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Heavy-Atom Isotope Effects on the Alkaline Hydrolysis of Methyl Formate: The Role of Hydroxide Ion in Ester Hydrolysis

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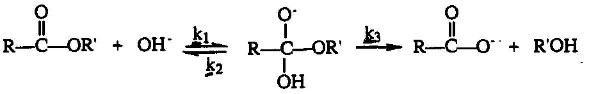
Abstract: Carbonyl carbon, carbonyl oxygen, and nucleophile oxygen isotope effects were measured for the alkaline hydrolysis of methyl formate in water at 25 °C. The carbonyl carbon isotope effect is $k^{12}/k^{13} = 1.0338$, the isotope effect for the carbonyl oxygen is $k^{16}/k^{18} = 0.999$, and that for the oxygen nucleophile is $k^{16}/k^{18} = 1.023$. These isotope effects are consistent with a stepwise mechanism in which the formation of the tetrahedral intermediate is largely rate-determining. The isotope effect on the oxygen nucleophile suggests that the attacking nucleophile in aqueous alkali is water with general base assistance from hydroxide.

Introduction

The alkaline hydrolysis of carboxylate esters is believed to occur by a two-step mechanism in which formation of the tetrahedral intermediate is largely rate-determining[1] (eq 1).



$$R{-}\overset{\overset{\large O}{\|}}{C}{-}OR' + OH^- \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R{-}\underset{OH}{\overset{O^-}{C}}{-}OR' \xrightarrow{k_3} R{-}\overset{\overset{\large O}{\|}}{C}{-}O^- + R'OH \qquad (1)$$

Although many physical methods have been employed to elucidate this mechanism, those utilizing stable oxygen isotopes played a central role. Labeling experiments with ^{18}O have demonstrated that the majority of esters hydrolyze with acyl rather than alkyl bond fission.[2] Classic experiments by Bender[3] and, subsequently, by Shain and Kirsch[4] showed that ^{18}O exchange between the carbonyl oxygen of the ester and water accompanies hydrolysis. This implicated the existence of the tetrahedral intermediate. The ratio of the rate of hydrolysis to the rate of exchange (k_h/k_{ex}) has been used to calculate the partitioning ratio, (k_3/k_2) for the tetrahedral intermediate. For esters, this partitioning ratio is greater than unity, indicating that the formation of the tetrahedral intermediate is more rate-determining than its breakdown. However, it is possible that not all ester hydrolyses follow the stepwise mechanism shown in eq 1. Evidence has been offered for a concerted pathway in cases where esters containing very good leaving groups react with poor nucleophiles.[5,6]

Information concerning the transition-state structure for the alkaline hydrolysis of esters has been obtained from heavy-atom kinetic isotope effects. The most extensively studied ester to date is methyl benzoate,[7] where development of a remote label method allowed measurement of the carbonyl carbon ($k^{12}/k^{13} = 1.04$), carbonyl oxygen ($k^{16}/k^{18} = 1.005$), and methoxyl oxygen ($k^{16}/k^{18} = 1.006$) isotope effects in water at 25 °C. The large carbonyl carbon isotope effect indicates this carbon undergoes considerable reaction coordinate motion on going from starting state to the transition state. The small methoxyl oxygen isotope effect suggests that formation of the tetrahedral intermediate is indeed largely rate-determining. This is consistant with the observation of carbonyl ^{18}O exchange of methyl benzoate ($k_h/k_{ex} = 27.7$) with water.[4] The surprisingly small carbonyl oxygen isotope effect has been tentatively interpreted as arising from a somewhat early transition state.[7]

The alkaline hydrolysis of methyl formate has also been studied by isotope-exchange and isotope-effect experiments.[8,9] Both the methoxyl oxygen isotope effect ($k^{16}/k^{18} = 1.009$ in water at 25 °C) and the oxygen exchange data ($k_h/k_{ex} = 18.3$ in water at 25 °C) are consistent with a largely rate-determining formation of the tetrahedral intermediate. In addition, Kirsch measured the formyl hydrogen isotope effect for methyl formate ($k_H k_D = 0.95$) and estimated that progress toward the tetrahedral geometry was ~36% complete the transition state.[9] In the present study, heavy-atom isotope effects are measured for the carbonyl oxygen, carbonyl carbon, and nucleophile oxygen during the alkaline hydrolysis of methyl formate. The isotope effects are rationalized within the context of the tetrahedral intermediate mechanism. The nature of the attacking oxygen nucleophile is also discussed.

Experimental Section

Materials. Methyl formate, obtained from Aldrich Chemical Co., was fractionally distilled, and the material boiling at 30.5–31.5 °C was collected. The distillate was stored refrigerated in a brown bottle under dry nitrogen. Water was purified with a Millipore Super Q water purification system. Iodine, resublimed crystals, was obtained from Spectrum Chemical Manufacturing Co. Triphenylmethane and anhydrous grade DMSO were obtained from Aldrich Chemical Co. The DMSO was further dried over freshly heated 4-Å molecular sieves and stored over dry, CO_2-free nitrogen.

Rates of reaction were determined with a Hitachi Model U3210 spectrophotometer using the published assay.[8] Isotope ratios were determined on a Finnigan Delta-E or a Finnigan 251 isotope ratio mass spectrometer. Isotopic compositions for carbon and oxygen are given as $\delta(^{13}C)$ and $\delta(^{18}O)$, respectively. Each δ value represents a per mil (‰) difference in the isotope ratio for the sample relative to a tank standard. This tank of CO_2 was standardized to PDB which is a primary isotope ratio standard.[10] Therefore, all δ values are relative to PDB. This relationship is shown in eq 2, where R(sample) is the isotope ratio of the sample (heavy/light) and R(standard) is the isotope ratio for the tank

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standard (or ultimately PDB). The values of $\delta(^{18}O)$ are given as per oxygen.

$$\delta = \left(\frac{R(\text{sample})}{R(\text{standard})} - 1\right) \times 1000 \tag{2}$$

Carbonyl Oxygen and Carbonyl Carbon Isotope-Effect Procedure. To measure the carbonyl carbon and carbonyl oxygen isotope ratios, formate produced during the hydrolysis was converted to CO_2 by the procedure reported by Hiller and Krueger[11] and modified by Hermes et al.[12] The particular details of the current experiments are as follows. To a 50-mL round-bottom flask equipped with a side arm were added enough aqueous 1.7 M KOH solution and enough aqueous 1.0 M methyl formate solution to bring the final concentration of these reagents into the 3–10 and 30–33 mM range, respectively. The total volume was approximately 25 mL. This solution was allowed to incubate at 25 °C for 0.5 h, then quenched with 1 mL of a 0.1 M sodium acetate–acetic acid solution (pH 5.8), and lyophilized for 24 h to remove the remaining water. Under dry, CO_2-free N_2, 5 mL of dry DMSO was added to this lyophilized material. This solution was further purged for 1 h with dry, CO_2-free N_2. In a separate flask, 250 mg of I_2 in 1 mL of dry DMSO was similarly purged. After purging was complete, the two solutions were mixed and the oxidation of formate was allowed to proceed for 1 h at room temperature.[12] The resulting CO_2 was isolated and analyzed as previously described.[13] These samples, with a limiting amount of hydroxide, served as the low-conversion samples.

High-conversion samples were obtained in a similar manner. A 5-mL solution containing approximately 500 μmol of KOH and approximately 165 μmol of methyl formate was incubated for 1.5 h at 25 °C. The reaction was quenched with acetate buffer, and the formate was oxidized to CO_2 in an identical manner to the low-conversion samples.

The above procedure was subjected to the following controls. A solution containing all reagents except methyl formate was subjected to the entire isotope-effect procedure. No CO_2 was detectable (<1 μmol measured manometrically). This ensured that only the CO_2 resulting from alkaline hydrolysis of methyl formate was present in the isotope-effect experiments. A similar control was performed on a solution containing methyl formate but no KOH. Again, no CO_2 was detectable.

Potassium formate (20 mg) was dried under vacuum with heat and then oxidized to CO_2 with I_2/DMSO in the absence of water. Other 20-mg samples of potassium formate were dissolved in the aqueous reaction medium used for the isotope-effect procedure. The water was then removed under vacuum. If the vacuum drying was allowed to proceed for at least 20 h, the $\delta(^{18}O)$ values were in agreement with those for the anhydrous sample. All samples were dried for a minimum of 20 h.

Isotopic Composition of the Carbonyl Oxygen of Methyl Formate. The carbonyl group of methyl formate was decarbonylated with (triphenylmethyl)sodium to carbon monoxide by a modification of the procedure of Powers.[14] (Triphenylmethyl)sodium was prepared by reaction of triphenylmethane (2.5 g, 10 mmol) with a solution of 0.40 g of NaH (60% oil dispersion, 10 mmol) in 25 mL of dry DMSO. The NaH–DMSO solution had been previously heated to 70 °C for 1 h under dry, CO_2-free nitrogen.

In a typical experiment, 1 mL of the (triphenylmethyl)sodium solution was transferred under nitrogen to one side of a Y-tube. A solution of 10 μL of methyl formate (162 mol) in 0.2 mL of dry DMSO was transferred under dry nitrogen to the other side of the Y-tube. The Y-tube was connected via a ground glass joint to a vacuum stopcock which, in turn, was connected to a high-vacuum line. The methyl formate side of the Y-tube was frozen with liquid nitrogen, and the apparatus was evacuated. After the stopcock was closed, the liquid nitrogen bath was removed and the contents of the two chambers were mixed. Vigorous bubbling was observed. After the reaction ceased, the CO was distilled through a liquid nitrogen trap into a tube containing 4-Å molecular sieves cooled to liquid nitrogen temperatures. The CO was then released with heating into the CO to CO_2 converter described by Crowe.[15] The CO was then converted to CO_2 with an electrical discharge. The resulting CO_2 was collected under vacuum and analyzed by isotope ratio mass spectrometry. During this conversion, half of the carbon from CO is lost as elemental C but all the oxygen atoms are converted to CO_2. As a control, samples from a tank standard of CO are converted to CO_2 with a precision of ±0.4‰ for the $\delta(^{18}O)$.

Table I. Carbon and Carbonyl Oxygen Isotope Effects on the Alkaline Hydrolysis of Methyl Formate in Water at 25 °C

fractn reactn	low-conversion (‰) $\delta(^{13}C)$	low-conversion (‰) $\delta(^{18}O)$	high-conversion (‰) $\delta(^{13}C)$	high-conversion (‰) $\delta(^{18}O)$	k^{12}/k^{13} [a]	k^{16}/k^{18} [a,b]
0.22	−61.6		−34.5		1.033	
0.20	−62.9		−34.8		1.034	
0.09	−65.3		−34.3		1.035	
0.23	−62.4		−34.3		1.034	
0.22	−60.8	−16.6	−34.1	−23.1	1.032	0.994
0.15	−64.1	−19.3	−34.4	−22.8	1.034	0.998
0.34	−58.7	−24.2	−34.2	−22.6	1.032	1.004
0.16	−64.0	−19.8	−34.4	−22.4	1.035	0.999
0.20	−63.3	−19.0	−34.4	−21.5	1.035	0.999
0.21	−63.4	−23.3	−34.3	−24.8	1.035	1.000
			mean −34.4	−22.9	1.034	0.999
			std dev ±0.2	±1.1	±0.001	±0.003

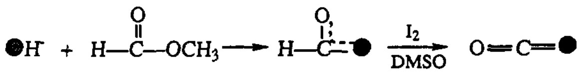
[a] Corrected for percent reaction. [b] Corrected for ^{18}O exchange with water (ref 18).

A small amount of saponification competed with the decarbonylation reaction. The presence of formate was quantified using the I_2 oxidation procedure described above. The amount of saponification could be minimized by the addition of methyl benzoate to the (triphenylmethyl)sodium solution. To be sure that competing saponification did not interfere with the isotopic analysis, samples with differing levels of saponification were generated. Samples in which 15–75% of methyl formate was hydrolyzed were subsequently decarbonylated. The extent of hydrolysis had no detectable effect on the $\delta(^{18}O)$ values of the resulting CO_2, which was formed from CO in these experiments.

Isotopic Composition of Water. The oxygen isotopic composition of the water used in all experiments was measured by exchanging the oxygen atoms of the water with those of a small amount of CO_2. A 20-mL aliquot of this water was sparged with CO_2-free nitrogen and then evacuated. A 150-μmol sample of CO_2 was transferred to the vessel containing the water, and the mixture was stirred overnight. The exchanged CO_2 was collected and analyzed by isotope ratio mass spectrometry. The $\delta(^{18}O)$ values for water and hydroxide ion were calculated from published fractionation factors.[16,17]

Results

The carbonyl carbon and carbonyl oxygen isotope effects on the alkaline hydrolysis of methyl formate (Table I) were measured in unbuffered aqueous solutions at 25 °C. The low-conversion samples contained limiting KOH so that the reaction would stop when the pH had dropped to near neutrality. The 100% conversion samples contained an excess of KOH. The formate produced by hydrolysis was oxidized to CO_2 by I_2 in dry DMSO. One oxygen atom of the CO_2 is derived from the carbonyl of methyl formate while the other is from the solvent. The isotopic composition of the latter is invariant during the time course of the reaction due to the essentially infinite pool of oxygen atoms in the solvent (eq 3).



$$\bullet\mathrm{H}^- + \mathrm{H{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}OCH_3} \longrightarrow \mathrm{H{-}\overset{\displaystyle O}{C}}{\cdots}\bullet \xrightarrow[\text{DMSO}]{I_2} \mathrm{O{=}C{=}}\bullet \tag{3}$$

The reliability of this protocol was tested by several controls. Samples which were missing either methyl formate or KOH produced no measurable CO_2 after being subjected to the entire isotope-effect procedure. Carbon isotope ratios for formate oxidized by the I_2/DMSO procedure or by combustion analysis agreed within ±0.2‰. Finally, the isotopic compositions of all 100% conversion samples were in good agreement for both carbon (±0.2‰) and oxygen (±1.1‰). The higher standard deviation

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Table II. Oxygen Isotopic Composition for Methyl Formate, Water, and Hydroxide

methyl formate carbonyl $\delta(^{18}O)$ (‰)	CO_2 exchanged with H_2O $\delta(^{18}O)$ (‰)
19.0	2.1
19.6	1.8
19.1	mean 2.0, range 0.3
19.4	
19.7	−39, H_2O (calcd, ref 16)
19.2	
19.2	
mean 19.3 std dev ±0.3	−79, OH (calcd, ref 17)

for the oxygen isotopic composition, though acceptable, reflects the difficulty of removing the last traces of water from the oxidation medium. The carbonyl isotope effects are corrected for the small amount of exchange with solvent during hydrolysis.[18]

Calculation of the oxygen isotope effect on the nucleophile required (1) measurement of the $\delta(^{18}O)$ of the water and hydroxide used in the hydrolysis and (2) measurement of the $\delta(^{18}O)$ of the carbonyl oxygen of methyl formate. The $\delta(^{18}O)$ for the CO_2 which was exchanged with the water used in the isotope effect experiments was 2.0‰ (Table II). Using known fractionation factors,[16,17] we calculated a $\delta(^{18}O)$ for water of −39.0‰ and for hydroxide of −79.0‰. The $\delta(^{18}O)$ for the carbonyl oxygen of methyl formate was measured by first decarbonylating the ester followed by conversion of the CO to CO_2 for isotope ratio analysis. The $\delta(^{18}O)$ for this carbonyl oxygen was found to be 19.3 ± 0.3‰ (Table II).

The $\delta(^{18}O)$ for formate after complete hydrolysis of methyl formate was −22.9‰ (Table I), which represents an average $\delta(^{18}O)$ of the two oxygens. One oxygen is from the solvent, and the other is from the carbonyl of methyl formate (see eq 3). After being corrected for a small amount of exchange with solvent, this average $\delta(^{18}O)$ becomes −21.3‰.[18] The measured $\delta(^{18}O)$ for the carbonyl oxygen of methyl formate (19.3‰, Table II) allows the $\delta(^{18}O)$ for the remaining oxygen atom in formate, the one derived from the solvent, to be calculated. The solvent-derived oxygen has a calculated $\delta(^{18}O)$ of −61.9‰. Since the $\delta(^{18}O)$ of water is −39.0‰ and that for hydroxide is −79.0‰, the difference between these two values and the measured −61.9‰ is due to an isotope effect on the nucleophile. If water is the nucleophile, the isotope effect is −22.9‰ or $k^{16}/k^{18} = 1.023$; if hydroxide is the nucleophile, the isotope effect is 17.1‰ or $k^{16}/k^{18} = 0.983$.

Discussion

Carboxylate esters, like methyl formate, are thought to undergo alkaline hydrolysis by the stepwise mechanism of eq 1. The evidence for this mechanism has been reviewed several times.[1] One of the principal lines of evidence is demonstration of carbonyl oxygen exchange with solvent, which for methyl formate is $k_h/k_{ex} = 18.3$.[8] If the tetrahedral intermediate is at protonic equilibrium, the partitioning ratio for this intermediate (k_3/k_2) would then be 9.2, consistent with a largely rate-determining attack of the nucleophile on methyl formate. Early transition states for the alkaline hydrolysis of these types of esters are also postulated on the basis of Bronsted correlations for the nucleophile and the leaving group.[19]

Although the alkaline hydrolysis of carboxylate esters has been studied for many years, very few esters have been investigated by heavy-atom isotope effects. The results of these isotope-effect studies proved useful in defining the transition-state structure within the context of an assumed stepwise mechanism. However, it is important to note that the alternative concerted mechanism cannot be rigorously eliminated on the basis of these isotope effects. Prior to the present investigation, methyl benzoate was the ester most extensively studied by this method.[7] Methyl benzoate is a useful model for interpretation of the current isotope effects for methyl formate because both esters are believed to react via the same stepwise mechanism and both esters share a common leaving group. The isotope effects for these esters[20] are summarized in Figure 1.

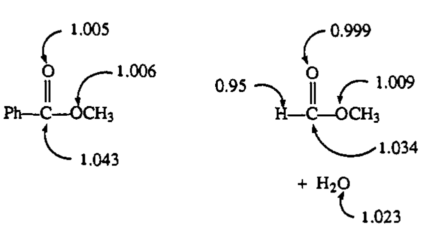

Figure 1. Summary of the isotope effects on the alkaline hydrolysis of methyl benzoate and methyl formate in water at 25 °C.

Carbonyl Carbon Isotope Effect. The carbonyl carbon isotope effect for the alkaline hydrolysis of methyl formate ($k^{12}/k^{13} = 1.034$) is similar in magnitude to that for methyl benzoate ($k^{12}/k^{13} = 1.043$).[7] These are primary isotope effects which mainly arise from motion of the carbonyl carbon along the reaction coordinate. Both carbon isotope effects are large which might indicate that the carbonyl carbon undergoes considerable reaction coordinate motion on going from ground state to transition state. Unfortunately, there is no known quantitative relationship between the magnitude of the carbonyl carbon isotope effect and the transition-state structure. In fact, recent experiments showed carbonyl carbon isotope effects to be quite insensitive to changes in the structure and reactivity of an ester.[21] When the structure of the ester was varied from bis(*p*-methoxyphenyl) carbonate to bis(*p*-nitrophenyl) carbonate, the relative reactivity toward alkali increased 5000-fold, while the carbonyl carbon isotope effect only decreased slightly from $k^{12}/k^{13} = 1.0493$ to $k^{12}/k^{13} = 1.0476$. If the change in relative reactivity is due to significant changes in the transition-state structure relative to the ground-state structure, then the carbonyl carbon isotope effect is not a sensitive tool for quantitatively defining a transition-state structure for this reaction. Theoretical calculations of heavy-atom isotope effects for a related reaction, carbonyl addition, are in general agreement with this premise.[22] For example, the calculated carbonyl carbon isotope effect for hydroxide addition to acetaldehyde (a large normal value) remains relatively constant over a wide range of early to moderately late transition-state structures.

Carbonyl Oxygen Isotope Effects. Because the carbonyl oxygen does not undergo significant reaction coordinate motion during hydrolysis, the observed isotope effect is mainly due to changes in bond order and can be treated as a secondary isotope effect.[23] The formyl hydrogen[9] ($k_H/k_D = 0.95$) isotope effect for this reaction qualitatively suggests some decrease in π-bond order as the transition state is reached. This, in turn, should lead to a normal carbonyl oxygen isotope effect. However, the observed carbonyl oxygen isotope effect is essentially unity ($k^{16}/k^{18} = 0.999$) and does not appear to reflect any decrease in π-bond order. Indeed, the carbonyl oxygen isotope effect on the alkaline

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(18) Marlier, J. F. Ph.D. Dissertation, University of Wisconsin–Madison, Madison, WI, 1978. The steady-state differential equations originally developed in this reference are used to correct the low-conversion and 100% conversion $\delta(^{18}O)$ values of the CO_2 for the presence of a small amount of carbonyl oxygen exchange with solvent ($k_h/k_{ex} = 18.3$; ref 8). These corrected $\delta(^{18}O)$ values are then used to calculate the carbonyl oxygen isotope effect and the oxygen isotope effect on the nucleophile. These corrections are so small that they are less than the uncertainty in measurement of the isotope effect.

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(20) The isotope effects in Figure 1 are from various sources. All methyl benzoate isotope effects are from ref 7; the methoxyl oxygen and formyl hydrogen isotope effects for methyl formate are from refs 8 and 9, respectively. The remaining isotope effects are from this study.

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hydrolysis of methyl benzoate[7] (k^{16}/k^{18} = 1.005), although normal, is also small. Similar results are obtained for the alkaline hydrolysis of *p*-nitrophenyl acetate (k^{16}/k^{18} = 1.004).[24]

There are at least three factors which explain the observed small normal or small inverse carbonyl oxygen isotope effects on the alkaline hydrolysis of esters. First, the alkaline hydrolysis may involve an early, ester-like transition state.[19] Such early transition states were used to explain the small formyl hydrogen isotope effect (k_H/k_D = 0.95) on the alkaline hydrolysis of methyl formate[9] and the small carbonyl oxygen isotope effect (k^{16}/k^{18} = 1.005) on the alkaline hydrolysis of methyl benzoate.[7] Once again, theoretical calculations of the carbonyl oxygen isotope effects for early transition states of carbonyl addition reactions are consistent with this hypothesis.[22]

The second factor is that the maximum carbonyl oxygen isotope effect for complete breaking of the carbonyl π-bond might be small. This would lead to a correspondingly smaller than expected observed oxygen isotope effect. Due to resonance, the carbonyl π-bond order is considerably lower than 2.0 in esters; therefore, breaking this π-bond does not lead to a full 1.0 bond order change. On the other hand, large carbonyl oxygen kinetic isotope effects, on the order of 1.03, are predicted by theoretical calculations for late transition states in ester hydrolysis[25] and carbonyl additions.[22] The only large, normal observed carbonyl oxygen isotope effect in aqueous solution is that for attack of hydrazine on methyl benzoate at pH 8 (k^{16}/k^{18} = 1.018), where breakdown of the tetrahedral intermediate is clearly rate-determining.[7] In this case, the observed carbonyl oxygen isotope effect is comprised of an equilibrium isotope effect upon formation of the tetrahedral intermediate and the kinetic isotope effect upon its decomposition.

In third factor is increased solvation of the carbonyl oxygen in the transition state. In the tetrahedral intermediate mechanism (eq 1), the carbonyl oxygen develops a partial negative charge in the transition state. In turn, this allows the formation of an additional hydrogen bond. Several models support the contention that increased hydrogen bonding will lower the magnitude of the expected normal oxygen isotope effect. First, the vapor pressure isotope effect for water is 1.0091.[26] The increased hydrogen bonding in liquid water causes the ^{18}O to favor this state. Second, the carbonyl oxygen isotope effect on the acid-catalyzed hydrolysis of methyl benzoate is notably inverse (k^{16}/k^{18} = 0.995), even though formation of the tetrahedral intermediate is largely rate-determining.[27] Although this case is not an example of solvation, it demonstrates that formation of a partial bond to hydrogen in the transition state can cause an expected normal oxygen isotope effect to become inverse. Finally, the isotope effect for complete desolvation of a carbonyl group has been estimated to be on the order of 1.01–1.02 for decarboxylation reactions.[12,28]

Nucleophile Oxygen Isotope Effect. The rate of alkaline hydrolysis of simple carboxylate esters exhibits first-order dependence on the concentrations of both ester and hydroxide ion. The simplest mechanism consistent with these data involves direct nucleophilic attack of hydroxide ion on the carbonyl carbon of the ester. This classical interpretation is based, in part, on the observation of inverse deuterium solvent isotope effects.[29] If this mechanism is correct, the $\delta(^{18}O)$ of the nucleophilic oxygen must change from −79‰ for aqueous hydroxide ion to −61.9‰ for the product, formate ion, during the course of hydrolysis. This would result in an inverse primary kinetic isotope effect of k^{16}/k^{18} = 0.983. However, a wealth of empirical evidence concerning heavy-atom isotope effects argues strongly against an inverse isotope effect and a role for hydroxide ion as the direct nucleophile in the reaction. In all but one case, observed primary heavy-atom isotope effects are normal because such isotope effects are dominated by reaction coordinate motion. The lone exception is the ^{37}Cl isotope effect for unsolvated chloride ion reacting as a nucleophile.[30] In this case, the isotope effect is inverse because chloride is more tightly bonded in the transition state than in the ground state where it is not bonded at all. In the present case, both hydroxide and water are highly solvated in the ground state. Consequently, there is no good reason to expect a large inverse primary oxygen isotope effect on the nucleophile in this reaction.

An alternate interpretation involves water acting as the actual nucleophile. Although hydroxide is a better nucleophile than water, it is highly hydrated in aqueous solution and may not be sterically free to attack the carbonyl carbon of the ester. If water is the actual nuclephile, the $\delta(^{18}O)$ of the nucleophilic oxygen changes from −39‰ for water to −61.9‰ for formate ion, resulting in a normal primary kinetic isotope effect of k^{16}/k^{18} = 1.023. In this alternative mechanism, the nucleophilic water is probably one of those hydrating a hydroxide ion. The hydroxide ion would then act more like a general base, removing a proton from the nucleophilic water in the transition state. The observation that methyl formate hydrolysis is catalyzed by succinate buffer[8] supports this conclusion. In addition, theoretical calculations for the addition of oxygen nucleophiles to the carbonyl group[22] predict normal primary kinetic isotope effects for early transition states like those expected for this reaction.

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(24) Hengge, A. C. Unpublished results.

(25) For calculations of the equilibrium carbonyl oxygen isotope effect for methyl benzoate, see footnote 16 in ref 7, above.

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