Multiple Isotope Effects on the Acyl Group Transfer Reactions of Amides and Esters

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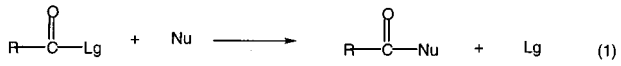
Received September 27, 2000

ABSTRACT

Acyl group transfer reactions, especially those to amides and esters, are important in biochemistry. Multiple kinetic isotope effects for the atoms at the reactive center of these molecules have provided the most detailed bonding picture of the transition state to date. These kinetic isotope effect studies are reviewed for several reactions of formamide, methyl benzoate, and methyl formate. In these cases all the evidence is consistent with a stepwise mechanism, involving tetrahedral intermediates. In the case of *p*-nitrophenyl acetate, the change to an excellent leaving group causes the tetrahedral intermediates to become kinetically unstable; the kinetic isotope effects are best fitted to a concerted mechanism.

Introduction

Acyl groups commonly refer to the carboxylic acid functional group and its major derivatives—acid halides (mostly chlorides), anhydrides, esters, amides, nitriles, and thioesters. The characteristic reaction of these functional groups is nucleophilic acyl substitution (or acyl transfer) which interconverts the various acyl groups (eq 1). Several



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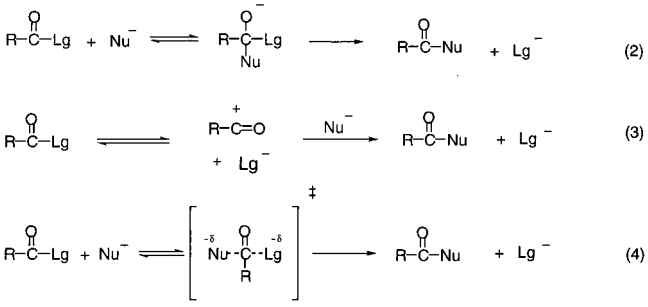
of these acyl groups play a central role in biology. For example, esters are one of the major functional groups of lipid chemistry, amides constitute the peptide bond in proteins, and thioesters serve as acyl donors and as intermediates in enzymatic catalysis.

This biochemical relevance has made acyl group transfer one of the most studied reactions in all of organic chemistry.[1] Organic chemists and biochemists have utilized an array of classic methods to probe the reaction mechanism, including kinetics, structure−reactivity studies, solvent effects, and stereochemistry.

Once stable and radioactive isotopes became readily available, they played a key role in mechanistic investigations. First, isotopes were used as tracers to locate the site of bond cleavage in esters.[2] Next, the stable isotopes of oxygen were used in positional isotope exchange (PIX) experiments.[3] These PIX experiments provided evidence for exchange between the carbonyl oxygen and water during hydrolysis, strongly implicating the existence of a symmetric tetrahedral intermediate (eq 2, where Nu = OH). The conclusions from these PIX experiments were so widely accepted that even today most organic textbooks tend to show all acyl group transfers occurring via this stepwise mechanism. However, more recent structure−reactivity studies offer considerable evidence for two additional mechanisms. Williams et al.[4] and others[5] provided evidence that the tetrahedral intermediate becomes unstable for molecules with very good leaving groups. For example, there is evidence that acid chlorides react by a dissociative mechanism (eq 3) and that the reaction of phenoxides with aryl acetates occurs by a concerted, S_N2-like mechanism (eq 4). The switchover from one mechanism to another depends on the nature of the leaving group and nucleophile. These alternative mechanisms are not universally accepted.[6]



Isotope effects are ideally suited to study these reactions because at least four different atoms undergo significant bonding changes during the reaction: the carbonyl carbon, the carbonyl oxygen, the leaving group heteroatom, and the nucleophilic atom. Measurement of the isotope effects for each of these reacting atoms has the potential to reveal a very complete bonding picture of the transition state. Despite this, isotope effect experiments initially played a relatively minor role in mechanistic studies of acyl group transfers. Many isotope effect experiments (especially those with elements heavier than hydrogen) are relatively difficult to perform; often the reacting atoms are buried too deeply within the molecule to be amenable to the methodology. The introduction of a remote label procedure[7] (discussed below) finally made it possible to measure isotope effects for nearly all of the reacting atoms during acyl group transfer.

This Account will be subject to several limitations. First, only isotope effects on nonenzymatic acyl transfers will be discussed. Biochemists were quick to adopt isotope effect methodology to study enzyme-catalyzed reactions; consequently, there are already several reviews on the general topic[8] and on the particular subject of enzyme-catalyzed ester hydrolysis.[9] Second, classic solvent isotope effects involving *hydrogen* will be omitted; this topic has also been extensively reviewed.[10] Finally, discussion will

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10.1021/ar000054d CCC: $20.00
Published on Web 01/18/2001

be limited to the reactions of amides and esters, since these are the only acyl groups studied by *multiple* isotope effects.

Some Isotope Effect Terminology

Before discussing the major methods for measuring isotope effects, it is useful to briefly review some terminology. Kinetic isotope effects (KIEs) are reported as a ratio of rate constants, $^{light}k/^{heavy}k$; equilibrium isotope effects (EIEs) are the ratio of equilibrium constants, $^{light}K/^{heavy}K$. If the ratio is greater than unity, the isotope effect is *normal*; if less than unity, it is *inverse*. It has become customary to report KIEs simply as $^{heavy}k$.[11a] For example, an oxygen isotope effect, $^{16}k/^{18}k$, is reported as ^{18}k. A similar convention holds for equilibrium isotope effects. Isotope effects fall into two categories, *primary* and *secondary*, based on the type of bond cleavage. Primary isotope effects are those in which the connection to the atom being studied is severed (or formed) during the course of the reaction. Secondary isotope effects (α and β) are those for atoms near the one actually undergoing bond breaking/forming (α for atoms directly attached and β for atoms which are two bonds removed from the reacting atom); the connection to these atoms is preserved during the reaction, despite changes in bonding.

KIEs reflect changes in bonding between the ground state and the transition state. Theorists have identified two contributions to isotope effects—a temperature-independent factor (TIF) and a temperature-dependent factor (TDF).[11b] The TIF (also called the imaginary frequency factor) arises from the motion of the heavy and light isotopes along the reaction coordinate; it is always normal. The TDF (also called the zero-point energy factor) is due to creation of new bond vibrational modes in the transition state; these can be normal (looser bonding to the isotope) or inverse (tighter bonding to the isotope). In the vast majority of cases, primary KIEs are normal because the TIF factor is dominant. For secondary KIEs there is usually little reaction coordinate motion, resulting in a negligible TIF factor. Consequently, secondary isotope effects can be either normal or inverse. For reversible reactions, the secondary KIE may be normal or inverse.

Measurement of Isotope Effects

Kinetic isotope effects are measured by two basic methods: the *direct method* and the *competitive method*. The direct method involves independent measurement of the individual rate constants for the light and heavy isotopomers. This method works reliably well for most primary and secondary hydrogen isotope effects because the ratio of rate constants is large enough that the ordinary precision of kinetic measurements (plus or minus a few percent) is not limiting. Although the direct method has been used to measure heavy-atom isotope effects,[12] it is seldom the method of choice. The magnitude of a typical heavy-atom isotope effect is only on the order of a few percent, putting high demands on the precision of measuring the individual rate constants.

The competitive method, pioneered by Bigeleisen and Wolfsburg,[13a] is usually chosen to measure heavy-atom KIEs. In this technique the heavier and lighter isotopes are allowed to compete for reagents in a single reaction mixture. A minimum of three data are required for a single determination of the KIE: (1) an isotope ratio (e.g., $^{18}O/^{16}O$) for unreacted starting material (R_0) or for the product after completion of the reaction (also R_0), (2) an isotope ratio for the starting material (R_s) or product (R_p) at some fraction of the total reaction, and (3) the actual fraction (f) of total reaction for R_s or R_p above. Equations 5 and 6 are used to calculate the KIE from analysis of either starting material or product, respectively.

$$\text{isotope effect} = \log(1 - f)/\log[(1 - f)(R_s/R_0)] \quad (5)$$

$$\text{isotope effect} = \log(1 - f)/\log[1 - f(R_p/R_0)] \quad (6)$$

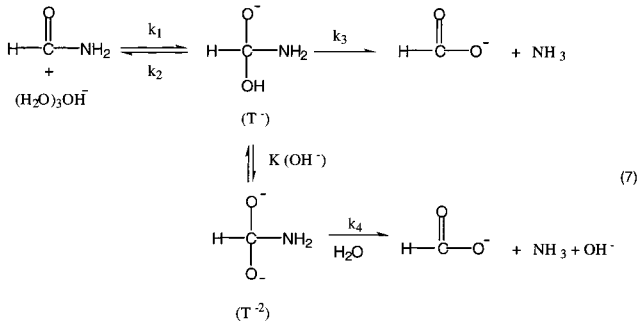
Because the differences in the reaction rates are very small for heavy atoms, a high degree of precision is required for measurement of the isotope ratios of the starting material or product. Fortunately, modern isotope ratio mass spectrometers (and other methods[13b]) are capable of such precision. However, the use of an isotope ratio mass spectrometer has one major drawback. The instrument requires analysis of pure gases (such as H_2, N_2, CO, and CO_2), and this, in turn, most often requires quantitative conversion of the atom of interest into one of these pure gases. Development of the analytical procedures is what makes heavy-atom isotope effect experiments so difficult. As mentioned earlier, the remote label method[7] alleviates some of these analysis problems. This method uses a substrate which is isotopically labeled in two positions: the first position is the atom of interest (which is not easily accessible), and the second position is an atom that can be easily analyzed (the remote label). The remote label atom must either have no isotope effect or have one that can be independently measured. One drawback of the remote label method is the necessity of difficult chemical syntheses using stable isotopes. However, it is usually worth the hard work, since multiple isotope effects at the reactive center of a molecule can pinpoint the rate-determining step of the mechanism and yield critical insights into the transition-state structure which are unavailable by other experimental methods.

Acyl Transfers of Amides

Despite being among the least reactive of the acyl groups, amides can undergo reaction with a variety of different nucleophiles. The most common and highly studied reaction is hydrolysis, which can occur at acidic, basic, or neutral pH. To date, the alkaline hydrolysis of formamide[14] is the sole amine reaction studied by multiple KIEs; the following section will concentrate on this one investigation.

Alkaline Hydrolysis. The kinetics of the alkaline hydrolysis for most amides is somewhat complex. Generally, the reaction is first-order in hydroxide at low base concentrations; for more highly reactive amides, a second-

order hydroxide term appears in the rate law under strongly alkaline conditions.[15] The proposed mechanism, which is consistent with these findings, involves formation of two tetrahedral intermediates (eq 7). The first, T^-, is formed by reaction of the amide with hydroxide, whereas the second, T^{-2}, is formed by removal of a proton from T^- by a second molecule of hydroxide. Alkaline hydrolysis of formamide follows the above second-order rate law.[16]



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A fairly large number of amides have been subjected to PIX experiments.[3e] In these experiments, the rate of hydrolysis is compared to the rate at which ^{18}O is exchanged between the carbonyl oxygen of the amide and water. The results are typically given as a ratio of the rate constants for hydrolysis to that for exchange, k_h/k_e. The existence of this exchange was taken as additional evidence for a stepwise mechanism involving a symmetric tetrahedral intermediate like T^-. In the particular case of formamide, k_h/k_e was shown to depend on the concentration of hydroxide.[14] Derivation of the steady-state rate laws for hydrolysis and exchange gave an expression (eq 8) from which the partition ratios (Kk_4/k_2 or k_3/k_2) for the tetrahedral intermediates were determined. Under dilute

$$k_h/k_e = (2k_3 + 2k_4K[OH^-])/k_2 \qquad (8)$$

alkaline conditions, the hydrolysis and exchange reactions of formamide occur at similar rates ($k_3/k_2 = 1.05$). At face value, this finding indicates that the transition states for breakdown of T^- to starting material (k_2) and to product (k_3) are of nearly equal energy. This result was not anticipated. A neutral nitrogen atom is a much worse leaving group than a neutral oxygen atom; as a result, k_2 was expected to be much larger than k_3. At higher hydroxide concentrations, more of the intermediate pool is trapped as T^{-2}, effectively slowing down exchange; the product is then formed via the k_4 pathway.

The nature of the transition states for alkaline hydrolysis was investigated by multiple KIE experiments performed under dilute alkaline conditions (the first-order reaction), where the concentration of T^{-2} is very low.[14] This low concentration of alkali was chosen because a mechanism involving only T^- might be a reasonable model for enzymatic reactions. To simplify the following discussion, all subsequent KIEs on formamide hydrolysis assume *only* a first-order reaction pathway.

The first KIE to be reported on the alkaline hydrolysis formamide was that for the formyl-H.[16a] This type of secondary KIE is sensitive to hybridization changes at the carbonyl carbon on going from the ground state to the transition state. A normal KIE is found when the reaction proceeds from an sp^3-hybridized ground state to an sp^2-hybridized transition state; an inverse KIE is observed for the opposite transition (sp^2 to sp^3). The magnitude of the KIE reflects the degree of hybridization change that has occurred (maximum inverse KIEs for these types of reactions are expected to be near 0.75[16a]). Kirsch found the magnitude of the observed KIE to be dependent on the hydroxide concentration, consistent with the mechanism in eq 7. Our determination[14] of this KIE at a single low concentration of hydroxide (0.20 M) was found to be $^Dk_{obs} = 0.80$, in reasonable agreement with Kirsch's reported determination at that hydroxide concentration. Since the PIX experiments gave a partitioning ratio for T^- ($k_3/k_2 = 1.05$), it is possible to estimate the isotope effects on the individual steps of the mechanism by using eq 9.

Table 1. Observed, Estimated, and Calculated Isotope Effects for the Individual Steps in the Mechanism of the Alkaline Hydrolysis of Formamide

	isotope effects		
isotope	observed[a]	estimated	calculated
formyl-H	$^Dk_{obs} = 0.80$	$^Dk_1 = 0.69$, $^Dk_{eq} = 0.69$	$^Dk_3 = 1.34$
carbonyl-C	$^{13}k_{obs} = 1.032$	$^{13}k_1 = 1.032$, $^{13}k_{eq} = 0.983$	$^{13}k_3 = 1.050$
nucleophile-O	$^{18}k_{obs} = 1.022$	$^{18}k_3 = 1.000$, $^{18}k_{eq} = 1.033$	$^{18}k_1 = 1.012$
leaving-N	$^{15}k_{obs} = 1.004$	$^{14}k_1 = 1.005$, $^{14}k_{eq} = 1.005$	$^{14}k_3 = 0.998$
carbonyl-O	$^{18}k_{obs} = 0.980$		

[a] Data from ref 14.

$$^*k_{obs} = [^*K_{eq}{}^*k_3 + {}^*k_1(k_3/k_2)]/(1 + k_3/k_2) \qquad (9)$$

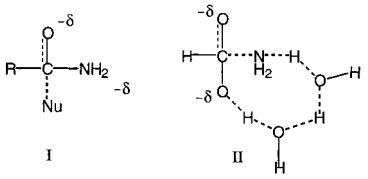
This equation is a general one and will be used in the subsequent discussions of isotope effects on the alkaline hydrolysis of formamide. The asterisk denotes an isotope effect on a particular step in the mechanism (e.g., $^*k_3$ is the isotope effect on the breakdown of T^-); $^*k_{obs}$ signifies the experimentally measured (or observed) KIE.

No exact solution to eq 9 is possible; there are too many unknowns. However, if there are reasonable ways to estimate two of the individual isotope effects, then the third can be calculated. In this case, formation (step 1) and breakdown (step 2) of T^- are of nearly equal energy. Consequently, the large observed inverse KIE appears to require the transition state for step 1 to resemble T^- (structure I, where Nu = OH^-), since any KIE on step 2 would be normal. Under these conditions, it is logical to assume that $^*K_{eq}$ is nearly the same as $^*k_1$, thereby simplifying eq 9. This simplification only holds for secondary KIEs on step 1. Whenever possible, we employed closely related model reactions to estimate the isotope effects on two of the individual steps; details of the models chosen are beyond the scope of this discussion but are given in ref 14. A summary of the results is given in Table 1. In the formyl-H case, the most intriguing result is the calculation of a large normal KIE on k_3, indicating a late sp^2-like transition state for this step (structure II). The role of water in structure II will be discussed later.

Table 2. Kinetic Isotope Effects on Alkaline Hydrolysis, Acid-Catalyzed Hydrolysis, and Hydrazinolysis of Methyl Formate (MF) and Methyl Benzoate (MB)

ester	nucleophile	formyl-H ($^{D}k_{obs}$)	carbonyl carbon ($^{13}k_{obs}$)	carbonyl oxygen ($^{18}k_{obs}$)	leaving oxygen ($^{18}k_{obs}$)	nucleophile ($^{15}k_{obs}$) or ($^{18}k_{obs}$)
MF	OH^-/H_2O	0.95[a]	1.034[b]	0.999[b]	1.009[c]	1.023[b]
MF	NH_2NH_2, pH 8	0.98[a]	1.038[d]	1.003[d]	1.062[c]	0.990[d]
MF	NH_2NH_2, pH 10	0.76[a]	1.020[d]	1.004[d]	1.005[c]	0.992[d]
MF	aqueous HCl	0.81[a]	nd	nd	1.0009[c]	nd
MB	OH^-/H_2O	nd	1.043[e]	1.005[e]	1.006[e]	nd
MB	NH_2NH_2, pH 8	nd	1.041[e]	1.018[e]	1.041[e]	nd
MB	aqueous H_2SO_4	nd	1.026[f]	0.995[f]	1.002[f]	nd

[a] Data from ref 23. [b] Data from ref 24. [c] Data from ref 22. [d] Data from ref 25. [e] Data from ref 7. [f] Data from ref 27.



The primary carbonyl-C KIE is difficult to interpret for this case and for most acyl group transfers. Since both steps in the mechanism involve considerable reaction coordinate motion on the part of the carbon atom, a large normal KIE is expected, regardless of which step is rate-determining (maximum primary KIEs for ^{13}C, ^{15}N, and ^{18}O are expected to be in the range 1.04−1.07[16c]). In fact, the carbonyl-C KIEs for all acyl group transfers fall in the same narrow range ($^{13}k_{obs}$ = 1.02−1.04, Tables 1−3). To make matters worse, this KIE seems quite insensitive to large differences in the structure of the reactant, the rate of the reaction, and possibly the structure of the transition state.[17] Consequently, the carbonyl-C KIE will not be discussed for subsequent reactions, except in the rare case where mechanistic insights are possible.

The carbonyl-O KIE on the alkaline hydrolysis of formamide is also difficult to interpret. Initially this KIE was expected to be quite important in the determination of transition-state structure. Breaking the carbonyl π bond should give a normal secondary KIE with a maximum magnitude estimated to be near $^{18}k_{obs}$ = 1.025−1.03.[18] Unfortunately, the situation is considerably more complex. The observed inverse KIE ($^{18}k_{obs}$ = 0.980) can only be explained by careful qualitative analysis of the new vibrational modes in the transition state. In the transition state, the carbonyl-O will gain new O−C−O bending and O−C−O−H torsional modes. This will stiffen bonding to the carbonyl-O in the transition state, thereby yielding a considerable inverse contribution to the observed KIE. Increased solvation of the negative charge generated on the carbonyl-O in the transition state has also been reported as a factor that will make this KIE more inverse.[19] In the case of formamide, these inverse contributions outweigh the one normal contribution. The complex nature of the carbonyl-O KIE often makes it an unreliable tool for delineating transition-state structure; in subsequent examples, this KIE will only be discussed for cases where it is possible to obtain transition-state bonding information.

The nucleophile-O KIE was a key to fully understanding the mechanism, but measurement of this KIE proved to be quite challenging. During hydrolysis, the original formamide molecule gains a second oxygen atom from the nucleophile which becomes chemically identical to the first. To this end we developed an analytical procedure to separately measure the isotopic composition of these two equivalent oxygen atoms. Once we accomplished this, the next problem was deciding which of the two candidates (hydroxide or water) was the actual nucleophile. The large known fractionation factor between water and hydroxide[20] provides a way to sort this out. If hydroxide is the nucleophile, the KIE turns out to be $^{18}k_{obs}$ = 0.982; if water is the nucleophile, the KIE is $^{18}k_{obs}$ = 1.022. As mentioned earlier, the vast majority of primary KIEs are normal because they are dominated by reaction coordinate motion. Therefore, the most likely nucleophile is water, presumably one of the water molecules solvating the hydroxide ion (structure I, where Nu = H_2O). Jencks reached a similar conclusion upon observation of general base catalysis of a series of formate esters.[5b]

Table 3. Kinetic Isotope Effects on the Reactions of *p*-Nitrophenyl Acetate with Various Nucleophiles[a]

nucleophile	secondary-H ($^{D}k_{obs}$)	carbonyl carbon ($^{13}k_{obs}$)	carbonyl oxygen ($^{18}k_{obs}$)	leaving oxygen ($^{18}k_{obs}$)
OH^-/H_2O	0.9562	1.0342	1.0039	1.0135
phenolate	0.9617	nd	1.0039	1.0182
$(CF_3)_2CHO^-$	0.9481	1.0294	1.0058	1.0210
$HO(CH_2)S^-$	0.9780	nd	1.0119	1.0219
$MeO_2C(CH_2)S^-$	0.9765	1.0380	1.0117	1.0172
$CH_3OCH_2CH_2NH_2$	0.9682	1.0279	1.0064	1.0330

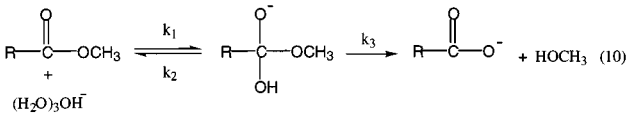
[a] Data from refs 9 and 26.

The small, normal leaving-N KIE ($^{15}k_{obs}$ = 1.004) was a bit of a surprise. The secondary nitrogen isotope effects on step 1 ($^{15}K_{eq}$ and $^{15}k_1$) should be small, reflecting the new torsional and bending modes in the transition state (inverse) and the breaking of the partial π bond to the nitrogen (normal). As a result, most of the observed KIE should arise from the k_3 step, which involves cleavage of the C−N bond. This primary KIE is expected to be in the range $^{18}k_{obs}$ = 1.02−1.03.[21] Why is the observed KIE so small? Development of a full negative charge on nitrogen during cleavage of the C−N bond is energetically unfavorable. An intramolecular proton transfer to the nitrogen (simultaneous to C−N bond breaking; structure II) probably aids in decreasing this unfavorable charge. This N−H bond formation in the transition state will stiffen the bonding to the leaving-N (an inverse contribution) and lower the observed KIE.

Acyl Group Transfers of Esters

The acyl group transfer reactions of esters has been more extensively studied via isotope effect methodology than the corresponding reactions of amides. Part of this interest was generated by the conclusions of structure−reactivity studies suggesting the possibility of a concerted, S_N2-like mechanism (eq 4) for reaction of reactive esters with certain nucleophiles.[4,5b] The published KIE studies will be discussed in the following order. First, the multiple KIE study of the hydrolysis and hydrazinolysis reactions of methyl benzoate[7] and methyl formate[22−25] will be presented. In these cases, all available evidence points to stepwise mechanisms involving tetrahedral intermediates; the KIEs will be interpreted within this framework. Later, the KIE work of Cleland and Hengge[9,26] on the transfer of various nucleophiles to *p*-nitrophenyl acetate will be discussed. These KIE studies are expected to span the change from a stepwise to a concerted mechanism.

Alkaline Hydrolysis of Methyl Formate and Methyl Benzoate. The kinetics of the alkaline hydrolysis of most alkyl esters, including methyl formate and methyl benzoate, are first-order in hydroxide.[1c] PIX experiments have been carried out ($k_h/k_e = 18.3$ for methyl formate;[22] $k_h/k_e = 27.7$ for methyl benzoate[3d]); these ratios are independent of hydroxide concentration, so the partition ratio (k_3/k_2) is simply $^1/_2(k_h/k_e)$. These results are consistent with a largely rate-determining attack of the nucleophile on the ester as shown in the mechanism of eq 10.



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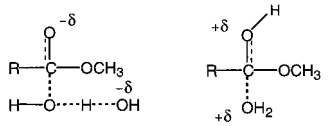
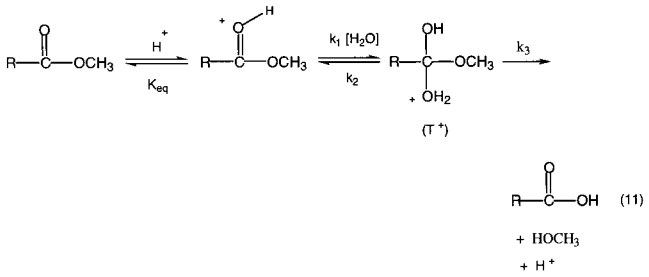
The observed formyl-H KIE for methyl formate is small and inverse ($^Dk_{obs} = 0.95$).[23] The conclusions of the PIX experiments (i.e., rate-determining first step), together with those from the formyl-H KIE experiments, point to an early, sp^2-like transition state. It is logical to assume that the mechanism for methyl benzoate alkaline hydrolysis will proceed through a similar rate-determining first step; whether the transition state is early or late cannot be reliably determined by the available isotope effects.

The leaving-O KIE provides another valuable piece of information in delineating the transition-state structure. These KIEs are quite small for both esters ($^{18}k_{obs} = 1.009$ for methyl formate;[22] $^{18}k_{obs} = 1.006$ for methyl benzoate[7]), consistent with a *mostly* (but not entirely) rate-determining first step. Why is the leaving-O KIE not unity? The observed KIE is attributable to two small normal contributions: a small one from step 2 of the mechanism and another from the weakening of the partial π bond to the leaving oxygen during step 1.

The nucleophile-O KIE is once again a key to fully understanding the transition-state structure. A method similar to that described above for formamide was used to independently measure the isotopic composition of each of the chemically equivalent oxygen atoms of formate.[24] The results of the isotopic analysis gave the same two possibilities—either water was the nucleophile with $^{18}k_{obs} = 1.023$, or hydroxide was the nucleophile with $^{18}k_{obs} = 0.982$. Once again, hydroxide can be rejected on the basis of the empirical observation that the vast majority of primary KIEs are normal because they are dominated by reaction coordinate motion. It is likely that the transition state for the reaction resembles structure III.



Acid-Catalyzed Hydrolysis. The kinetics of acid-catalyzed hydrolysis of most esters is first-order in acid.[1c] The accepted mechanism involves protonation of the carbonyl-O in the first step, followed by addition of water and breakdown of the tetrahedral intermediate to product (eq 11). The partition ratio from PIX experiments favors the overall hydrolysis ($k_h/k_e = 11.4$) for methyl formate,[22] consistent with rate-determining formation of T^+.



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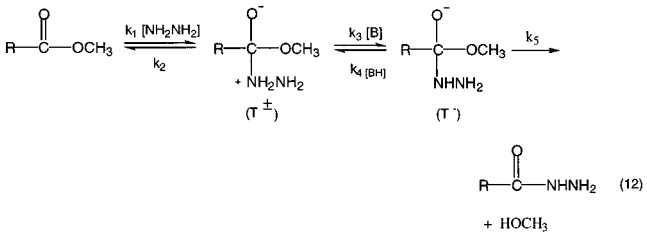
Only two significant KIE studies of the acid-catalyzed hydrolysis of esters have been reported, again for methyl benzoate and methyl formate. The formyl-H KIE for methyl formate is significantly inverse ($^Dk_{obs} = 0.81$);[23] indicating a transition state with considerable sp^3 character. The leaving-O KIEs are very small for both esters (Table 2), indicating some step prior to breakdown of the neutral tetrahedral intermediate is rate-determining. Taken together, these KIEs argue for a rate-determining step 2 (attack of water). The carbonyl-O KIE on the acid-catalyzed hydrolysis of methyl benzoate ($^{18}k_{obs} = 0.995$)[27] is significantly more inverse than the corresponding isotope effects on the alkaline hydrolysis ($^{18}k_{obs} = 1.005$) and hydrazinolysis ($^{18}k_{obs} = 1.018$) for this compound.[7] A qualitative argument based on increased vibrational modes in the transition state was offered earlier as an explanation for the smaller than expected carbonyl oxygen KIEs on the alkaline hydrolysis. In the present case, addition of a proton to the carbonyl oxygen will serve to further stiffen the bonding to the carbonyl oxygen in the transition state and cause the observed KIE to be even more inverse. All the above information strengthens the case for rate-determining attack of water (structure IV).

Hydrazinolysis. The hydrazinolysis of esters exhibits a break in the log *k* vs pH profile, indicative of a change in mechanism. Satterthwait and Jencks proposed[28] that the rate-determining step of the mechanism changed from

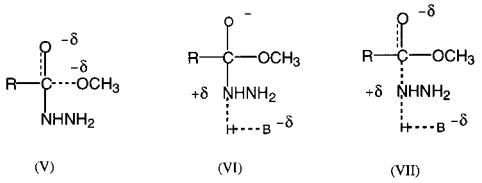
breakdown of T^- at low pH to the general-base-catalyzed formation of T^- at high pH (eq 12). Isotope effect studies



(12)

of the hydrazinolysis of both methyl benzoate and methyl formate have been reported.[7,22,23,25] Only the methyl formate case will be discussed here because the KIEs for this ester were measured at both low and high pH.

Two KIEs played a major role in defining the transition-state structure—those for the formyl-H and leaving-O. At pH 8, the formyl-H KIE was small and inverse ($^{D}k_{obs}$ = 0.98),[23] indicating a transition state with nearly the same sp^2 hybridization as the ground state. There are two ways to rationalize this. Either the rate-determining step is formation of $T^{\pm}$ and the transition state is very early or the breakdown of T^- is rate-determining with a late transition state which resembles the product (also sp^2-like). The large leaving-O KIE ($^{18}k_{obs}$ = 1.062)[22] indicates considerable C−O bond breaking to the leaving group in the transition state and argues convincingly for the latter proposal (structure V).



(V) (VI) (VII)

The nucleophile-N KIE ($^{15}k_{obs}$ = 0.990)[25] is also worthy of note. Why is this primary KIE inverse when all primary KIEs are expected to be normal? The answer lies in a careful consideration of all the factors leading up to the transition state. If k_5 is rate-determining, the mechanism simplifies to two steps—the equilibrium formation of T^- and its decomposition. On the basis of models, the equilibrium isotope effect for the formation of T^- is expected to be inverse, in the neighborhood of $^{15}K_{eq}$ = 0.976−0.981. The KIE on breakdown of T^- is a secondary one resulting from weakening of bending (N−C−O) and two torsional (N−C−O−C and N−N−C−O) modes (a normal KIE). Combining this normal KIE with the large inverse equilibrium isotope effect for formation of T^- will yield an observed KIE with a magnitude between the two. Thus, $^{15}k_{obs}$ = 0.990 seems reasonable.

The formyl-H KIE at pH 10 is more inverse than at pH 8 ($^{D}k_{obs}$ = 0.76),[23] consistent with an sp^3-like transition state. At the same time, the leaving-O KIE falls to $^{18}k_{obs}$ = 1.005,[22] indicating that some step prior to breakdown of T^- is rate-determining. For acetate esters, Jencks argued that this step was the general-base-catalyzed formation of T^- from $T^{\pm}$ (structure VI). Surprisingly, the carbonyl-C KIE was decisive in testing this hypothesis. If the general base catalysis step were rate-determining, the observed carbonyl-C KIE would be largely an equilibrium isotope effect on formation of $T^{\pm}$ (no bonds are broken or formed to the carbonyl carbon during the proton transfer step). Although there are no outstanding models in the literature for the equilibrium formation of $T^{\pm}$, a crude model can be constructed from known fractionation factors.[29] All estimates give a large inverse $^{13}K_{eq}$, in stark contrast to the observed large normal KIE ($^{13}k_{obs}$ is 1.020).[25] Thus, our results seem to favor a concerted mechanism, with nucleophilic attack of the amine and proton transfer occurring simultaneously (structure VII). However, Singleton and Merrigan[30] recently calculated the carbonyl-C EIE between formamide and $T^{\pm}$. The calculated EIE is normal ($^{13}K_{eq}$ = 1.022), due to a large hyperconjugation effect. This theoretical EIE is consistent with the stepwise mechanism involving $T^{\pm}$.

The Reactions of *p*-Nitrophenyl Acetate (PNPA). The work of Cleland and Hengge on acyl transfers to PNPA is an important contrast to the reactions discussed above because these isotope effects provide compelling evidence for an alternate, concerted mechanism. Most of the nucleophiles in the study had a pK_a in the 9.3−9.9 range (hydroxide was the lone exception). With an excellent leaving group like *p*-nitrophenol, the tetrahedral intermediate was predicted to become unstable, favoring the concerted mechanism.[4] Only a few of the nucleophiles studied are given in Table 3, just enough to present the major evidence for the existence of the concerted mechanism.

The β-H (methyl group) and the leaving-O KIEs provided the most important evidence for the concerted mechanism; for succinctness the discussion will center on these two KIEs. The β-H KIE is a secondary one (similar to the formyl-H KIE); it can also be used to measure hybridization changes at the carbonyl-C. With oxyanions, sulfur anions, and amine nucleophiles, a small inverse secondary KIE ($^{D}k_{obs}$ = 0.95−0.98) is typically observed; the expected maximum for a fully sp^3-hybridized transition state is about $^{D}k_{obs}$ = 0.89.[26] Therefore, significant sp^3 character is lacking in the transition state for all these reactions, consistent with either a concerted mechanism or an early transition state for formation of a tetrahedral intermediate.

The leaving-O KIE provides strong supporting evidence for the former possibility. The magnitude of these KIEs ($^{18}k_{obs}$ = 1.0135−1.0335) indicates significant C−O bond cleavage to the leaving group in the transition state. As a comparison, the leaving-O KIEs on the alkaline hydrolysis of methyl formate and methyl benzoate, where formation of T^- is rate-determining, are only $^{18}k_{obs}$ = 1.006−1.009. The *p*-nitrophenol group (pK_a = 7.15) is a much better leaving group than methanol (pK_a = 15.5). Consequently, if the mechanism for PNPA were stepwise, breakdown of the tetrahedral intermediate (involving C−O bond cleavage to *p*-nitrophenol) would be expected to be much faster than return to ester. This would lead to a predicted leaving-O KIE which is significantly smaller than that for the alkaline hydrolysis of methyl benzoate and methyl

formate. In contrast, the large observed leaving-O KIEs strongly support the existence of a concerted mechanism for most reactions of PNPA. The amine case is the lone exception. The large leaving-O KIE is similar to that observed for hydrazinolysis of methyl formate[25] and methyl benzoate[7] at pH 8, where breakdown of T^- is rate-determining. In fact, the required proton transfer from the nitrogen nucleophile seems to make a mechanism involving a tetrahedral intermediate a good possibility for alkylamines.

Summary

Multiple KIE experiments for atoms at the reactive center of esters and amides have provided detailed pictures of the transition-state structures during acyl group transfers. Amides and esters with poor leaving groups (formamide, methyl formate, and methyl benzoate) appear to undergo acyl transfer via a stepwise mechanism, involving one or more tetrahedral intermediates. For alkaline hydrolysis of formamide, the transition states for formation and breakdown of T^- are of nearly equal energy; both are relatively late. The transition state for alkaline hydrolysis of methyl benzoate and methyl formate is early and occurs during formation of T^-. The transition state for acid-catalyzed hydrolysis of methyl formate involves attack of water during formation of T^+. Hydrazinolysis of methyl formate exhibits a change in mechanism with pH. The transition state at pH 10 occurs during formation of T^-; this changes to breakdown of T^- at pH 8. When the leaving group is changed to *p*-nitrophenol (in PNPA), the tetrahedral intermediate appears to become kinetically unstable, and a concerted mechanism becomes the most viable alternative for most acyl group transfers.

Research in the author's laboratory was supported by a Cottrell College Science award from Research Corporation and by State Faculty Support Grant (summer fellowships) from Cal Poly. I am grateful to Professors W. W. Cleland and M. H. O'Leary for providing summer research space and supplies.

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AR000054D