Cyclic α-Acetoxynitrosamines: Mechanisms of Decomposition and Stability of α-Hydroxynitrosamine and Nitrosiminium Ion Reactive Intermediates

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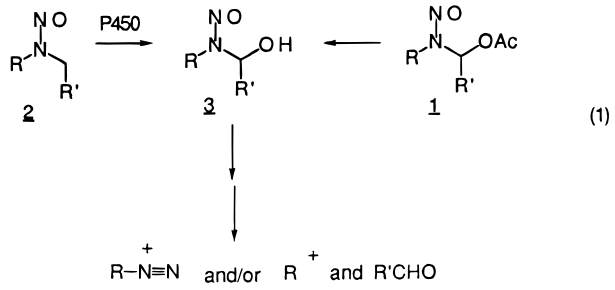
Received January 29, 1999

Abstract: A study of the kinetics and mechanism of the decay of α-acetoxy-*N*-nitrosopyrrolidine and α-acetoxy-*N*-nitrosopiperidine are reported. The compounds differ in reactivity by more than 2 orders of magnitude at physiological pH. On the basis of thermodynamic parameters, common ion inhibition and azide ion trapping experiments, both compounds appear to decompose under these conditions by the formation of *N*-nitrosiminium ion intermediates. The differences in reactivity are rationalized on the basis of results from an ab initio study, described in the accompanying paper. The first direct study of the kinetics of decay of cyclic α-hydroxynitrosamines of nitrosopiperidine and nitrosopyrrolidine and a third compound, 2-hydroxy-2-methylnitrosopyrrolidine is also summarized. These prove to be highly unstable reactive intermediates, in contrast to what might be expected on the basis of earlier reports concerning cyclic α-hydroxynitrosamines.

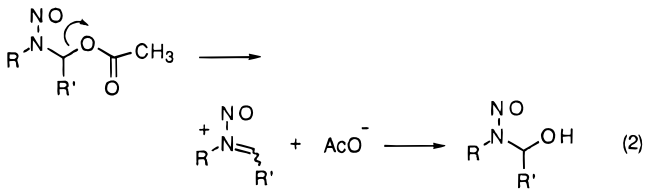
Introduction

α-Acetoxynitrosamines (**1**, eq 1) are widely employed in elucidating the mechanisms of nitrosamine (**2**, eq 1) carcinogens.[1,2] They are purported precursors to α-hydroxydialkylnitrosamines (**3**, eq 1) that are believed to be the products of metabolic activation of many nitrosamines, as indicated schematically in eq 1.



(1)

α-Hydroxydialkylnitrosamines subsequently give rise to electrophiles that covalently modify DNA. The mechanism by which α-acetoxynitrosamines decompose has been the subject of differing reports,[3−5] but it has most recently been claimed[6] that the simplest acyclic members of the family undergo S_N1 solvolysis to give *N*-nitrosiminium ions that subsequently hydrate, as in eq 2.



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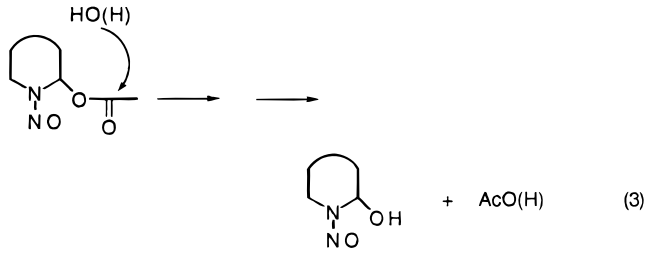
Cyclic α-acetoxynitrosamines have likewise been used for the generation of reactive intermediates of a number of important carcinogens including tobacco specific nitrosamines[7] and nitrosopyrrolidine and nitrosopiperidine.[8,9] These esters have enabled important progress in determining the structure of DNA and nucleoside adducts from the ultimate electrophiles. However, the cyclic esters have been less-studied with respect to mechanism. Mainly on the basis of differences in the rates of hydrolysis, the mechanism of eq 3—the more "typical" carbonyl attack mechanism of ester hydrolysis—has been proposed in the case of α-acetoxynitrosopyrrolidine.[7] A mixture of the two mechanisms, eqs 2 and 3, has also been suggested.[4]



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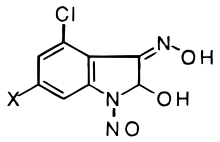
10.1021/ja9902975 CCC: $18.00
Published on Web 05/20/1999

Reports concerning cyclic α-hydroxynitrosamines are puzzling in the context of what is known of the corresponding acyclic compounds. A number of the latter α-hydroxy methyl compounds, (**3**, eq 1, R′ = H), were generated ~20 years ago, characterized in aprotic organic solvents, and shown to have maximum half-lives of seconds to minutes in aqueous media.[10,11] More recently, similar methods were employed to produce α-methylene compounds, (**3**, eq 1, R′ = CH_3, $CH(CH_3)_2$, and Ph) that were shown to have maximum half-lives of seconds in aqueous media.[12] In contrast, the cyclic α-hydroxynitrosamines, **4** (X = H and OMe) were isolated as stable solids from aqueous nitrosation of the indoles and fully characterized.[13] Their stability was attributed to both the formation of an intramolecular hydrogen bond involving the oxygen of the α-hydroxyl and the steric inhibition of ring opening. Similarly, the α-hydroxy derivative of nitrosomorpholine, **5**, was claimed to have been isolated after 21 h (5 °C) of aqueous nitrosation of 3-hydroxy-



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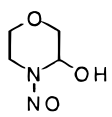

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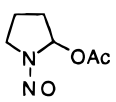
morpholine.[14] The solid compound **5** was reportedly stable for weeks but was characterized only by field desorption mass spectrometry. It might therefore be presumed on the basis of the extant literature that cyclic structure either inherently, or in concert with other structural features, imbues a stability on the α-hydroxynitrosamine.

In view of these uncertainties, we have undertaken a kinetic study of the cyclic α-acetoxypyrrolidine **6** and α-acetoxypiperidine **7**. In addition, we have generated and studied the corresponding α-hydroxy derivatives, **8** and **9**, and a third α-hydroxy compound, **10**, which is the first methinyl α-hydroxynitrosamine to be kinetically characterized.



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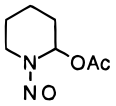

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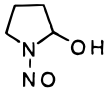

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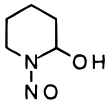

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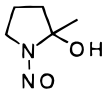

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In summary, **6** and **7** decompose via the formation of *N*-nitrosiminum ions at physiological pH, as in the mechanism of eq 2, but with surprisingly different reactivities. Further, the α-hydroxynitrosamines prove to be highly unstable in aqueous media with half-lives of seconds to milliseconds at physiological pH.

Experimental Section

Warning! Many nitrosamines are powerful carcinogens. Precautions taken in handling include use of frequently changed double pairs of disposable gloves and 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). Dilution of concentrated nitroso compounds is recommended prior to treatment as the pure materials may react violently with this mixture.

Materials. Organic solvents were dried and purified by distillation before use. The chemicals for synthesis and kinetics were ACS grade or better. Water was distilled in glass.

Methods. Kinetics. Kinetics of decay were generally monitored at 230 nm using a Hewlett-Packard 8452A diode array spectrophotometer or an Applied Photophysics DX17MV stopped-flow spectrophotometer. Both instruments were thermostated at 25 °C by circulating water baths. Reaction media were maintained at ionic strength μ = 1.0 M, unless otherwise noted, using $NaClO_4$. Values of pH were obtained using an Orion model 720 pH meter with attached combination electrode. Two point calibrations were done before recording pH values at the end of reactions. Calibrations were carried out using commercially available standards, or standards prescribed by the *Merck Index*, 8th edition.[15]

Product Quantitation. Products were quantitated in some cases as 2,4-dinitrophenylhydrazones after separation by HPLC using Waters pumps and UV/vis detector, a Wisp-Ultra injector, Axxiom pump controller/data system, and a C18 column (Waters μ-Bondapac or Keystone Beta-sil). Standard curves were generated from authentic standards. The 2,4-dinitrophenylhydrazones were synthesized from commercially available materials. The hydrazones were stable to the reaction conditions as indicated by injection of the reaction products after 10 and 20 half-lives of reaction which indicated a change in the amount of hydrazone of less than 5%.

Synthesis. The α-acetoxy-*N*-nitrosopyrrolidine **6** and α-acetoxy-*N*-nitrosopiperidine **7** were synthesized as described by Saavedra.[16] **6**: ^{1}H NMR ($CDCl_3$) 7.19 (1 H, t), 3.60 (2H, m), 2.16 (2H, m), 2.10 (3H, s), 2.05 (2H, m); ^{13}C NMR ($CDCl_3$) δ 169.43, 83.81, 44.78, 31.00, 20.91, 19.54 ppm. **7**: ^{1}H NMR ($CDCl_3$) 7.3 (1 H, t), 4.80 (1H, q), 2.68 (1H, m), 2.22 (1H, m), 2.1 (3H, s), 1.89 (5H, m).

α-Phenoxy-α-methyl-*N*-nitrosopyrrolidine, 11. A mixture of phenol (6.03 mmol) and dry triethylamine (6.03 mmol) in 5 mL of dry methylene chloride was added with stirring to a solution of 3,4-dihydro-5-methyl-2*H*-pyrrole[17] (0.5 g, 6.03 mmol) in 10 mL of methylene chloride, maintained at −10 °C. $NOBF_4$ (1.1 equiv) was added with a spatula slowly. The brown solution was stirred for 10 min and then washed 3 times with equal volumes of cold water. The organic layer was dried over $MgSO_4$ and filtered, and the methylene chloride was evaporated off under a stream of argon. The product was purified by preparative TLC on silica gel using methylene chloride as the mobile phase: ^{1}H NMR ($CDCl_3$) 7.3 (5 H, Ph), 3.75 (2H, m), 2.70 (1H, m), 2.14 (3H, m), 2.15 (3H, s) ppm; ^{13}C NMR ($CDCl_3$) δ 139.34, 134.68, 132.51, 126.65, 101.64 (C2), 48.11 (C5), 39.06 (C3), 24.77 (C4), 20.85 (CH_3) ppm.

α-Hydroperoxy-*N*-nitroso Compounds. The synthesis of α-hydroperoxy-*N*-nitrosopyrrolidine **12**, α-hydroperoxy-*N*-nitrosopiperidine **13**, and α-hydroperoxy-α-methyl-*N*-nitrosopyrrolidine **14** was accomplished by the following procedure. To a solution of **6**, **7**, or **11** (0.7 mmol) in 3 mL of acetonitrile was added 3 mL of 50 wt % H_2O_2 in water. The mixture was stirred at 40 °C for 2 h, washed with water three times, and extracted with methylene chloride. The organic layer was dried over $MgSO_4$ and filtered, and the solvent was evaporated. The products were purified by preparative TLC using 9.5/0.5 methylene

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chloride/ether in the cases of **12** and **13**. The compound **14** was similarly purified using methylene chloride as eluant. **12**: ^{1}H NMR (CD_3CN) δ 10.00 (1H, s), 6.22 (1H, m), 3.50 (2H, m), 2.10 (4H, m) ppm; ^{13}C NMR (CD_3CN) δ 95.03, 45.18, 29.14, 20.97 ppm. Anal. Calcd: C, 36.36; H, 6.10; N, 21.20. Obsd: C, 36.56; H, 6.14; N, 21.04. **13**: ^{1}H NMR ($CDCl_3$) δ 8.32 (1H, s), 6.25 (1H, t), 4.85 (1H, q), 2.75 (1H, m), 2.25 (1H, m), 1.8 (5H, m) ppm; ^{13}C NMR ($CDCl_3$) δ 91.84, 36.10, 28.15, 23.69, 18.94 ppm. Anal. Calcd: C, 41.09; H, 6.90; N, 19.17. Obsd: C, 41.34; H, 6.85; N, 18.89. **14**: ^{1}H NMR ($CDCl_3$) δ 8.53 (1H, b), 3.69 (2H, m), 2.46 (1H, m), 2.07 (3H, m), 1.99 (3H, s) ppm; ^{13}C NMR ($CDCl_3$) δ 101.44, 47.94, 37.83, 23.39, 21.16 ppm.

α-Hydroxynitrosamines. In a NMR tube, the α-hydroperoxy compound was converted to the α-hydroxy compound by addition of triphenylphosphine in 0.7 mL of $CDCL_3$ or CD_3CN. Conversion occurred within the time required to shake the tube, shim the instrument, and record the spectrum. **8**:^{1}H NMR (CD_3CN) δ 6.10 (1H, t), 4.88 (*OH*, d), 3.50 (2H, m), 2.10 (4H, m) ppm; ^{13}C NMR (CD_3CN) δ 84.60, 44.89, 33.03, 20.49 ppm. **9**: ^{1}H NMR ($CDCl_3$) δ 6.22 (1H, b), 4.75-(1H, q), 4.60 (*OH*, b), 2.80 (1H, m), 2.00 (6H, m) ppm; ^{13}C NMR ($CDCl_3$) δ 81.62, 35.77, 31.66, 24.12, 18.23 ppm. **10**: ^{1}H NMR ($CDCl_3$) δ 3.49 (1H, t), 2.59 (2H, m), 2.37 (1H, m), 2.16 (3H, s), 1.95 (2H, m) ppm.

2,4-Dinitrophenyl Hydrazones 15, 16, and 17.[18] To a solution of 0.2 g of 2,4-dinitrophenylhydrazine in 50 mL of HCl (2N) at 60 °C was added 0.1 g of 2,3-dihydrofuran, 3,4-dihydro-2*H*-pyran or crotonaldehyde to generate **15**, **16,** and **17**, respectively. The solution was cooled at 0 °C for 30 min and filtered. The yellow solid was washed with HCl (2 N) and purified by recrystallization in EtOH. **15**:^{1}H NMR ($CDCl_3$) δ 11.20 (1H, b), 9.10(1H, d), 8.30 (1H, q), 7.90 (1H, d), 7.60 (1H, t), 3.80 (2H, t), 2.60 (2H, m), 1.95 (2H, m), 1.55 (1H, b) ppm. **16**: ^{1}H NMR ($CDCl_3$) δ 11.10 (1H, b), 9.20 (1H, d), 8.30 (1H, q), 8.00 (1H, d), 7.60 (1H, t), 3.80 (2H, t), 2.55 (2H, m), 1.8 (4H, m), 1.30 (1H, t) ppm. **17**: ^{1}H NMR ($CDCl_3$) δ 11.10 (1H, b), 9.12(1H, d), 8.33 (1H, q), 8.27 (1H, d), 7.97 (1H, d), 7.75 (1H, d), 6.31 (2H, m), 1.96 (3H, d) ppm; ^{13}C NMR ($CDCl_3$) δ 150.06, 144.79, 140.81, 138.81, 129.93, 128.5, 127.83, 123.49, 116.59, 18.84 ppm.

α-Azido-*N*-nitrosopyrrolidine, 18. α-Acetoxy-*N*-nitrosopyrrolidine (0.16 g) was added to 10 mL of water containing 0.65 g of NaN_3 and 0.146 g of cacodyllic acid, and the reaction was gently stirred for 40 h at room temperature. The reaction was extracted into ether and dried with Na_2SO_4. Final purification was carried out by column chromatography using silica gel and CH_2Cl_2 as eluant. Yield: 30%. ^{1}H NMR ($CDCl_3$): E(87%) 6.25 (b, 1H); 3.4−3.8 (d of m, 2H); 1.95−2.2 (m, 4H). Z(13%) 5.71 (b,1H); 4.35 (m, 2H); 1.95−2.2 (m). Anal. Calcd: C, 34.04; H, 5.00; N, 49.62. Obsd: C, 34.32; H, 5.00; N, 49.36.

α-Azido-*N*-nitrosopiperidine, 19. This was isolated using a prodcedure analogous to that above for the pyrrolidine compound from 0.035 g of the corresponding α-acetoxy compound. The purified material (preparative TLC) migrated as a single spot on TLC and was >97% pure on HPLC analysis monitoring at 230 nm. ^{1}H NMR ($CDCl_3$): 6.45 (s, 1H), 2.93 (t of d, 2H), 2.05 (m, 2H); 1.70−1.90 (m, 4H).

Results

α-Acetoxynitrosamines. In the case of α-acetoxynitrosopyrrolidine, **6**, at all values of pH, and α-acetoxynitrosopyrrolidine, **7**, above pH = 6, the kinetics of decay of the N−NO chromophore exhibited good first-order behavior for 3−5 half-lives of reaction. The values of k_{obsd} varied only slightly with buffer concentration at constant buffer ratio, typically changing less than 10% over a change in concentration that ranged between 0.03 and 0.3 M. Plots of k_{obsd} versus buffer concentration, usually containing three points, were extrapolated to zero buffer concentration and the corresponding rate constant was taken as k_o, the buffer-independent rate constant. The largest effect of buffer concentration was observed with the reaction α-acetoxynitrosopyrrolidine in hydrazine buffer (80% monocation). The value of k_{obsd} in 0.15 M buffer solution increased 104% above k_o. The change in k_o as a function of pH is indicated in Figure 1.

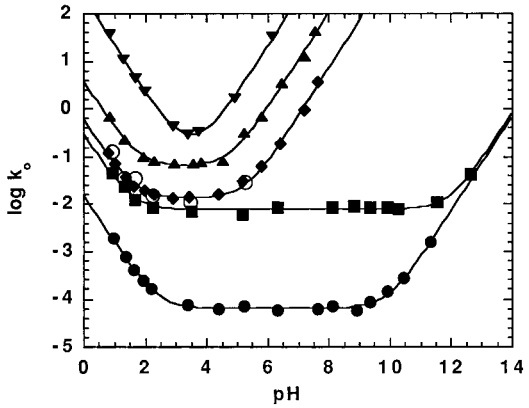

Figure 1. Plot of the log of k_o, the buffer independent rate constant, against pH for the decay of cyclic α-acetoxynitrosamines **6** (solid circles) and **7** (squares) and cyclic α-hydroxynitosamines **8** (triangles), **9** (diamonds), and **10** (inverted triangles) at 25 °C ionic strength 1M ($NaClO_4$). Open circles are for **9** derived from the decay kinetics of **7** (see text).

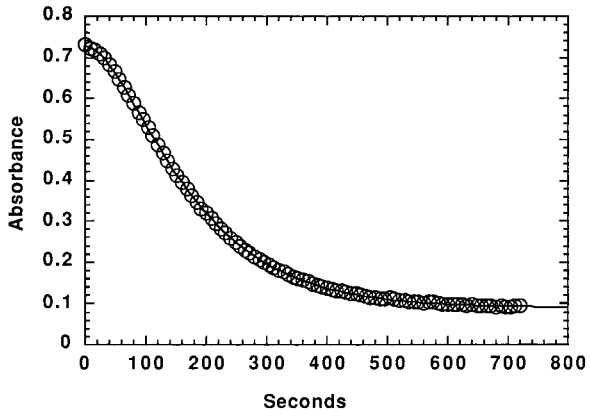

Figure 2. Plot of absorbance at 230 nm versus time for the decay of **7** at pH 5.2, 0.02 M acetate buffer 25 °C, ionic strength 1 M ($NaClO_4$). Solid line is a best fit to eq 14.

In the case of α-acetoxynitrosopiperidine, below pH 6, the kinetics of decay were not first order, as typified by the absorbance versus time plot in Figure 2. Such behavior was reminiscent of our earlier observation that some acyloxydialkylnitrosamines have a reactivity comparable with the product α-hydroxydialkylnitrosamine so that the latter is formed as a non-steady-state intermediate.[6b] Treatment of these data is explained in the Discussion section.

Activation parameters for the decay of the cyclic α-acetoxynitrosamines **6** and **7** were obtained from measurements of k_{obsd} in 0.05 M cacodylic acid buffers containing 50% buffer base form. The decay curves in all cases exhibited clean first-order behavior over 5 half-times of reaction. At 25 °C, plots of k_{obsd} against buffer concentration showed increases in k_{obsd} of less than 3% at 0.2 M buffer above the value of k_o. Eyring plots of $\ln(h(k_{obsd}/k_BT))$ against $1/T$ are linear for **6** and **7** (six data points each, $r^2 = 0.999$ and 0.998, respectively). The values of the slopes permit calculation of $\Delta H^\dagger = 21.8 \pm 3.4$ and 17.8 ± 0.8 kcal/mol and the values of the intercepts permit calculation of $\Delta S^\dagger = -4.8 \pm 1.1$ and -8.2 ± 1.2 cal/deg mol.

The effect of acetate concentration on the values of k_{obsd}/k_o is indicated in Figure 3. The experiments were carried out in 0.05 M cacodylic acid buffers containing 50% buffer base form.

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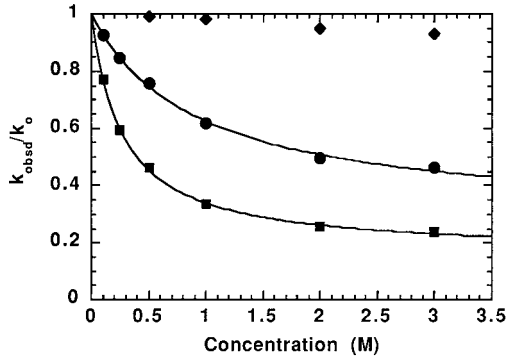


Figure 3. Plot of k_{obsd}/k_o against acetate ion (squares and circles) or trifluroacetate ion (diamonds) concentration in 0.05 M cacodylic acid buffer pH 6.53, 4M ionic strength ($NaClO_4$) for the decay of **6** (circles) and **7** (squares and diamonds).

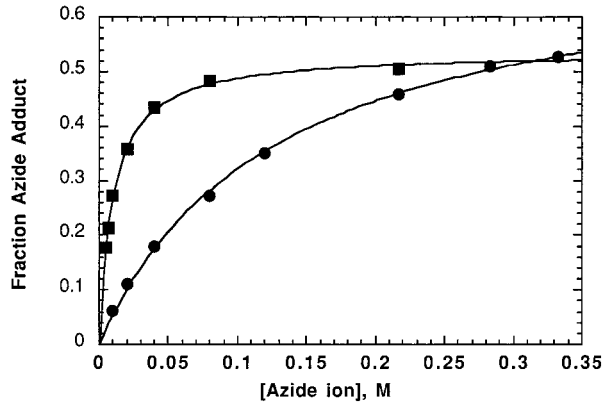
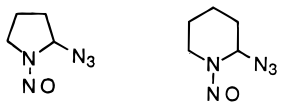


Figure 4. Fractional yield of azide ion adducts **18** (circles) and **19** (squares) from **6** and **7**, respectively at 25 °C, ionic strength 1 M ($NaClO_4$).

The effect, "common ion inhibition", was relatively weak so that high concentrations of sodium acetate were used to demonstrate this effect. Ionic strength was maintained at 4 M with $NaClO_4$ in these experiments. The effect of exchange of salt-type on k_{obsd} was investigated using sodium trifluoroacetate and α-acetoxynitrosopiperidine. The results of this experiment are also included in Figure 3.

The formation of α-azido adducts, **18** and **19**, of the two cyclic nitrosamines during the decomposition of the parent α-acetoxynitrosamines was quantitated as a function of azide ion concentration. The data are summarized in Figure 4. In the case of the pyrrolidine derivative the azide product, **18**, was

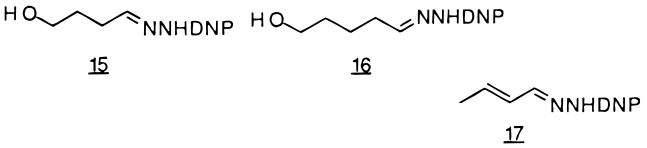


18 19

quantified after one half-time of reaction. The amount of unreacted α-acetoxy-*N*-nitrosopyrrolidine was also determined and varied in a given run from the mean value for all runs by less than ±2%. Direct measurements of k_{obsd} for the decay of the α-acetoxy-*N*-nitrosopiperidine indicated an increase in k_{obsd} of less than 5% over the entire range of azide ion concentration. Determination of the yield of α-azido-*N*-nitrosopiperidine, **19**, after 10 and 20 half-times gave yields that were the same within ±2% in two cases in which it was checked. In the case of the α-azido-*N*-nitrosopyrrolidine, **18**, the stability of the product was indicated by the following control. A solution of α-azido-*N*-nitrosopyrrolidine standard, which was used to generate standard curves, was incubated under identical reaction conditions for the decay of the α-acetoxy-*N*-nitrosopyrrolidine. The amount of α-azido-nitrosopyrrolidine determined after one and two half-lives of decay of the corresponding α-acetoxy compound was the same within 3%.

α-Hydroxydialkylnitrosamines. These compounds were generated following the method first employed by Okada and co-workers[11,12] involving triphenylphosphine reduction of the hydroperoxy compounds. The compounds studied here are considerably less stable but NMR characterization was obtainable. However, even in deuterated acetonitrile solutions dried by distillation from CaH_2, the α-hydroxycompounds slowly decompose. Certain distinguishing spectroscopic changes indicate the formation of the α-hydroxydialkylnitrosamines. In the ^1H NMR spectrum, the hydroxyl protons of the hydroperoxides are sharp singlets in the region of 8−10 ppm, whereas those of the α-hydroxy compounds are shifted upfield considerably, at 4−5 ppm and are broadened or clearly coupled in the case of the pyrrolidine derivative. The C2 atom experiences the expected upfield shift, ∼11 ppm, in the ^{13}C NMR spectrum upon reduction of the peroxide in the case of both the pyrrolidine (**8**) and piperidine (**9**) derivatives. The α-methyl-α-hydroxynitrosopyrrolidine (**10**) was too unstable to obtain a ^{13}C NMR spectrum.

The products of decay of **8** and **9** were partially characterized. In the case of **8**, 25 volumes of 0.04 M $HClO_4$ were mixed with one volume of an acetonitrile solution containing the hydroperoxy precursor (**12**) that had been treated with 1 equiv of tributylphosphine. Subsequent derivatization with dinitrophenylhydrazine gave 47%/35% ratio of yields of the hydrazones **15**/**17**. A similar analysis for the piperidine derivative gave a 45% yield of the hydrazone **16**. The hydrazones were stable to the reaction and analysis conditions as indicated by the fact that doubling the reaction time changed the yield by less than 1%. Other products were evident in the chromatograms in both analyses, as might be expected from the published work on the decay of the solvolyses of the corresponding α-acetoxy compounds,[8,9] but a complete analysis was not attempted.



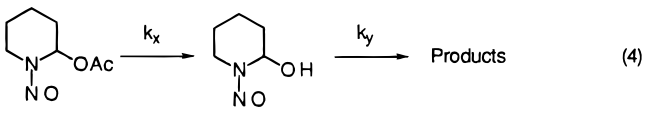
15 16 17

The kinetics of decay of the α-hydroxynitrosamines were monitored at 230 nm using stopped-flow methods by mixing one volume of an acetonitrile solution containing the hydroperoxy compound, which had been treated with a 20% excess of tributylphosphine, with 25 volumes of aqueous solution. The absorbance decay exhibited good first-order behavior over five half-lives of reaction in all cases. In buffer dilution experiments analogous to those described above for the α-acetoxy compounds, variable extents of increases in k_{obsd} with increasing buffer concentration were observed. Plots of k_{obsd} against buffer concentration, typically containing three points were linear and extrapolation to zero buffer concentration yielded the buffer-independent rate constant k_o. The largest increase in k_{obsd} occurred with phosphate buffers (160% at 0.3 M 80% dianion with **8**), all other buffers gave appreciably smaller effects. Values of k_o are plotted as a function of pH in Figure 1 for the three cyclic α-hydroxynitrosamines studied.

Discussion

α-Acetoxynitrosamines. In the case of α-acetoxynitrosopyrrolidine (**6**), at all values of pH studied, and in the case of α-acetoxynitrosopiperidine (**7**), above pH 5.8, the kinetics of the decay of absorbance at 230 nm exhibited good first-order behavior. The values of the buffer-independent rate constant, k_o, were accurately obtained from buffer dilution plots which showed only small changes in k_{obsd} over the range of buffer examined. The values of log k_o are plotted against pH in Figure 1.

In the case of α-acetoxynitrosopiperidine (**7**), below pH 6, the decay of absorbance was distinctly non-first order (Figure 2), consistent with the formation of a non-steady-state intermediate. We have encountered examples of this behavior previously and assumed that the non-steady-state intermediate might be the α-hydroxynitrosamine.[6b] Indeed the data give a good fit (solid line, Figure 2) to the model in eq 4 using the appropriate eq 5 with the assumption that the extinction coefficient of the ester and the α-hydroxy compound are



(4)

$$\mathrm{Abs} = A_o e^{-k_x t} + A_o[k_x/(k_y - k_x)][e^{-k_x t} - e^{-k_y t}] + C_\infty \quad (5)$$

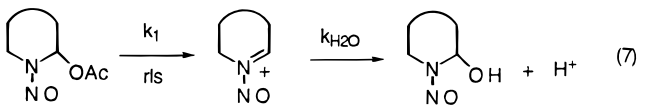
identical. The smaller rate constant was taken as that for the decay of α-acetoxypiperidine (k_x in eqs 4 and 5). The larger rate constant was taken as that for the α-hydroxypiperidine (k_y in eqs 4 and 5). Values for the buffer independent constants, k_o, for these reactions were obtained from buffer dilution plots, as described earlier (Results). These values are plotted in Figure 1 as solid squares below pH 6 for the ester (**7**) and as open circles for the α-hydroxy compound (**9**). The α-hydroxy compounds are discussed in a later section, but it suffices to note here that the latter values give reasonable agreement with those measured directly for the α-hydroxynitrosopiperidine generated by reduction of the hydroperoxide.

The pH rate profiles for decay of the α-acetoxynitrosamines in Figure 1 are consistent with a three-term rate law for the buffer-independent decomposition reaction, as in eq 6, including hydrogen ion dependent (k_{H^+}), hydroxide ion dependent (k_{OH^-}), and pH independent (k_1) terms. Values for the rate constants in eq 6 were determined by fitting to the data in Figure 1. The

$$k_{obsd} = k_1 + k_{H^+}[H^+] + k_{OH^-}[OH^-] \quad (6)$$

values are summarized in Table 1 and the fits are represented by the solid lines in Figure 1. As with a number of acyclic α-acetoxynitrosamines,[6] the dominant mechanism at physiological pH is that of the pH-independent term, k_1.

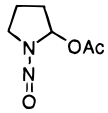
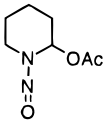
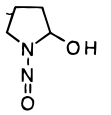
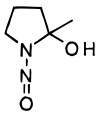
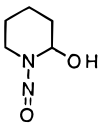
Three pieces of evidence are consistent with the mechanism of the pH-independent reaction involving the rate-limiting formation of nitrosiminium ions, as in eq 7.



(7)

(1) Entropy of Activation. Values of $\Delta S^\dagger = -8.2$ (±1.5) and −4.8 (±1.1) cal deg^{-1} mol^{-1} were determined at pH = 6.30 for the α-acetoxypiperidine and α-acetoxypyrrolidine, respectively from Eyring plots (Results). Such small negative values are not consistent with the values ranging from −30 to −50 cal deg^{-1} mol^{-1} that typify reactions involving hydrolysis by carbonyl attack of esters and related compounds.[19] Similarly small negative and positive values of $\Delta S^\dagger$ have been reported for classical S_N1 solvolysis of halides and sulfonates[20] and for the reactions of acyclic α-acyloxynitrosamines that decompose by formation of nitrosiminium ions.[6a]

Table 1. Rate Constants for the Decay of Cyclic α-Acetoxynitrosamines and α-Hydroxynitrosamines, 25 °C, Ionic Strength 1 M ($NaClO_4$)[a]

	k_{H+} M^{-1} s^{-1} (SE)[b]	$10^2 \times k_1$ s^{-1} (SE)[b]	k_{OH-} M^{-1} s^{-1} (SE)[b]
	0.0153 (0.0007)	0.0064 (0.0002)	0.74 (0.05)
	0.290 (0.029)	0.78 (0.03)	0.87 (0.11)
	3.7 (0.5)	6.4 (0.5)	1.08×10^8 (0.08×10^8)
	220 (12)	16 (2)	2.2×10^9 (0.2×10^9)
	0.59 (0.05)	1.35 (0.08)	7.3×10^6 (0.5×10^5)

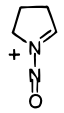
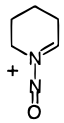
[a] Reactions with a-hydroxy compounds contained 4% acetonitrile by volume, parameters obtained by best fits of the data in Figure 1 to eq 6. [b] Standard error in parentheses.

(2) Common Ion Inhibition. The cyclic α-acetoxynitrosamines decompose in a manner that can be inhibited by added acetate ion, presumably through capture of a free nitrosiminium ion intermediate. Figure 3 shows that the values of k_{obsd} divided by the acetate independent rate constant k_o decrease with increasing acetate concentration at constant ionic strength for both **6** and **7**, circles and squares, respectively. High concentrations of acetate ion are required for substantial inhibition so the experiments in Figure 3 were carried out at 4 M constant ionic strength, maintained with $NaClO_4$. Control experiments for the medium effect of exchanging a high concentration of $NaClO_4$ for acetate salt were carried out by measuring the effect of increasing concentrations of sodium trifluoroacetate at constant ionic strength (Figure 3, diamonds). The data for the reaction of the piperidine derivative are shown. This indicates that the medium effect is negligible compared to the effect exerted by the common ion acetate in the other two experiments.

(19) Jencks, W. P.; Carrioulo, J. *J. Am. Chem. Soc.* **1961**, *83*, 1743. Johnson, S. L. *Adv. Phys. Org. Chem.* Gold, V., Ed. **1967**, 236.

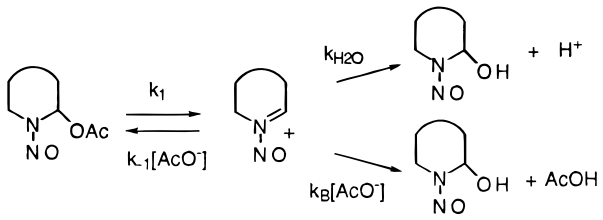
(20) Maskill, H. *The Physical Basis of Organic Chemistry*; Oxford University Press: New York, NY; 1985, Chapter 6.

Table 2. Experimentally Determined Rate Constant Ratios for Reaction of Cyclic *N*-Nitrosiminium Ions With Azide and Acetate Ions and Water

Cation	Azide Ion Reactions [a]		Acetate Ion Reactions [b]	
	k_{az}/k_{H2O} (M^{-1})	k_N/k_C	k_{-1}/k_{H2O} (M^{-1})	k_b/k_{H2O} (M^{-1})
	7.9 ±0.4	0.37 ± 0.03	0.79 ± 0.05	0.31 ± 0.05
	101 ± 3	0.87 ± 0.02	3.12 ± 0.06	0.61 ±0.03

[a] Rate constant for processes in eq 10. Ionic strength 1 M with $NaClO_4$. [b] Rate constants for processes in eq 8. Ionic strength 4 M with $NaClO_4$.

Inspection of Figure 3 indicates that the effect of increasing acetate ion concentration appears to reach a limit beyond which the rate constant ratio shows no further decrease; and, this is interpreted as being due to a competitive reaction of the nitrosiminium ion with acetate ion to give products. A kinetic scheme for such a mechanism is described in eq 8. The k_B step that is competitive with return to starting materials, k_{-1}, could represent a general base-catalyzed addition of water to give the



(8)

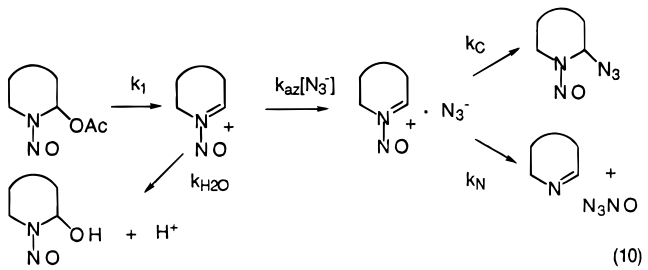
α-hydroxy product or an acetate-stimulated denitrosation of the cation. We have previously favored the latter.[21] The appropriate expression for k_{obsd}/k_o as a function of acetate anion concentration is given in eq 9.

$$k_{obsd}/k_o = \frac{\left[\dfrac{k_b[AcO^-]}{k_{H_2O}} + 1\right]}{\dfrac{(k_{-1} + k_b)[AcO^-]}{k_{H_2O}} + 1} \qquad (9)$$

The best fit of the data to this expression is indicated by the solid lines in Figure 3 and the values of parameters that characterize the fits are given in Table 2. At infinite acetate ion concentration, the limiting relative rate constant, k_{obsd}/k_o, is calculated to be 25% and 16% of the acetate ion independent ratio for **6** and **7**, respectively. Thus, ~80% of the cation partitions back to starting material while ~20% undergoes acetate-assisted product formation.

There are alternative interpretations for the limited ability of acetate to inhibit the reactions but there is experimental evidence that militates against these alternatives. The possibility has been suggested[4] that there is a competitive carbonyl attack mechanism of decay. However, even the smaller of the limiting rate constants required by such an explanation, using the above 20% of the observed pH independent rate constant for α-acetoxypyrrolidine, is more than 2 orders of magnitude larger than that observed for other acetate esters with alkyl alcohol leaving groups.[19] In addition, the carbonyl attack mechanism for such compounds is dominated by a hydroxide ion term above pH = 6 and this again is inconsistent with the pH rate profiles in Figure 1. Finally, the linear Eyring plots give no evidence of curvature that would signify the existence of more than one mechanism with a single rate-controlling step. We cannot rule out that acetate reacts at the nitroso nitrogen, but there is evidence against this in related systems.[22]

(3) Azide Ion Trapping. The conversion of appreciable fractions of the α-acetoxy compounds **6** and **7** to α-azido adducts **18** and **19**, respectively, by azide ion—in the absence of a measurable acceleration of the decay of the α-acetoxy compounds—is consistent with trapping of the nitrosiminium ion intermediate after the rate-limiting step of the reaction. Figure 4 indicates the fractional yields of the azide adducts as a function of azide ion concentration. This range of concentrations of sodium azide increases the value of k_{obsd} by less than 5% (Results). Thus the azide adducts must be formed by a reaction after the rate-limiting step. The limiting yield of azide adduct with increasing azide ion concentration is likely due to a denitrosation reaction of azide at the nitrosiminium nitrogen that is competitive with reaction at carbon that yields the azide adduct, as in eq 10. Azide ion stimulated denitrosation of acyclic



(10)

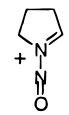
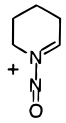
nitrosiminium ion has recently been reported.[23] The limiting yield of azide adduct is not due to general base-catalyzed hydration. If such a reaction occurred, acetate ion should be a more effective catalyst due to its greater basicity and should

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

(22) Chahoua, L.; Fishbein, J. C. *Can. J. Chem.* **1999**, in press.

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

Table 3. Estimated Rate and Equilibrium Constants for Reactions Involving Cyclic *N*-Nitrosiminium Ions Based on the Azide Ion "Clock"[a]

Cation	$10^{-8}k_{H2O}$ [b] (s^{-1})	$10^{-8}k_{-1}$ [c] ($M^{-1}\,s^{-1}$)	$10^{-8}k_b$ [c] ($M^{-1}\,s^{-1}$)	$10^{13}K_1$ [d] (M)
	6.3	5.0	1.95	1.28
	0.49	1.5	0.30	520

[a] Value for k_{az} (eq 10) taken as 5×10^9 M^{-1} s^{-1}. [b] Calculated from the ratio in Table 2. [c] Rate constants as in eq 8, calculated from the ratios in Table 2 and the value of k_{H_2O} in this Table. [d] Refers to the equilibrium in eq 8. Calculated from the value of $10^{-8}k_{-1}$ in this Table and the value of k_1 in Table 1.

limit the common ion inhibition at much lower concentrations than observed in Figure 3.

The data in Figure 4 can be fit to the appropriate expression, eq 11, which is derived for the mechanism of eqn 10:

$$F_{az} = \frac{\left[\frac{1}{1 + k_N/k_C}\right]}{1 + \frac{k_{H_2O}}{k_{az}[N_3^-]}} \qquad (11)$$

Values for the parameters that gave the best fits are collected in Table 2, and the fits are represented by the solid lines in Figure 4. The values of these parameters are discussed in more detail later.

In summary, both of the cyclic α-acetoxynitrosamines appear, as in other cases,[6,22] to decompose predominantly, if not exclusively, through the formation of *N*-nitrosiminium ion intermediates at physiological pH. They do so with markedly different rate constants, the piperidine derivative being greater than 100 times more reactive than the pyrrolidine derivative, as might have been deduced.[24,25] The reason for the differences in propensity of the two compounds to undergo solvolysis is addressed below.

***N*-Nitrosiminium Ions.** Rate constants for the various reactions of the nitrosiminium cations can be obtained from the "azide clock" method of Jencks[26] and the ratios in Table 2 and are summarized in Table 3. Azide ion reacts with highly unstable carbocations with rate constants that are diffusion limited. McClelland and Steenken[27] have confirmed by direct measurements that the value of the diffusion-limited reaction of azide varies over a fairly narrow range of $(5 \pm 2) \times 10^9$ M^{-1} s^{-1} with a fairly large range of cation bulkiness. The reaction of azide ion appears to be diffusion-limited when the k_{az}/k_{H_2O} ratio reaches a value of 10^3 M^{-1} or less. It has been shown recently by direct measurement that azide can react with a nitrosiminium ion with a nearly diffusion-limited rate constant of 2×10^9 M^{-1} s^{-1} for which the value of $k_{az}/k_{H_2O} \sim 1000$.[21] Inspection of the relevant values of k_{az}/k_{H_2O} in Table 2 indicates that the ratio is indeed small by comparison and it is concluded that the reaction of azide ion is diffusion limited. For the cyclic nitrosiminium ions in the present study the value of 5×10^9 M^{-1} s^{-1} is taken as a reasonable value for k_{az}. From this, and the k_{az}/k_{H_2O} values in Table 2, the values of k_{H_2O}, for the reaction of the cations with water, can be closely estimated. These values are summarized in Table 3. Cyclic nitrosiminium cations react with solvent water with half-lives of nanoseconds, the pyrrolidine derivative being more reactive toward water by a factor of ~12.

Rate constants for the reactions of the cations with acetate (Table 3) can be estimated from k_{H_2O} and the ratios summarized in Table 2. There is some uncertainty in these absolute values due to the fact that the rate constant for reaction with water was obtained from experiments at 1 M ionic strength while the rate constant ratios relevant to the acetate ion reactions were obtained at 4 M ionic strength. The calculation assumes that the rate constant for capture of the cation by water is similar in magnitude at the two different ionic strength conditions. This is likely a reasonable assumption since the water reaction involves formation of a cation (protonated alcohol) from the cationic iminium ion and effects upon activity of the cations due to changes in ionic strength is likely similar.[21]

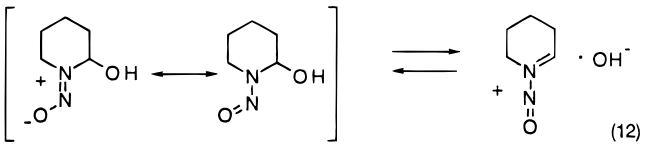
The values of the rate constants for the acetate reactions in Table 3 indicate that like the reactions with water, the cation of the pyrrolidine derivative is the more reactive. The differences are smaller but within a kilocalorie of the differences observed for the water reaction. The smaller differences are consistent with a Hammond effect due to the greater nucleophilicity of acetate ion compared to water in the k_{-1} (eq 8) reaction and the larger driving force due to the abstraction of the proton from the attacking water molecule by acetate in the reaction proposed for k_b (eq 8) compared to the unassisted or water assisted hydration reaction. The smaller difference in the k_{-1} reaction (eq 8) could also be due to the approach, in the case of the cation from the pyrrolidine reaction, of an upper limit value for capture by acetate that is due to the requirement for desolvation of the acetate ion in the solvent separated acetate anion−cation pair.[28]

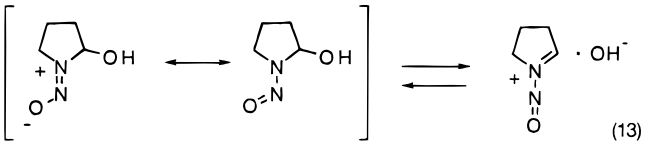
Values for the rate constants for capture of the cations by acetate ion, k_{-1} (eq 8), permit calculation of the equilibrium constant for *N*-nitrosiminium ion formation from the starting acetoxy compounds, K_1, and these are included in Table 3. The rate constants k_1, also required for the calculations, are taken as the values for the pH independent solvolysis constants, k_1 in Table 1. The values of K_1 in Table 3 indicate that formation of the *N*-nitrosiminum ion is 3.6 kcal/mol more favorable in the case of the six-member ring system than in the five-member ring system. Most of this difference is due to the ~100-fold difference in rate constants for formation of the cation. The differences in equilibrium and rate constants are inconsistent with a claim made previously that the five-member ring cation is more stable than the six-member ring cation.[16]

High level ab initio calculations on a very similar system, detailed in the accompanying paper,[29] are qualitatively consistent with the experimental observations made here. In the reactions below, calculations at the MP4(SDTQ)/6-31G*//RHF/6-31G* level indicate that the equilibrium for the six-membered ring system in eq 12 is favored over the five-membered ring system

(24) Hecht, S. S.; Chen, C. B.; Hoffmann, D. *Cancer Res.* **1978**, *38*, 215.
(25) Reference 9, Hecht et al.
(26) Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1982**, *104*, 4689.
(27) McClelland, R. A.; Kanagasabapathy, V. M.; Banait, N. S.; Steenken, S. *J. Am. Chem. Soc.* **1991**, *113*, 1009.
(28) Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 1373.
(29) Glaser, R. *J. Am. Chem. Soc.* **1999**, *121*, 5170−5175 (accompanying manuscript).

in eq 13 by 7.96 kcal/mol. This difference appears to be largely


(12)


(13)

accounted for by a weaker N−N double-bond character in the α-hydroxynitrosopiperidine (eq 12) compared to the α-hydroxynitrosopyrrolidine (eq 13), presumably due to the greater instability of the exocyclic double bonding in the former.

In essence, the theoretical calculations indicate that the larger equilibrium constant for formation of the *N*-nitrosiminium ion in the case of the piperidinyl system is due to greater electron density on the amino nitrogen in the neutral species. It is reasonable that the same is true in the case of the α-acetoxynitrosamines. Thus, this increased electron density on the amino nitrogen in the case of α-acetoxy-*N*-nitrosopiperidine engenders a greater driving force for expulsion of the acetate ion leaving group (k_1, eq 8) and, in the reverse reaction, a greater stability of the iminium ion from the piperidine derivative because the product of the k_{-1} step (eq 8) is not so strongly stabilized by electron delocalization into the nitroso group.[29]

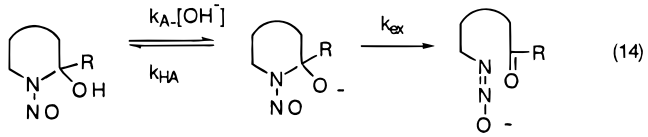
α-Hydroxynitrosamines. As before,[13] these compounds were generated by reduction using tributylphosphine in acetonitrile and were subsequently subjected to kinetic study by mixing in a stopped flow spectrophotometer with a 25-fold excess of aqueous solution.

There are a number of observations consistent with the formation of the cyclic α-hydroxynitrosamines by this method. These include (a) ^{1}H and ^{13}C NMR spectra (Results); (b) the concordance of rate constants observed for the decay of the product of reduction of the α-hydroperoxide of *N*-nitrosopiperidine with those of the non-steady-state intermediate in the decay of α-acetoxy-*N*-nitrosopiperidine below pH 6; and (c) formation in reasonable yields (45−80%) of the dinitrophenylhydrazones of some aldehyde products expected for the decay of the α-hydroxynitrosamines.

Rate constants for the decay of the α-hydroxynitrosamines are plotted as a function of pH in Figure 1. The shapes of the profiles are qualitatively similar to those previously published for acyclic methylene α-hydroxynitrosamines[13] and are consistent with the three-term rate law in eq 6. Values for each of the rate constants in eq 6 were obtained from a best fit of the data to eq 6 and are summarized in Table 1. The fits using the parameters in Table 1 are indicated by the solid lines in Figure 1. The pH-independent reaction, k_1, in the case of the α-hydroxy-α-methylnitrosopyrrolidine contributes to the overall rate constant for decay, k_{obsd}, over a very narrow range of pH but is not negligible; a fit of the data to a two-term rate law containing only k_{H^+} and k_{OH^-} underestimates the value of k_{obsd} for the smallest measured values of k_{obsd} by 61%.

Most significantly, the reactivity of these cyclic compounds is quite similar to that reported for the acyclic methylene α-hydroxynitrosamines.[13] The half-lives at physiological pH = 7.4 are between 1 and 400 ms. This is in sharp contrast to the report[15] that α-hydroxynitrosomorpholine, **5**, could be isolated from aqueous acid after 21 h at 5 °C and kept as a stable solid. Under conditions of maximum stability, pH ∼3, the most stable compound studied here, **9**, has a half-life of ∼50 s at 25 °C.

The hydroxide ion catalyzed reaction is the dominant pathway for decay of the cyclic α-hydroxynitrosamines at physiological pH of ∼7.4; and, for α-hydroxynitrosopyrrolidine and α-hydroxynitrosopiperidine, the mechanism of this reaction likely involves the rate-limiting expulsion of the diazoate leaving group from the conjugate base of the α-hydroxycompound, as in eq 14 (R = H). The undetectable-to-weak catalysis by most buffers


(14)

(Results) is consistent with this stepwise mechanism, and militates against a concerted proton abstraction and expulsion of the leaving group. A similar conclusion was reached previously in the case of acyclic dialkylnitrosamines.[13] Notably, though, phosphate buffers do appear to substantially stimulate decomposition of both α-hydroxynitrosopyrrolidine and α-hydroxynitrosopiperidine, and the reason for this, and possible mechanism, are the subject of current investigations.

A second step, k_{A^-} (eq 14), could be partly rate limiting in the case of α-hydroxy-α-methylnitrosopyrrolidine. The second-order rate constant for the hydroxide ion reaction in this case ($k = 2.2 \times 10^9$ M^{-1} s^{-1}) approaches the value expected for a thermodynamically favorable diffusion-limited proton transfer ($k \approx 10^{10}$ M^{-1} s^{-1}).[31] The proton transfer between hydroxide ion and the α-hydroxynitrosamine is expected to be diffusion limited because the pK_a of the latter is lower than that of water, and is likely similar to that for a hemiacetal, p$K_a \approx 13$.[31]

The α-hydroxy-α-methylnitrosopyrrolidine is the first tertiary α-hydroxynitrosamine studied and is the most reactive of the cyclic compounds. The greater reactivity toward hydroxide ion, by a factor of 20, of the α-hydroxy-α-methylnitrosopyrrolidine compared to α-hydroxy-*N*-nitrosopyrrolidine is likely specifically due to an accelaration of the k_{ex} step (eq 14) due to the additional methyl group in the former compound. This acceleration is expected because of the strong stabilization, relative to its tetrahedral precursor, of the product carbonyl group by the methyl group compared to hydrogen.[34,35] To the extent that there is carbonyl group character in the transition state, this stabilizing interaction of the methyl group will lower the energy of the transition state, thus increasing the rate constant for leaving group expulsion. Stabilization of the product carbonyl group by substitution of hydrogen by a methyl or a phenyl group has been noted previously as an important factor affecting the stability of acyclic α-hydroxynitrosamines.[13] For the α-hydroxynitrosamines, the greater reactivity of the pyrrolidine compared to the piperidine can be speculated upon in light of the ab initio computations presented in the accompanying paper.[29] As described earlier, these calculations indicate a larger interaction between the amino nitrogen and the nitroso group in the case

(30) Eigen, M. *Angew. Chem., Int. Ed.* **1964**, *3*, 1.
(31) Funderburk, L. H.; Aldwin, L.; Jencks, W. P. *J. Am. Chem. Soc.* **1978**, *100*, 5444. The pK_a is likely this low due to the similarity of the electron-withdrawing power of the nitrosamino group compared to an alkoxy moiety in a hemiacetal: $\sigma_I = 0.25$ for RO− and $\sigma_I > 0.28$ has been estimated for the nitrosamino group.[33,34]
(32) Exner, O. In *Correlation Analysis in Chemistry, Recent Advances*; Chapman, N. B., Shorter, J. Eds., Plenum: New York, 1978.
(33) Wichems, D. N.; Nag, S.; Mills, J.; Fishbein, J. C. *J. Am. Chem. Soc.* **1992**, *114*, 8846.
(34) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, Harper & Row: New York, 1987; Chapter 8.

of the pyrrolidine compared to the piperidine compound. It is ultimately the ability of the nitroso group to accept electron density that makes the nitrosamino group a relatively good nucleofuge compared to the amine anion. To the extent that delocalization of electron density is more advanced in the ground state, or easier to achieve in reaching the transition state, this will enhance reactivity. Thus, the more delocalized pyrrolidine might be expected to be more reactive, consistent with experimental observation. This expectation assumes there is bond cleavage with the leaving group in the rate-limiting step of each particular reaction (k_1, k_{H^+}, and k_{OH^-} in eq 6). This is clearly the case in the hydroxide ion catalyzed reaction (k_{OH^-}), as discussed above. It is also very likely in the pH independent (k_1) and hydrogen ion dependent (k_{H^+}) reactions, both of which exhibit the aforementioned acceleration with methyl- or phenyl-for-hydrogen substitution that is due to stabilization of the penultimate C═O group in the product.

Acknowledgment. This work was supported by grants RO1 CA52881 and KO4 CA62124 from the National Cancer Institutes. The authors wish to thank Professor Rainer Glaser for illuminating discussions.

JA9902975