Evidence for the Formation of α-Hydroxydialkylnitrosamines in the pH-Independent Solvolysis of α-(Acyloxy)dialkylnitrosamines

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A kinetic study of the decay of α-(acyloxy)dialkylnitrosamines in aqueous solutions, at 25 °C, ionic strength 1 M ($NaClO_4$), and 4% acetonitrile by volume, is reported. Rate constants for disappearance of the N−NO chromophore or appearance of benzaldehyde product increase with the introduction of electron-withdrawing groups into the acyloxy moiety. For some compounds, in some regions of pH, the kinetics are non-first-order. For these compounds, in other regions of pH, the rate constants are coincident with those previously reported for the decay of the corresponding α-hydroxydialkylnitrosamines. These data provide direct evidence that α-hydroxydialkylnitrosamines are intermediates in the pH-independent decomposition of the α-(acyloxy)dialkylnitrosamines studied.

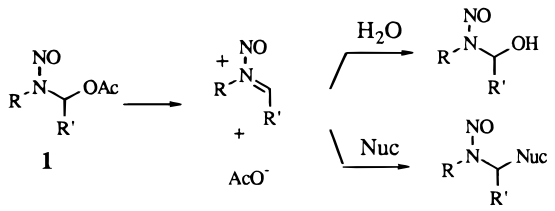
Introduction

α-(Acyloxy)dialkylnitrosamines, **1** (Scheme 1), have been widely employed in studies of nitrosamine carcinogenesis.[1] A number of these compounds have been shown to be mutagenic or carcinogenic. They are believed to be sources of α -hydroxydialkylnitrosamines, **2** (Scheme 1). As in Scheme 1, α-hydroxydialkylamines are the likely products of enzymatic α-hydroxylation of dialkylnitrosamines **3**, and they decompose to yield electrophilic species that are purportedly responsible for the alkylating activity, and thus biological activity, of dialkylnitrosamines.

The mechanism of solvolytic decay of simple α-(acyloxy)dialkylnitrosamines was recently reexamined.[2] In the pH range between pH = 3−13 it is dominated by the two-term rate law of eq 1. It was concluded that the pH independent reaction involves the formation of nitros-

$$k_{obsd} = k_1 + k_{OH}[OH^-] \quad (1)$$

iminium ion intermediates, as in eq 2. It was also shown that these can be trapped by added nucleophiles, and it has been explicitly assumed that they react with solvent



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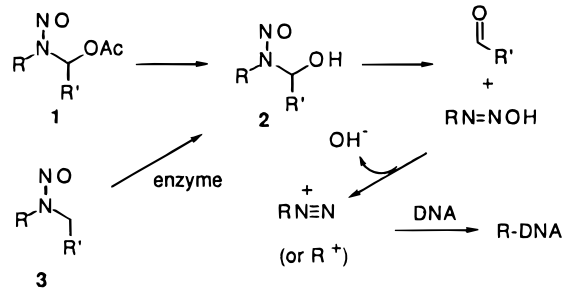
water to yield α-hydroxydialkylnitrosamines, as in eq 2.[3]

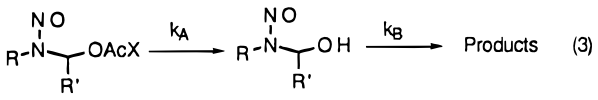
There is in fact no evidence that α-hydroxy-*N*,*N*-dialkylnitrosamines are intermediates in the pH independent (k_o, eq 1) solvolysis of α-(acyloxy)dialkylnitrosamines. The notion that they might not be arises from

Scheme 1



the suggestion[4] that α-(acyloxy)dialkylnitrosamines decompose with "anchimeric assistance" by the nitroso group. There is evidence consistent with the formation of α-hydroxydialkylnitrosamines in the base-catalyzed reaction, k_{OH} (eq 1), for which a carbonyl attack mechanism was originally proposed, and the esterase-catalyzed hydrolysis, another carbonyl attack mechanism.[5] Thus, it was shown that the stereochemical course of formation of 1-phenylethanol was similar in the hydrolysis of (−)-1-(acetoxymethyl)(1-phenylethyl)nitrosamine both in aqueous buffered solution, pH 8.5, and in the hog liver esterase-catalyzed reaction. While the rate constants k_1 and k_{OH} are not known for this substrate, it seems likely by comparison with similar systems that the nonenzymatic hydrolysis at pH = 8.5 involves predominantly the base-catalyzed reaction (k_{OH}, eq 1).

A test for mechanisms that require the intermediacy of α-hydroxydialkylnitrosamines involves the generation of the α-hydroxydialkylnitrosamines from highly reactive esters with good leaving groups. In the general mechanism of eq 3, under the condition in which k_A is fast relative to k_B, it is predicted that the esters should



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decompose more rapidly than the α-hydroxydialkylnitrosamines such that the latter become non-steady-state intermediates. The lifetimes of a few α-hydroxydialkyl-

⊗ Abstract published in *Advance ACS Abstracts,* March 15, 1997.

(1) Lawley, P. D. In *Chemical Carcinogens*; Searle, C. D., Ed.; ACS Monograph 182, American Chemical Society: Washington, DC, 1984. Lijinsky, W. *Chemistry and Biology of N-Nitroso Compounds*; Cambridge University Press: Cambridge, 1992.

(2) Revis, C.; Rajamäki, M.; Fishbein, J. C. *J. Org. Chem.* **1995**, *60* 7733.

(3) Rajamäki, M.; Vigroux, A.; Chahoua, L.; Fishbein, J. C. *J. Org. Chem.* **1995**, *60*, 2324.

(4) Wiessler, M. *N-Nitrosamines*; Anselme, J.-P., Ed.; American Chemical Society: Washington, DC, 1979; p 57.

(5) Gold, B.; Linder, W. B. *J. Am. Chem. Soc.* **1979**, *101*, 6772.

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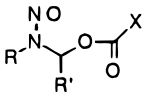
nitrosamines were reported some years ago,[6] and we have recently reported the exalted reactivity of some others.[7] In every case these compounds are substantially more reactive than the acetate ester precursors so that they are never more than steady state intermediates in the decay of the α-acetoxydialkylnitrosamines. However, with better leaving groups, for which k_A increases while k_B remains constant, it should be possible to reach the point at which $k_A > k_B$.

This report summarizes results of mainly kinetic experiments involving the compounds **4**−**6**. This study indicates that there is a large dependence of α-(acyloxy)-dialkylnitrosamine reactivity upon the pK_a of the conju-



4; R = -$CH_2CH_2CH_2CH_3$, **5**; R = -CH_2CH_3, **6**; R = -CH3,
R' = H R' = -CH_3 R' = Ph
a ; X = -CH_3 **a**; X = -CH_3 **a**; X = -CH_3
b; X = -CH_2Cl **b**; X = -CH_2Cl **b**; X = -CH_2Cl
c; X = -$CHCl_2$ **c**; X = -$CHCl_2$

gate acid of the leaving group acetate anion. Thus α-(acyloxy)dialkylnitrosamines have been synthesized that are more reactive than the reactivity reported for the corresponding α-hydroxydialkylnitrosamines. A direct test of the mechanism of eq 3 is afforded, and the results require the intermediacy of α-hydroxydialkylnitrosamines in the decay of α-(acyloxy)dialkylnitrosamines in aqueous media.

Experimental Section

Warning! Many α-acetoxydialkylnitrosamines are proven to be powerful direct acting carcinogens! Procedures must be carried out with due precautions. Manipulations were carried out by personnel wearing frequently-changed double pairs of disposable gloves and in a well-ventilated hood. Contaminated and potentially-contaminated materials were treated with 50% aqueous sulfuric acid containing the commercially available oxidant "No Chromix" (Aldrich Chemical).

Materials. Organic solvents were purified by distillation before use. Organic chemicals for synthesis were ACS grade or better. Inorganic chemicals were ACS grade or better and were used without further purification. Water was distilled in glass.

Kinetics. All kinetic runs were carried out using a Hewlett Packard 8452A diode array spectrophotometer or an Applied Photophysics DX17MV stopped-flow spectrophotometer, both thermostated at 25 °C by a circulating water bath. Kinetic runs were initiated when solutions containing esters dissolved in acetonitrile were injected into the cuvettes or injected mechanically into the observation cell of the stopped-flow instrument, to give final substrate concentrations of $1-2 \times 10^{-4}$ M. The final acetonitrile concentration was 4% by volume. Routinely, values of k_{obsd} were calculated from absorbance decay or appearance data using a commercially available fitting program (Enzfitter) or the software routine on the stopped-flow. In cases where non-first-order kinetics were observed, the data were handled as described in the Results and Discussion sections. The pH values were obtained after the kinetic runs using a Corning Model 501 pH meter with attached combination electrode. Two point calibrations were done before recording pH values. Calibrations were carried out using commercially available standards or standards prescribed by the Merck Index, 8th edition.[8]

Synthesis. α-Acetoxydialkylnitrosamines 4a−6a. These were prepared essentially as reported previously[2] from the appropriate imine (or cyclic trimer of the imine) and nitrosonium tetrafluoroborate, in the presence of triethylammonium acetate. Physical data for **4a** and **5a** were reported previously. **6a**: ^{1}H NMR ($CDCl_3$) δ (*E*) isomer (96%): 8.32 (1 H, s), 7.42 (5 H, s), 2.82 (3H, s), 2.26 (3H, s); (*Z*) isomer (4%): 3.56 (3H, s), 2.19 (3H, s). Anal. Calcd: C, 57.69; H, 5.81; N, 13.45. Found: C, 58.34; H, 5.85; N, 13.2.

α-(Chloroacetoxy)dialkylnitrosamines 4b−6b. These were prepared in a manner analogous to that for the acetates, but the electrophilic addition was carried out at 0−5 °C. Subsequent to addition of the $NOBF_4$, the reaction was stirred an additional 20 min and was washed in a separatory funnel three times with an equal volume of water each time. The organic layer was dried over $MgSO_4$ and filtered, and the methylene chloride was removed by evaporation with argon. The crude material was freed of aldehyde by washing with pentane. The compounds **4b** and **5b** were purified by chromatography on silica with ether/hexane (1/5) and ether, respectively, used as solvents. The compound **6b** was recrystallized from pentane/methylene chloride (9/1). **4b**: ^{1}H NMR ($CDCl_3$) δ: (*E*) isomer (88%): 0.90 (t, 3H); 1.28, (m, 2H); 1.45, (m, 2H); 3.55, (t, 2H); 4.10, (s, 2H); 6.25 (s, 2H); (*Z*) isomer (12%): 1.00, (t, 3H); 1.45, (m, 2H); 1.78, (m, 2H); 4.30, (t, 2H); 4.05, (s, 2H); 5.42, (s, 2H). **5b**: ^{1}H NMR ($CDCl_3$) δ 1.09 (3H, t), 1.51 (3H, d), 3.59 (2H, m), 4.07 (2H, s), 7.21 (4 H, q). **6b**: ^{1}H NMR ($CDCl_3$) δ (*E*) isomer (96%): 8.39 (1H, s), 7.45 (5 H, s), 4.23 (2H, s), 2.83 (3H, s); (*Z*) isomer (4%): 3.60 (3H, s) ppm. Anal. Calcd: C, 49.5; H, 4.6; N, 11.55. Found: C, 49.74; H, 4.63; N, 11.62.

α-(Dichloroacetoxy)dialkylnitrosamines 4c, 6c. A mixture of dichloroacetic acid (3.5 mmol) and dry triethylamine (3.5 mmol) in 5 mL of dry methylene chloride was added at −10 °C to a stirred solution of imine (3.5 mmol) in 15 mL of dry methylene chloride. $NOBF_4$ (1.1 equiv) was added with a spatula slowly. The solution bubbled and turned yellow-orange. The clear solution was stirred for 10 min and was washed rapidly three times with cold water. The organic layer was dried over $MgSO_4$ and filtered, and the methylene chloride was evaporated off under an argon stream. The crude material was washed with pentane to get rid of aldehyde and was stored over dry ice. **4c**: ^{1}H NMR ($CDCl_3$), (*E*) isomer (87%): δ 0.90 (t, 3H); 1.28 (m, 2H); 1.45 (m, 2H); 3.60 (t, 2H); 6.00 (s, 1H); 6.35 (s, 2H); (*Z*) isomer (13%): δ 1.00 (t, 3H); 1.45 (m, 2H); 1.78 (m, 2H); 4.30 (t, 2H); 5.50 (s, 2H); 5.94 (s, 1H). **6c**: ^{1}H NMR ($CDCl_3$) δ 8.36 (1H, s); 7.44 (5H, s); 6.12 (1H, s); 2.83 (3H, s).

Results

For compounds **4a**−**c** and **5a,b**, the kinetics of disappearance of the N−N=O chromophore were monitored at 230 nm and exhibited good first-order behavior over most of the range of pH = 2−13 (see exceptions below). In general, changes in buffer concentration from 0.05−0.30 M did not have much effect on the value of k_{obsd} with typical increases being 10−25% for variation over this concentration range. Changes in bicarbonate buffer concentrations (at pH > 9) effected the largest increases in k_{obsd}, these being as much as 50% increases, at ∼0.2 M buffer, above the value of k_{obsd} extrapolated to a buffer concentration equal to zero.

For compounds **6a**−**c**, the kinetics of appearance of the product benzaldehyde were monitored at 254 nm and

(6) Mochizuki, M.; Anjo, T.; Okada, M. *Tetrahedron Lett.* **1980**, *21*, 3693. Okada, M.; Mochizuki, M.; Anjo, T.; Sone, T.; Wakabayashi, Y.; Suzuki, E. *IARC Sci. Publ.* **1980**, *31*, 71. Mochizuki, M.; Anjo, T.; Takeda, K.; Suzuki, E.; Sekiguchi, N.; Huang, G. F.; Okada, M. *IARC Sci. Publ.* **1982**, *41*, 553.

(7) Mesić, M.; Revis, C.; Fishbein, J. C. *J. Am. Chem. Soc.* **1996**, *118*, 7412.

(8) *The Merck Index*, 8th ed.; Stecher, P. G., Ed., Merck & Co.: Rahway, NJ, 1968.

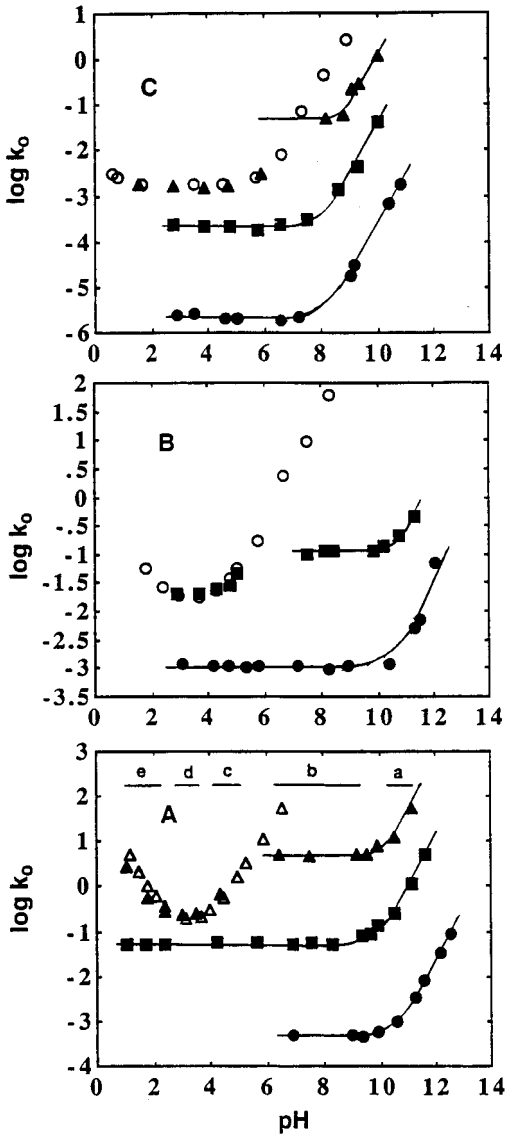


Figure 1. Plots of log k_o, the buffer independent rate constants for decay or increase of absorbance in the decomposition of α-(acyloxy)dialkylnitrosamines (solid symbols) and α-hydroxydialkylnitrosamines (open symbols), against pH in aqueous solutions: 25 °C, ionic strength 1 M ($NaClO_4$), 4% acetonitrile by volume. Panel A, compounds **6**: (●), **a**; (■), **b**; (▲), **c**; and (△), the corresponding α-hydroxy derivative. Panel B, compounds **5**: (●), **a**; (■), **b**; and (○), the corresponding α-hydroxy derivative. Panel C, compounds **4**: (●), **a**; (■), **b**; (▲), **c**; and (○), the corresponding α-hydroxy derivative.

exhibited good first-order behavior over most of the range of pH between pH = 2−13 (see exceptions below). The effects of changing buffer concentration on the value of k_{obsd} were similar to those described for compounds **4** and **5** above. Slightly larger effects with bicarbonate buffers were observed for compounds **6** with <150% increases, at 0.2 M buffer, above the value of k_{obsd} extrapolated to zero buffer concentration being typical.

Values of k_o, the buffer independent rate constant for either disappearance of the N−N=O chromophore (compounds **4** and **5**) or the appearance of benzaldehyde (compounds **6**), were obtained as the *y*-intercept values of the lines from plots of k_{obsd} against buffer concentration. The slight effects of buffer concentration on the values of k_{obsd} (above) enabled values of k_o to be obtained with typical uncertainties of se = ±5%. Plots of log k_o against pH are presented in Figure 1.

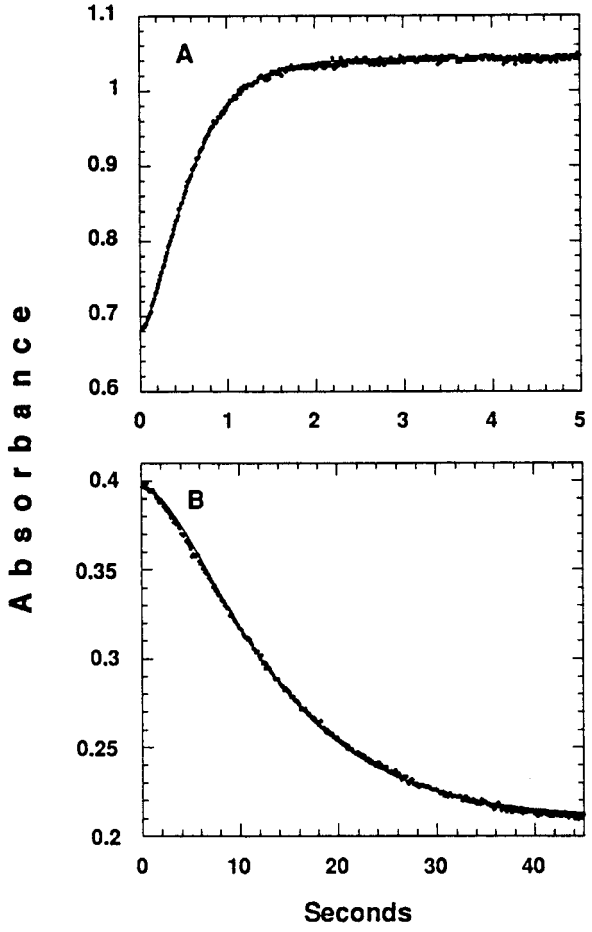


Figure 2. Plots of absorbance against time for the decay of **6c**, in panel A, and **5b**, in panel B, under the reaction conditions described in Figure 1. Fits to the data were obtained using eqs 6 or 7 and the parameters given in the text.

Table 1. Yields of Azide Adduct and Values of k_{obsd} in the Decay of 6b as a Function of Azide Ion Concentration in Aqueous Solution, 25 °C, 4% Acetonitrile, Ionic Strength 1 M ($NaClO_4$), 0.05 M Cacodylic Acid Buffer, 50% Anion

azide ion concentration (M)	k_{obsd} (s^{-1})	azide adduct % yield
0	0.042	0
0.0002	0.043	42
0.001	0.043	80
0.005	0.043	99
0.008	0.042	102

In the case of **4c**, **5b**, and **6c** the kinetics were non-first-order in narrow regions of the pH range studied. The ranges were **4c**, pH ∼ 6−7.5; **5b**, pH ∼ 5.5−7; and **6c**, pH ∼ 4.5−6. Examples of the data for such runs for compounds **4c** and **6c** are given in Figure 2.

The yields of azide ion adduct as a function of azide ion concentration in the reaction of **6b** are summarized in Table 1. Also in Table 1 are rate constants for the first-order appearance of benzaldehyde in solutions of varying azide ion concentration determined spectrophotometrically at 250 nm.

Discussion

α-Hydroxydialkynitrosamine Intermediates. We previously studied the pH dependence of the buffer-independent decay of some simple α-(acyloxy)dialkylnitrosamines and observed that in the range of pH = 3−13,

Table 2. Rate Constants for the Decay of α-(Acyloxy)dialkylnitrosamines in Aqueous Solutions at 25 °C, Ionic Strength 1 M ($NaClO_4$), 4% Acetonitrile by Volume

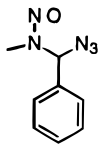
compound	k_1,[a] s^{-1}	k_{OH},[a] M^{-1} s^{-1}
4a[b]	2.0×10^{-6}	2.4
4b	2.1×10^{-4}	320
4c	0.017	1.25×10^4
5a[b]	0.00103	3.2
5b	0.112	87
6a	0.00044	1.3
6b	0.053	632
6c	4.4	2.1×104

[a] As defined in eq 1. Standard errors for all entries $<\pm 10\%$. [b] Previously reported in ref 2.

the rate law of eq 1 was obeyed.[2] It was concluded that the pH-independent term, k_1, involved rate-limiting formation of *N*-nitrosiminium ions, as in eq 2, on the basis of the near-zero values of $\Delta S^{\ddagger}$, structure−activity relations, and the trapping of putative *N*-nitrosiminium ions after the rate-limiting step by azide ion to yield adducts such as in eq 2.[2,3] Others[16a] have previously characterized the reaction chemistry of **6a** and obtained qualitatively similar evidence for the involvement of an *N*-nitrosiminium ion. It was also concluded, in accord with earlier suggestions, that the pH-dependent term, k_{OH}, involves hydroxide ion attack at the carbonyl group.

The kinetic data for the acetate esters **5a** and **4a** (Figure 1, parts B and C, respectively) were presented as part of the previous study,[2] and the kinetic data for the acetate ester **6a**, Figure 1A, also shows the biphasic dependence indicated for the rate law of eq 1 over the limited range studied. Fits to the rate law of eq 1, using values for the rate constants summarized in Table 2, are indicated by the solid lines in Figure 1.

Substitution of electron-withdrawing groups into the acetate moiety of the esters is expected to increase the rate constants for both the pH dependent and pH independent reactions, and this is in fact observed. The increases in rate constants k_1 and k_{OH} are expected due to the improved leaving group ability, for the k_1 process, and increased electrophilicity, in the k_{OH} process, upon introduction of the electron-withdrawing group. In the case of the chloroacetates **6b** and **4b**, the data in Figure 1 (panels A and C, respectively) show the expected increases in k_1 and k_{OH}. In the cases of the k_1 process for **4b** and **6b**, the observed reactions are too fast to involve rate-limiting reactions of the carbonyl group. Reported rate constants for such reactions involving substrates which should have similar reactivity are too small to account for the observed reactivity of **4b** and **6b**. Thus, the pH independent decay of ethyl chloroacetate is $\sim 10^{-7}$ s^{-1}.[9] Additional evidence that there is no change in mechanism for the pH independent reaction in the case of **6b** is found in Table 1 which indicates a quantitative yield of the azide adduct **7** when **6b** is decomposed in



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the presence of 8 mM sodium azide in the absence of an increase in k_{obsd} of greater than 5%. This data requires that the azide adduct is formed in a reaction subsequent to the rate-limiting step, presumably involving capture of the *N*-nitrosiminium ion formed in the rate-limiting step of the k_1 process, as in eq 2.[3,10]

Inspection of Figure 1 shows that in the case of the chloroacetate **5b** (Figure 1B, solid squares) and the dichloroacetates **4c** (Figure 1C, solid triangles) and **6c** (Figure 1A, solid triangles) the log k_o−pH profiles are more complex. The behavior is qualitatively similar for all three compounds and is most clearly observed for **6c** (Figure 1A, solid triangles) using the five bars in the figure, labeled a−e, that indicate pH ranges that exhibit distinct pH dependences. The rate constants in regions a and c are inversely proportional to hydrogen ion concentration while those in b and d are pH independent and those in region e are hydrogen ion dependent.

In the pH range between b and c the kinetics of benzaldehyde formation are non-first-order, as illustrated in Figure 2A. Non-first-order behavior in the disappearance of the N−NO chromophore in the analogous regions of pH is observed for compounds **4c** and **5b**. An example of this behavior is indicated in the case of **5b** in Figure 2B. The non-first-order behavior means that the apparent downward break in the pH rate profiles between regions b and c cannot be ascribed to a change in rate-determining step involving a steady state intermediate which would give clean first-order kinetic behavior at every pH.

The observation of non-first-order behavior indicates the accumulation of a non-steady-state intermediate that is concluded to be the α-hydroxydialkylnitrosamines. This conclusion is based on the coincidence of the pH-rate profiles for decay of α-hydroxydialkylnitrosamines that were recently reported[7] with parts of the pH-rate profiles for **4c**, **5b**, and **6c** that are reported here. The pH-rate profiles for decay of the α-hydroxydialkylnitrosamines are included in Figures 1A−C (open symbols). The kinetic behavior of the α-hydroxydialkylnitrosamines accounts qualitatively for the kinetic behavior observed in regions c, d and e of the pH-rate profiles for benzaldehyde formation, in the case of **6c**, and for the pH-rate profiles for N−NO group disappearance, starting from **4c** and **5b**. The behavior over the complete range of pH studied can be understood by the kinetic scheme in eq 3 in which k_A and k_B are further defined as in eq 4 and eq 5.[11] In regions a and b (Figure 1A), $k_B \gg k_A$ so that the α-hydroxydialkylnitrosamine does not build up to any

$$k_A = k_1 + k_{OH}[OH^-] \quad (4)$$

$$k_B = k°_1 + k°_2/[H^+] + k°_3[H^+] \quad (5)$$

extent and good first-order kinetic behavior is observed. The upward break in the pH-rate profile between region a and b indicates a change in mechanism from hydroxide ion attack of the carbonyl group (k_{OH}, region a) to pH-independent formation of the nitrosiminium ion (k_1, region b). Best fits of the data to such a kinetic scheme using the parameters summarized in Table 2 are indicated as solid lines in the Figure 1. Throughout regions

(9) Jencks, W. P.; Carrioulo, J. *J. Am. Chem. Soc.* **1961**, *83*, 1743.

(10) Vigroux, A.; Kresge, A. J.; Fishbein, J. C. *J. Am. Chem. Soc.* **1995**, *117*, 4433.

(11) The rate constants $k°_1$, $k°_2$, and $k°_3$ used here are, respectively, identical with k_1, k_2, and k_3 given in ref 7. The new symbols were employed to avoid ambiguity with other rate constant names in the present report.

c, d, and e, $k_A > k_B$ so that good first-order kinetics of the reactions involving the α-hydroxydialkylnitrosamines are observed and the rate law is adhered to that was previously determined in these cases.[7,11]

The non-first-order kinetic behavior between regions b and c is expected for a system such as that in eq 3 when $k_A \sim k_B$, and the observed non-first-order kinetic behavior is quantitatively consistent with the interpretation in terms of the eq 3. Kinetic expressions for the appearance of benzaldehyde from **6c** and the disappearance of the N−NO chromophore in the case of compounds **4c** or **5b** are given in eqs 6 and 7, respectively.[12]

$$\text{Abs} = A_o[1 - (1/(k_B - k_A)[k_B e^{-k_A t} - k_A e^{-k_B t}]] + C_o \quad (6)$$

$$\text{Abs} = A_o e^{-k_A t} + A_o[k_A/(k_B - k_A)][e^{-k_A t} - e^{-k_B t}] + C_\infty \quad (7)$$

Values of k_B for the decay of the α-hydroxydialkylnitrosamines were computed from the best fit of the data in Figure 2, parts A and B, to eqs 6 and 7 using values of k_A for the decay of the α-acyloxy compounds equal to the values of k_o in Table 2, the observed absorbance at $t = 0$ (C_o) or $t = \infty$ (C_∞), and the total change in absorbance (A_o) from the absorbance at $t = 0$ and the absorbance at $t = \infty$. This analysis yields a rate constant k_B for the α-hydroxydialkylnitrosamine derivative of **6c** of 2.4 s^{-1} (using eq 6) while the value calculated from the rate constants determined from direct measurement of the decay of the α-hydroxydialkylnitrosamine, reported previously,[7] yields $k_B = 2.3$ s^{-1}. A similar analysis using eq 7 and the data for **5b** in Figure 2B gives $k_B = 0.19$ s^{-1} for the decay of the α-hydroxydialkylnitrosamine which compares well with the value of $k_{obsd} = 0.19$ s^{-1} that was measured directly from the α-hydroxy-*N*,*N*-diethylnitrosamine under identical conditions of pH and buffer concentration.[13]

Ultraviolet spectral analysis of the decay of **5b** is consistent with the non-steady-state formation of the α-hydroxydialkylnitrosamine. A solution (0.10 M methoxyacetic acid buffer, 50% anion, $\mu = 1$, 25 °C) of **5b** with an initial absorbance at λ_{max} (232 nm) of 0.969 was allowed to decay for 50 s (~8 half-lives of ester decay) after which the absorbance at λ_{max} (230 nm) was 0.474. This absorbance subsequently decayed to a final value of 0.046 units. The λ_{max} at 230 nm of the transient remaining after >99% decay of the ester is consistent with that expected for the N−N=O chromophore of the α-hydroxydialkylnitrosamine.[6]

Effects of Leaving Group. The values of k_1 in Table 2 indicate that the *N*-nitrosiminium ion-forming reaction is quite sensitive to the basicity of the carboxylate ion leaving group. Plots of log k_1 against the conjugate acid pK_a of the leaving group have slopes, β_{lg}, of −1.13, −1.05, and −1.15 for compounds **4a**−**c**, **5a**−**b**, and **6a**−**c**, respectively. The values of β_{lg} indicate that the substituents detect a build-up of a full negative charge in proceeding from the ground state to the transition state. Such large values β_{lg} suggest an advanced transition state with considerable C−O bond fission, but complete scission of the bond is not required in the absence of knowledge of the "effective charge" on the oxygen in the ester, which could be considerable.[14]

The notion that there is considerable C−O bond cleavage in the rate-limiting step is consistent with the progress in the transition state that is indicated by other measures. Substantial rehybridization at the central carbon of the penultimate iminium ion is suggested by the secondary α-deuterium kinetic isotope effects of $k^H/k^D \sim 1.15$ (per hydrogen) that has recently been determined.[15]

Biological Relevance. For most of the compounds reported in this study, and in the case of a number of others that have been studied in this laboratory, the dominant mechanism of decomposition at physiological pH involves the pH independent (k_1) process. It is frequently assumed that α-(acyloxy)dialkylnitrosamines are converted to α-hydroxydialkylnitrosamines via esterase action in vivo; however, in a number of cases, esterases fail to accelerate the decay of α-(acyloxy)-dialkylnitrosamines.[16] In these cases, the predominant reaction likely involves the same pH independent (k_1) mechanism and the intermediacy of *N*-nitrosiminium ions and α-hydroxydialkylnitrosamines.

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(12) Espenson, J. H. *Chemical Kinetics and Reaction Mechanisms*; McGraw-Hill Book Co.: New York, 1981.

(13) Allowing both rate constants to vary in the fit, as requested by a referee, gives $k_A = 0.11$ s^{-1} and $k_B = 0.20$ s^{-1} for **5b**. The former value is in good agreement with the experimentally determined value (Table 2) of k_1 (= k_A, under these conditions) = 0.11 s^{-1}.

(14) Williams, A. *Acc. Chem. Res.* **1984**, *17*, 425.

(15) Cai, H.; Fishbein, J. C. Unpublished results.

(16) (a) Baldwin, J. E.; Scott, A.; Branz, S. E.; Tannenbaum, S. R.; Green, L. *J. Org. Chem.* **1978**, *43*, 2427. (b) Hecht, S. S.; Young-Sciame, R.; Chung, F.-L. *Chem. Res. Toxicol.* **1992**, *5*, 706.