Heavy-Atom Isotope Effects on the Hydrazinolysis of Methyl Formate

John F. Marlier,*,† Brandy A. Haptonstall,† Amanda J. Johnson,† and Katherine A. Sacksteder‡

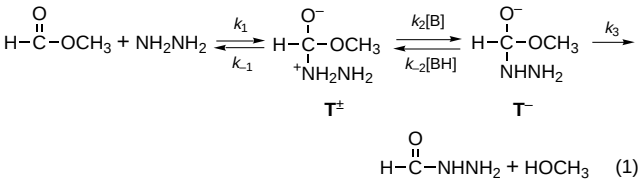
Contribution from the Department of Chemistry and Biochemistry, California Polytechnic State University, San Luis Obispo, California 93407, and Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706

Received March 28, 1997[⊗]

Abstract: The carbonyl carbon, carbonyl oxygen, and nitrogen nucleophile isotope effects were measured for the hydrazinolysis of methyl formate at pH 8 and 10. At pH 8, where breakdown of a tetrahedral intermediate to products is rate-determining, the carbonyl carbon isotope effect is $k^{12}/k^{13} = 1.038$, the carbonyl oxygen isotope effect is $k^{16}/k^{18} = 1.003$, and the nitrogen nucleophile isotope effect is $k^{14}/k^{15} = 0.990$. The isotope effects at pH 8 are consistent with a late transition state, which greatly resembles the hydrazide product. At pH 10, where formation of a tetrahedral intermediate is rate-determining, the carbonyl carbon isotope effect is $k^{12}/k^{13} = 1.020$, the carbonyl oxygen isotope effect is $k^{16}/k^{18} = 1.004$, and the nitrogen nucleophile isotope effect is $k^{14}/k^{15} = 0.9917$. These isotope effects are best rationalized in terms of a concerted general base catalyzed nucleophilic attack of hydrazine on methyl formate as the rate-determining step.

Introduction

The reaction of alkyl esters with amines in aqueous solution is subject to general base catalysis by a second molecule of amine; a reaction not involving such catalysis occurs to a lesser extent.[1−3] Kinetic investigations of the hydrazinolysis of methyl formate and other alkyl esters showed a change in rate-determining step with varying pH.[4] These results implied the existence of tetrahedral addition intermediates. The most widely accepted mechanism is the one proposed by Jencks,[5] which is given in eq 1. The rate-determining step is believed to change from breakdown of $\mathbf{T}^-$ (step 3) at pH 8 to general base catalyzed formation of $\mathbf{T}^-$ (step 2) at pH 10.[5,6]



Information about the transition state structures for the hydrazinolysis of methyl formate and related alkyl esters was obtained previously from isotope effect studies. For the hydrazinolysis of methyl formate at pH 8 a large methoxyl oxygen isotope effect ($k^{16}/k^{18} = 1.062$) together with a small inverse formyl hydrogen isotope effect ($k_H/k_D = 0.98$) were consistent with a late transition state, largely resembling the bonding in the hydrazide product.[7,8] At the same pH, the hydrazinolysis of methyl benzoate gave a slightly smaller methoxyl oxygen isotope effect ($k^{16}/k^{18} = 1.040$) and a considerable carbonyl oxygen isotope effect ($k^{16}/k^{18} = 1.018$).[9] Assuming a similar mechanism for the hydrazinolysis of the two esters, these results imply a slightly earlier transition state for the methyl benzoate case, where the π bond order is not completely restored to the carbonyl oxygen in the transition state. At pH 10, the methoxyl oxygen isotope effect for methyl formate fell to $k^{16}/k^{18} = 1.005$ and the formyl hydrogen isotope effect became more inverse ($k_H/k_D = 0.72$).[7,8] These results are consistent with a change in rate-determining step and with a transition state that is largely tetrahedral. A comparison of the transition states for methyl formate and methyl benzoate is not possible at pH 10 because the isotope effects on the hydrazinolysis of methyl benzoate at pH 10 were not reported.

In the present paper we present isotope effects for the carbonyl carbon, carbonyl oxygen, and nitrogen nucleophile on the hydrazinolysis of methyl formate. The results of these experiments are used to more fully characterize the bonding for the transition states at pH 8 and 10.

Experimental Section

Materials. Methyl formate, hydrazine hydrate, DMSO (anhydrous), chloramine-T, Dowex 50 W ×8-100 resin, and formic hydrazide were obtained from Aldrich Chemical Co. Iodine, sublimed, was from Spectrum Chemical Manufacturing Co. HEPES and CAPS buffers were from Sigma Chemical Co. All chemicals were of reagent grade or better and were used without further purification.

Kinetics. The rates of reaction for hydrazinolysis at pH 8 and 10 were determined by following absorbance changes at 242 nm with a Hitachi model U3210 spectrophotometer with a cell compartment that was temperature equilibrated at 25 °C.[7,8] To correct for the amount of hydrolysis at pH 10, the total absorbance change at 242 nm was measured for a reaction containing a large excess of hydrazine (where no appreciable hydrolysis occurred). The percent of hydrolysis was calculated from this absorbance change and the changes measured during the actual isotope effect runs. The percent hydrolysis and the known isotope effect on alkaline hydrolysis[10] were then used to calculate the isotope effect on hydrazinolysis.

† California Polytechnic State University.
‡ University of Wisconsin.
[⊗] Abstract published in *Advance ACS Abstracts,* August 15, 1997.

(1) Bannett, J. F.; Davis, G. T. *J. Am. Chem. Soc.* **1960**, *82*, 665.
(2) Jencks, W. P.; Carriuolo, J. *J. Am. Chem. Soc.* **1960**, *82*, 675.
(3) Bruice, T. C.; Mayahi, M. F. *J. Am. Chem. Soc.* **1960**, *82*, 3067.
(4) Blackburn, G. M.; Jencks, W. P. *J. Am. Chem. Soc.* **1968**, *90*, 2638.
(5) Satterthwait, A. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 7018.
(6) Satterthwait, A. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 7031.
(7) Sawayer, C. B.; Kirsch, J. F. *J. Am. Chem. Soc.* **1973**, *95*, 7375.
(8) Bilkadi, Z.; deLorimier, R.; Kirsch, J. F. *J. Am. Chem. Soc.* **1975**, *97*, 4317.
(9) O'Leary, M. H.; Marlier, J. F. *J. Am. Chem. Soc.* **1979**, *101*, 3300.
(10) Marlier, J. F. *J. Am. Chem. Soc.* **1993**, *115*, 5953.

S0002-7863(97)00993-1 CCC: $14.00

Isotope ratios were determined on a Finnigan Delta-E isotope ratio mass spectrometer. Isotopic compositions are given as $\delta(^{13}C)$ for carbon, $\delta(^{18}O)$ for oxygen, and $\delta(^{15}N)$ for nitrogen. The δ value represents a per mil difference in the isotope ratio relative to a tank standard.

Carbonyl Carbon and Carbonyl Oxygen Isotope Effect Procedure. The procedure used was essentially that reported in an earlier paper, but modified to allow analysis of residual substrate.[10] The reaction at pH 8 was initiated by addition of 1 mL of a 0.5 M aqueous methyl formate solution to 22 mL of a solution containing 0.47−0.57 M total hydrazine at pH 8. The reaction at pH 10 was initiated by addition of 1 mL of a 0.5 M methyl formate solution to 20 mL of a solution that contained 0.04 M total hydrazine and 0.4 M total CAPS buffer in a 100-mL round-bottom flask. An aliquot was withdrawn for monitoring by the UV assay. At the appropriate fraction of reaction (the fraction of reaction was varied between 0.46 and 0.73), 2.5 M $NaHSO_4$ was added to quench the reaction (pH 4). The contents of the flask were distilled under vacuum, and the unreacted methyl formate (plus some water) was collected into a 50-mL round-bottom flask containing an excess of KOH. After complete saponification of the ester, the flask was equipped with a sidearm containing a stopcock that was capped at the end with a septum. A 1.0-mL sample of a 1 M HEPES solution, pH 7, was added to the formate and the contents were dried at 70 °C under high vacuum for 3 h. Finally, 2 mL of anhydrous DMSO was added through the sidearm, followed by a solution of 200 mg of I_2 in anhydrous DMSO. The reaction was stirred and the resulting CO_2 was collected immediately by vacuum distillation as it was being formed. The isotopic composition of the starting material (which is needed to determine the isotope effect) was determined by quantitative hydrolysis of methyl formate in excess KOH, followed by oxidation to CO_2 in DMSO/I_2.

The entire procedure was checked by several controls. A reaction mixture that contained all reagents except methyl formate failed to produce detectable amounts of CO_2; similar results were obtained when all the methyl formate was allowed to react with hydrazine. In a separate experiment, sodium formate was incubated with water enriched in ^{18}O. This formate was then carried through the entire oxidation procedure. The $\delta(^{18}O)$ value for the resulting CO_2 showed no detectable exchange of the oxygen atoms with the enriched water. When methyl formate of known isotopic composition was subjected to the entire isolation procedure, the $\delta(^{13}C)$ and $\delta(^{18}O)$ remained unchanged.

Nitrogen Nucleophile Isotope Effect Procedure. The reaction was initiated by adding an aqueous methyl formate solution to an equal volume of a solution containing hydrazine and either HEPES buffer (Li^+-form) at pH 8 or CAPS buffer (Li^+-form) at pH 10. All reaction mixtures contained a molar excess of hydrazine to methyl formate (varied from a 2:1 to a 1.25:1 ratio) so that the reaction was limited by methyl formate. When all the methyl formate had reacted, the reaction mixture was adjusted to pH 5.0 with 1 M lithium acetate buffer and the unreacted hydrazine was isolated by ion exchange chromatography on Dowex 50 resin (Li-form). The product, formic hydrazide, was first washed off the column with H_2O, followed by elution of the hydrazine with 0.1 M LiOH (6.5-mL fractions). A 1-mL aliquot of each fraction was titrated with chloramine-T to an iodide end point.[11] This allowed determination of the amount of hydrazine in each fraction and calculation of the fraction of hydrazine consumed in the reaction. The pooled fractions of hydrazine were placed in a 250-mL round-bottom flask containing 4 mL of 1.0 M HEPES, pH 7.1, to neutralize the LiOH. This flask had a sidearm equipped with a stopcock and a small vessel containing at least a 5-fold molar excess of aqueous chloramine-T. Atmospheric N_2 was removed by several freeze−thaws and then the chloramine-T was added to the hydrazine solution. The resulting N_2 was collected under vacuum over 4 Å molecular sieves at liquid N_2 temperature, after first passing through one dry ice trap and two liquid N_2 traps. The N_2 sample was then analyzed by isotope ratio mass spectrometry. The concentrations of hydrazine and buffer (HEPES or CAPS) were varied in some of the runs. No detectable change in the isotope effect was observed.

As a control, hydrazine of known isotopic composition was subjected to the isolation procedure. The resulting $\delta(^{15}N)$ was unchanged. A reaction mixture that contained all reagents except hydrazine failed to yield detectable amounts of N_2.

(11) Singh, B.; Sing, R. *Anal. Chim. Acta* **1954**, *11*, 408.

Table 1. Carbonyl Carbon, Carbonyl Oxygen, and Nitrogen Nucleophile Isotope Effects on the Hydrazinolysis of Methyl Formate in Water at 25 °C[a,b]

pH	carbonyl C isotope effect[c] (k^{12}/k^{13})	carbonyl O isotope effect (k^{16}/k^{18})	N nucleophile isotope effect[d] (k^{14}/k^{15})
8.0	1.038 ± 0.001 (9)	1.003 ± 0.001 (9)	0.990 ± 0.001 (8)
10.0	1.020 ± 0.001 (5)	1.004 ± 0.001 (4)	0.9917 ± 0.0003 (6)

[a] All isotope effects corrected for percent reaction. [b] Numbers in parentheses are the number of determinations. [c] Corrected for the amount of alkaline hydrolysis (ref 10). [d] The isotope effect at pH 8 is corrected for the equilibrium isotope effect of the pK_a of hydrazine (see text).

Results

The carbonyl carbon and carbonyl oxygen isotope effects for hydrazinolysis of methyl formate were measured in aqueous solution at pH 8 and 10 at 25 °C. The unreacted methyl formate was collected, hydrolyzed to formate ion, and then oxidized to CO_2 by anhydrous DMSO-I_2. The resulting CO_2 was analyzed by isotope ratio mass spectrometry. One of the oxygen atoms of CO_2 is derived from the carbonyl oxygen of methyl formate, the other is from the solvent. The isotopic composition of the latter oxygen does not change during alkaline hydrolysis because the solvent is essentially an infinite pool of oxygen atoms. All hydrolyses were performed by using a single source of 1 M aqueous KOH that was tightly sealed between reactions. The control experiments to test the reliability of the procedure are described in the Experimental Section. The hydrazinolysis at pH 10 was too rapid at high concentrations of hydrazine (>0.1 M) to allow accurate determination of the fraction of reaction by the UV assay. Consequently, the concentration had to be lowered and a significant amount of alkaline hydrolysis occurred. The fraction of hydrolysis was determined for each run, and the observed carbonyl carbon and carbonyl oxygen isotope effects were corrected by using the published isotope effects on alkaline hydrolysis.[10] The isotope effects are summarized in Table 1.

Nitrogen nucleophile isotope effects were determined at pH 8 and 10 under similar conditions to those for the carbonyl carbon and carbonyl oxygen isotope effects. Both sets of experiments involved analysis of residual hydrazine. The unreacted total hydrazine (containing a mixture of hydrazine, and its conjugate acid) was isolated and oxidized to N_2 for isotopic analysis. The concentration of hydrazine and buffers (HEPES or CAPS) was varied in several runs, but no change in the nitrogen isotope effect was detected. Controls for this procedure are described in the Experimental Section. The isotope effect at pH 8 had to be corrected for the equilibrium isotope effect on the pK_a of hydrazine. This equilibrium isotope effect is technically difficult to measure so the $^{15}K_{eq}$ of 1.0167 for protonation of phenylalanine (a primary amine) was used as an approximate value.[12] Because the masses of the atoms attached to the nitrogen being protonated are similar in both cases, the $^{15}K_{eq}$ of a primary amine should serve as a good approximation for that of hydrazine. It should be noted that substitution of a more electronegative atom (nitrogen) for a carbon atom of a primary amine might have some additional small effect on estimation of the $^{15}K_{eq}$ for hydrazine. Calculation of the isotope ratio for free hydrazine was accomplished by using eq 2, where x_1 is the [product]/[reactant] ratio at equilibrium, y_1 is the $^{15}N/^{14}N$ ratio of the product at equilibrium, and y_2 is the mass ratio of the initial reactant.[12] This isotope ratio was subsequently converted to a $\delta(^{15}N)$ value.

(12) Hermes, J. D.; Weiss, P. M.; Cleland, W. W. *Biochemistry* **1985**, *24*, 2959.

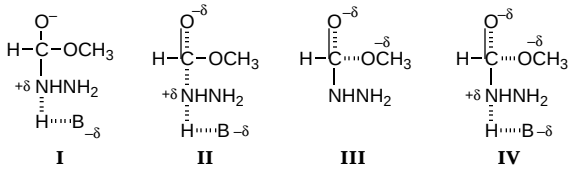
$$^{15}K_{eq} = y_2/y_1 + x_1(y_2/y_1 - 1) \quad (2)$$

This yielded a $\delta(^{15}N)$ for free hydrazine of −10.4 at pH 8.0. The measured $\delta(^{15}N)$ in the residual substrate (total hydrazine) did not change during the course of the reaction, remaining at 0.2. Since the $\delta(^{15}N)$ did not change for the total pool of hydrazine, the $\delta(^{15}N)$ for the product must also be 0.2. Thus the observed isotope effect must be (−10.4 − 0.2) or $k^{14}/k^{15} = 0.990$. These isotope effects are also summarized in Table 1.

Discussion

The hydrazinolysis of methyl formate has been shown to occur by a stepwise mechanism involving one or more tetrahedral intermediates (eq 1). To more fully characterize the transition state for this reaction, we have measured the carbonyl carbon, carbonyl oxygen, and nitrogen nucleophile isotope effects at pH 8 and 10. These results, as well as the earlier methoxyl oxygen and formyl hydrogen isotope effects measured by Kirsch et al.,[7,8] are summarized in Figure 1. The isotope effects are discussed within the framework of this stepwise mechanism.

Isotope Effects at pH 10. A proton transfer step (step 2, eq 1) is reported to be rate-determining at pH 10; the transition state for this step is shown as structure **I**.[5,6] Taken together the reported large inverse formyl hydrogen isotope effect[8] and the small normal methoxyl oxygen isotope effect[7] (see Figure 1) were offered as additional evidence for transition state structure **I**. However, a very late (and nearly tetrahedral) transition state for a concerted, general base-catalyzed formation of $\mathbf{T}^-$ (structure **II**) cannot be rigorously ruled out on the basis of these reported isotope effects.



I **II** **III** **IV**

(a) Carbonyl Carbon Isotope Effects. The carbonyl carbon isotope effect at pH 10 presented in this study is slightly smaller ($k^{12}/k^{13} = 1.020$) than most of those reported for the reaction of alkyl esters with basic nucleophiles ($k^{12}/k^{13} = 1.03-1.04$).[9,10,13] Interpretation of carbonyl carbon isotope effects for many acyl group transfer reactions is difficult because of the considerable reaction coordinate motion of this atom and because carbonyl carbon isotope effects appear to be insensitive to changes in the structure and the reactivity of esters.[13] However, in the present study this carbonyl carbon isotope effect provides crucial information on the transition state structure.

The small leaving group oxygen isotope effect and the large inverse formyl hydrogen isotope effect (see Figure 1) argue for a transition state that is largely sp^3 hybridized, with very little bond reduction to the leaving oxygen. Jencks contends that the rate-determining step for the aminolysis of esters with poor leaving groups (like methoxy) is a proton transfer from $\mathbf{T}^\pm$ to a general base. This conclusion can be tested for the hydrazinolysis of methyl formate by the carbonyl carbon isotope effect. If the rate-determining step is removal of a proton from $\mathbf{T}^\pm$, it follows that $\mathbf{T}^\pm$ must be in equilibrium with methyl formate and hydrazine. Removal of a proton from the nitrogen of $\mathbf{T}^\pm$ will cause a negligible change in the observed carbonyl carbon isotope effect. Therefore, the observed carbonyl carbon isotope effect should arise solely from the equilibrium between $\mathbf{T}^\pm$ and methyl formate. On the other hand, if the nucleophilic addition and proton removal occurred in a concerted fashion (transition state structure **II**), then the carbonyl carbon isotope effect will be a primary one. The question is as follows: Will a primary and a secondary isotope effect be different enough to distinguish between the two possibilities?

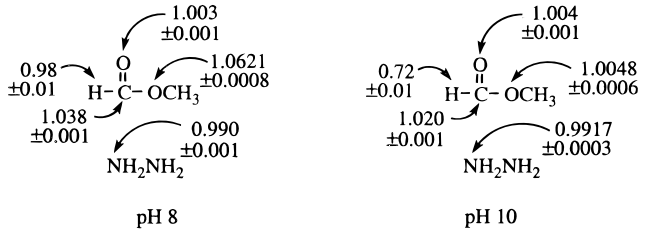


Figure 1. Summary of the isotope effects on the hydrazinolysis of methyl formate. All isotope effects are given as k_{light}/k_{heavy}.

Although we are not aware of any direct models in the literature for this equilibrium isotope effect, an inverse isotope effect is clearly expected since bonding to the carbon in the tetrahedral intermediate will be stiffer than that in the ground state ester. There are at least two examples that allow a reasonable approximation of the magnitude of this inverse isotope effect. The first example is the equilibrium formation of ethyl hydrazine from ethene (eq 3). In this model a C−C

$$NH_2NH_2 + CH_2{=}CH_2 \rightleftarrows CH_3CH_2NHNH_2 \quad (3)$$

double bond (instead of a C−O double bond) is replaced with a C−N single bond. Although an alkene and a carbonyl group have different polarities, the masses of the reacting atoms are similar. The data used to estimate this $^{13}K_{eq}$ have been tabulated.[14] The reasoning is as follows: The equilibrium isotope effect for forming ethane from ethene is $^{13}K_{eq} = 0.998$.[15] Replacement of a hydrogen on the isotopic carbon atom of ethane with a heavy atom, such as nitrogen, decreases the value to 0.983. Addition of a second nitrogen (to make the hydrazine) will further reduce this equilibrium isotope effect to around 0.979. Since the carbonyl carbon isotope effect on the ionization of benzoic acid is small ($k^{12}/k^{13} = 1.001$),[16] protonation of the inner nitrogen (effectively the difference between $\mathbf{T}^-$ and $\mathbf{T}^\pm$) is not expected to significantly change the above carbon isotope effect. Therefore, $^{13}K_{eq} = 0.979$ is a rough estimate of the carbonyl carbon equilibrium isotope effect for formation of $\mathbf{T}^\pm$ (or $\mathbf{T}^-$) from methyl formate and hydrazine. The second example is a theoretical calculation of the equilibrium carbonyl carbon isotope effect for addition of hydroxide to acetaldehyde (addition of a heavy atom to a carbonyl).[17] This calculated isotope effect is $^{13}K_{eq} = 0.962$ at 25 °C. Both of the above examples clearly lead to the expectation of a large inverse carbonyl carbon isotope effect for the equilibrium between methyl formate and a tetrahedral species (either $\mathbf{T}^-$ or $\mathbf{T}^\pm$) of between 2 and 4%. By contrast, a primary carbon isotope effect for a concerted mechanism would be expected to be large and normal, due to the considerable reaction coordinate motion experienced by the carbonyl carbon. There are many examples of this type of kinetic isotope effect in the literature; one pertinent example is the carbonyl carbon isotope effect on the alkaline hydrolysis of methyl formate ($k^{12}/k^{13} = 1.034$).[10] In this case carbonyl oxygen exchange and a small leaving group isotope effect clearly established attack of the nucleophile on the ester to be rate-determining.[7] The present carbonyl carbon

(13) Marlier, J. F.; O'Leary, M. H. *J. Am. Chem. Soc.* **1990**, *112*, 5996.

(14) Cleland, W. W. In *Methods Enzymol.* **1980**, *64*, 104. Especially pertinent are the rules in footnote r on page 110.

(15) Hartshorn, S. R.; Shiner, V. J. *J. Am. Chem. Soc.* **1972**, *94*, 9002.

(16) Bayles, J. W.; Bron, J.; Paul, S. O. *J. Chem. Soc., Faraday Trans. 1* **1976**, *7*, 1546.

(17) Hogg, J. L.; Rodgers, J.; Kovach, I.; Schowen, R. L. *J. Am. Chem. Soc.* **1980**, *102*, 79.

isotope effect on the hydrazinolysis of methyl formate at pH 10 is normal and rather large, making it impossible that methyl formate and $\mathbf{T}^{\pm}$ are in equilibrium, thereby requiring a concerted, general base mechanism for this reaction. The observation of general base catalysis[1−3] and a large primary isotope effect argue strongly in favor of a mechanism involving a concerted, general base catalyzed formation of $\mathbf{T}^{-}$ (transition state structure **II**) as the rate-determining step. In addition, the large formyl hydrogen isotope effect[8] (Figure 1) indicates this transition state is rather late and sp^3-like.

Can this conclusion be extended to include the aminolysis of other esters? The concerted mechanism will occur whenever $\mathbf{T}^{\pm}$ does not have a finite lifetime. Jencks has postulated that a concerted mechanism is possible for catalysis by strong bases of weakly basic amines (giving a $\mathbf{T}^{\pm}$ with a relatively low pK_a) or for esters containing very good leaving groups.[5] Because methyl formate is such a reactive ester (making it necessary to have a low level of hydrazine to follow the reaction), the present carbonyl carbon isotope effect experiments at pH 10 employed a ratio of total buffer (CAPS) to total hydrazine of 10/1. This makes it likely that CAPS ($pK_a = 10.4$), rather than hydrazine ($pK_a = 8.3$), served as the general base in the reaction. Therefore, these conditions involve catalysis by a relatively strong base (CAPS) of the attack on methyl formate by weakly basic (but highly nucleophilic) hdyrazine. In fact, the pK_a of the nitrogen of $\mathbf{T}^{\pm}$ has been estimated[5] to be near 9.6, making proton transfer from $\mathbf{T}^{\pm}$ to CAPS thermodynamically favorable. This would not be true when hydrazine is acting as a general base. The experimental conditions employed by Jencks and co-workers at or above pH 10 had a ratio of total buffer to total hydrazine that was 10 to 30 times lower than that used in our experiments. Our conditions are much closer to those outlined above where a concerted mechanism becomes possible. Therefore, at this time it is not possible to rigorously extend the concerted mechanism to the acetate esters studied by Jencks without further isotope effect studies.

(b) Carbonyl Oxygen Isotope Effect. The measured carbonyl oxygen isotope effect ($k^{16}/k^{18} = 1.004$) is a secondary kinetic isotope effect because the connection between the carbon and the oxygen is not severed during the reaction. The largest measured carbonyl oxygen isotope effects reported in the literature are those for the hydrazinolysis of methyl benzoate[9] at pH 8 ($k^{16}/k^{18} = 1.018$) and the reaction of sulfur anions with *p*-nitrophenyl acetate[18] ($k^{16}/k^{18} = 1.012$). In contrast, primary oxygen isotope effects (where an O−C σ bond is completely severed) can be much larger. For example, the methoxyl oxygen isotope effect on the hydrazinolysis of methyl formate[7] at pH 8 is $k^{16}/k^{18} = 1.062$. This is considerably smaller than the theoretical maximum of $k^{16}/k^{18} = 1.19$ calculated by Biegeleisen and Wolfsberg[19] for a primary oxygen isotope effect. The corresponding theoretical maximum for the secondary isotope effect on breaking a carbonyl π bond is not known, but may be close to that calculated for the addition of hydroxide to acetaldehyde ($k^{16}/k^{18} = 1.03$).[17]

Three reasons have been given to account for the lesser magnitude of the secondary carbonyl oxygen isotope effects:[10] (1) The breaking of a π bond to oxygen will inherently give smaller isotope effects than breaking a σ bond. A more detailed explanation for the hydrazinolysis of methyl formate involves a qualitative analysis of vibrational modes in the ground state and transition state for this reaction. Formation of a new C−N bond adds bending (O−C−N) and torsional (O−C−N−N) modes in the transition state which are not present in the ground state. This will serve to stiffen the bonding in the transition state (an inverse isotope effect) and will counter the normal isotope effect for breaking the carbonyl π bond, leading to an observed effect that is smaller than expected. This stiffening is more important in methyl formate than in higher molecular weight esters such as methyl benzoate. Methyl formate does not have the C−C−C−O torsional mode present in methyl benzoate, and the H−C−O bending mode of methyl formate is less ^{18}O sensitive than the C−C−O mode present in methyl benzoate. Empirical data seem to fit this explanation; the carbonyl oxygen isotope effects on the alkaline hydrolysis of methyl benzoate[9] ($k^{16}/k^{18} = 1.0046$), *p*-nitrophenyl acetate[18] ($k^{16}/k^{18} = 1.0039$), and methyl formate[10] ($k^{16}/k^{18} = 0.999$) decrease in the direction predicted. (2) The transition states for all these reactions may occur either very early during formation of the tetrahedral intermediate or very late during breakdown of a tetrahedral species. Both cases would give a transition state structure containing a large degree of sp^2 character (like the starting ester or amide product) and should yield a small oxygen isotope effect. This may explain the small isotope effects for alkaline hydrolysis, but in the present case, the large inverse formyl hydrogen isotope effect argues for a transition state that contains considerable sp^3 character. (3) The anionic oxygen in the transition state is more strongly solvated than the ground state, and this increased hydrogen bonding in the transition state lowers the observed isotope effect. Several models that support this conclusion include the vapor pressure isotope effect for water (1.0091),[20] the inverse carbonyl oxygen isotope effect on the acid-catalyzed hydrolysis of methyl benzoate ($k^{16}/k^{18} = 0.995$),[21] and the estimated isotope effect on desolvation of the carboxyl group ($k^{16}/k^{18} = 1.01-1.02$) during decarboxylations.[22]

Nitrogen Isotope Effect. Isotope effects on entering nucleophiles, like all primary isotope effects, are composed of two principal factors—the temperature-independent factor (TIF) and the temperature-dependent factor (TDF).[23] The TIF is always normal, whereas the TDF is normal when bonds are being broken to the isotopic atom in the transition state and inverse when these bonds are being formed. This usually makes interpretation of nucleophile isotope effects somewhat complex.

As outlined in the preceding sections, the most likely transition state for hydrazinolysis at pH 10 is one like that shown in structure **II**. Furthermore, this transition state is late and sp^3-like. Although nearly all observed primary kinetic isotope effects for bond formation are normal (they are dominated by reaction coordinate motion), calculations suggest that as the transition state occurs later (i.e. greater bond formation to the nucleophile) the observed isotope effect will tend to become smaller.[17,24] In the hydrazinolysis reaction the nucleophilic nitrogen gains a new C−N bond but simultaneously loses a N−H bond. As a result it is reasonable to expect the primary nitrogen isotope effect to be a small normal one. The outer, or nonnucleophilic, nitrogen experiences additional N−N−C bending and N−N−C−O torsional modes in the transition state (structure **II**) and will show an inverse secondary isotope effect (perhaps as large as 0.5−1.0%). Since both the nucleophilic and outer nitrogens are analyzed together, this secondary isotope effect will make the observed isotope effect more inverse.

Isotope Effects at pH 8. The reported large methoxyl oxygen isotope effect[7] (Figure 1) indicates considerable C−O bond breaking in the transition state. The small formyl hydrogen isotope effect[8] (Figure 1) is consistent with a late transition state

(18) Hengge, A. C.; Hess, R. A. *J. Am. Chem. Soc.* **1994**, *116*, 11256.
(19) Bigeleisen, J.; Wolfsberg, M. *Adv. Chem. Phys.* **1958**, *1*, 15.
(20) Szapiro, S.; Steckel, F. *Trans. Faraday Soc.* **1967**, *63*, 883.
(21) Marlier, J. F.; O'Leary, M. H. *J. Org. Chem.* **1981**, *46*, 2175.
(22) Headley, G. W.; O'Leary, M. H. *J. Am. Chem. Soc.* **1990**, *112*, 1894.
(23) Melander, L. *Isotope Effects on Reaction Rates*; Ronald Press: New York, 1960.
(24) Paneth, P.; O'Leary, M. H. *J. Am. Chem. Soc.* **1991**, *113*, 1691.

in which the carbonyl carbon has considerable sp^2 hybridization (like that of the hydrazide product). Thus, at pH 8 the formation of products (i.e. decomposition of a tetrahedral intermediate) is clearly rate-determining. The following isotope effects are rationalized within this framework.

(a) Carbonyl Carbon Isotope Effect. The measured carbonyl carbon isotope effect (Figure 1) is similar to that reported for methyl benzoate at the same pH ($k^{12}/k^{13} = 1.040$).[9] Assuming that breakdown of the tetrahedral species $\mathbf{T}^-$ to products is fully rate-determining, the observed isotope effect is composed of an equilibrium isotope effect for formation of $\mathbf{T}^-$ and a kinetic isotope effect on its decomposition. If proton transfer from the nitrogen atom has a negligible effect on this equilibrium carbon isotope effect, it follows that the equilibrium isotope effect for formation of $\mathbf{T}^-$ should be near the 0.979 inverse value calculated earlier for $\mathbf{T}^\pm$ (see discussion for pH 10). Consequently, the carbonyl carbon kinetic isotope effect on the decomposition of $\mathbf{T}^-$ can be estimated to be on the order of $k^{12}/k^{13} = 1.06$. If the product forming step is not fully rate-determining, the estimated kinetic isotope effect on decomposition of $\mathbf{T}^-$ will be somewhat smaller.

(b) Carbonyl Oxygen Isotope Effect. Although there is strong evidence for a change in the rate-determining step on going from pH 8 to 10, the carbonyl oxygen isotope effect does not exhibit a significant change (see Figure 1). Once again, the stronger solvation of the anionic oxygen in the transition state as compared to the ground state may partly account for these similar, small isotope effects. Interestingly, the carbonyl oxygen isotope effect for hydrazinolysis of methyl benzoate at pH 8 is significantly larger ($k^{16}/k^{18} = 1.018$)[9] than that for methyl formate. As pointed out earlier, this trend toward larger carbonyl oxygen isotope effects for methyl benzoate is also true for other reactions like alkaline hydrolysis, where $k^{16}/k^{18} = 1.0046$ for methyl benzoate and $k^{16}/k^{18} = 0.999$ for methyl formate. The reason for this trend seems to be the additional bending (O−C−N) and torsional (O−C−N−N) modes present in the transition state but not in the ground state. As mentioned previously, methyl benzoate already has similar bending and torsional modes to the benzene ring making the effect of adding new ones to the incoming nucleophile proportionally smaller than in the methyl formate case.

(c) Nitrogen Isotope Effects. At pH 8 the conjugate acid of hydrazine (pK_a 8.3) is the dominant molecular species, and the equilibrium nitrogen isotope effect on this pK_a must be considered. This equilibrium isotope effect is technically difficult to measure but should be similar to that for a primary amine such as phenylalanine ($^{15}K_{eq} = 1.0167$).[12] Correction of the observed nitrogen isotope effect for this equilibrium (using the phenylalanine model) gives an overall isotope effect of $k^{14}/k^{15} = 0.990$ (see Results section) for formation of the hydrazide product from free hydrazine.

If the product-forming step is fully rate-determining, the observed nitrogen isotope effect would be composed of an equilibrium isotope effect on tetrahedral intermediate formation and a kinetic isotope effect on its breakdown. Assuming for the moment that $\mathbf{T}^-$ is the only tetrahedral intermediate that leads directly to products,[5] the equilibrium isotope effect for its formation can be estimated by utilizing the known equilibrium isotope effect for formation of phenylalanine ($-NH_2$ form) from free NH_3 and cinnamate ($^{15}K_{eq} = 0.984$).[12] This model involves replacement of a hydrogen of ammonia with a secondary carbon atom from phenylalanine, whereas the formation of $\mathbf{T}^-$ involves replacement of a hydrogen of hydrazine with a carbon containing two heteroatoms (each with nearly the same mass as carbon). The presence of the outer nitrogen on hydrazine (versus ammonia) will alter the estimated equilibrium isotope effect in two ways. First, the equilibrium isotope effect for protonation of a primary amine such as phenylalanine is $^{15}K_{eq} = 1.0167$ and that for ammonia is $^{15}K_{eq} = 1.0192$.[12] By analogy deprotonation of the secondary amine in $\mathbf{T}^\pm$ should slightly increase the equilibrium isotope effect for formation of $\mathbf{T}^-$ by about the same 0.2%, giving $^{15}K_{eq} = 0.986$. Second, the outer nitrogen will make this value even lower by approximately 0.5−1.0% because of the aforementioned gain of one new bending and one new torsional mode. Therefore, one would reasonably expect the equilibrium isotope effect for formation of $\mathbf{T}^-$ to be in the range $^{15}K_{eq} = 0.976-0.981$. The nitrogen kinetic isotope effect on decomposition of $\mathbf{T}^-$ (transition state structure **III**) is a secondary one resulting from the weakening of one bending mode (N−C−O) and two torsional modes (N−C−O−C and N−N−C−O) in the transition state as a result of breaking the bond to the leaving group. This loss of three vibrational modes will give a normal isotope effect, which will cause the observed isotope effect to be larger (more normal) than the above estimated equilibrium isotope effect of $^{15}K_{eq} = 0.976-0.981$. Seen in this light the observed isotope effect of $k^{14}/k^{15} = 0.990$ is a reasonable result.

The nitrogen isotope effect (as well as the other isotope effects) is consistent with a second mechanism in which deprotonation of the inner nitrogen of $\mathbf{T}^\pm$ occurs simultaneously with bond breaking to the leaving group (transition state structure **IV**). This is possible because any general base species present at pH 8 may not be strong enough to remove the proton from $\mathbf{T}^\pm$. However, as methoxide begins to leave the amide nitrogen will become more acidic making removal of the proton more facile. If this is the case, we must estimate the equilibrium nitrogen isotope effect on the formation of $\mathbf{T}^\pm$ (instead of $\mathbf{T}^-$) from hydrazine. This isotope effect will be lower than that estimated above for the formation of $\mathbf{T}^-$ ($^{15}K_{eq} = 0.976-0.981$) due to the proton on the inner nitrogen. Once again we can employ the isotope effect for protonation of the amino group of phenylalanine as a model.[12] Protonation will lower the equilibrium isotope effect on formation of $\mathbf{T}^\pm$ to approximately $^{15}K_{eq} = 0.959-0.964$. If we assume product formation is fully rate-determining and an equilibrium isotope effect on formation of $\mathbf{T}^\pm$ near $^{15}K_{eq} = 0.96$, the kinetic isotope effect for product formation (including deprotonation) must be approximately $k^{14}/k^{15} = 1.03$ for this alternative mechanism.

Summary

The isotope effects presented here complete a study of all the reacting atoms in the hydrazinolysis of methyl formate and provide further refinement of the transition state structures. Strong evidence is offered in support of a concerted, general base catalyzed formation of a tetrahedral intermediate ($\mathbf{T}^-$) as the rate-determining step at pH 10. This differs from the rate-determining proton transfer step proposed earlier for other aliphatic esters.[5,6] At pH 8 the isotope effects are consistent with rate-determining breakdown of an anionic tetrahedral intermediate ($\mathbf{T}^-$) to products, as reported in the literature[5,6] or with a concerted, general base catalyzed decomposition of $\mathbf{T}^\pm$ to products.

Acknowledgment. I am grateful to Professor W. W. Cleland (GM18938) of the University of Wisconsin−Madison for helpful discussions and for providing summer laboratory space and supplies. Additionally, I wish to thank Professor Marion H. O'Leary of Sacramento State University for useful comments on this work and Isabel Treichel of the University of Wisconsin−Madison for help with mass spectral analysis.

JA970993+