\text{Abs}) = kt + \text{constant}$, and the slope of the linear plot of $-\ln(\text{Abs}) - t$ was taken as the pseudo-first-order rate constant (k^{obs}) of the reaction. The linearity of the plots was usually of a regression coefficient of $R^2 > 0.996$. The kinetic determinations were repeated at least three times in most cases, and the standard deviations were reported.

The procedure used to determine the rate constants of the MA^+ reaction at refluxing temperature of *i*-PrOH (82 °C) has been reported in the Supporting Information section of our previous communication.[13]

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