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Steric Effects on the Primary Isotope Dependence of Secondary Kinetic Isotope Effects in Hydride Transfer Reactions in Solution: Caused by the Isotopically Different Tunneling Ready State Conformations?

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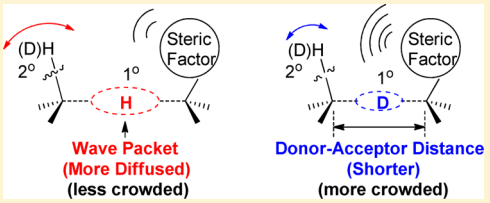
Supporting Information

ABSTRACT: The observed 1° isotope effect on 2° KIEs in H-transfer reactions has recently been explained on the basis of a H-tunneling mechanism that uses the concept that the tunneling of a heavier isotope requires a shorter donor−acceptor distance (DAD) than that of a lighter isotope. The shorter DAD in D-tunneling, as compared to H-tunneling, could bring about significant spatial crowding effect that stiffens the 2° H/D vibrations, thus decreasing the 2° KIE. This leads to a new physical organic research direction that examines how structure affects the 1° isotope dependence of 2° KIEs and how this dependence provides information about the structure of the tunneling ready states (TRSs). The hypothesis is that H- and D-tunneling have TRS structures which have different DADs, and pronounced 1° isotope effect on 2° KIEs should be observed in tunneling systems that are sterically hindered. This paper investigates the hypothesis by determining the 1° isotope effect on α- and β-2° KIEs for hydride transfer reactions from various hydride donors to different carbocationic hydride acceptors in solution. The systems were designed to include the interactions of the steric groups and the targeted 2° H/D's in the TRSs. The results substantiate our hypothesis, and they are not consistent with the traditional model of H-tunneling and 1°/2° H coupled motions that has been widely used to explain the 1° isotope dependence of 2° KIEs in the enzyme-catalyzed H-transfer reactions. The behaviors of the 1° isotope dependence of 2° KIEs in solution are compared to those with alcohol dehydrogenases, and sources of the observed "puzzling" 2° KIE behaviors in these enzymes are discussed using the concept of the isotopically different TRS conformations.



INTRODUCTION

The rule of the geometric mean formulated by Bigeleisen in 1955 states that there should be no isotope effects on isotope effects in chemical reactions.[1] For H-transfer reactions the transferring ("in-flight") primary (1°) isotopes of H/D/T do not affect the isotope effects at the "in-place" secondary (2°) H/D/T positions in the H-donor or acceptor, and vice versa.[2] However, violations of the rule were often seen in the enzyme-catalyzed H-transfer reactions.[3−9] Currently, there are two explanations in vogue, both of which use the concept of 1° H-tunneling. One explanation uses the model of 1°/2° H coupled motions in a traditional H-tunneling mechanism,[3,9,10] while the other uses the concept that the donor−acceptor distance (DAD) is shorter in D-tunneling than in H-tunneling.[11−13] The latter concept has been described in the Marcus-like H-tunneling model and in the large-curvature H-tunneling model.[14−18] In the coupled motions model, the 2° C−H bond out-of-plane bending vibrational modes are coupled with the vibrations of the transferring 1° H thus are a component of the reaction coordinate. This results in an inflated 2° kinetic isotope effect (KIE).[4,19−22] The inflation of the 2° KIEs is larger in a tunneling mechanism, and since H-tunneling is more probable than D-tunneling, more inflation in H-tunneling processes is expected.[4,10] Thus, the 1° isotope has an effect on 2° KIEs. In the Marcus-like and large-curvature H-tunneling models, the shorter DAD required for D-tunneling, as compared to H-tunneling, could bring about spatial crowding that in turn stiffens the vibrations of the 2° H and reduces the 2° KIE.[12,13,18] This also explains the 1° isotope effect on 2° KIEs. The model of 1°/2° H coupled motions combined with a H-tunneling mechanism was proposed in early 1980s and has been widely used in analyzing enzyme-catalyzed H-transfer reactions.[5,9,23−26] It, however, encountered challenges in explaining the 2° KIEs that are smaller than the semiclassically expected value (i.e., deflated 2° KIEs) in D-tunneling processes of hydride transfer reactions mediated by a series of alcohol dehydrogenases and mutants.[11−13] While both explanations work for the understanding of the observed unequally inflated 2° KIEs in H- and D-tunneling, the observed deflated 2° KIEs appear to require using the concept that the DAD is shorter in D-tunneling resulting in a more pronounced steric isotope effect.[11−13]

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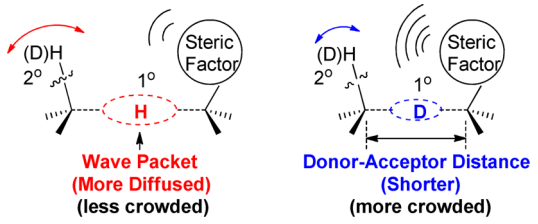
The explanation that uses the concept of a shorter DAD for D-tunneling than for H-tunneling leads to a new physical organic research direction. Namely, "how does structure affect the 1° isotope dependence of 2° KIEs?" and "how, in turn, does this dependence provide information about the structure of the tunneling ready states?" The hypothesis is that H-tunneling and D-tunneling have tunneling ready state structures which have different DADs, and that pronounced 1° isotope effect on 2° KIEs should be observed in tunneling systems that are sterically hindered (Scheme 1). This paper will test the hypothesis by

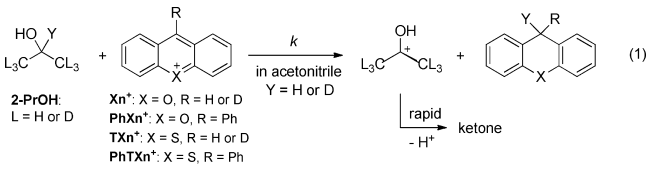
Scheme 1[a]



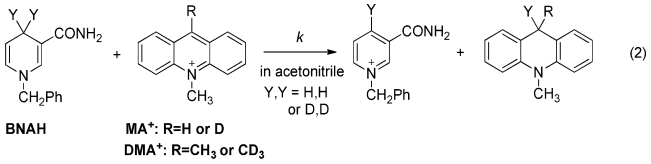
[a]It is hypothesized that the steric/crowding factors will increase the 1° isotope effect (i.e., the DAD effect) on the 2° KIEs. The 2° H(D) are at the α- or the β-position. The oval-shaped area is meant to show the wave packet of the transferring H/D in the tunneling ready states. DAD stands for donor−acceptor distance.

determining the 1° isotope effect on 2° KIEs for hydride transfer reactions from various hydride donors to different carbocationic hydride acceptors. Systems are designed to include close approach of the steric groups and the targeted 2° H/D's in the tunneling ready states (Scheme 1). Moreover, these systems are also designed with the idea that they might facilitate understanding the behaviors of the 1° isotope dependence of 2° KIEs in alcohol dehydrogenases.

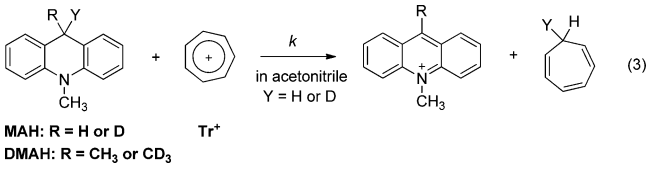
One of our designs uses hydride transfer reactions from 2-propanol (2-PrOH) to xanthylium ion (Xn^+), thioxanthylium ion (TXn^+) and their 9-phenylsubstituted analogues ($PhXn^+$ and $PhTXn^+$) (counterions: BF_4^- or ClO_4^-) in acetonitrile (eq 1). These reactions use a sequential hydride-proton transfer



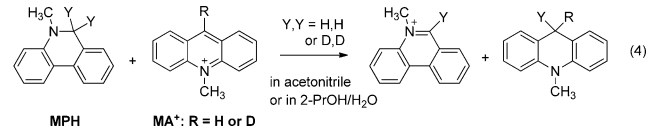
mechanism in which the hydride transfer is rate-determining.[27−29] The 2° KIEs are those on both the 2-propanol (β-H_6/D_6) and the (T)Xn^+ (α-9-H/D).[28,30,31] Since the cations with the 9-Ph group has a high steric hindrance at the reaction center, a comparison of the effect of the 1° isotope on the β-2° KIEs on 2-PrOH in its reactions with Xn^+ vs $PhXn^+$ and with TXn^+ vs $PhTXn^+$ can examine the effect of steric hindrance on the 1° isotope dependence of the β-2° KIEs. Furthermore, since the relatively bulky β-2° CH_3 group has more spatial interactions than α-2° H with other structures in the reactive complex, it is expected that the β-2° KIEs will be more sensitive to the 1° isotope effect. Therefore, a comparison of the 1° isotope dependence of β-2° KIEs with that of α-2° KIEs can also provide information about the steric effect on the 1° isotope effect on the 2° KIEs. To further understand the latter, we will also study the hydride transfer reactions from 1-benzyl-1,4-dihydronicotinamide (BNAH) to 10-methylacrdinium ion (MA^+) and 9,10-dimethylacrdinium ion (DMA^+) (counterions: BF_4^-) in acetonitrile (eq 2); and the hydride transfer reactions



from the reduced forms of MA^+ and DMA^+, i.e., MAH and DMAH, to a more reactive tropylium ion ($Tr^+BF_4^-$) in the same solvent (eq 3). The 1° isotope effects on 2° KIEs at the 9-



α-H/D position of the MA^+/or MAH and that at the bulkier 9-β-CH_3/CD_3 position of the DMA^+/or DMAH will be determined and compared. Furthermore, our predictions are inconsistent with the reported pronounced 1° isotope dependence of α-2° KIEs on MA^+ in its reaction with another dihydropyridine hydride donor (5-methyl-5,6-dihydrophenathrine (MPH)) by Ostovic, Roberts and Kreevoy (eq 4), which



has been cited frequently in the discussion of the 1° isotope effect on 2° KIEs in literature.[19] We will reinvestigate the 2° KIEs of the reaction both in the 2-PrOH/H_2O solvent that those authors used and in acetonitrile that we use, and compare the results with observations from our reactions. We will show that our results, including especially from the unprecedented study of 1° isotope effect on the β-type 2° KIEs, are consistent with our hypothesis, and furthermore discuss why our observations cannot be explained by the traditional model of 1°/2° H coupled motions combined with a tunneling mechanism. We propose that this study should be extended to other types of H-transfer reactions to examine how general the concept of isotopically different tunneling ready state conformations in explaining the 1° isotope effect on 2° KIEs in this class of reactions might be. Then the observed behaviors of the 1° isotope dependence of 2° KIEs are compared to the previously observed 2° KIEs in the alcohol dehydrogenases, and the origins of the latter observations are discussed on the basis of the tunneling DAD concept.

RESULTS

β-CH_3/CD_3 2° KIEs Are More Sensitive to the 1° Isotope Effect than the α-H/D 2° KIEs. Table 1 lists the β-2° KIEs at the H_6/D_6 position of 2-PrOH for both the hydride and deuteride transfers to the (T)Xn^+ and Ph(T)Xn^+ (1). Table 2 lists the α-2° KIEs at the 9-H/D position of the Xn(9-L)$^+$ and TXn(9-L)$^+$ (L = H or D) (1). The α-2° KIEs at the benzylic H/D position of the benzyl alcohol in its reaction with NAD^+ in an alcohol dehydrogenase are also included in Table 2

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Table 1. β-H_6/D_6 2° KIEs on 2-PrOH for Its Reaction with the Carbocations[a,b]

alcohol	$CL_3CY(OH)CL_3$			
cations	Xn^{+c}	$PhXn^{+c,d}$	TXn^+	$PhTXn^+$
Y = H	1.05 (0.02)	1.05 (0.03)	1.05 (0.03)	1.04 (0.01)
Y = D	1.04 (0.02)	1.00 (0.02)	1.04 (0.01)	0.98 (0.01)

[a]In acetonitrile at 60 °C; numbers in parentheses are standard deviations. [b]Y is 1° isotope; L is 2° isotope (α-C changes from sp^3 to sp^2). [c]From refs 28, 30. [d]See SI for a detailed analysis of the raw data.

Table 2. α-2° H/D KIEs on Carbocations for Their Reactions with Alcohols and That on Benzyl Alcohol for Its Oxidation by NAD^+[a,b]

alcohols	$CH_3CY(OH)CH_3$		PhCYLOH
cations	$Xn(9\text{-}L)^{+c}$	$TXn(9\text{-}L)^+$	NAD^{+d}
Y = H	0.99(0.01)[e]	0.97(0.02)[e]	1.20[f]
Y = D	0.98(0.03)[e]	0.96(0.03)[e]	1.12[f]

[a]In acetonitrile at 60 °C; numbers in parentheses are standard deviations. [b]Y is 1° isotope; L is 2° isotope. [c]From ref 30. [d]Converted from the observed H/T KIEs from ref 13 using KIE(H/T) = [KIE(H/D)][1.42,2] catalyzed by the yeast alcohol dehydrogenase. [e]At the 9-H/D positions of Xn^+ or TXn^+ (9-α-C changes from sp^2 to sp^3). [f]At the benzylic H/D position of PhCYLOH (α-C changes from sp^3 to sp^2).

for comparison (see later discussions in this paper).[13] All the α-2° KIEs in solution reactions are 1° isotope independent. The β-2° KIE on 2-PrOH is 1° isotope independent in the reactions of both Xn^+ and TXn^+, but is 1° isotope dependent in the reactions of both of their bulky 9-Ph substituted derivatives. This shows that the steric factor enhances the effect of the 1° isotope on β-2° KIEs in these systems. The trends are *consistent* with the observations in the hydride transfer eqs 2−4. Table 3

Table 3. α- and β-2° KIEs on (D)MA^+ for Their Reactions with BNAH[a,b]

H-donor	BNAH-4,4′-Y,Y	
cations	$MA(9\text{-}L)^{+c}$	$DMA(9\text{-}CL_3)^{+d}$
	α-H/D 2° KIE	β-H_3/D_3 2° KIE
Y = H	0.99 (0.02)	1.07 (0.03)
Y = D	0.99 (0.01)	0.99 (0.01)

[a]In acetonitrile, Y is 1° isotope, L is 2° isotope; numbers in parentheses are standard deviations. [b]On cation (9-α-C changes from sp^2 to sp^3). [c]At 24 °C. [d]At 32 °C.

shows that 1° isotope has no effect on α-2° KIEs at the 9-H/D position of the MA^+ and significant effect on the β-2° KIE at the 9-CH_3/CD_3 positon of the DMA^+ in their reactions with BNAH (2). The significant effect of the 1° isotope on the β-2° KIEs at the 9-CH_3/CD_3 positon of DMAH in its hydride transfer reactions to Tr^+ (3) are included in Table 4. Table 5 shows no 1° isotope effect on the α-2° KIEs on MA^+ in its reaction with MPH both in acetonitrile and in 2-PrOH/H_2O (4/1, v/v). The literature results from Ostovic, Roberts and Kreevoy that *deviate significantly* from ours are also included in Table 5 for comparison. The 1° KIEs of eqs 1−3 are listed in the following Table 6 to indicate that the hydride transfers are involved in the rate-limiting steps.

Activation Parameters and Calculation Provide Evidence about the Steric Effect on the Targeted 2° H's Vibrations in Tightly Associated Reactive Complexes. Activation parameters for H-transfer processes in eqs 1 to 3 are listed in Table 6. The observed higher activation enthalpies in the crowded systems of $PhXn^+$, DMA^+, DMAH (vs Xn^+, MA^+, MAH) suggest that the steric effects most likely play a role in decreasing the rate of these reactions. The observed large negative activation entropies especially in the crowded systems suggest that the transition states are tightly associated complexes, which in turn suggests that the steric factor likely hinders/stiffens the 2° H's vibrations in the reactive complexes.

The tightly associated reactive complexes may result from the n−π and π−π interactions between the O in 2-PrOH (in eq 1) or between the π-bonds on the dihydropyridine rings (in eq 2 to 4) with the electron-deficient heteroaromatic carbocations. In order to acquire the geometric structure of the tunneling reactive complex (also called tunneling ready state or TS for H-tunneling) and to gain information regarding how the group interaction affects the vibrations of the 2° H/D during the formation of the tunneling ready state, we carried out gas-phase calculations to obtain the classical TS structures for the 2-PrOH/Xn^+ and 2-PrOH/$PhXn^+$ systems (Figure 1 and SI for details). Note that the differences caused by the phenyl for H substitution in the classical TS's is expected to be similar for the tunneling ready states (even though the two differ in DAD, the hybridization of the reaction centers and the trajectory of the H-transfer).[32] Calculation confirms that these are tight complexes in the sense that the electron clouds from the reacting entities are mutually penetrating even in areas that are not forming covalent bonds (i.e., the OH group with the central ring of the (Ph)Xn^+). Importantly, there is apparent steric interaction between the Ph-group in $PhXn^+$ and the β-CH_3 groups in 2-PrOH, but little steric interaction can be seen in the reaction of Xn^+.

The formation of π−π charge-transfer complex between nitrogen heterocycles and electron deficient cyclic π systems similar to the substrates in eqs 2−4 have been reported.[33−36] These strongly suggest tightly associated tunneling ready state structures in our dihydropyridine oxidation reactions.

Table 4. α- and β-2° KIEs on (D)MAH for Their Reactions with the Tropylium Ion (Tr^+)[a,b]

H-donors	$MAH(9,9'\text{-}Y,L)^+$	$DMAH(9\text{-}Y\text{-}9'\text{-}CL_3)^+$
	α-H/D 2° KIE	β-H_3/D_3 2° KIE
Y = H	1.09 (0.04)[c]	0.97 (0.03)
Y = D	−[c]	0.91 (0.02)

[a]In acetonitrile at 35 °C, Y is 1° isotope, L is 2° isotope; numbers in parentheses are standard deviations. [b]On H-donor (9-α-C changes from sp^3 to sp^2). [c]Estimated indirectly from the rates of the reactions of MAH(9,9′-H,H), MAH(9,9′-H,D) and MAH(9,9′-D,D) by assuming 2° KIE is 1° isotope independent (see the derivation from SI) .

Table 5. Comparison of the α-H/D 2° KIEs on MA^+ for Its Reduction by MPH-6,6′-Y,Y with Those Reported in Literature[a,b]

solvents	acetonitrile	2-PrOH/H_2O (4/1, v/v)	
Y = H	0.98 (0.02)	1.00 (0.03)	1.05[c]
Y = D	0.99 (0.03)	1.01 (0.03)	0.96[c]

[a]At 22 °C (unless otherwise indicated), numbers in parentheses are standard deviations; Y is 1° isotope, L is 2° isotope. [b]On $MA(9\text{-}L)^+$ which reaction center changes from sp^2 to sp^3. [c]From ref 19, at 25 °C.

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Table 6. 1° KIEs and Activation Parameters for the Hydride Transfer Reactions in Acetonitrile

reaction system	2-PrOH/Xn^+ (eq 1)	2-PrOH/$PhXn^+$ (eq 1)	BANH/MA^+ (eq 2)	BNAH/DMA^+ (eq 2)	MAH/Tr^+ (eq 3)	DMAH/Tr^+ (eq 3)
temp (°C)	22.5−68.5	22.0−67.0	4.5−29.5	17.0−48.0	15.0−55.0	15.0−55.0
1° KIE	2.57 ± 0.31 (68.5 °C)	3.03 ± 0.12 (67.0 °C)	3.96 ± 0.03 (29.5 °C)	3.49 ± 0.18 (48.0 °C)	4.02 ± 0.16 (55.0 °C)	3.42 ± 0.20 (55.0 °C)
$\Delta H^{\ddagger}$ (kJ/mol)	44.8 ± 0.6	56.9 ± 0.8	21.2 ± 1.1	25.5 ± 1.0	41.7 ± 1.0	47.3 ± 1.1
$\Delta S^{\ddagger}$ (J/mol K)	−123.1 ± 1.9	−141.9 ± 2.4	−137.9 ± 3.7	−148.2 ± 3.2	−92.4 ± 3.4	−110.5 ± 3.4

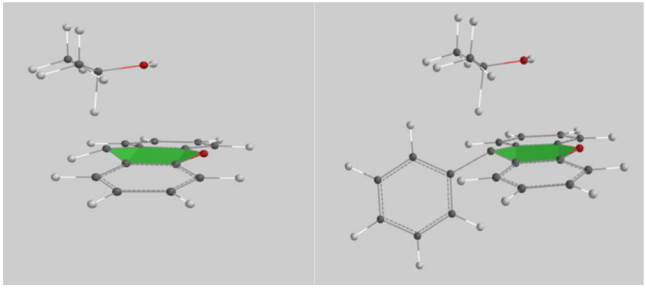

Figure 1. Calculated classical TS structures for the reactions of 2-PrOH with Xn^+ (left) and $PhXn^+$ (right) in gas phase. The tunneling ready state structures are expected to have similar geometric differences. Steric interaction between the β-CH_3 groups in 2-PrOH and the 9-Ph group in $PhXn^+$ is apparent.

DISCUSSION

Theoretical H-Tunneling Models That Require a Shorter DAD for Transfer of a Heavier Isotope. Prior to the discussion of the structural effects on the 1° isotope dependence of 2° KIEs, it is necessary to describe the newly established Marcus-like H-tunneling model that invokes the DAD concept. This full tunneling model was established on the basis of the Marcus theory of electron tunneling to include the short DAD sampling necessary for H-tunneling to occur.[12,14−17,37−40] Achieving a tunneling-ready state requires that (i) the system must reach a point of energy degeneracy between the reactant and product, and (ii) the system must reach a DAD short enough that the barrier to tunneling is sufficiently narrow. Both processes are assisted by heavy atom motions. Process (i), sometimes referred to as reorganization, has skeletal changes in both the donor and the acceptor. This involves hybridization changes and thus determines the 2° KIEs. Process (ii), known as gating, samples a range of conformations at various DADs. It determines the extent of 1° H wave function overlap between the reactant and product states, and thus the extent of H-tunneling and the 1° KIE. Since the D wave function is more localized than the H wave function, a shorter DAD is required for D-tunneling. This DAD difference results in different tunneling ready state structures for the hydrogen isotopes.[13] A schematic description of the new Marcus-like model that clearly shows the different DADs in H- and D-tunneling processes can be found in ref 13. It should be emphasized that this DAD difference is also required in the large-curvature H-tunneling model.[18] Therefore, the effect of the 1° isotope on 2° KIEs is brought about by the effect of the DAD on 2° KIEs within both models. Note that calculations following the Marcus-like model that reproduce the 2° KIEs on benzyl alcohol oxidation by the yeast alcohol dehydrogenase have shown that the DAD difference between H-tunneling and D-tunneling is about 0.2 Å.[41]

"Unusual" 2° KIEs and the Steric Effect on the 1° Isotope Dependence of β-Type 2° KIEs. *Hydride Transfer from 2-PrOH to (T)Xn^+ and Ph(T)Xn^+ (Eq 1, Tables 1 and 2).* The β-2° KIEs on 2-PrOH are generally normal (>1) and those on the cations are inverse (<1), consistent with the semiclassical predictions for the donor hybridization change from sp^3 to sp^2 and the acceptor hybridization change from sp^2 to sp^3. However, the magnitudes of the 2° KIEs are "unusual". In a classical mechanism, 2° KIEs fall within the range from unity to the corresponding equilibrium isotope effect (EIE).[42−44] When the 2° KIE is close to unity an early TS is suggested, and when it is close to the EIE a late TS is suggested. We have estimated the β-H_6/D_6 2° EIE for the conversion from $CL_3CH(OH)CL_3$ to $CL_3C^+(OH)CL_3$ to be 1.51, and we take the EIE of 0.89 previously reported for the C-4 H/D position of the NAD^+ (for its conversion to NADH) to be the EIE for the α-9-H/D in the conversion from (T)Xn^+ to (T)XnH.[30,31] Thus, both the β-H_6/D_6 2° KIEs on 2-PrOH and the α-H/D KIEs on Xn^+ and TXn^+ are close to unity and far away from the respective EIEs, suggesting that the TS's are early in terms of the rehybridization of the reaction centers. This, however, is inconsistent with the endothermic nature of the hydride transfer processes that produce a highly reactive $CL_3C^+(OH)$-CL_3 carbocation product, for which a late TS is predicted by the Hammond's Postulate. It is also inconsistent with the large negative Hammett constant ($\rho = -2.67$) reported by us[31] for the reactions of substituted benzyl alcohols with $PhXn^+$, which also suggests a late TS. Therefore, the observed β-H_6/D_6 2° KIEs on 2-propanol are deflated with respect to the semiclassical prediction of close to 1.51, and the observed α-H/D KIE on (T)Xn^+ are inflated with respect to the semiclassical prediction of close to 0.89. These 2° KIE results are inconsistent with a classical H-transfer mechanism.

Within the Marcus-like H-tunneling model, the observed 2° KIEs (close to unity for both 2-PrOH and the (T)Xn^+ cations) can be explained in terms of the small degree of rehybridization/reorganization of the reaction centers during the formation of the tunneling ready state. In the reactions of Xn^+ and TXn^+, the steric hindrance effect at both reaction centers is insignificant; so a decrease in DAD resulted from the change from H- to D-tunneling does not change the degree of rehybridization/reorganization, and hence the size of 2° KIEs, to an extent that can be observed experimentally (cf., Figure 1, the left structure) (Scheme 2 (a)). Therefore, the 2° KIEs at both the α-H/D position of the (T)Xn^+ and that at the β-H_6/D_6 position of the 2-PrOH show little dependence on the 1° isotope. In the reactions of Ph(T)Xn^+, however, the steric hindrance effect between the Ph-group of the cations and the β-CH_3 groups of 2-PrOH becomes significant, thus hindering the reorganization of the 2° CH_3 groups that are expected to swing toward the Ph-group during the sp^3 to sp^2 conversion (cf., the right structure in Figure 1). Hence, a decrease in DAD for the reactions involving the Ph-containing cations will hinder the β-$CH_3(CD_3)$ reorganization (or stiffen the β-H/D vibrations to increase the 2° H/D zero point energy difference) more than in the case of (T)Xn^+. As a result, the decrease of the 2° KIEs from H- to D-tunneling is pronounced (Scheme 2 (b)). The

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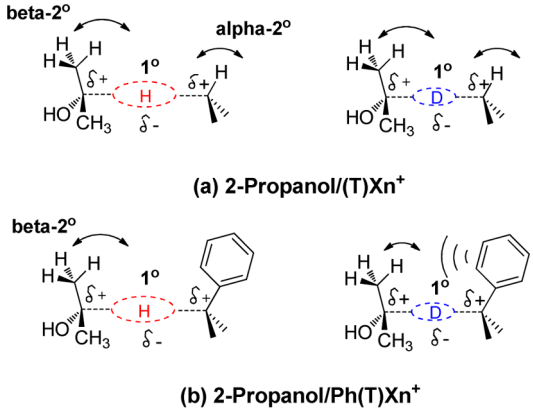
Scheme 2. Schematic Description of the Tunneling Ready States for the Alcohol Oxidation[a]



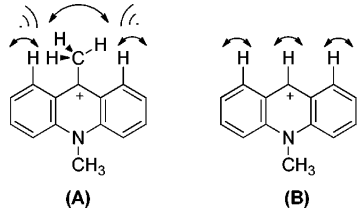
[a](a) The vibrations of the β-CH_3 bonds of 2-PrOH and the 9-α-H of $(T)Xn^+$ do not interfere with each other in either H- or D-tunneling. (b) The Ph group in $Ph(T)Xn^+$ significantly hinders the vibrations of the sterically readily accessible β-CH_3 group in 2-PrOH more in the D-tunneling ready state that requires a shorter DAD.

decreased 2° KIEs due to the steric effect (here brought about by a decrease in DAD) can also be understood using the concept that the 2° C−D bond is slightly shorter than the C−H bond (by about 0.001 Å) due to the difference in the vibrational extent of the C−H(D) bonds.[45−47] That is, the shorter 2° C−D bond encounters less steric hindrance during the formation of the tunneling ready state, making the rate decrease due to the steric effect less than that for the 2° C−H substrate reaction and resulting in a diminished 2° H/D KIE.[47−51] Note that this isotopic bond-length difference is too small to differentiate the DADs for the 1° H- and D-tunneling and thus to affect the 1° KIEs significantly (the latter DAD difference is about 0.2 Å (see above)). The above inference has been recently substantiated by our computations (using the Marcus-like H-tunneling model) that reproduce the 2° KIE behaviors in the reactions of both Xn^+ and $PhXn^+$.[32,52]

Hydride Transfer from BNAH to MA^+ and DMA^+ (Eq 2, Table 3). The hydride affinities of BNA^+ (the oxidized form of BNAH) and MA^+ from literature are 243 and 318 kJ/mol (in DMSO), respectively.[53] This indicates that the reaction is highly exothermic which, according to the Hammond's Postulate, suggests an early TS. The hybridization of the reaction center at the MA^+/DMA^+ changes from sp^2 to sp^3. Therefore, the 2° KIEs are expected to be inverse and close to unity. The observed α-2° KIE on MA^+ for the hydride transfer process is indeed inverse (<1) and close to unity, but the β-2° KIE on DMA^+ is inflated to be >1 (1.07), and it becomes inverse for deuteride transfer. While the loss of the conjugative effect between the β-$CH(D)_3$ and the 9-C^+ as a result of conversion from sp^2 to sp^3 should lead to an inverse 2° KIE, the 2° KIE behaviors in these systems appear to be inconsistent with the classical H-transfer process.

The α-2° KIE on MA^+ (inverse and close to unity) can be rationalized in terms of a small degree of rehybridization/reorganization at the reaction center during the formation of the tunneling ready state, similar to the explanation for the observed small α-2° KIEs on $(T)Xn^+$ in their oxidation of alcohols. Knowing the size difference between the 9-H of MA^+ and the 9-CH_3 of DMA^+, the inflation in the β-2° KIE (from an expected inverse value to 1.07) on the latter cation may be explained in terms of a release of the spatial crowding interaction between vibrations of its 9-CH_3 group and 4,5-H groups during a sp^2 to sp^3 conversion (structure (**A**) in Scheme 3). The release of the crowding effect is more for a longer C−H

Scheme 3. [a]



[a]Release of intramolecular spatial crowding effect between vibrations of the 9-$CH(D)_3$ group and the 4,5-H's in DMA^+ for an sp^2 (structure (**A**)) to sp^3 conversion results in a normal β-2° KIE. Conversely, a gain of the steric effect for an sp^3 (in DMAH) to sp^2 (DMA^+) conversion will result in an inverse 2° KIE (see Discussion for the reaction of DMAH). No such steric release effect is involved between vibrations of 9-H and 4,5-H's in MA^+ (structure (**B**)).

bond (than for a C−D bond) thus resulting in an inflated β-CH_3/CD_3 2° KIE. So the observed inflated 2° KIE of 1.07 suggests that the KIE caused by the intramolecular steric hindrance release[54] (should be >1) wins over the effect of the hyperconjugative loss (<1) in determining the 2° KIE. In the reaction of MA^+, however, the 9-H group on MA^+ does not have an intramolecular crowding effect with 4,5-H's (structure (**B**) in Scheme 3), so an expected inverse 2° KIE for an sp^3 to sp^2 conversion is observed.

In the reaction of DMA^+ the significantly decreased β-2° KIE on D-tunneling (0.99) as compared to H-tunneling (1.07) can be explained as a consequence of a decreased DAD in D-tunneling. The intermolecular steric hindrance with BNAH becomes important, somehow hindering the reorganization of the β-2° $CH(D)_3$ group in the DMA moiety of the tunneling ready state, decreasing the 2° KIE (Scheme 4(a), Sub = BNAH). There is no such steric effect between the inaccessible/"hidden" α-9-H on MA^+ and the BNAH structure. Therefore, a change in DAD does not affect the corresponding α-2° KIE on MA^+, resulting in no 1° isotope effect on 2° KIEs (Scheme 4(b)).

Hydride Transfer from MAH and DMAH to Tr^+ (Eq 3, Table 4). These reactions use the reduced forms of MA^+ and DMA^+, i.e., MAH and DMAH, as hydride donors. The hybridization changes on the donor centers are from sp^3 to sp^2, which is opposite to those in the reductions of $(D)MA^+$ in eq 2. Therefore, including a comparison of the 2° KIE behaviors in the two systems can avoid the overexplanation that might result from considering only one reaction direction. From literature, the hydride affinity of the hydride acceptor of Tr^+ is 690 kJ/mol (in DMSO).[53] A comparison of this value with that of MA^+ (632 kJ/mol) suggests that the reactions are highly exothermic, which also suggests an early TS. Therefore, the 2° KIE is expected to be normal and close to unity for the sp^3 to sp^2 conversion from (D)MAH to $(D)MA^+$. The observed α-2° KIE on MAH (1.09) is indeed normal but far from unity, whereas the β-2° KIE on DMAH (0.97 for H-transfer) is even inverse showing significant deflation. As expected, the "signs" (normal or inverse) of the 2° KIEs are opposite to those observed in eq 2. While both reactions are highly exothermic, the α-2° KIE on MAH in eq 3 is close to the reaction EIE of 1.12, whereas the

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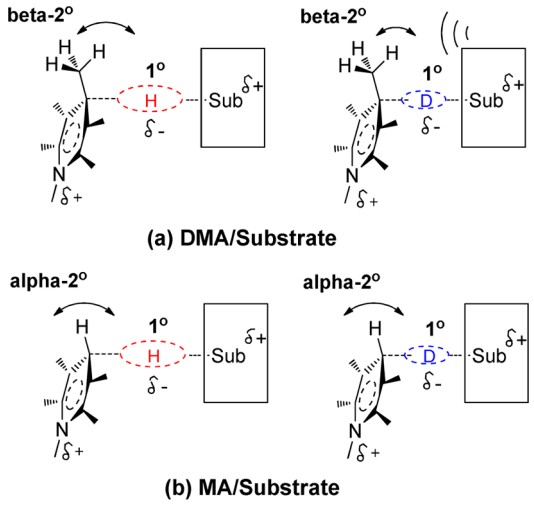
Scheme 4. Schematic Description of the Tunneling Ready States for the Hydride Transfers from Substrate BNAH to DMA^+ and MA^+[a]



[a](a) The vibrations of the sterically readily accessible 9-β-CH_3 in DMA interfere with the other substrate BNAH more in D-tunneling than in H-tunneling. (b) The interference does not happen for the reaction of MA^+ where the isotope effect of a "hidden" 9-α-H is concerned.

observed 0.99 on MA^+ in the reverse hybridization change in eq 2 is far away from the corresponding EIE of 0.89. Note again, the EIEs of 1.12/0.89 are taken from previous reports at the C-4 H/D position of the NADH/NAD^+ in their interconversion process. The unexpectedly large α-2° KIE on MAH is not consistent with the classical H-transfer mechanism. However, it can be explained in terms of a large degree of rehybridization/reorganization of the reaction center during the formation of the tunneling ready state in a tunneling mechanism.

The observed deflated, even inverse, β-2° KIE (0.97) on DMAH can result from the spatial crowding between its 9-CH_3/CD_3 group and 4,5-H groups, which increases in the sp^3 to sp^2 activation process (cf. structure (**A**) in Scheme 3); i.e., gain of the steric effect stiffens the CH_3 vibrations thus reducing the 2° KIE. Therefore, the intramolecular isotope effect that results from increased hindrance (2° KIE < 1) wins over the hyperconjugative effect (>1 due to sp^3 to sp^2 conversion) in determining the 2° KIE. This is consistent with the explanation for the inflated normal β-2° KIE on DMA^+ in its oxidation of BNAH (2) where the hybridization change is in the opposite direction.

As compared to the H-tunneling, the more inverse β-2° KIE (0.91) in the D-tunneling can be explained in terms of a larger intermolecular steric effect between the DMAH and Tr moieties due to a decreased DAD, which adds to the intramolecular steric effect between vibrations of 9-CH_3 and 4,5-H's that arises in the tunneling ready state. That is, the intermolecular steric hindrance somehow hinders the reorganization of the β-2° $CH(D)_3$ group in the DMAH moiety in the tunneling ready state, making the 2° KIE more inverse (cf. Scheme 4(a) where Sub = Tr). In the reaction of MAH, however, the 9-H group on MAH does not have an intramolecular crowding effect with 4,5-H's (cf. structure (**B**) in Scheme 3) and does not have an intermolecular crowding effect with the Tr^+; so the α-2° KIE is normal and does not depend upon the 1° isotope (cf. Scheme 4(b) where Sub = Tr).

Hydride Transfer from MPH to MA^+ (Eq 4, Table 5). Ostovic, Roberts and Kreevoy reported that the α-2° KIE on MA^+ is 10% higher in H-transfer than in D-transfer in this reaction in 2-PrOH/H_2O (4/1, v/v).[19] The significant effect of the 1° isotope on the 2° KIEs reported was very influential at the time. It was interpreted as results from the solution reactions strongly supporting the model of 1°/2° H coupled motions and H-tunneling, which had been proposed on the basis of enzymatic reaction studies and supported by a subsequent theoretical investigation.[3,10] Unfortunately, the results were not reproducible in our lab. Our results show that the 2° KIEs are independent of the 1° isotope not only in the aqueous 2-PrOH solvents that those authors used but also in acetonitrile. Our results are consistent with the trends observed in eqs 1−3, which is that the α-type 2° KIEs are not sensitive to the 1° isotope effect in solution reactions.

1°/2° H Coupled Motion Model Cannot Explain the Observed Effect of 1° Isotope on 2° KIEs. As described in the Introduction, currently there are two models that have been used to explain the 1° isotope effect on 2° KIEs. The need to use the tunneling DAD concept to explain the effect of 1° isotope on 2° KIEs exposes the difficulties inherent to a possible analysis based on the traditional model of H-tunneling and 1°/2° H coupled motions.[10,20,22,55,56] Within the coupled motions model, the 1° isotope independence of α-2° KIEs would be explained in terms of little or no 1° H-tunneling, whereas the observed pronounced 1° isotope dependence of β-2° KIEs would be interpreted in terms of a greater degree of tunneling. According to this analysis, it can be concluded that the reactions of BNAH/MA^+, MAH/Tr^+ and 2-PrOH/Xn^+ take place with little or no tunneling effect, which would imply that the temperature dependence of 1° KIEs will not fall outside of the semiclassical limits predicted by the traditional Bell H-tunneling theory that has been used in combination with the coupled motions model to explain the 1° isotope effect on 2° KIEs, whereas those of BNAH/DMA^+, DMAH/Tr^+ and 2-PrOH/$PhXn^+$ take place with significant tunneling effects, which would imply that the temperature dependence of 1° KIEs will be most likely outside of the semiclassical limits. However, the temperature dependence of 1° KIEs that we observed in these systems (Table S2 in SI) is not consistent with such an analysis. The reactions of BNAH/MA^+ and BNAH/DMA^+ (2) give rise to similar Arrhenius isotopic preexponential ratios (A^H/A^D = 0.2 vs 0.1), and these are well outside of the semiclassical limits (The semiclassical limits of A^H/A^D = 0.7 to 1.4).[16,56,57] So do the reactions of MAH/Tr^+ and DMAH/Tr^+ (3) (A^H/A^D = 0.4 vs 0.4). In contrast, the reactions of 2-PrOH/Xn^+ and 2-PrOH/$PhXn^+$ (1) give rise to A^H/A^D values (0.9 and 1.4), neither of which fall outside of the semiclassical limits. These results show that the extent of H-tunneling is similar in the three pairs of reactions irrespective of α-2° H or β-2° CH_3 groups and the steric factors in the substrates. (Note that the magnitude of the A^H/A^D values in the Marcus-like full-tunneling model is ascribed to the effect of thermal motions on the ease of the tunneling DAD samplings. The analysis of the temperature dependence of the 1° KIEs using this model is not a focus of discussion in this paper.)

Moreover, within the model of H-tunneling and 1°/2° H coupled motions, the 2° KIEs are expected to be inflated with respect to the values predicted by the semiclassical KIE theory. This is not always true in our observations. For example, in the reactions of 2-PrOH with $(T)Xn^+$ and $Ph(T)Xn^+$, the β-H_6/D_6 KIEs on 2-PrOH are in the range of 0.98 to 1.05 for the

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hydride/deuteride transfer processes (Table 1). As discussed earlier, however, the expected values should be close to an EIE of 1.51 because of the endothermic nature of the reactions. So all of these observed 2° KIEs, in either H- or D-tunneling process, are actually deflated. Moreover, while the β-2° KIEs on 2-PrOH in its reactions with (T)Xn^+ are deflated, the α-2° KIEs on (T)Xn^+ (0.97 and 0.99, Table 2) are inflated (also see the earlier discussion). It is not possible that the 1°/2° H coupled motions occur with a 2° H in one reactant but not with a 2° H in the other. Also, in the exothermic reactions between 1,4-dihydropyridines and carbocations, the observed α-2° KIE on MA^+ in its reaction with BNAH (Table 3) and the β-2° KIE on DMAH in its reaction with Tr^+ (Table 4) appear not to be inflated either, even though the temperature dependence of 1° KIEs study strongly suggests a traditional H-tunneling mechanism (A^H/A^D = 0.2 and 0.4, respectively).

α-2° KIEs: 1° Isotope Independent in Solution vs Dependent in Alcohol Dehydrogenases. Interestingly, the 1° isotope effect on the α-type 2° KIEs is not significant in the solution reactions, but it is significant in the hydride transfer reactions catalyzed by alcohol dehydrogenases.[11−13] Klinman and co-workers determined the α-2° KIEs on the oxidation of benzyl alcohol ($PhCL_2OH$, L = isotopes of H) by NAD^+ for H- and D-transfers catalyzed by both wild-type alcohol dehydrogenases and mutants. It was observed that the α-2° H/T KIEs on H-transfers were inflated and invariant no matter what type of alcohol dehydrogenases were used, but the 2° D/T KIEs on D-transfers were deflated to different degrees when using different enzymes.[5−8,41] Roston and Kohen recently determined the 2° H/T KIEs on both H- and D-transfers catalyzed by the yeast alcohol dehydrogenase, directly showing a significantly deflated 2° KIE with D-transfer.[13] Table 2 lists the latter 1° isotope dependence of α-2° KIE results. Our results show that steric hindrance from the reaction system deflates the 2° KIEs on the sterically readily accessible β-CH_3/CD_3 groups but not on the "hidden" α-H/D groups. Therefore, the observed significant 1° isotope effect on the α-2° KIEs in the alcohol dehydrogenase reactions implies that the reorganization of the "hidden" α-2° H/D's encounters a significant resistance during the D-transfer rehybridization process which can be attributed to the restrictive protein environment of the active site rather than to steric hindrance between the two moieties of the tunneling ready state itself. It has been proposed from the alcohol dehydrogenases data that the H- and D-transfers have isotopically different tunneling ready state conformations and a shorter DAD necessary for D-tunneling would induce a less optimal fit in the active site thus increasing the environmental crowding effect and decreasing the α-2° KIEs.[11−13] Our results confirm the proposition.

CONCLUSIONS

The 1° isotope effects on 2° KIEs were determined in hydride transfer reactions from various hydride donors to different carbocationic hydride acceptors in acetonitrile. The purposes of the study are (i) to test the hypothesis that H-tunneling and D-tunneling have tunneling ready state structures which have different donor−acceptor distances and thus different conformations, and that therefore a pronounced 1° isotope effect on 2° KIEs should be observed in the tunneling systems that are sterically hindered, and (ii) to compare the 2° KIE behaviors in solution with those in alcohol dehydrogenases in order to provide insight into the mechanism of the enzyme catalyzed H-transfer reactions.

Our findings include the following: (1) most 2° KIE values do not conform to the KIE values predicted from the semiclassical transition state theory; (2) the steric effect can augment the 1° isotope dependence of 2° KIEs, and the β-CH_3/CD_3 2° KIE is more sensitive to the 1° isotope effect than is the α-H/D 2° KIE; (3) the 1° isotope dependence of 2° KIEs can imply information about the structure of the tunneling ready states; (4) the significant 1° isotope dependence of α-2° KIEs reported by Ostovic, Roberts and Kreevoy in 1983 for a hydride transfer reaction similar to ours (and which has been frequently cited in the 1° isotope dependence of 2° KIE studies in biological H-transfer reactions) is not reproduced in our work; (5) both the 2° KIE values and their 1° isotope dependences cannot be explained by the traditional model of H-tunneling and 1°/2° H coupled motions. Our results support our hypothesis. Further studies should be extended to other types of H-transfer reactions including proton and hydrogen atom transfers.

Sources of the observed "puzzling" 1° isotope dependence of α-type 2° KIEs in alcohol dehydrogenases were also analyzed. It has been proposed in literature that D-tunneling that requires a shorter DAD induces a densely packed protein active site environment, which significantly hinders the α-2° H/D's reorganization and results in deflated 2° KIEs and hence the effect of the 1° isotope on the α-2° KIEs. Our results are consistent with this idea.

EXPERIMENTAL SECTION

General Procedures. *Alcohol Oxidations.* 9-Phenylthioxanthylium tetrafluoroborate ($PhTXn^+BF_4^-$) and thioxanthylium perchlorate ($TXn(9\text{-}H)^+ClO_4^-$) and 9-deuterated thioxanthylium perchlorate ($(TXn(9\text{-}D)^+ClO_4^-)$) were synthesized from the dehydration reactions of the corresponding alcohol precursors with HBF_4 or $HClO_4$ in propionic anhydride or acetic anhydride according to a published procedure.[58] The procedure is the same as the one that we used to synthesize the 9-phenylxanthylium tetrafluoroborate ($PhXn^+BF_4^-$) and the xanthylium perchlorate ($Xn(9\text{-}H)^+ClO_4^-$, $Xn(9\text{-}D)^+ClO_4^-$).[30] The 9-phenyl-thioxanthen-9-ol (PhTXnOH) was synthesized by reacting the thioxanthone with the Grignard reagent PhMgBr in dry THF. The 9H(D)-thioxanthen-9-ol (TXn(9-H)OH or TXn(9-D)OH) was synthesized from the reduction of thioxanthen-9-one by $NaBH_4$ or $NaBD_4$ according to a literature procedure.[59] 2-Propanol-β-d_6 ($(CD_3)_2CHOH$), 2-propanol-α-d_1 ($(CH_3)_2CDOH$) and 2-propanol-α-d_1-β-d_6 ($(CD_3)_2CDOH$) were prepared by the reduction of acetone or acetone-d_6 with $NaBH_4$ or $NaBD_4$ according to a literature procedure.[28,60] In order to make a direct kinetic comparison, the normal 2-propanol was also synthesized by using the same procedure, i.e., reduction of acetone with $NaBH_4$. The densities of the alcohols were determined (see Table S1 in SI). Acetonitrile was distilled over $KMnO_4/K_2CO_3$ and then P_2O_5 in nitrogen atmosphere before use.

1,4- and 1,2-Dihydropyridine Oxidations. 1-Benzylnicotinamide bromide (BNA^+), 1-benzyl-1,4-dihydronicotinamide (BNAH) and its deuterated analogue (BNAH-4,4′-d,d, 96% D content (from NMR data)) were prepared according to the commonly used procedure.[53,61] The normal 10-methylacridinium ion ($MA(9\text{-}H)^+BF_4^-$) and its 9-deuterated analogue ($MA(9\text{-}D)^+BF_4^-$) were synthesized from the oxidation of 10-methylacridan (MAH) and 9,9′-dideuterio-10-methylacridan (MAH-9,9′-d,d) by slight excess of the tropylium tetrafluoroborate ($Tr^+BF_4^-$) (molar ratio = 1:1.1) in acetonitrile and recrystallized in an acetonitrile−ether mixture. The $Tr^+BF_4^-$ was commercially available and was recrystallized from acetonitrile-ether for both synthesis and kinetic study uses. The 10-methylacidan (MAH) was synthesized from the reaction of $NaBH_4$ with MA^+I^- that was made from the reaction of acridine with CH_3I in acetone.[53] The MAH-9,9′-d,d was prepared from the reduction of the commercially available 10-methylacridone by $LiAlD_4$ according to a literature

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method.[53] Likewise, the 9,10-dimethylacridinium ion (DMA(9-CH_3)$^+BF_4^-$) and its 9-CD_3 analogue (DMA(9-CD_3)$^+BF_4^-$) were synthesized from the hydride transfer reactions from 9,10-dimethylacridan (DMAH) and 9-deuterated methyl-10-methylacrdan to $Tr^+BF_4^-$. The latter two 9-methyl substituted acridans were prepared from the reactions of MA^+I^- with Grignard reagents CH_3MgI and CD_3MgI in dry ether, following a literature procedure.[62] The 5-methyl-5,6-dihydrophenanthridine (MPH-6,6′-h,h) was synthesized by the hydride reduction of 5-methylphenanthridinium iodide (MP^+I^-) with $NaBH_4$. The latter salt was directly prepared by the reaction of phenanthridine with CH_3I in ether. The MPH-6,6′-d,d was synthesized by reduction of the 5-methyl-phenanthridin-6(5*H*)-one using $LiAlD_4$ in dry ether, and the ketone was prepared by the direct oxidation of MP^+I^- by KO_2 according to a literature procedure.[63]

Kinetic and KIE Measurements. *Alcohol Oxidations.* The procedure for the kinetic and KIE determinations of the reactions of $PhXn^+$ and Xn^+ in pure acetonitrile was reported previously.[28,30] The pseudo-first-order rate constant (k^{pfo}) was determined by following the decay of the cations spectroscopically. The observed effect of [2-propanol] on the k^{pfo} of the reactions, in which the [2-propanol] range was chosen for the convenience of kinetic measurements, are consistent with the second-order rate law within the experimental error.[28] The second-order rate constant (k) was calculated by k^{pfo} dividing by [2-propanol] and the KIE was calculated from the k values ($= k^H/k^D$). Use of the normal 2-propanol that we synthesized following the same procedure to synthesize its isotopologues minimizes the errors in KIEs determined.

The kinetic and KIE determinations of the reaction of $PhTXn^+$ used the same procedure for the kinetic determination of the $PhXn^+$ reaction.[28,30] Typically, 40 μL of 0.207 M stock solution of $PhTXn^+$ in acetonitrile was added to 4 mL of acetonitrile solution of large excess of 2-propanol (0.314 M) in a sealed 10 mL reaction vial placed in a water bath set at 60 °C. Aliquots of about 0.25 mL were periodically withdrawn from the reaction into the sample vials which were precooled in ice. The samples were immediately placed in a freezer (~ −20 °C). For each kinetic experiment, 7 to 8 reaction aliquots were collected within 1−3 half-lives of the reaction. For UV−vis spectral analysis, 60 μL aliquots of the reactions were diluted into 1.94 mL acetonitrile. The corresponding UV−vis spectra at different reaction times, i.e., the kinetic scans, were obtained (SI). The absorbance (A) decrease at 372 nm due to the $PhTXn^+$ consumption was recorded as a function of time (t). The obtained A *vs* t data were fitted to the integrated first-order rate equation, $-\ln(A) = k^{obs}\cdot t$ + constant, to give the k^{pfo} ($R^2 > 0.999$). The kinetic solutions of a pair of isotopologues were placed side by side in the water bath. The rate measurements were repeated 4 times and the standard deviations are small.

The kinetic and KIE determinations of the reaction of TXn^+ used the same procedure as used for the reaction of Xn^+. 2 mL 0.0120 M alcohol solution in acetonitrile was placed in a cuvette sitting in a thermostated cell holder with temperature maintained at 60 °C. 32 μL of 0.00250 M TXn^+ stock solution (in acetonitrile) was rapidly transferred to the alcohol solution and the decay in absorbance at 373 nm due to TXn^+ with time (t) was recorded for about 2−3 half-lives. The obtained $-\ln(A) - t$ data were plotted, and the slope of the linear correlation (R^2, mostly >0.999) was taken as the k^{pfo}. The measurement was repeated three to four times, and the whole procedure was further repeated at least four times using the new solutions.

Oxidations of BNAH and MPH by (D)MA$^+$. The kinetics of the BNAH oxidation by MA^+ and DMA^+ were determined spectroscopically by following the decay of the cations at 440 nm. For the oxidations of MPH (1.20×10^{-2} M) by MA^+ (6.00×10^{-4} M), the determination was made by following the decay of the cation at 373 nm. A typical determination of the kinetics for the reaction of DMA^+ with BNAH is as follows. To the 1.90 mL 3.99×10^{-3} M BNAH solution in acetonitrile with certain temperature in a cuvette, 100 μL of 4.70×10^{-3} M DMA^+ stock solution (in acetonitrile) was rapidly added. The decay in absorbance at 440 nm due to DMA^+ with time (t) was recorded for at least 10 half-lives to determine A_∞ and for 2−3 half-lives to determine the rate constant. The obtained $-\ln(A - A_\infty) \sim t$ data were plotted and the slope of the linear correlation ($R^2 > 0.999$) was taken as the k^{pfo}. Determination of the effect of [BNAH] on k^{pfo} showed that the reaction follows the second-order rate law. Second-order rate constants (k) and the β-d_3 2° KIE on DMA^+ were calculated.

For the fast MA^+ reaction kinetic determinations, equal volumes of 6.00×10^{-3} M BNAH solution and 6.00×10^{-4} M MA^+ solution (both in acetonitrile) were mixed in a specially designed thermostated cuvette connected to a rapid stopped-flow mixer equipped with a pneumatic drive unit (a Hi-Tech Scientific SFA-20 fast kinetic determination kit). The decay in absorbance at 440 nm due to MA^+ with time (t) was recorded. The obtained $-\ln(A - A_\infty) \sim t$ data were plotted (R^2 is within the range of 0.9980−0.9999) and the k^{pfo} was obtained. Second-order rate constants (k) and the α-D 2° KIE on MA^+ were calculated.

The measurements were repeated three times, and the whole procedure was further repeated three times using the new solutions.

(D)MAH Oxidations by Tr$^+$. The kinetics of the (D)MAH oxidations by Tr^+ were determined spectroscopically by following the formation of the (D)MA^+ at 373 nm. Detailed kinetic determination procedures are similar to those for the oxidations of BNAH and MPH except that the formation of the cations was monitored. In the reaction of MAH/Tr^+, [MAH] = 7.32×10^{-5} M, [Tr^+] = 2.00×10^{-2} M; in the reaction of DMAH/Tr^+, [DMAH] = 7.32×10^{-5} M, [Tr^+] = 8.00×10^{-2} M, at 35 °C. The A_∞ was determined and $-\ln(A_\infty - A) \sim t$ was plotted to derive the k^{pfo}. The reactions follow the second-order rate law. The second-order rate constants and the KIEs on (D)MA^+ were calculated. The measurements were repeated three times, and the whole procedure was further repeated three times using the new solutions.

Calculation of the TS Geometry. The gas-phase TS (activated complex) structure was determined by Hartree−Fock calculations with a 6-31G** basis set. It is only meant to show the qualitative geometry of the TS and infer the information about the spatial interactions of groups in the actual tunneling ready state. The detailed calculation procedure is included in the SI.

■ ASSOCIATED CONTENT

Supporting Information

Kinetic scans, densities of the 2-PrOH and its isotopologues, temperature dependence of 1° KIEs, effect of [2-PrOH] and [H^+] on the β-2° KIEs on 2-PrOH in its reaction with $PhXn^+$, reaction rates and 2° KIEs from typical determinations, raw kinetic data examples to show the method to derive the standard deviations in 2° KIEs, and computational method and procedure are included. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b03085.

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Notes

The authors declare no competing financial interest.

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