Multiple Isotope Effect Study of the Acid-Catalyzed Hydrolysis of Methyl Formate

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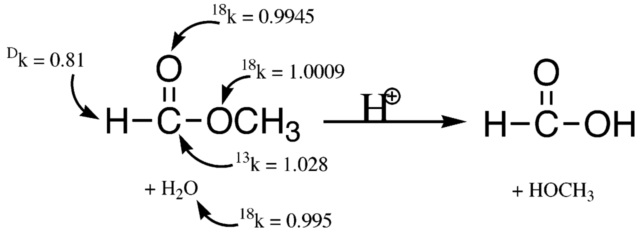

Multiple isotope effects have been measured for the acid-catalyzed hydrolysis of methyl formate in 0.5 M HCl at 20 °C. The isotope effects in the present investigation include the carbonyl carbon ($^{13}k = 1.028 \pm 0.001$), the carbonyl oxygen ($^{18}k = 0.9945 \pm 0.0009$), the nucleophile oxygen ($^{18}k = 0.995 \pm 0.001$), and the formyl hydrogen ($^{D}k = 0.81 \pm 0.02$). Determination of the carbonyl carbon, carbonyl oxygen, and formyl hydrogen isotope effects was performed via isotopic analysis of residual substrate. However, determination of the oxygen nucleophile isotope effect required analysis of the oxygen atoms of the product (formic acid), which exchange with the solvent (water) under acid conditions. This necessitated measurement of the rate of exchange of these oxygen atoms under the conditions for hydrolysis ($k_{ex} = 0.0723$ min^{-1}) and correction of the raw isotope ratios measured during the nucleophile-O isotope effect experiment. These results, along with the previously reported isotope effect for the leaving oxygen ($^{18}k = 1.0009$) and the ratio of the rate of hydrolysis to that of exchange of the carbonyl oxygen with water ($k_h/k_{ex} = 11.3$), give a detailed picture of the transition-state structure for the reaction.

Introduction

For well over half a century, chemists have investigated the reaction mechanisms of the carboxylic acid functional group and its derivatives.[1] The characteristic reaction of these functional groups is nucleophilic acyl substitution (also called acyl transfer). Some of these substitutions are of great importance in biology. For example, the hydrolysis of esters, which is the subject of this investigation, is central to the reactions of lipids and of the neurotransmitter, acetyl choline.

The hydrolysis of esters occurs under both acidic and basic conditions. Although these reactions have been studied by a host of physical organic methods, the use of stable isotopes has played a central role. First, classic ^{18}O labeling experiments by Polanyi and Szabo[2] established that hydrolysis occurs in almost all cases by cleavage of the acyl-O bond rather than the alkyl-O bond. Next, Bender and others[3] devised PIX (positional isotope exchange) experiments demonstrating that most alkyl esters undergo both acidic and alkaline hydrolysis faster than they exchange the carbonyl-O with solvent. The PIX results are reported as a ratio of rate constants, k_h/k_{ex}. For alkaline and acid-catalyzed hydrolysis of methyl formate,[4] these ratios are 18.3 and 11.3, respectively; for the alkaline hydrolysis of methyl benzoate,[3c] the ratio is 27.7. These PIX experiments are strong evidence for the presence of a symmetrical tetrahedral intermediate during hydrolysis, and the magnitude of the rate ratio further implies that formation of the tetrahedral species is slower than its breakdown. The mechanism which is

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[1]) (a) Bender, M. L. *Chem. Rev.* **1960**, *60*, 53. (b) Johnson, S. L. *Adv. Phys. Org. Chem.* **1967**, *5*, 237. (c) Jencks, W. P. *Chem. Rev.* **1972**, *72*, 705.

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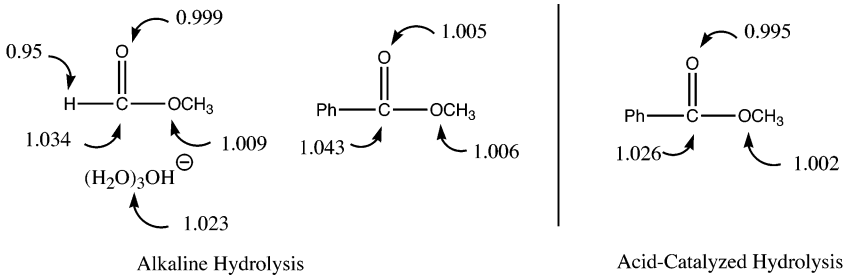
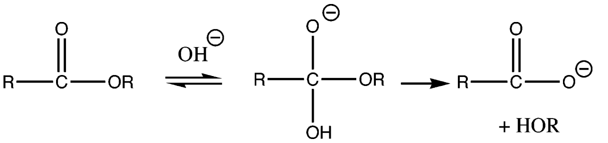
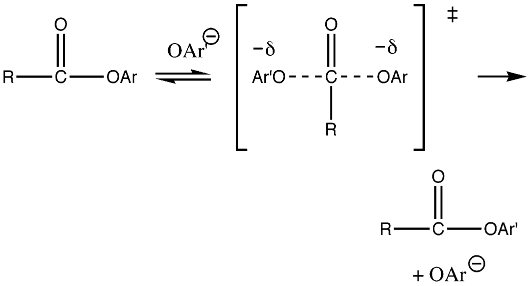


FIGURE 1. Summary of the multiple KIEs for the hydrolysis of methyl formate and methyl benzoate.

most consistent with these findings is referred to as the stepwise mechanism; it is shown for alkaline hydrolysis in eq 1. The results of these PIX experiments were so widely accepted that many introductory organic chemistry texts still treat the acyl transfer of all esters as occurring by this stepwise pathway. However, tetrahedral intermediates for esters with very good leaving groups may become so unstable that an alternative mechanism is possible. For example, structure−reactivity studies by Williams[5] and others[6] offer evidence that the reaction of phenoxides with aryl acetates may occur via an associative (S_N2-like) mechanism, as shown in eq 2. A KIE (kinetic isotope effect) study of the acyl transfer reaction of *p*-nitrophenyl acetate[7] also supports this type of mechanism. It appears that the concerted mechanism occurs when the pK_a of the leaving group is much less than 15.



$$\mathrm{R{-}C({=}O){-}OR} \overset{OH^\ominus}{\rightleftharpoons} \mathrm{R{-}C(O^\ominus)(OH){-}OR} \longrightarrow \mathrm{R{-}C({=}O){-}O^\ominus} + \mathrm{HOR} \quad (1)$$



$$\mathrm{R{-}C({=}O){-}OAr} \overset{OAr'^\ominus}{\rightleftharpoons} \left[\overset{-\delta}{\mathrm{Ar'O}} \cdots \mathrm{C}({=}O)(R) \cdots \overset{-\delta}{\mathrm{OAr}} \right]^\ddagger \longrightarrow \mathrm{R{-}C({=}O){-}OAr'} + \mathrm{OAr}^\ominus \quad (2)$$

Multiple KIEs involve the measurement of isotope effects for all the atoms of the reactive center of a molecule. This has the potential to reveal a more detailed transition-state structure than any single KIE. Multiple KIE studies were first completed for alkaline hydrolysis of several carboxylate esters, including methyl benzoate[8] and methyl formate.[4,9] A complete set of five KIEs has been measured for the attack on *p*-nitrophenyl acetate by hydroxide and other groups with pK_a's > 9 (ref 7). The KIEs for the alkaline hydrolysis of methyl benzoate and methyl formate show some striking similarities. Both are characterized by very small leaving-O KIEs, small carbonyl-O KIEs, and large carbonyl-C KIEs (see Figure 1). Together with the results of the PIX experiments, these KIEs are interpreted as arising from a stepwise mechanism, where the rate-determining step is the formation of a negatively charged tetrahedral intermediate. The uniquely simple molecular structure of methyl formate also allowed for the additional measurement of the formyl-H and nucleophile-O KIEs. The secondary formyl-H KIE is small and inverse ($^{D}k = 0.95$).[10] This result further defines the transition-state structure as being early and sp^2-like. More surprising are the results for the nucleophile-O KIE, which allowed for two possible interpretations.[9] First, hydroxide might be the direct nucleophile, but this would make the observed KIE a large inverse one ($^{18}k = 0.982$). Alternatively, the nucleophile might be one of the water molecules coordinated to a hydroxide, leading to a normal KIE ($^{18}k = 1.023$). The latter option was proposed to be the most likely of the two choices because most observed primary KIEs are dominated by reaction coordinate motion, which results in a normal KIE. Wolfsberg−Stern type calculations by Schowen[11] show this is especially true for early transition states such as those in the alkaline hydrolysis case. This general base mechanism has been both challenged[12] and supported.[6b,13]

The acid-catalyzed hydrolysis of esters has not been as extensively studied as the alkaline case. The kinetics of hydrolysis is first-order in hydronium ion for most alkyl esters. There is additional evidence for a mechanism which includes the rate-determining attack of two water molecules[14] on the ester (eq 3). The only multiple KIE reported is for the acid-catalyzed hydrolysis of methyl benzoate[15] (see Figure 1). The leaving-O KIE is small, but normal, indicating breakdown of the tetrahedral intermediate is fast compared to its formation. Interestingly, the carbonyl-O KIE becomes significantly inverse,

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(5) (a) Ba−Saif, S.; Luthra, A. K.; Williams, A. *J. Am. Chem. Soc.* **1987**, *109*, 6362. (b) Ba−Saif, S.; Luthra, A. K.; Williams, A. *J. Am. Chem. Soc.* **1989**, *111*, 2647. (c) Ba−Saif, S.; Colthust, M.: Waring, M. A.; Williams, A. *J. Chem. Soc., Perkin Trans. 2.* **1991**, 1901.

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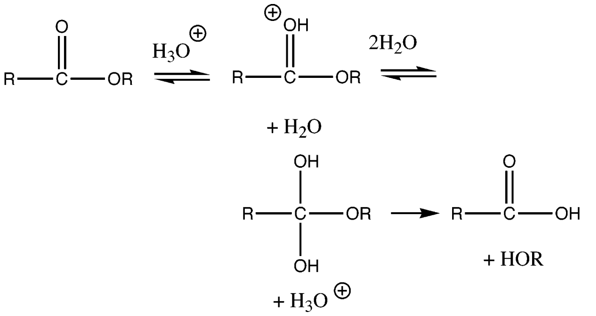


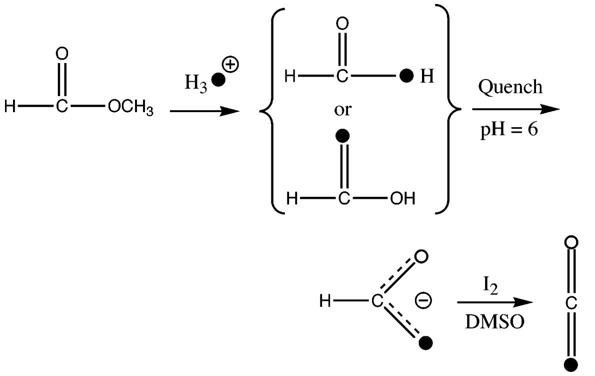
(3)

consistent with the addition of a proton to the carbonyl oxygen, which stiffens the bonding to this atom in the transition state. A formyl-H KIE is not possible for this substrate, and an analytical procedure to measure the nucleophile-O KIE has proven difficult.

The relative ease of isotopic analysis for all the atoms at the reactive center of methyl formate makes methyl formate an ideal candidate for a multiple KIE study of the acid-catalyzed hydrolysis. In addition, some experimental information is already available from the literature, such as the leaving-O KIE ($^{18}k = 1.0009$) and the carbonyl-O PIX ($k_h/k_{ex} = 11.3$).[4] Although the above results are consistent with the mechanism of eq 3, these results say little about bonding to the nucleophile, the carbonyl-C, and the carbonyl-O during hydrolysis. KIEs for these atoms and the requisite analytical procedures are reported in this paper.

Results

The carbonyl-C and carbonyl-O KIEs were measured in a single experiment because the $^{13}\delta$ and the $^{18}\delta$ can be determined simultaneously on the isotope ratio mass spectrometer. The meaning of δ is defined in the Supporting Information. These two isotope effects were measured by isolation of residual methyl formate, followed by quantitative alkaline hydrolysis to formate and subsequent oxidation to CO_2 (eq 4). Analysis of residual substrate avoided a correction for the known oxygen exchange of the product, formic acid, with water. The carbonyl-O still had to be corrected for the exchange of the carbonyl-O of methyl formate with water during the hydrolysis. PIX experiments[4] have been reported ($k_h/k_{ex} = 11.3$) that allow this correction to be calculated; the correction equations[16] have already been published and are also given in the Supporting Information. No correction was necessary for the oxygen derived from the nucleophile because both the residual and unreacted (R_0) substrates were quantitatively converted to formate by alkaline hydrolysis. Thus, the isotopic composition of this oxygen will change by the same amount in both cases. All isotope effects are shown in Table 1.



(4)

TABLE 1. Isotope Effects on the Acid-Catalyzed Hydrolysis of Methyl Formate in 0.5 M Aqueous HCl at 20 °C

atom	isotope effect, k(light)/k(heavy)[a,b]
carbonyl-C	1.028 ± 0.001 (6)
carbonyl-O[c]	0.9945 ± 0.0009 (6)
formyl-H	0.81 ± 0.02 (6)
leaving-O[d]	1.0009 ± 0.0004
nucleophile-O[e]	0.995 ± 0.001

[a] Corrected for fraction of reaction. [b] The standard deviation is given for each isotope effect, and the number of determinations is shown in parentheses. [c] Corrected for carbonyl-O exchange of methyl formate with solvent. [d] Data from ref 4. [e] See text.

The formyl-H KIE was also measured by analysis of residual substrate. This was largely because of the ease of analysis. Kirsch[10] reported this isotope effect earlier, but the conditions were 1.0 M HCl, instead of the 0.5 M HCl in the present study. Both KIEs are identical ($^{D}k = 0.81$) and are shown in Table 1.

Measurement of the nucleophile-O KIE requires analysis of the product, because only the product, not the reactant, contains the oxygen atom derived from the solvent. Previous measurements of the nucleophile-O KIE for the alkaline hydrolysis of methyl formate[9] were done after complete hydrolysis, where the product, formate ion, does not undergo oxygen exchange with solvent. However, the product of acid-catalyzed hydrolysis is formic acid, not formate, and the oxygen atoms of formic acid do exchange with water. Thus, the reaction cannot be allowed to proceed to completion but must be quenched early in the reaction before too much ^{18}O exchange has occurred. This makes the determination of the nucleophile-O KIE for the acid-catalyzed hydrolysis a much more difficult experimental procedure.

To determine the nucleophile-O KIE, the reaction is quenched early and the actual fraction of the reaction determined. The quenched reaction mixture is kept at a pH greater than 5.8 so that formate, rather than formic acid, is present. Formate is oxidized to CO_2, and the $^{18}\delta$ of CO_2 is determined by isotope ratio mass spectrometry. One oxygen of CO_2 (the open ○ in eq 4) is derived from the carbonyl-O of methyl formate; the other (the closed ●, eq 4) is derived from the nucleophile. Calculation of the actual nucleophile-O KIE requires two corrections to the $^{18}\delta$ for the oxygen (○) which is derived from the carbonyl-O of methyl formate. These corrections are for (1) exchange of the carbonyl-O of *methyl formate* during hydrolysis, and (2) the known carbonyl-O KIE for acid-catalyzed hydrolysis (Table 1). The first correction has been described above for measurement of the carbonyl-O KIE. The second correction requires the independently determined carbonyl-O KIE, the fraction of reaction at quench, and the $^{18}\delta$ for the carbonyl-O of the starting methyl formate (−6.3).[16] These requisite data are then

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TABLE 2. Corrections to the $^{18}\delta$ for CO_2 Because of the Oxygen Exchange of Formic Acid[a]

fraction reaction	$^{18}\delta_{(obs)}$	$^{18}\delta_{(unexch)}$[b]
0.18	−16.3	−17.6
0.19	−16.6	−17.9
0.19	−16.6	−17.9
0.18	−16.0	−17.2
0.19	−17.5	<u>−18.9</u>
		−17.9 ± 0.6

[a] All hydrolysis reactions are at 0.5 M HCl and 20 °C. [b] Correction equations described in Supporting Information; standard deviation (±) given for data in column 3.

inserted into Bigeleisen and Wolfsberg's equation[17] for the competitive method to obtain the corrected $^{18}\delta$ for the oxygen of CO_2 at the time of quench. Correction factor 1 changes the $^{18}\delta$ of CO_2 from −6.3 to −6.6; application of correction factor 2 further changes it to −1.6. The $^{18}\delta$ of −1.6 is the final isotopic composition for the oxygen of CO_2 derived from the carbonyl-O of methyl formate; it is subsequently called $^{18}\delta_{(carbonyl)}$.

A final correction factor (3) is to the $^{18}\delta$ of both oxygen atoms of CO_2 for the amount of ^{18}O exchange into *formic acid* prior to quenching. A kinetic investigation of the rate of ^{18}O exchange between formic acid and water under the conditions of the isotope effect experiment was necessary to make this final correction. Formic acid was allowed to incubate in ^{18}O-enriched water at 20 °C in 0.5 M HCl. The $^{18}\delta$ for formic acid was determined after quenching and oxidizing to CO_2. The exchange was found to follow first-order kinetics with a rate constant of 0.072 min^{-1}, while the first-order rate constant for overall hydrolysis was 0.042 min^{-1} under the same conditions. The amount of exchange into formic acid at the time of quench was calculated from the integrated rate law for two sequential first-order reactions (see Supporting Information). The correction data are summarized in Table 2.

Application of correction factor 3 gives the unexchanged $^{18}\delta$ for *formic acid* at the time of quench ($^{18}\delta_{(unexch)} = -17.9$). The isotopic composition of the single oxygen atom of CO_2 that was derived from the carbonyl-O of *methyl formate* (eq 4) at the time of quenching is $^{18}\delta_{(carbonyl)} = -1.6$. Since half of the oxygen atoms of CO_2 are derived from the carbonyl-O of methyl formate, the $^{18}\delta$ for the remaining oxygen atom (from the nucleophile) can be calculated by solving for $^{18}\delta_{(nucl)}$ in eq 5; it was found to be $^{18}\delta_{(nucl)} = -34.2$.

$$^{18}\delta_{(unexch)} = (0.5)^{18}\delta_{(carbonyl)} + (0.5)^{18}\delta_{(nucl)} \qquad (5)$$

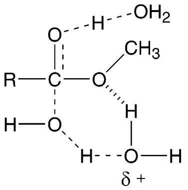
The remaining $^{18}\delta$ needed to calculate the nucleophile-O KIE is that for water. This can be measured by exchanging the oxygen atoms of a small CO_2 sample in the acidified water used in the isotope effect procedures. After correction for the known fractionation factor[18] between CO_2 and water (−41.0‰), the $^{18}\delta_{(water)}$ was found to be $^{18}\delta_{(water)} = -39$. Since the solvent (nucleophile) is in very large molar excess over the substrate, the $^{18}\delta_{(water)}$ does not change appreciably during the reaction. Thus the overall nucleophile-O KIE is then equal to the difference between $^{18}\delta_{(water)}$ and $^{18}\delta_{(nuc)}$ or [(−39.0) − (−34.2) = −4.8]. The nucleophile-O KIE then becomes $^{18}k = 0.995 \pm 0.001$.

Discussion

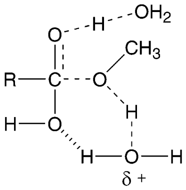
Kinetic evidence indicates that the rate expression for the acid-catalyzed hydrolysis of most alkyl esters should include one molecule of ester, one hydronium ion, and two water molecules. This information is consistent with the mechanism shown in eq 3. Previously determined formyl-H KIE ($^{D}k = 0.81$), leaving-O KIE ($^{18}k = 1.0009$), and PIX ($k_h/k_{ex} = 11.3$) experiments pointed to the formation of a neutral tetrahedral intermediate as the rate-determining step.[4,10]

The small amount of carbonyl-O exchange has been difficult to explain in light of the similarity of the pK_a's for water (15.7) and methanol (15.5), if one accepts the assumption of rapid proton transfers. Under this assumption, the expectation is for a roughly equal partitioning of the tetrahedral intermediate between ester and carboxylic acid. There is evidence that the rates of expulsion for hydroxide and methoxide are not the same under the alkaline conditions for nucleophilic aromatic substitution and related reactions.[19] However, these findings are not applicable to the current acid-catalyzed reaction, where any developing positive charges are shared by the solvent as a whole.[14] Under these conditions, where proton transfer to oxygen is concomitant with C−O bond cleavage, the leaving group ability is expected to more closely correlate with pK_a.

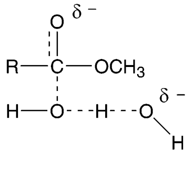
A plausible alternative to the mechanism of eq 3 is one where proton transfer to the carbonyl-O is concerted with the formation of the carbon−nucleophile bond (eq 6). In this mechanism the original carbonyl-O of methyl formate is shown as a closed letter O (●). TI_1 and TI_2 are identical except for the pattern of isotopic labeling. The mechanism in eq 6 allows the developing δ+ charges in the transition state to be as far apart as possible (see structures I and II) and offers a plausible explanation for the low amount of observed carbonyl-O exchange. Unlike the tetrahedral intermediate of eq 3, the first-formed tetrahedral intermediate (TI_1) is not symmetrical, and carbonyl-O exchange would require the extensive reorganization of the hydrogen-bonding network to form TI_2. If this solvent reorganization is kinetically significant, it would lead to a diminished amount of exchange. Arguments for kinetically significant proton transfers have been posed for the alkaline hydrolysis of benzoate esters[3b,3c] and for the neutral hydrolysis of ethyl trifluoroacetate.[3a]



Structure I



Structure II



Structure III

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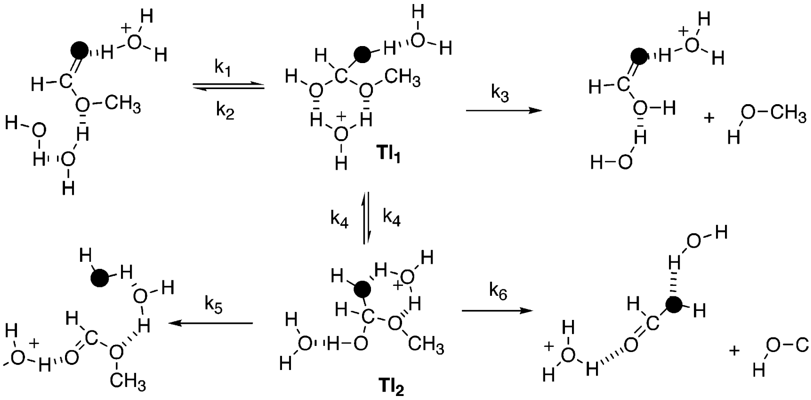

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This concept can be treated on a semiquantitative basis by deriving the steady-state expression for the observed rate constants for exchange and hydrolysis in mechanism 6 as shown in eq 7 and 8, respectively.

$$k_{\mathrm{ex}} = \frac{\dfrac{k_1k_4k_5}{k_4 + k_5 + k_6}}{k_2 + k_3 + \dfrac{k_4k_5 + k_4k_6}{k_4 + k_5 + k_6}} \tag{7}$$

$$k_{\mathrm{h}} = \frac{k_1\left(k_3 + \dfrac{k_4k_6}{k_4 + k_5 + k_6}\right)}{k_2 + k_3 + \dfrac{k_4k_5 + k_4k_6}{k_4 + k_5 + k_6}} \tag{8}$$

Assuming nearly equal rates for the expulsion of $-$OH and $-OCH_3$ (i.e. k_2, k_3, k_5, and k_6 are all equal), eqs 7 and 8 simplify to eqs 9 and 10. The resulting expression for $k_{\mathrm{h}}/k_{\mathrm{ex}}$ is given in eq 11. This allows calculation of the partitioning ratio k_2/k_4 (and k_3/k_4), which is equal to 4.65.

$$k_{\mathrm{ex}} = \frac{k_1}{4}\left(\frac{k_4}{k_2 + k_4}\right) \tag{9}$$

$$k_{\mathrm{h}} = \frac{k_1}{2} \tag{10}$$

$$11.3 = \frac{k_{\mathrm{h}}}{k_{\mathrm{ex}}} = \frac{2(k_2 + k_4)}{k_4} \tag{11}$$

The implication of this partitioning ratio is that solvent reorganization leading to carbonyl-O exchange is 4.65 times slower than product formation, making this solvent reorganization kinetically significant.

If the above analysis is correct, the transition state for formation of the tetrahedral intermediate is similar in energy to that for its breakdown to formic acid, and both transition states (structures I and II) will contribute to the observed KIEs. The large inverse formyl-H KIE and the small leaving-O KIE require the transition state for the breakdown step (k_3) to be nearly tetrahedral. Consequently, the bond order changes when going from the tetrahedral intermediate to the transition state of the k_3 step (structure II) must be very small. Therefore, the observed KIEs are largely due to the bonding changes which occur during the k_1 step. As a result, structure I can be used to qualitatively compare the KIEs and the transition-state structures for acidic hydrolysis to those for alkaline hydrolysis of methyl formate (structure III) and to those calculated for the addition of hydroxide to acetaldehyde.

The Formyl-H Isotope Effect. The mechanism of eq 6 can be simplified to describe the formyl-H KIE. It is not necessary to include the bottom branch of the mechanism (the k_4, k_5, and k_6 steps) because the formyl-H KIE is not sensitive to exchange of the carbonyl-O. The simplified mechanism is shown in eq 12. The k_{obs} for eq

$$\text{methyl formate} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{TI} \xrightarrow{k_3} \text{formic acid} \tag{12}$$

12 is $k_1k_3/(k_2 + k_3)$, which further reduces to $k_1/2$, assuming $k_2 = k_3$. As a result ${}^{\mathrm{D}}k_{\mathrm{obs}}$ is equal to the KIE on step 1 (${}^{\mathrm{D}}k_1$) and, as described above, the transition state best resembles structure I.

Secondary hydrogen KIEs can be used to estimate the degree of progress toward tetrahedral geometry in the transition state for formyl transfer reactions.[10] In both acidic and alkaline hydrolysis, the transition state occurs somewhere between the trigonal ground state of methyl formate and the tetrahedral geometry of the intermediate. From a qualitative standpoint, the large inverse formyl-H KIE observed for acid-catalyzed hydrolysis of methyl formate (${}^{\mathrm{D}}k = 0.81$) argues for a later, more sp^3-like transition state than does that for alkaline hydrolysis (${}^{\mathrm{D}}k = 0.95$). Is it possible to put this increase in sp^3 character on a semiquantitative basis? One way to accomplish this is through theoretical KIE calculations. However, to date there are no reliable isotope effect calculations for acyl group transfers such as those reported in this manuscript. However, Schowen and coworkers have published detailed Wolfsberg$-$Stern type calculations for a related reaction, namely, the addition of hydroxide to acetaldehyde.[11] This reaction is actually quite a good model for the acidic and basic hydrolysis of methyl formate, because the rate-determining step for both hydrolyses is addition of an oxygen nucleophile to a carbonyl, just like in the addition of hydroxide to acetaldehyde. In addition, methyl formate and acetaldehyde share structural characteristics that are important for isotope effect calculations; both have the two "heavy atoms" plus one hydrogen attached to the carbonyl carbon in the trigonal ground state, and both are forming a new C$-$O bond in the transition state. Because the model is a good one, it should be possible to estimate carbon$-$nucleophile bond orders (a measure of progress along the reaction coordinate) for acidic and alkaline hydrolysis using the observed formyl-H KIEs and the table of calculated bond orders of the model in ref 11.

Using the calculations of Schowen[11] as a guide, a $^{D}k = 0.95$ would correspond to a carbon−nucleophile bond order of about 0.2 for alkaline hydrolysis, and a $^{D}k = 0.81$ would correspond to a bond order of about 0.7 for acid hydrolysis. It must be noted that Kirsch[10] independently estimated progress toward tetrahedral geometry for the alkaline hydrolysis of methyl formate to be about 36% complete at the transition state and "very close to tetrahedral" for acidic hydrolysis. For purposes of the discussions that follow, we will use a carbon−nucleophile bond order of 0.2 for alkaline hydrolysis and 0.7 for acid hydrolysis, as inferred from the Schowen paper.

The Carbonyl-C Isotope Effect. The carbonyl-C KIE can be discussed within the framework of the simplified mechanism of eq 12, because this atom is also not sensitive to carbonyl-O exchange. As a result, the $^{13}k_{obs}$ is largely equal to $^{13}k_1$, which is the KIE on step 1. The large magnitude of this KIE is mostly due to reaction coordinate motion and, to a lesser extent, loss of the carbonyl π-bond. The carbonyl-C and the nucleophile-O must move toward each other during formation of the tetrahedral intermediate, and the latter is constricted by hydrogen bonds, so most of the motion will be by the carbon.

Theoretical studies indicate that the carbonyl-C KIE is quite insensitive to changes in transition-state structure. The calculated carbonyl-C KIEs in ref 11 change only slightly from $^{13}k = 1.029$ to $^{13}k = 1.015$ as the carbon−nucleophile bond order increases from 0.2 to 0.7. KIE studies of the hydrolysis of carbonate esters seem to agree with this conclusion.[20] Although the relative rate increased 5000-fold from bis(p-methoxyphenyl) carbonate to bis(p-nitrophenyl) carbonate, the observed carbonyl-C KIE decreased only slightly from $^{13}k = 1.0493$ to $^{13}k = 1.0476$. If this large rate change reflects a significant change in transition-state structure relative to ground-state structure, then the carbonyl-C is indeed not very sensitive to such changes.

To be more confident about this hypothesis, it is desirable to have an empirical example where the transition-state structures for two closely related reactions differ mainly by their position along the reaction coordinate. Assuming the above carbon−nucleophile bond orders apply to alkaline (0.2) and acidic (0.7) hydrolysis, how do the observed carbonyl-C KIEs correspond to the calculated values given above? The empirical values fall from $^{13}k = 1.034$ (alkaline) to $^{13}k = 1.028$ (acid). Like the calculated carbonyl-C KIEs, the observed KIEs decrease as the transition state becomes later. In addition, the magnitude of this decrease is small in both cases, consistent with the original hypothesis that the carbonyl-C KIE is indeed quite insensitive to large changes in transition-state C−O bond order for these types of acyl group transfers. Of course, it would require more than two experimentally determined KIEs to argue empirically that the change in KIE is linear with the change in bond order.

The Leaving Oxygen Isotope Effect. The simplified mechanism of eq 12 also applies to the leaving-O. In this case, the isotope effect is on k_3 so that $^{18}k_{obs}$ is given by eq 13.

$$^{18}k_{obs} = \frac{^{18}k_3 + k_3/k_2}{1 + k_3/k_2} \tag{13}$$

If $k_2 = k_3$, the actual (isotope effect − 1) is twice the observed value ($^{18}k_{obs} = 1.0009$).[4] Thus, $^{18}k_3 = 1.0018$ and the small magnitude of this KIE is consistent with an early transition state for C−O bond cleavage, which is expected because the transition state for formation of the tetrahedral intermediate is late.

The Carbonyl Oxygen Isotope Effect. The carbonyl-O KIE should be interpreted using the full mechanism of eq 6 and the resulting steady-state rate constants of eq 8. This is because a certain fraction of the carbonyl-O atoms will exchange with solvent via the bottom pathway of the mechanism in eq 6 and, as a result, will experience a different set of isotope effects. However, this expanded analysis is complicated by a lack of knowledge of the expected fractionation factors and KIEs for the individual steps of the mechanism. In general, the small inverse KIE ($^{18}k = 0.9945$) reflects a normal contribution from breaking the carbonyl-O π-bond and an inverse one due to introduction of new torsional and bending modes as the bond to the nucleophile is formed.

The bonding to the carbonyl-O in the transition state for the acid-catalyzed hydrolysis of methyl formate (structure I) is significantly different than that proposed for either the alkaline hydrolysis of methyl formate (structure III) or the addition of hydroxide to acetaldehyde. As a result, it is not possible to compare the magnitude of these carbonyl-O KIEs in the manner described above. The most striking difference is the addition of a proton to the carbonyl-O in the acid-catalyzed case, which results in a significant reduction in the carbonyl π-bond order. Unlike the normal KIEs predicted by calculation, the observed carbonyl-O KIE for alkaline hydrolysis ($^{18}k = 0.999$) is essentially unity. This has been discussed in detail elsewhere.[9] Despite a later transition state, the observed inverse carbonyl-O KIE ($^{18}k = 0.995$) on the acid-catalyzed hydrolysis of methyl formate reflects the increased stiffness caused by the protonation of the oxygen and by the introduction of new bending/torsional modes in the transition state. A similar observation was noted in the acid-catalyzed hydrolysis of methyl benzoate.[15] In contrast, it is interesting to note that the calculated isotope effects for the addition of hydroxide to acetaldehyde are always normal, becoming larger for later transition states. This is expected because the carbonyl π-bond order is decreasing and the carbonyl-O is becoming negatively charged.

The Nucleophile Oxygen Isotope Effect. Nucleophile-O KIEs are very difficult to measure. The competitive method requires an analysis of the isotopic composition of either the reactant or the product during the reaction. Technically this means one must monitor the isotopic composition of the solvent (reactant) or formic acid (product). It is not practical to monitor the isotopic composition of the solvent's oxygen atoms during the reaction, because the solvent is in very large molar excess

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over the reactant, resulting in a negligible change during the course of the reaction. Therefore, an analytical procedure must be developed to track the isotopic composition of the single oxygen atom in the product, formic acid, which is derived from the solvent nucleophile. To make matters worse, formic acid undergoes oxygen exchange with water under acidic conditions, as does the carbonyl-O of the reactant, methyl formate. The difficult analytical procedure, with three required correction factors (see Results section), is worth the effort because heavy-atom solvent isotope effects offer a simple and direct view into the bonding changes that occur to the solvent nucleophile when going to the transition state. As mentioned in the Introduction, it was a heavy-atom solvent isotope effect study that first uncovered the possibility of a general base mechanism in the alkaline hydrolysis of esters.[9] In this case, one of the water molecules that hydrates the hydroxide ion is postulated to be the direct nucleophile.

Deuterium solvent isotope effects have long been used to gather similar information concerning bonding to the solvent nucleophile in the transition state. However, this method depends on knowledge of the degree of hydrogen bonding between the substrate and the solvent in the ground state and in the transition state (and the resulting fractionation factors), in addition to the number of protons in flight. There has been some controversy in resolving the results of heavy-atom nucleophile and deuterium solvent isotope effects for the alkaline hydrolysis of esters and amides. Two independent analyses of the same proton inventory data for the alkaline hydrolysis of ethyl acetate have arrived at two different conclusions, one supporting direct attack of hydroxide[12] and the other in agreement with the general base mechanism.[13a]

These two mechanisms were also investigated by a recent theoretical calculation[21] using the cluster-continuum method. Although the transition state involving direct attack by hydroxide was calculated to be lower in energy, the general base mechanism was a mere 1 kcal higher. Given the assumptions inherent in such a theoretical study, it is not possible to decide the issue on the basis of theory alone. It further appears that the role of hydroxide as a direct nucleophile or as a general base must be argued on a case by case basis. For example, a general base mechanism involving hydroxide has been proposed for nucleophilic aromatic substitution of chlorotriazines,[13b] but direct attack of hydroxide was proposed for the alkaline hydrolysis of phosphodiesters.[22]

The nucleophile-O KIE must be analyzed using the full mechanism of eq 6, because the nucleophile-O becomes a carbonyl-O, instead of an −OH group, in the bottom portion of this mechanism. Several assumptions allow the rate constant expression for $^{18}k_{\mathrm{obs}}$ to be simplified. First, it is likely that the KIEs on the k_3, k_5, and k_6 steps are near 1.0, because the transition state in either direction from the tetrahedral intermediate will closely resemble the tetrahedral intermediate. Second, it is assumed that the magnitudes of the rate constants k_2, k_3, k_5, and k_6 are equal, based on the previously mentioned similarity of the pK_a's for methanol and water. Third, as shown previously in eq 11, $k_4 = k_2/4.65$. Since it is assumed that $k_2 = k_3$, it follows that $k_4 = k_3/4.65$. With these assumptions, eq 14 can be derived for $^{18}k_{\mathrm{obs}}$, where $^{18}K_{\mathrm{eq1}}$ is the equilibrium constant on step 1. Although there is no

$$0.995 = {}^{18}k_{\mathrm{obs}} = \frac{{}^{18}K_{\mathrm{eq1}} + 1.19({}^{18}k_1)}{2.19} \tag{14}$$

precise model for $^{18}K_{\mathrm{eq1}}$, a crude estimate of the magnitude of $^{18}K_{\mathrm{eq1}}$ comes from the known fractionation factor between water and a secondary alcohol ($^{18}K_{\mathrm{eq}}$= 0.968).[23] The actual magnitude of this equilibrium isotope effect will be affected by both the slightly heavier mass and the considerably higher electronegativity of oxygen in the tetrahedral intermediate versus carbon in a secondary alcohol.

Substitution of 0.968 for $^{18}K_{\mathrm{eq1}}$ gives a value for $^{18}k_1$ of 1.017. In this case, the nucleophile-O $^{18}k_1$ is normal even though $^{18}k_{\mathrm{obs}}$ is inverse, because of the very inverse value of $^{18}K_{\mathrm{eq1}}$. This result does not agree with the results of theoretical calculations[11] which predict that $^{18}k_1$ will be inverse. This prediction is based on the assumption that the inverse contribution of the TDF (temperature dependent factor) will be larger than the normal contribution from the TIF (temperature independent factor) for late transition states such as those observed in this reaction.[24] The actual reason for this discrepancy is not clear. It is possible that primary KIEs for reactions in solution (vs those in the gas phase used for most calculations) are always normal because they are dominated by reaction coordinate motion (i.e. TIF). This point is borne out by the fact that almost all primary KIEs in the literature involving bond formation are normal. A second reason for the fairly large normal value of $^{18}k_1$ is that the estimate of $^{18}K_{\mathrm{eq1}}$ might be too inverse. More experimental work on the determination of relevant fractionation factors needs to be done to address this latter concern.

Conclusions

Multiple isotope effect studies have been completed for the alkaline and acid-catalyzed hydrolysis of methyl formate. The two reaction mechanisms share many commonalities. The rate-determining step in both cases involves the formation of a tetrahedral intermediate. Furthermore, for both reactions the carbonyl-C undergoes a very similar bonding change when going to the transition state. The one major difference is the placement of the two transition states along the reaction coordinate. The small inverse formyl-H KIE for the alkaline hydrolysis is evidence for an early, sp^2-like transition state, whereas the large inverse KIE for acid-catalyzed hydrolysis indicates a later, sp^3-like transition state. The carbonyl-C KIEs for these two reactions support the theoretical prediction that the magnitude of observed carbonyl-C KIEs changes little despite a change from an early to a late transition state. The magnitude of the carbonyl-C KIEs for alkaline and acidic hydrolysis also supports the conclusion that the carbonyl-C is in motion

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in the transition state. The nucleophile-O KIE on the formation of the tetrahedral intermediate is normal for acidic hydrolysis because of the large assumed equilibrium isotope effect on this step. This result does not agree with theoretical calculations in the gas phase, where the TDF is predicted to dominate this KIE, leading to an inverse value for $^{18}k_1$. It is hoped that the empirical observations in the paper will spur the development of theoretical investigations of isotope effects for this important class of reactions.

Experimental Section

Materials and Methods. Anhydrous DMSO, 1-*d*-methyl formate, and anhydrous 1-*h*-methyl formate were obtained from a commercial supplier. The isotopic composition of the carbonyl oxygen of the methyl formate ($^{18}\delta = -6.3$) was determined previously.[16] MES, HEPES, NAD$^+$, and formate dehydrogenase were obtained from a commercial supplier and were of the highest purity available. Sublimed I_2 was also obtained from a commercial supplier. Isotope ratios for carbon and oxygen were measured on an isotope ratio mass spectrometer. The *m/z* 61/60 ratios of methyl formate for measurement of the formyl-H KIE were determined on a GC-MS with an XT-1 nonpolar column.

Formate Assay. The fraction of reaction was determined via a formate dehydrogenase assay. Typically, a 50 μL aliquot of the reaction mixture was quenched in 4950 μL of 0.10 M phosphate buffer at pH 7.6. A 50 μL aliquot of this diluted solution was added to a solution composed of 100 μL of 15 mM NAD$^+$ and 650 μL of the phospate buffer at pH 7.6 in a 1 mL quartz glass cuvette. Reaction was initiated by addition of 200 μL of a formate dehydrogenase solution (~50 U/mL in the phospate buffer), and the increase in absorbance at 340 nm was determined. Methyl formate was not reactive under these conditions.

Carbonyl Carbon and Carbonyl Oxygen Isotope Effect Procedures. A solution containing 750 μL of water and 25 μL of methyl formate (400 μmol) was incubated at 20 °C. Then a 250 μL aliquot of a 2.0 M HCl solution was added with stirring. Before quenching, a 50 μL aliquot was withdrawn and added to 250 μL of 1.0 M NaOH. After allowing at least 1 h for quantitative alkaline hydrolysis, 250 μL of this solution was added to 4750 μL of the phosphate buffer and the total amount of methyl formate present in the initial reaction mixture was determined via the formate dehydrogenase assay. At a designated time, the reaction was quenched with a solution containing 1 mL of a 1.0 M MES solution (basic form) and 100 μL of 1.0 M NaOH. The resulting pH was between 5.7 and 6.0. Control experiments show that there was negligible further reaction under these conditions. A 50 μL aliquot of this solution was subjected to the above formate dehydrogenase assay in order to determine the amount of formate produced at the time of quench. The remaining quenched solution was placed in a small round-bottom flask, which was equipped with a vacuum stopcock. The unreacted methyl formate was collected under high vacuum by passing the vapor first through a dry ice/isopropanol trap (which collected the water) and then through a liquid N_2 trap (which collected methanol and methyl formate). Methyl formate was then distilled under vacuum into a round-bottom flask containing 1 mL of a 1.0 M NaOH solution (at liquid N_2 temperature). This round-bottom flask was equipped with two stopcocks. One stopcock was on a sidearm that was capped with a septum. The second stopcock was for attachment to the high vacuum line. The solution was warmed to ambient temperature and allowed to undergo complete alkaline hydrolysis (1−2 h). This solution was quenched to pH 7 with 2.0 M HEPES (H$^+$ form) and dried under high vacuum at 70 °C overnight. While under vacuum, 2 mL of anhydrous DMSO containing 250 mg of I_2 was added through the sidearm to the dried formate and the resulting CO_2 was collected into a liquid nitrogen trap as previously described.[16] Isotopic analysis gave the δ for both the oxygen and carbon atoms.

Kinetics of ^{18}O Exchange of Formate with Water. A hydrolysis solution was prepared as described above, except the total volume was 5.0 mL. The hydrolysis was allowed to go to completion (overnight). The solution was then incubated at 20 °C, and the exchange reaction was initiated by addition of 10 μL of ^{18}O-enriched water (0.47 atom % ^{18}O). At various time intervals, 100 μL aliquots were withdrawn and immediately quenched to pH 7 with 2.0 M HEPES (Na-form). The formate was then dried and oxidized to CO_2 as described above.

Nucleophile Oxygen Isotope Effect Procedure. Measurement of this isotope effect requires analysis of the product, rather than of the residual substrate. The solutions employed, the quenching procedure, and the formate assay were the same as described above. However, the quenched solution was immediately dried under high vacuum at 70 °C overnight so that all unreacted methyl formate was pumped away. The dried solution was then oxidized to CO_2 as described above in the carbonyl carbon/oxygen procedure.

Formyl Hydrogen Isotope Effect Procedure. The formyl-H KIE was measured by the same residual substrate isolation procedure as described for the carbonyl carbon/oxygen, using a 1:1 mixture of 1-*d*-methyl formate to 1-*h*-methyl formate. The collected methyl formate was then dissolved in methanol (as a carrier) and the D/H ratio (*m/z* 61/60) was directly measured by GC-MS, without prior oxidation.

Determination of the $^{18}\delta$ for Water ($^{18}\delta_{(water)}$). A small sample of CO_2 (<100 μmol) was added to an evacuated round-bottom flask equipped with a stopcock containing 20 mL of degassed water and 1 drop of concentrated H_2SO_4. The mixture was stirred overnight, and the CO_2 was isolated and analyzed by isotope ratio mass spectrometry. The $^{18}\delta$ for Madison, WI, water has not changed significantly over a 10 year period ($^{18}\delta = -39.3 \pm 0.3$).

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Supporting Information Available: Equations are given to correct (1) the isotopic composition of the carbonyl-O of methyl formate for exchange with water during hydrolysis and (2) the isotopic composition of formic acid for exchange with water under acidic conditions. Also provided is the definition of δ. This information is available free of charge via the Internet at http://pubs.acs.org.

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