

## A Heavy-Atom Isotope Effect Study of the Hydrolysis of Formamide

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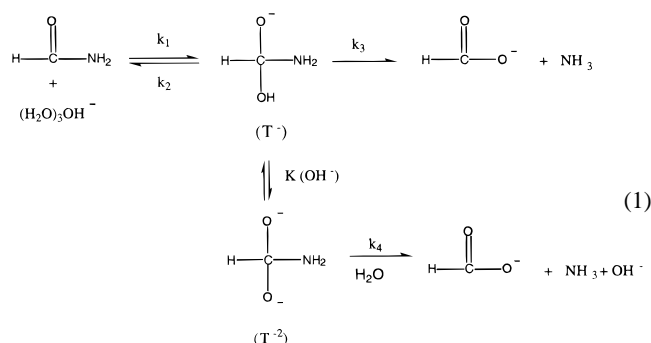
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**Abstract:** Isotope effects were measured for all the atoms at the reactive center of formamide during hydrolysis in dilute alkaline solution. Most of the reaction proceeds by a pathway that is first-order in hydroxide, although a small amount proceeds by a pathway that is second-order in hydroxide. For alkaline hydrolysis at 25 °C the carbonyl carbon isotope effect is  $^{13}k_{\text{obs}} = 1.0321$ , the carbonyl oxygen isotope effect is  $^{18}k_{\text{obs}(\text{C}=\text{O})} = 0.980$ , the formyl hydrogen isotope effect is  $^{\text{D}}k_{\text{obs}} = 0.80$ , and the nitrogen leaving group isotope effect is  $^{15}k_{\text{obs}} = 1.0040$ . The ratio of the rate of hydrolysis to the rate of exchange for the alkaline hydrolysis of formamide was shown to be linearly dependent on the hydroxide concentration, ranging from an extrapolated value of  $k_{\text{h}}/k_{\text{ex}} = 2.1$  at very low hydroxide concentrations to  $k_{\text{h}}/k_{\text{ex}} = 8.4$  at 1.5 M hydroxide. This is consistent with a mechanism in which an increasing fraction of the tetrahedral intermediate pool is trapped as a dianion at high pH, effectively lowering the rate of exchange. These results also indicate that the transition states leading into and out of the tetrahedral intermediate are of comparable energy for the pathway which is first-order in hydroxide. The solvent nucleophile isotope effect is  $^{18}k_{\text{obs}(\text{nuc})} = 1.022$  for water as the attacking nucleophile or  $^{18}k_{\text{obs}(\text{nuc})} = 0.982$  for hydroxide as the attacking nucleophile. These results strongly suggest that one of the water molecules hydrating the hydroxide ion is the actual attacking nucleophile instead of hydroxide ion itself.

## Introduction

Amides play a key role in both organic chemistry and biochemistry. Important biological reactions such as the degradation of proteins, as well as other metabolic reactions, underscore the importance of understanding the mechanisms of amide hydrolysis. Amides are the least reactive of the common acyl groups toward basic nucleophiles, due to the poor ability of an unprotonated nitrogen to act as a leaving group. The most widely accepted mechanism under basic conditions involves tetrahedral intermediates<sup>1–3</sup> as shown in eq 1.



Kinetic studies have shown that the alkaline hydrolysis of many amides with simple aliphatic amines usually follows a rate law which is first-order in hydroxide.<sup>1,2,4</sup> On the other hand, some anilides, and other amides containing strong electron-

withdrawing substituents, show a rate law term which is second-order in hydroxide.<sup>4</sup> One explanation is that electron-withdrawing groups on the amine serve to lower the  $\text{p}K_{\text{a}}$  of the  $-\text{OH}$  group of the tetrahedral intermediate, facilitating the creation of an oxydianion which can eliminate the amine with or without prior protonation.<sup>4</sup> General-base catalysis, although uncommon, has been observed for reactive anilides such as trifluoroacetanilides and formanilides.<sup>3</sup> Formamide alkaline hydrolysis has been studied at several concentrations of hydroxide<sup>5,6</sup> and found to have a rate law containing both first- and second-order terms in hydroxide, consistent with the mechanism in eq 1.

Amides are known to exchange the carbonyl oxygen with solvent during alkaline hydrolysis.<sup>4</sup> The results of such exchange experiments are usually reported as a ratio of the rate constant for hydrolysis to that for exchange ( $k_{\text{h}}/k_{\text{ex}}$ ). In turn, this result is used to determine the partitioning ratios ( $Kk_4/k_2$  or  $k_3/k_2$ , eq 1) for the tetrahedral intermediate, assuming the proton-transfer steps are rapid. It was initially expected that the tetrahedral intermediate would partition back to amide much faster than formation of products ( $k_{\text{h}}/k_{\text{ex}} < 1$ ) because the hydroxyl group is a better leaving group than most amines. This is true for benzamide, where  $k_{\text{h}}/k_{\text{ex}} = 0.21$  at 109 °C, but increasing the number of alkyl groups on the nitrogen decreases the relative amount of exchange from  $k_{\text{h}}/k_{\text{ex}} = 0.74$  for *N*-methylbenzamide to an undetectable level of exchange in *N,N*-dimethylbenzamide.<sup>7,8</sup> It was postulated that tertiary amides are

(4) Brown, R. S.; Bennet, A. J.; Slebocka-Tilk, H. *Acc. Chem. Res.* **1992**, 25, 481 and references therein.

(5) Kirsch, J. F. In *Isotope Effects on Enzyme-Catalyzed Reactions*; Cleland, W. W., O'Leary, M. H., Northrup, D. B., Eds.; University Park Press: Baltimore, MD, 1977; pp 100–121.

(6) Hine, J. S.; King, R. S.; Midden, W. R.; Sinha, A. *J. Org. Chem.* **1981**, 46, 3186.

(7) Bender, M. H.; Ginger, R. D.; Unik, J. P. *J. Am. Chem. Soc.* **1958**, 80, 1044.

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(1) O'Conner, C. J. *Q. Rev. Chem. Soc.* **1971**, 24, 553.

(2) Bender, M. L.; Thomas, R. J. *J. Am. Chem. Soc.* **1961**, 83, 4183.

(3) (a) Schowen, R. L.; Jayarmann, H.; Kershner, L. *J. Am. Chem. Soc.* **1966**, 88, 3373. (b) Mader, P. M. *J. Am. Chem. Soc.* **1965**, 87, 3191. (c) DeWolfe, R. H.; Newcomb, R. C. *J. Org. Chem.* **1971**, 36, 3870.

incapable of exchange because at least one hydrogen must be present on the nitrogen of the tetrahedral intermediate to catalyze proton transfer between oxygen atoms. Recent studies by Brown and co-workers<sup>4</sup> on a series of toluamides included several tertiary amides that did exchange the carbonyl oxygen with the solvent. The current interpretation is that the extent of carbonyl oxygen exchange of an amide is mostly dependent on the basicity of the amine.<sup>4</sup>

The nature of the steps for decomposition of the tetrahedral intermediates ( $k_3$  and  $k_4$ ) is not fully understood. Solvent isotope effects, which are often complicated to interpret, tend to indicate that the nitrogen of fairly basic amines (such as aliphatic amines) is fully protonated prior to leaving;<sup>4</sup> however, a concerted proton transfer with a late transition state remains a possible alternative.

Isotope effects have provided detailed information concerning the transition-state structure of many acyl group transfer reactions. However, only a few such studies have been reported for the reactions of amides. Kirsch determined the formyl hydrogen isotope effect on the alkaline hydrolysis of formamide.<sup>5</sup> This isotope effect is dependent on the concentration of hydroxide, consistent with a term in the rate law that is both first- and second-order in hydroxide. Kirsch fitted these data to the mechanism of eq 1 and calculated the kinetic isotope effects for formation of  $T^-$  ( $Dk_1 = 0.65$ ), breakdown of  $T^-$  ( $Dk_3 = 1.58$ ), and breakdown of  $T^{2-}$  ( $Dk_4 = 1.41$ ), indicating fairly late transition states in all cases. There are only a few reports of heavy-atom isotope effects on amide hydrolysis. Leaving group nitrogen isotope effects for chymotrypsin-catalyzed hydrolysis of *N*-acetyl-L-tryptophanamide<sup>9</sup> ( $^{15}k_{\text{obs}} = 1.010$  at pH 8) and papain-catalyzed hydrolysis of *N*-benzoyl-L-argininamide<sup>10</sup> ( $^{15}k_{\text{obs}} = 1.023$  at pH 8) were carried out by O'Leary. The difference between the isotope effects for the two enzyme-catalyzed reactions appears to be due to different partitioning ratios of the intermediates. The leaving group nitrogen isotope effect on the alkaline hydrolysis of benzamide ( $^{15}k_{\text{obs}} = 1.004$ ) was reported as an unpublished result in ref 9.

In this paper we present isotope effects for all of the atoms at the reactive center of formamide during hydrolysis in dilute alkaline solution, where the reaction is largely first-order in hydroxide ion concentration. We are primarily interested in the mechanism of the first-order reaction because of its similarity to the mechanism of enzyme-catalyzed amide bond hydrolysis and, hence, its suitability as a model. The results of these experiments allow a detailed picture of the transition-state structure for this reaction. In addition, the solvent oxygen nucleophile isotope effect allows determination of the actual nucleophile (water or hydroxide) for the reaction.

## Experimental Section

**Materials and Methods.** Formamide and HEPES buffer were from Sigma Chemical Co. Methyl formate (anhydrous), DMSO (anhydrous), triphenylmethane, sodium hydride (60% oil suspension), and 2.0 M ammonia in methanol were obtained from Aldrich Chemical Co. Iodine (sublimed) was from Mallinckrodt Chemical Co. All were of reagent grade or better and were used without further purification. Water containing 99 atom %  $^{18}\text{O}$  was obtained from Isotec Inc. for the GC-MS method, and that containing 96 atom %  $^{18}\text{O}$  was obtained from Icon Services Inc. for the NMR method. *l*-Methyl formate (99 atom %) was obtained from Aldrich Chemical Co.

The UV spectra were measured on a Cary 4 UV-vis spectrophotometer. A UV assay at 240 nm was used to determine the fraction of

reaction needed in the calculation of the nitrogen isotope effect, the formyl hydrogen isotope effect, and the carbonyl oxygen exchange with solvent. For the carbonyl carbon and carbonyl oxygen isotope effects the fraction of reaction was determined by manometric measurements of the amount of  $\text{CO}_2$  produced via oxidation of formate. In separate control experiments the two methods of measuring the fraction of reaction were shown to be in close agreement.

Isotope ratios for the heavy-atom isotope effects were determined on a Finnigan Delta-E isotope ratio mass spectrometer; isotopic compositions are given as  $\delta(^{13}\text{C})$  for carbon,  $\delta(^{18}\text{O})$  for oxygen, and  $\delta(^{15}\text{N})$  for nitrogen. The  $\delta$  value represents a per mil (‰) difference in the isotope ratio compared to a tank standard. In the oxygen exchange experiments the  $^{18}\text{O}/^{16}\text{O}$  ratio of the carbonyl oxygen of unreacted formamide was measured on a Shimadzu QP or a Hewlett-Packard 1800A GC-MS, equipped with an XT-1 nonpolar column; the  $^{18}\text{O}/^{16}\text{O}$  ratio of the product, formate, was measured with a Bruker 360 NMR spectrometer, utilizing the isotope shift of the carbonyl carbon. The ratio of *l*-*d*-formamide to *l*-*h*-formamide in the formyl hydrogen isotope effects was determined on a Hewlett-Packard Series II (model 5890) gas chromatograph, equipped with a 60 m carbowax column.

**Syntheses.** Knowledge of the isotopic composition of the carbonyl oxygen of formamide is needed to determine the oxygen nucleophile isotope effect. This  $\delta(^{18}\text{O})$  cannot be determined directly. Consequently, formamide was synthesized from methyl formate, for which the isotopic composition of the carbonyl oxygen can be determined by a published decarbonylation procedure.<sup>11</sup> To synthesize this formamide, a dry methanolic solution which was 0.89 M in methyl formate and 1.88 M in ammonia was allowed to react under dry nitrogen at room temperature for 21 h. The methanol and ammonia were removed by rotary evaporation, and the remaining material (crude yield 100%) was distilled under reduced pressure. A sample of this purified formamide was hydrolyzed completely and then oxidized by  $\text{I}_2/\text{DMSO}$ . Finally, the  $\delta(^{13}\text{C})$  of the carbonyl carbon was determined by isotope ratio mass spectrometry of the resulting  $\text{CO}_2$  (see below). The fact that the isotope ratio of the carbonyl carbon of formamide did not change significantly (<1 per mil) from that of the starting methyl formate infers that the conversion was complete and the isotope ratio for the carbonyl oxygen must also be unchanged during the synthesis and purification of formamide.

*l*-*d*-Formamide was synthesized in an identical manner from *l*-*d*-methyl formate. The purified product was mixed in a 1:1 ratio with natural *l*-*h*-formamide and used in the formyl hydrogen isotope effect experiments.

**Carbonyl Carbon and Carbonyl Oxygen Isotope Effect Procedures.** The carbonyl carbon and carbonyl oxygen isotope effects were measured by a slight modification of the previously published procedure.<sup>11</sup> In a typical experiment a low conversion sample contained 0.10–0.20 M KOH and 0.20 M formamide in a total volume of 2.0 mL. After a reaction time of 15 min (10–32% reaction) the reaction was quenched by addition of 1.0 mL of HEPES buffer, pH 7.5. This solution was transferred to a round-bottom flask equipped with a side arm which contained a stopcock. Water was removed by heating this flask to 85 °C under high vacuum for a minimum of 3 h, after which 2.0 mL of anhydrous DMSO containing 300 mg of  $\text{I}_2$  was added through a septum attached to the side arm. The  $\text{CO}_2$  was collected into a U-tube at liquid nitrogen temperature after first being passed through two liquid nitrogen-pentane traps. Control experiments with formamide in the absence of KOH failed to produce any  $\text{CO}_2$  when subjected to the drying and oxidation procedure, making removal of unreacted formamide unnecessary. The  $\delta(^{18}\text{O})$  and  $\delta(^{13}\text{C})$  of the resulting  $\text{CO}_2$  were determined by isotope ratio mass spectrometry. High conversion samples were prepared by allowing a solution which was 0.20 M formamide and 1.4 M KOH to react for a minimum of 50 min. This solution was quenched with 0.6 mL of 1.0 M HCl and 1.0 mL of 1.0 M HEPES, pH 7.5. The solution was then dried, oxidized, and analyzed as above.

**Leaving Nitrogen Isotope Effect Procedure.** Low conversion samples for the nitrogen isotope effects were prepared by allowing a 5.0 mL solution which was 0.20 M in formamide and 0.10–0.20 M in

(8) Bunton, C. A.; Nayak, B. O'Conner, C. *J. Org. Chem.* **1968**, *33*, 572.

(9) O'Leary, M. H.; Kluetz, M. D. *J. Am. Chem. Soc.* **1972**, *94*, 3585.

(10) O'Leary, M. H.; Urberg, M.; Young, A. P. *Biochemistry* **1974**, *13*, 2077.

(11) Marlier, J. F. *J. Am. Chem. Soc.* **1993**, *115*, 5953.

KOH to react until enough KOH was consumed that reaction effectively had ceased (39–57% reaction by the UV assay). A 1.0 mL aliquot was immediately applied to a 2 mL Dowex 50 column (H<sup>+</sup>-form) and eluted with water. Fractions of 3.0 mL volume were collected. In separate control experiments it was shown that the unreacted formamide eluted in the first three fractions, whereas the ammonium ion was tightly bound to the column. The pooled fractions containing the unreacted formamide were treated with enough 10 M KOH to bring the final concentration of hydroxide to 1.0 M. The hydrolysis to ammonia and formate was allowed to proceed for 1.75 h, after which the solution was made mildly acidic (pH paper) with 9 M H<sub>2</sub>SO<sub>4</sub>. The reaction mixture was then placed in a round-bottom flask equipped with a side arm which was attached to a small glass vessel containing a several-fold molar excess of a concentrated NaOBr solution.<sup>9</sup> After several freeze–thaw cycles under high vacuum to remove atmospheric nitrogen, the NaOBr was added from the side arm to the main flask. The nitrogen produced by oxidation of the ammonia was collected under vacuum into molecular sieves at liquid nitrogen temperature after passing through two liquid nitrogen traps. The  $\delta(^{15}\text{N})$  of the collected nitrogen was then determined by isotope ratio mass spectrometry. High conversion samples were generated from solutions that were 0.20 M in formamide and 1.4 M in KOH. After complete hydrolysis the ammonium ion produced was oxidized and analyzed as above. A column was not necessary in the case of the high conversion samples because no unreacted formamide remained.

**Nucleophile Oxygen Isotope Effect Procedure.** The oxygen nucleophile isotope effect was measured by reacting a solution that was 0.27 M in formamide of known  $\delta(^{18}\text{O})$  at the carbonyl oxygen (see above synthesis section) and 1.7 M in KOH for at least 1 h. The formate produced by this hydrolysis was neutralized by addition of 1.0 mL of 1.0 M HEPES (acid form) and then dried and oxidized by I<sub>2</sub>/DMSO as described above. The isotope ratio of the water (and hydroxide) used in these experiments was determined by exchanging the oxygen atoms of the water (10 mL) with a 0.1 mmol sample of CO<sub>2</sub> for 20 h with stirring. The exchanged CO<sub>2</sub> was then collected under vacuum and analyzed by isotope ratio mass spectrometry in the usual manner.

**Formyl Hydrogen Isotope Effect Procedure.** The formyl hydrogen isotope effect was measured using a 1:1 mixture of 1-*d*-formamide/1-*h*-formamide (see synthesis section). In these experiments a solution that was 0.20 M in total formamide and 0.20–0.40 M in KOH was followed by the UV assay at 240 nm. After the reaction nearly stopped due to low levels of KOH (31–68% reaction), the solution was immediately extracted three times with 2 mL of ethyl acetate. The pooled ethyl acetate was dried with anhydrous MgSO<sub>4</sub> and analyzed by capillary GC. The two isotopic formamides separated on the 60 m carbowax column, with the deuterium-containing isotopomer eluting 5 s later than natural abundance material. The area under each peak was determined by a Grams 95 program. The isotope effect was then calculated in the usual way from the fraction of reaction and the isotope ratios.

**Carbonyl Oxygen Isotope Exchange Procedures.** The extent of carbonyl oxygen isotope exchange was measured by two methods. In the first method a solution containing 0.40 mL of 1.0 M formamide, 0.20 mL of H<sub>2</sub>O (99 atom % <sup>18</sup>O), and 0.100–0.300 mL of 1.0 M KOH in a total volume of 1.000 mL was allowed to react and then extracted with ethyl acetate and dried just as in the formyl hydrogen isotope effect experiment (see preceding paragraph). In this case the <sup>18</sup>O/<sup>16</sup>O ratio of the unreacted formamide was determined on the Shimadzu QP GC–MS. The isotope exchange at 2.0 M hydroxide was determined in a similar manner, except the Hewlett-Packard GC–MS was used for isotopic analysis. The extent of reaction was determined by either the UV assay or integration of the formyl hydrogen resonances in the proton NMR.

The extent of carbonyl oxygen exchange was also measured by a novel method utilizing the <sup>18</sup>O isotope shift on the carbonyl carbon of [<sup>13</sup>C]formate. For this exchange experiment 10  $\mu\text{L}$  of 9.4 M KOH, 10  $\mu\text{L}$  of [<sup>13</sup>C]formamide, 480  $\mu\text{L}$  of [<sup>18</sup>O]water (96 atom %), and a few crystals of EDTA were sealed in an NMR tube and allowed to sit for 30 min, after which the <sup>13</sup>C NMR spectrum was taken. The extent of reaction was determined by integration of the carbonyl carbon

**Table 1.** Carbonyl Oxygen Exchange with Solvent during the Alkaline Hydrolysis of Formamide in Water

av [OH <sup>-</sup> ], M <sup>a</sup>	GC–MS method $k_{\text{h}}/k_{\text{ex}}^b$	<sup>13</sup> C NMR method $k_{\text{h}}/k_{\text{ex}}^c$
0.092	2.5	2.7
0.11		
0.22	2.9	
1.46	8.3	

<sup>a</sup> (Initial + final hydroxide concentration)/2. <sup>b</sup> At 25 °C, unbuffered. <sup>c</sup> At room temperature: 22–24 °C, unbuffered.

resonances for formamide and formate. A *T*<sub>1</sub> study established the optimum setting for the integration of the carbonyl carbon resonances; a control experiment containing equal amounts of formate and formamide yielded the expected 1:1 integration. The extent of isotope exchange was determined by separate integration of the <sup>13</sup>C carbonyl carbon resonances containing zero, one, and two <sup>18</sup>O atoms.

## Results

Formamide was shown to exchange its carbonyl oxygen with the solvent during alkaline hydrolysis in water. Two methods were employed to measure the ratio of the rate constant for hydrolysis to that for exchange ( $k_{\text{h}}/k_{\text{ex}}$ ); the results are given in Table 1. In the first method (GC–MS) the fraction of reaction was determined either by the UV assay or by integration of the formyl hydrogen NMR resonances for formamide and formate; the ratio of <sup>18</sup>O/<sup>16</sup>O of the carbonyl oxygen in unreacted formate was determined by GC–MS. The ratio,  $k_{\text{h}}/k_{\text{ex}}$ , was calculated using equations published elsewhere.<sup>7</sup>

In the second method the oxygen isotope shift on the <sup>13</sup>C resonance for the isotopically enriched carbonyl carbon was used to determine the <sup>18</sup>O/<sup>16</sup>O ratio in the carbonyl oxygen of the product, formate. In the NMR method, formate containing two <sup>18</sup>O atoms was produced exclusively via exchange with solvent; formate with one <sup>18</sup>O and one <sup>16</sup>O arose mostly from hydrolysis (a small amount appears from exchange with the small amount of <sup>16</sup>O water); the small amount of formate with two <sup>16</sup>O atoms came exclusively from the hydrolysis reaction with the small amount of <sup>16</sup>O water in the reaction mixture. Simple integration of the carbonyl carbon resonances of formamide and formate served to determine the fraction of reaction. From the limited data collected, the <sup>13</sup>C NMR method appears to have an error limit of approximately  $\pm 5\%$ , which is only slightly higher than the error limit for determining  $k_{\text{h}}/k_{\text{ex}}$  by the GC–MS method ( $\pm 2\text{--}3\%$ ). Combining the results of both methods,  $k_{\text{h}}/k_{\text{ex}}$  was found to be linearly dependent on the average concentration of hydroxide (see Table 1 and Discussion).

The carbonyl carbon and carbonyl oxygen isotope effects on the alkaline hydrolysis were measured in aqueous solution at 25 °C by a slight modification of our published procedure.<sup>12</sup> This procedure was subjected to controls that ensured unreacted formamide did not interfere with collection and oxidation of formate produced during hydrolysis. The carbonyl carbon isotope effect was determined directly from the measured  $\delta(^{13}\text{C})$  for low and complete conversion samples (Table 2). Calculation of the carbonyl oxygen isotope effect from the measured  $\delta(^{18}\text{O})$  was not as straightforward. The solvent (water), representing a vast molar excess of oxygen atoms compared to the carbonyl oxygen of formamide, maintains a constant  $\delta(^{18}\text{O})$  during the course of hydrolysis. One of the oxygen atoms of formate (which is subsequently converted to CO<sub>2</sub>) was derived from the solvent and the other from either the original carbonyl oxygen of formamide or the solvent via the exchange reaction.

(12) Marlier, J. F.; Haptonstall, B. A.; Johnson, A. J.; Sacksteader, K. A. *J. Am. Chem. Soc.* **1997**, *119*, 8838.

**Table 2.** Isotope Effects on the Alkaline Hydrolysis of Formamide in Water at 25 °C

atom	$k(\text{light})/k(\text{heavy})^{a,b}$
carbonyl carbon	1.0321 ± 0.0008 (6)
carbonyl oxygen <sup>c</sup>	0.980 ± 0.001 (5)
leaving nitrogen	1.0040 ± 0.0005 (6)
formyl hydrogen	0.80 ± 0.02 (4)

<sup>a</sup> Corrected for fraction of reaction. <sup>b</sup> Number of determinations in parentheses. <sup>c</sup> Corrected for carbonyl oxygen exchange (see the text).

Therefore, calculation of the carbonyl oxygen isotope effect required a correction for  $\delta(^{18}\text{O})$  of those oxygens which entered formate either via exchange with solvent (i.e.,  $k_h/k_{ex}$ ) or by direct attachment of the nucleophile. Differential equations to accomplish this correction<sup>13</sup> are given in detail in the Supporting Information; the oxygen isotope effects in Table 2 reflect this correction. The rather high standard error on the carbonyl oxygen isotope effect reflects not only the normal uncertainty in measuring a heavy-atom isotope effect, but also the additional uncertainty on measuring the extent of oxygen exchange with solvent. The observed kinetic isotope effects did not vary with pH within the narrow range of hydroxide ion concentrations used in these experiments.

The nitrogen isotope effect was measured by first separating unreacted formamide from ammonia on a Dowex 50 (H<sup>+</sup>-form) column and then hydrolyzing this formamide quantitatively to formate plus ammonia. The ammonia was subsequently oxidized to nitrogen with NaOBr by a published procedure.<sup>9</sup> Control experiments demonstrated that formamide was not fractionated on this short column and that unreacted formamide was quantitatively separated from the ammonia formed during hydrolysis. The isotope effects are also given in Table 2. The observed kinetic isotope effects did not vary with pH within the narrow range of hydroxide ion concentrations used in these experiments.

The formyl hydrogen isotope effect (Table 2) was measured at an average 0.2 M hydroxide ion concentration by a capillary GC method. The 1-*d*-formamide was shown to elute 5 s after the natural abundance formamide on a 60 m carbowax capillary GC column. Therefore, the area under the peaks was used to calculate the change in the ratio of these two isotopically substituted formamides at various fractions of reaction. The fraction of reaction was determined by the UV assay.

Measurement of the oxygen isotope effect on the nucleophile required synthesis of formamide with a known  $\delta(^{18}\text{O})$  for the carbonyl oxygen. This was accomplished by measuring the  $\delta(^{18}\text{O})$  for the carbonyl oxygen of methyl formate<sup>11</sup> and then quantitatively converting this methyl formate to formamide in the presence of dry methanolic ammonia. This synthesis preserved the known isotopic composition of the carbonyl oxygen during conversion of methyl formate to formamide because there was no source of exchangeable oxygen atoms and because the conversion was quantitative. The carbonyl oxygen of this methyl formate (and hence formamide) was found to have a  $\delta(^{18}\text{O})$  of  $-6.3$  (Table 3).

A sample of this formamide was completely hydrolyzed in aqueous 1.7 M KOH, dried, and oxidized to CO<sub>2</sub> by I<sub>2</sub>/DMSO. This higher hydroxide concentration was chosen because at the lower hydroxide concentrations quantitative conversions are more difficult (slow reaction rates) and because a large amount of carbonyl oxygen exchange makes determination of the  $\delta(^{18}\text{O})$  inherently less precise. The isotopic composition of this

**Table 3.** Isotopic Composition of Oxygen Atoms Involved in Calculation of the Nucleophile Isotope Effect on the Alkaline Hydrolysis of Formamide in Water at 25 °C

oxygen source	$\delta(^{18}\text{O})^a$
CO <sub>2</sub> from complete hydrolysis of formamide	$-37.2 \pm 1.0$ (6)
CO <sub>2</sub> from carbonyl oxygen of formamide	$-6.3 \pm 0.3$ (6)
CO <sub>2</sub> exchanged with water	$+1.4$ , range 0.4 (2)
water	$-39.6$ (calc)
hydroxide	$-79.6$ (calc)

<sup>a</sup> Compared to a PDB standard.

CO<sub>2</sub> was found to have a  $\delta(^{18}\text{O})$  of  $-37.2$  (Table 3). The origin of the two oxygen atoms of the CO<sub>2</sub> (O<sup>1</sup>=C=O<sup>2</sup>) produced in this manner was as follows: (1) one of the oxygen atoms (O<sup>1</sup>) was completely derived from the solvent nucleophile (water or hydroxide). The second oxygen atom (O<sup>2</sup>) was derived from either (2) the solvent nucleophile via exchange or (3) the original unexchanged carbonyl oxygen of formamide (still having a  $\delta(^{18}\text{O})$  of  $-6.3$  because hydrolysis was quantitative).

In the present experiment it is not necessary to measure the isotopic composition of the oxygen atoms of CO<sub>2</sub> at both high and low conversions, as is typical in a competitive experiment; the above-mentioned quantitative hydrolysis is sufficient. This is due to the fact that the ultimate source of O<sup>1</sup> is the solvent oxygen nucleophile (water or hydroxide), which is incorporated into formamide during hydrolysis. Since the solvent is present in a very large excess over the substrate (formamide), it can be treated as essentially an infinite reservoir of oxygen atoms, which is of constant concentration and isotopic composition. All that is required to determine the nucleophile isotope effect is a way to determine the  $\delta(^{18}\text{O})$  for O<sup>1</sup> and to compare it to the invariant  $\delta(^{18}\text{O})$  of the possible nucleophiles (water or hydroxide).

This calculation can be accomplished using eq 2, where the per mil isotopic abundances  $\delta_{(\text{obs})}$ ,  $\delta^1$ ,  $\delta^2_{(\text{ex})}$ , and  $\delta^2_{(\text{un})}$  are, respectively, those for the entire CO<sub>2</sub> molecule, for O<sup>1</sup> (which

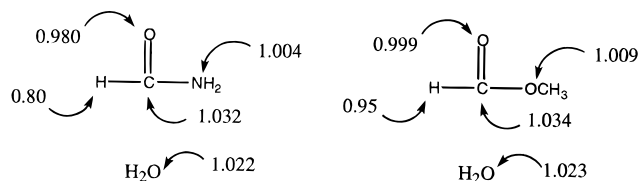
$$\delta_{(\text{obs})} = 0.5\delta^1 + 0.5\delta^2_{(\text{ex})}f_{(\text{ex})} + 0.5\delta^2_{(\text{un})}f_{(\text{un})} \quad (2)$$

is directly from the nucleophile), for O<sup>2</sup> (which is from the carbonyl oxygen of formamide after exchange with solvent), and for O<sup>2</sup> (which is from the carbonyl oxygen of formamide without exchange with the solvent). Additionally,  $f_{(\text{ex})}$  is the fraction of formamide molecules which has undergone oxygen exchange, whereas  $f_{(\text{un})}$  is the fraction that has not exchanged. The one assumption in this calculation is that the isotope ratio of the oxygen atoms entering formate via the exchange pathway is the same as that for the nucleophile (i.e.,  $\delta^1 = \delta^2_{(\text{ex})}$ ). This is a reasonable assumption since the exchange reaction begins with the same nucleophilic attack step ( $k_1$ , eq 1) as the hydrolysis pathway and because the amount of exchange is so small ( $k_h/k_{ex} = 8.7$ ) at the high level of hydroxide employed for these particular experiments. Using this assumption and solving for  $\delta^1$  gives eq 3.

$$\delta^1 = [\delta_{(\text{obs})} - 0.5\delta^2_{(\text{un})}f_{(\text{un})}]/[0.5 + 0.5f_{(\text{ex})}] \quad (3)$$

The magnitude of  $\delta_{(\text{obs})}$  is  $-37.2$  per mil (Table 3); that for  $\delta^2_{(\text{un})}$  is  $-6.3$  per mil. The values of  $f_{(\text{un})}$  and  $f_{(\text{ex})}$  can be calculated from the differential equations cited earlier<sup>13</sup> (and detailed in the Supporting Information), plus the known value of  $k_h/k_{ex}$  (Table 1). The above information allows the  $\delta(^{18}\text{O})$  of the attacking nucleophile (or  $\delta^1$ ) to be calculated, which is  $-62.3$  per mil. The  $\delta(^{18}\text{O})$  values of  $-39.6$  per mil for the water and  $-79.6$  per mil for the hydroxide used in these experiments were

(13) Marlier, J. F. Ph.D. Dissertation, University of Wisconsin—Madison, Madison, WI, 1978.



**Figure 1.** Summary of the isotope effects on alkaline hydrolysis of formamide and methyl formate.<sup>11,17,18</sup>

determined by completely exchanging the oxygen atoms of water with  $\text{CO}_2$  and then correcting for the known fractionation factors between  $\text{CO}_2$  and water ( $-41.0$  per mil)<sup>14</sup> and between water and hydroxide ( $-40.0$  per mil).<sup>15</sup> As a result, either water is the attacking nucleophile with an isotope effect of  $^{18}k_{\text{obs}(\text{nuc})} = 1.022 \pm 0.001$  or hydroxide is the nucleophile with an isotope effect of  $^{18}k_{\text{obs}(\text{nuc})} = 0.982 \pm 0.001$ .

## Discussion

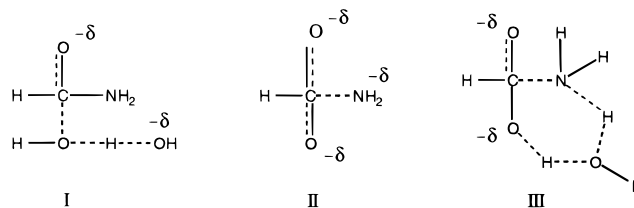
The alkaline hydrolysis of amides is widely believed to occur by the mechanism shown in eq 1. The product is formed by pathways which are either first-order or second-order in hydroxide. At the low initial hydroxide concentrations used in the present isotope effect experiments (0.1–0.2 M) the hydrolysis proceeds mainly through the pathway which is first-order in hydroxide (involving only  $\text{T}^-$ ). By contrast only a very few esters<sup>16a</sup> were thought to undergo alkaline hydrolysis by a second-order pathway in a mechanism analogous to that given in eq 1. Recent studies have cast doubt on these earlier findings;<sup>16b</sup> the current evidence supports only the first-order pathway for ester alkaline hydrolysis. To more fully characterize the bonding in the transition state for the alkaline hydrolysis of amides, we have measured isotope effects for all the reacting atoms of formamide under conditions where the first-order pathway greatly predominates. The solvent oxygen nucleophile isotope effect was measured under highly alkaline conditions, where the attack of the nucleophile (step 1, eq 1) was largely rate-determining. This nucleophile isotope effect was important in correcting the carbonyl oxygen isotope effect for the presence of exchanged oxygen atoms from the solvent and for determination of the actual nucleophile (water or hydroxide) in the hydrolysis reaction. In addition, the extent of carbonyl oxygen exchange with solvent was determined at various hydroxide concentrations. The results of the isotope effect study are summarized in Figure 1, and compared to those previously published for the alkaline hydrolysis of methyl formate.<sup>11,17,18</sup>

**Carbonyl Oxygen Isotope Exchange.** Formamide undergoes carbonyl oxygen exchange with solvent; this exchange is dependent on the concentration of hydroxide. These results are consistent with the mechanism in eq 1, where higher concentrations of hydroxide effectively trap more of the intermediate as  $\text{T}^{2-}$ , increasing hydrolysis relative to exchange (assuming  $k_4 > k_3$ ). The steady-state equations for the hydrolysis and exchange reactions of eq 1 have been published.<sup>4</sup> From these equations an expression for  $k_{\text{H}}/k_{\text{ex}}$  as a function of hydroxide concentration can be developed and is given in eq 4, assuming rapid proton transfers. The oxygen isotope exchange results (Table 1) were

$$k_{\text{H}}/k_{\text{ex}} = (2k_3 + 2k_4K[\text{OH}^-])/k_2 \quad (4)$$

fitted to eq 4; linear regression analysis gave an excellent fit ( $r^2 = 0.999$ ). The y-intercept of this plot ( $2k_3/k_2$ ) equals 2.10, making the partition ratio for  $\text{T}^-$  ( $k_3/k_2$ ) equal to 1.05. The small magnitude of  $k_3/k_2$  normally indicates the transition states leading to and from  $\text{T}^-$  are of comparable energy and, therefore, resemble  $\text{T}^-$  in structure. However, the proton transfer required to make the nitrogen a better leaving group appears to cause the transition state for decomposition of  $\text{T}^-$  to become less  $\text{sp}^3$ -like than expected (see below). In contrast, esters such as methyl formate show much less oxygen exchange ( $k_{\text{H}}/k_{\text{ex}} = 18.3$ ), arguing for a largely rate-determining formation of  $\text{T}^-$ . The slope of the above plot ( $2k_4K/k_2$ ) equals 4.29. From these results it follows that, under conditions of 0.086–0.15 M average hydroxide concentrations used in the present work, only 14–23% of the formate is produced via the pathway which is second-order in hydroxide. Therefore, the subsequent simplification of the discussion of the remaining isotope effects solely in terms of the first-order pathway is justified.

**Formyl Hydrogen Isotope Effect.** The secondary formyl hydrogen isotope effect is sensitive to changes in the hybridization of the adjacent carbonyl carbon atom. Kirsch reported an inverse formyl hydrogen isotope effect on the alkaline hydrolysis of formamide<sup>5</sup> which was dependent on the initial hydroxide concentration (in the range 0.05–1.0 M). This result was interpreted in terms of an increasing proportion of the reaction proceeding through  $\text{T}^{2-}$  instead of  $\text{T}^-$ , in agreement with the observed hydroxide dependence of  $k_{\text{H}}/k_{\text{ex}}$  (see above). From the observed isotope effects Kirsch calculated the isotope effects on the individual steps of the mechanism. The isotope effect on formation of  $\text{T}^-$  was found to be  $^{\text{D}}k_1 = 0.65$ , arguing for a very late  $\text{sp}^3$ -like transition state (structure I). The isotope effect on breakdown of  $\text{T}^-$  (structure III) was calculated to be  $^{\text{D}}k_3 = 1.58$ ; that for breakdown of  $\text{T}^{2-}$  (structure II) was  $^{\text{D}}k_4 = 1.41$ ,



indicating late,  $\text{sp}^2$ -like transition states in both cases. The transition state for the breakdown of  $\text{T}^-$  (structure III) is shown as involving a simultaneous proton transfer (through water) from the  $-\text{OH}$  of  $\text{T}^-$  to the leaving nitrogen. On the basis of solvent deuterium isotope effects, Brown<sup>4</sup> has proposed an alternative mechanism involving stepwise protonation of the nitrogen prior to leaving. These solvent isotope effects are also consistent with a late transition state III. The transition states for the breakdown of  $\text{T}^-$  in both mechanisms avoid the unlikely formation of the highly basic  $\text{NH}_2^-$  ion. Neither mechanism can be strictly eliminated on the basis of the isotope effects presented in this paper.

Our determination of  $^{\text{D}}k_{\text{obs}} = 0.80$  (Table 2) was performed only at an average hydroxide concentration of 0.20 M and is in general agreement with those reported by Kirsch at that hydroxide concentration. The relationship between the isotope effects on the individual steps of the mechanism and the observed isotope effect is given by eq 5. In eq 5, the asterisk

$$*k_{\text{obs}} = [*K_{\text{eq}}*k_3 + *k_1(k_3/k_2)]/(1 + k_3/k_2) \quad (5)$$

(14) Friedman, I.; O'Neill, J. R. U.S. Geological Survey Professional Paper No. 440-KK, 1977.

(15) Green, M.; Taube, H. *J. Phys. Chem.* **1963**, *67*, 1565.

(16) (a) Khan, M. N.; Olagbemi, T. O. *J. Org. Chem.* **1982**, *47*, 3695.

(b) Kellogg, B. A.; Brown, R. S.; McDonald, R. S. *J. Org. Chem.* **1994**, *59*, 4652.

(17) Sawyer, C. B.; Kirsch, J. F. *J. Am. Chem. Soc.* **1973**, *95*, 7375.

(18) Bilkadi, Z.; deLorimier, R.; Kirsch, J. F. *J. Am. Chem. Soc.* **1975**, *97*, 4317.

**Table 4.** Calculated Kinetic Isotope Effects on the Various Steps in the Mechanism of Alkaline Hydrolysis of Formamide (See the Text)

isotope effect	assumed	calculated
formyl H	$^Dk_1 = ^DK_{eq} = 0.69$	$^Dk_3 = 1.34$
carbonyl C	$^{13}k_{1(C=O)} = 1.032, ^{13}K_{eq(C=O)} = 0.983$	$^{13}k_{3(C=O)} = 1.050$
nucleophilic O	$^{18}k_{3(nuc)} = 0.995, ^{18}K_{eq(nuc)} = 1.033$	$^{18}k_{1(nuc)} = 1.017$
	$^{18}k_{3(nuc)} = 1.000, ^{18}K_{eq(nuc)} = 1.033$	$^{18}k_{1(nuc)} = 1.012$
	$^{18}k_{3(nuc)} = 1.005, ^{18}K_{eq(nuc)} = 1.033$	$^{18}k_{1(nuc)} = 1.007$
leaving N	$^{15}k_1 = ^{15}K_{eq} = 1.000$	$^{15}k_3 = 1.008$
	$^{15}k_1 = ^{15}K_{eq} = 1.005$	$^{15}k_3 = 0.998$
	$^{15}k_1 = ^{15}K_{eq} = 1.010$	$^{15}k_3 = 0.988$

refers to an isotope effect which is always given as a ratio of the rate (or equilibrium) constants for the light/heavy isotopes (i.e., in the case of hydrogen isotope effects  $*k$  symbolizes  $^Dk$ , which in turn refers to  $^Hk/^Dk$ ). In particular,  $*k_{obs}$  is the observed isotope effect,  $*K_{eq}$  is the equilibrium isotope effect on step 1, and  $*k_1$  and  $*k_3$  are the kinetic isotope effects on formation and decomposition of  $T^-$ , respectively. The ratio  $k_3/k_2 = 1.05$  results from the isotope exchange experiments extrapolated to very low hydroxide concentrations (see above).

One can obtain a reasonable estimate of  $^DK_{eq}$  from the hydrogen fractionation factor between the formyl hydrogen of formic acid and the hydrogen of a secondary alcohol, which is  $^DK_{eq} = 0.69$ .<sup>19</sup> The equilibrium isotope effect on formation of  $T^-$  should be of a similar magnitude. Because the formyl hydrogen isotope effect is a secondary one and because the transition state for formation of  $T^-$  appears to be a late one, it is reasonable to expect that  $^DK_{eq}$  should be similar in magnitude to  $^Dk_1$ . Using these estimated values,  $^Dk_3$  is calculated to be 1.34 (Table 4), in approximate agreement with that calculated by Kirsch ( $^Dk_3 = 1.58$ ). Finding a late,  $sp^2$ -like transition state for breakdown of  $T^-$  is somewhat of a surprise because it is expected that the transition state would resemble the reactive tetrahedral intermediate. However, the results of these formyl hydrogen isotope effect studies strongly suggest any viable transition state for breakdown of  $T^-$  must involve considerable C–N bond cleavage.

**Carbonyl Carbon Isotope Effect.** Carbonyl carbon isotope effect data on the hydrolysis of amides are lacking in the literature. However, several carbonyl carbon isotope effects have been measured for the alkaline hydrolysis of esters. The carbonyl carbon isotope effect for the alkaline hydrolysis of methyl formate<sup>11</sup> ( $^{13}k_{obs} = 1.0324$ ) compares closely with that reported here for formamide ( $^{13}k_{obs} = 1.0321$ ). The isotope effects on the individual rate constants can be approximated from eq 5. An estimate of  $^{13}K_{eq} = 0.979$  has been reported for the addition of hydrazine to methyl formate.<sup>12</sup> After the small secondary contribution of the outer nitrogen is removed (making  $^{13}K_{eq} = 0.983$ ), this will serve as a crude model for the present equilibrium carbon isotope effect. The best model for the carbonyl carbon isotope effect on  $k_1$  is the alkaline hydrolysis of methyl formate, where  $^{13}k_{obs} = 1.0324$ . In the methyl formate case the first step (attack of hydroxide) is largely rate-determining, making  $^{13}k_{obs}$  nearly equal to the kinetic isotope effect on step 1 ( $^{13}k_1$ ). Unfortunately, the transition state is considerably earlier for the alkaline hydrolysis of methyl formate when compared to formamide. However, the carbonyl carbon isotope effect for the alkaline hydrolysis of a large number of esters appears to be rather insensitive to large changes in the structure of the ester (and hopefully the transition state);<sup>11,12,20</sup> all such carbonyl carbon isotope effects are in the range  $^{13}k_{obs} = 1.03–1.04$ . Using these estimated isotope effects in eq 5 gives

a calculated magnitude of  $^{13}k_3 = 1.05$  (Table 4). This calculated isotope effect is a bit higher than expected. Given the difficulty of accurately estimating  $^{13}K_{eq}$  and  $^{13}k_1$ , the calculated magnitude of  $^{13}k_3$  is at least in the ballpark. In general, large normal kinetic isotope effects such as these on  $k_1$  and  $k_3$  are indicative of steps involving a high degree of reaction coordinate motion on the part of the carbonyl carbon atom as expected in both steps of the mechanism. However, this large normal observed isotope effect effectively eliminates any mechanism in which  $T^-$  (or an N-protonated form of  $T^-$ ) is at equilibrium with the ground state (formamide). If this were the case, the observed carbonyl carbon isotope effect would need to be inverse, approximately in the  $^{13}K_{eq} = 0.983$  range.<sup>12</sup>

**Carbonyl Oxygen Isotope Effect.** The carbonyl oxygen isotope effect is a secondary isotope effect because the connection between atoms is not severed during the reaction. A mathematical calculation of the oxygen isotope effects on the individual steps from eq 5 is not possible at this time because there are no reasonable models to help estimate  $^{18}K_{eq(C=O)}$ ,  $^{18}k_{1(C=O)}$ , or  $^{18}k_{3(C=O)}$ . However, a qualitative description is possible. The bond order to the carbonyl oxygen is reduced on going to either of the possible transition states (structure **I** or **III**), leading to the expectation of a normal isotope effect. In fact, most of the carbonyl oxygen isotope effects reported for the reaction of esters with basic nucleophiles are small, but normal; the alkaline hydrolysis of methyl formate<sup>11</sup> is an exception, where  $^{18}k_{obs(C=O)} = 0.999$ . Therefore, the moderate inverse isotope effect observed for formamide is somewhat of a surprise, and the explanation must involve more than simple  $\pi$  bond breaking.

A more detailed explanation requires a qualitative analysis of the vibrational modes in the ground state and in transition states **I** and **III** (recall that the transition states for formation and destruction of  $T^-$  are of nearly equal energy). In either transition state **I** or **III** there is a gain of new O–C–O bending and O–C–O–H torsional modes which stiffens the bonding to the carbonyl oxygen. This stiffening gives an inverse isotope effect which counters the normal isotope effect on carbonyl  $\pi$  bond breaking. Why is the carbonyl oxygen isotope effect on the alkaline hydrolysis of formamide ( $^{18}k_{obs(C=O)} = 0.980$ ) considerably more inverse than that of the methyl formate case ( $^{18}k_{obs(C=O)} = 0.999$ )? The answer must be due to significantly different changes in bonding on going to the transition states. The transition state for step 1 ( $k_1$ ) in formamide hydrolysis has more  $sp^3$  character than that for methyl formate (see Formyl Hydrogen Isotope Effects, Figure 1), and consequently, the bond to the nucleophile will be more fully developed in this transition state. Therefore, any bond stiffening in the transition state resulting from introduction of new vibrational modes (an inverse effect) will be proportionally greater in the formamide case. On the other hand, one might expect the corresponding reduction in  $\pi$  bond order (a normal effect) to also be greater in the later transition state of formamide hydrolysis, giving more comparable observed isotope effects. However, the nitrogen lone pair in amides is more delocalized by resonance than the corresponding lone pair on the alkoxy oxygen of esters, which gives the amide ground state a lower carbonyl  $\pi$  bond order and leads to a lower than expected normal isotope effect. Thus, in formamide hydrolysis the bond stiffening created by the new vibrational modes in the transition state contributes more to the overall observed isotope effect than in the case of methyl formate hydrolysis. Similar effects are seen in secondary

(20) (a) Marlier, J. F.; O'Leary, M. H. *J. Am. Chem. Soc.* **1990**, *112*, 5996. (b) O'Leary, M. H.; Marlier, J. F. *J. Am. Chem. Soc.* **1979**, *101*, 3300.

(19) Cleland, W. W. *Methods Enzymol.* **1980**, *64*, 104.

hydrogen isotope effects for acyl group transfers, where the carbonyl carbon goes from an  $sp^2$  ground state to an  $sp^3$ -like transition state.<sup>5,11,12,18</sup>

**Oxygen Nucleophile Isotope Effect.** The oxygen of formate which is derived from the solvent during the alkaline hydrolysis is 22 per mil lighter than that for water and 18 per mil heavier than that for hydroxide (see Table 3). Therefore, water as the nucleophile gives an isotope effect of  $^{18}k_{\text{obs}(\text{nuc})} = 1.022$ ; hydroxide as the nucleophile gives an isotope effect of  $^{18}k_{\text{obs}(\text{nuc})} = 0.982$ . Which of these is the actual nucleophile? In either case a new bond is formed to the nucleophilic oxygen, making the isotope effect on the nucleophile a primary one. Primary isotope effects are composed of a temperature-independent factor (TIF) which is due to reaction coordinate motion and a temperature-dependent factor (TDF) which is due to creation of new vibrational modes in the transition state.<sup>21</sup> In almost all known cases, isotope effects on attacking nucleophiles are normal because they are dominated by reaction coordinate motion (the TIF). One known exception is the reaction of unhydrated chloride ion as a nucleophile.<sup>22</sup> The observed nitrogen nucleophile isotope effect on the hydrazinolysis of methyl formate is slightly inverse and appears to be another exception.<sup>12</sup> However, this observed isotope effect is inverse because of a secondary isotope effect on the nonnucleophilic (or outer) nitrogen of hydrazine, which is analyzed along with the nucleophilic one. There is no way to quantitatively correct for the secondary isotope effect of this outer nitrogen and for the concerted proton transfers in the transition state, but such effects would tend to make the observed isotope effect appear more inverse than it actually is. In light of these results a large inverse isotope effect of  $^{18}k_{\text{obs}(\text{nuc})} = 0.982$  is clearly not expected, making water (probably one of the water molecules hydrogen bonded to hydroxide ion) the actual nucleophile for alkaline hydrolysis.

Water was also proposed to be the nucleophile for the alkaline hydrolysis of esters, on the basis of a nearly identical nucleophile isotope effect of  $^{18}k_{\text{obs}(\text{nuc})} = 1.023$  for methyl formate hydrolysis<sup>11</sup> and the observation of general-base catalysis of the hydrolysis of a series of formate esters.<sup>23</sup> In the methyl formate case the carbonyl oxygen exchange data ( $k_{\text{H}}/k_{\text{ex}} = 18.3$ ) and the lack of a sizable leaving group oxygen isotope effect ( $^{18}k_{\text{obs}(\text{lg})} = 1.009$ ) clearly indicated that the first step (attack of the nucleophile) was rate-determining. Furthermore, the small inverse formyl hydrogen isotope effect indicated the transition state for attack of the nucleophile was quite early. Theoretical calculations have indicated that early transition states for nucleophilic attack will give large, normal isotope effects, whereas later transition states tend to give smaller normal or, in extreme cases, inverse isotope effects.<sup>24,25</sup>

The alkaline hydrolysis of formamide at low hydroxide concentrations is more complex than that of methyl formate because the transition states for formation and destruction of  $T^-$  are of comparable energy. In addition, transition states for both formation and destruction of  $T^-$  are relatively late (see the formyl hydrogen isotope effect discussion). At this point it is only possible to do a crude analysis of the three individual isotope effects ( $^{18}K_{\text{eq}(\text{nuc})}$ ,  $^{18}k_{1(\text{nuc})}$ , and  $^{18}k_{3(\text{nuc})}$ ) using eq 5 and appropriate models. One must start with the assumption that

the nucleophile isotope effect measured at high hydroxide concentration is similar to that at low hydroxide concentration. The known oxygen fractionation factor between water and a secondary alcohol ( $^{18}K_{\text{eq}(\text{nuc})} = 1.033$ ) is a reasonable model for the equilibrium isotope effect on formation of  $T^-$ . Estimation of the magnitude of  $^{18}k_{1(\text{nuc})}$  or  $^{18}k_{3(\text{nuc})}$  is more difficult, but estimation of  $^{18}k_{3(\text{nuc})}$  is probably easiest. The isotope effect on  $k_{3(\text{nuc})}$  is composed of (1) a small normal primary isotope effect on the breaking of the O–H bond, (2) a small inverse secondary isotope effect on formation of the partial  $\pi$  bond of formate, and (3) a small normal isotope effect from loss of a vibrational mode (O–C–N) as the leaving group departs. Since all of these effects are expected to be small, it is likely that the overall isotope effect will be a small inverse or small normal one, perhaps in the range  $^{18}k_{3(\text{nuc})} = 0.995$ – $1.005$ . Application of eq 5 gives a magnitude of  $^{18}k_{1(\text{nuc})}$  in the range 1.007–1.017 (Table 4).

Are these calculated magnitudes for  $^{18}k_{1(\text{nuc})}$  reasonable? During formation of  $T^-$  the nucleophilic oxygen loses an O–H bond to the general base (hydroxide) and simultaneously gains a new C–O bond, giving a substantial normal isotope effect. How large can this normal isotope effect on  $k_1$  be? The nucleophile oxygen isotope effect on the alkaline hydrolysis of methyl formate ( $^{18}k_{\text{obs}(\text{nuc})} = 1.023$ ) is probably near the upper limit.<sup>11</sup> On the other hand, formation of new vibrational modes in the transition state for formation of  $T^-$ , particularly the new O–C–O and O–C–N bending modes, will give an inverse contribution to the overall isotope effect. In the methyl formate case discussed above, the transition state for formation of  $T^-$  is early and the inverse contribution of forming new bending modes is relatively unimportant. However, the transition state for formation of  $T^-$  in formamide hydrolysis is later and the inverse contribution from creation of these new vibrational modes is greater, leading to a smaller observed isotope effect. Seen in this light, the calculated range for  $^{18}k_{1(\text{nuc})} = 1.007$ – $1.017$  seems quite reasonable.

One final point underscores the earlier choice of water as the nucleophile for alkaline hydrolysis with  $^{18}k_{\text{obs}(\text{nuc})} = 1.022$  over hydroxide with  $^{18}k_{\text{obs}(\text{nuc})} = 0.982$ . If one uses the latter observed isotope effect in eq 5, the resulting calculated magnitude of  $^{18}k_{1(\text{nuc})}$  is in the range 0.931–0.940. Inverse oxygen isotope effects of 6–7% would be among the largest such effects observed (near the theoretical maximum) and are clearly not possible in this case.

**Nitrogen Isotope Effect.** The observed leaving group nitrogen isotope effect is surprisingly small ( $^{15}k_{\text{obs}} = 1.004$ ) for a primary isotope effect involving C–N bond breaking. The isotope effects on the individual steps of the mechanism are again given in eq 5. Good models for estimation of the individual isotope effects are once again lacking. Nevertheless, a rough estimation is possible. Nitrogen isotope effects for reactions where breaking a C–N bond is rate-determining have been estimated to be in the range of  $^{15}k_{\text{obs}} = 1.01$ – $1.03$ .<sup>10</sup> What makes interpretation of the present nitrogen isotope effect difficult is the possibility that proton transfer to the nitrogen may occur either simultaneous with (structure **III**) or prior to C–N bond breaking; either case will lower the observed isotope effect below the above stated range. As a result it is probably better to estimate  $^{15}k_1$  from the oxygen leaving group isotope effects on the analogous hydrolysis reactions of methyl formate, where formation of  $T^-$  is known to be largely rate-determining. The observed methoxyl oxygen isotope effects for methyl formate range from  $^{18}k_{\text{obs}(\text{lg})} = 1.009$  for alkaline

(21) Melander, L. *Isotope Effects on Reaction Rates*; Ronald Press: New York, 1960.

(22) Cromartie, T. H.; Swain, G. G. *J. Am. Chem. Soc.* **1976**, *98*, 2962.

(23) Stefandis, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1993**, *115*, 6045.

(24) Paneth, P.; O'Leary, M. H. *J. Am. Chem. Soc.* **1991**, *113*, 1691.

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

hydrolysis to  $^{18}k_{\text{obs(lg)}} = 1.001$  for acid-catalyzed hydrolysis.<sup>17,18</sup> These models may actually overestimate the kinetic isotope effect on formation of  $\text{T}^-$  because breakdown of  $\text{T}^-$  (which can show a large normal isotope effect near 1.06)<sup>17</sup> might still contribute slightly to the overall rate and the observed isotope effect. During formation of  $\text{T}^-$  the leaving oxygen experiences a loss of  $\pi$  bond character on going to tetrahedral geometry (a normal effect) and gains a new O–C–O bending mode (an inverse effect). Both effects are small and tend to cancel one another. For formamide the isotope effect on the loss of  $\pi$  bond character to the nitrogen should be larger than in the methyl formate case because amides are more highly conjugated than esters and because the transition state is later in the formamide case. On the other hand, this later transition state will also increase the effect of creating new bending modes in the transition state, thereby increasing the inverse contribution to the overall observed nitrogen isotope effect. In addition, a difference of 1 amu for the nitrogen isotopes (vs 2 amu for the oxygen isotopes) will make maximum observed nitrogen isotope effects smaller than those for oxygen. Therefore, it is not unreasonable to assume that  $^{15}k_1$  will be in the range 1.00–1.01, similar in magnitude to those listed above for hydrolysis of methyl formate.

Since the transition state for formation of  $\text{T}^-$  is somewhat late and the nitrogen kinetic isotope effect on step 1 is a secondary one, it is again possible to make the crude assumption that  $^{15}K_{\text{eq}}$  and  $^{15}k_1$  are of comparable magnitude, which allows estimation of  $^{15}k_3$  from eq 5. The calculated isotope effect on step 2 becomes  $^{15}k_3 = 0.988\text{--}1.008$  (see Table 4). Are the calculated isotope effects for breakdown of  $\text{T}^-$  reasonable? As mentioned above breaking a C–N bond will give an estimated nitrogen isotope effect of 1.01–1.03, depending on the degree of bond breaking in the transition state. The simultaneous formation of the N–H bond in the transition state is expected to give an inverse contribution to the overall observed isotope effect because almost all of the reaction coordinate motion will be on part of the proton. This will lower the observed isotope effect considerably, again depending on the degree of N–H bond formation in the transition state. Therefore, it is safe to propose that  $^{15}k_3$  will be considerably lower than the 1.01–

1.03 range. In this light the calculated isotope effects in Table 4 seem reasonable.

**Summary.** The results of the extensive isotope effect study in this paper allow a detailed picture of the transition-state structure for the alkaline hydrolysis of formamide under conditions where the reaction is first-order in hydroxide. Isotope exchange experiments demonstrate that the tetrahedral intermediate partitions almost equally between formamide and formate, indicating that the transition states for both steps of the mechanism are of nearly equal energy. The transition state for formation of the tetrahedral intermediate is rather late and  $\text{sp}^3$ -like. The nucleophile is water, presumably one of the water molecules in the hydration sphere of hydroxide. This is in agreement with earlier findings for alkaline hydrolysis of esters. The most likely transition state for decomposition of the tetrahedral intermediate is also somewhat late, having considerable  $\text{sp}^2$  character and a high degree of C–N bond cleavage. It is likely that C–N bond cleavage is accompanied by a concerted proton transfer to the nitrogen (via water) and that this proton transfer is nearly complete in the transition state. A stepwise protonation of the nitrogen prior to leaving is also possible.

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**Supporting Information Available:** Differential equations for determining the fraction of formamide molecules which have undergone carbonyl oxygen exchange with solvent during alkaline hydrolysis. (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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