Copper-Catalyzed Redox Deacylation of Isomeric *N*- and *O*-Benzoylhydroxylamines

Steven P. Wathen and Anthony W. Czarnik*

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

Received May 21, 1992 (Revised Manuscript Received August 3, 1992)

N- and *O*-benzoylhydroxylamines are, in principle, capable of forming five-membered metal chelates structurally similar to those of α-amino esters. Because α-amino esters are hydrolyzed by many metal ions, the metal-promoted or -catalyzed reactions of *N*- and *O*-benzoylhydroxylamines were examined. Two metals that are active with α-amino esters—Ni(II) and Zn(II)—show no reaction with either benzohydroxamic acid or *O*-benzoylhydroxylamine in aqueous solution. Cu(II) accelerates the rate of benzoic acid formation with both compounds by factors of up to 60- and 40-fold, respectively, under the conditions evaluated. Both reactions give benzoic acid as product, but hydroxylamine is not a product in either reaction. We observe that *O*-benzoylhydroxylamine reacts with Cu(II) with net oxidation and with Cu(I) with net reduction of the nitrogenous product. When a substoichiometric amount of Cu(II) is used, copper serves as a catalyst to effect deacylation with redox disproportionation of the starting material.

Introduction

Metal-catalyzed ester and amide hydrolyses continue to be of interest due to the desirability of making abiotic esterases and peptidases, and mechanistically distinct demonstrations of metal ion promotion and catalysis of hydrolysis have been described.[1] We have been involved in the design of monomeric systems demonstrating M^{2+} catalysis in the hydrolyses of unactivated esters[2a] and amides,[2b] specifically those in which the product does not coordinate so tightly to the metal that severe product inhibition results. The earliest report of metal-promoted ester hydrolysis is that of Kroll, who observed acceleration by Cu(II) in the hydrolysis of α-amino esters such as methyl glycinate (**1**).[3] This reaction, however, shows virtually complete inhibition by the product α-amino acid. It appeared to us that the same type of metal-accelerated hydrolysis might be observed using isomeric *N*- and *O*-acylhydroxylamines (e.g., **2** and **3**), which have the potential of forming isostructural metal chelates (Figure 1). Furthermore, because the products of their hydrolyses are expected to bind metals less tightly than the starting materials, turnover behavior is expected in the reactions of **2** and **3** while not in that of **1**.

The chelating propensity of hydroxamic acids is well documented, and the characteristic color of the ferric ion complex is used as a colorimetric assay.[4] Hydroxamic acids (e.g., **2**) form 5-membered chelates;[5] by analogy, *O*-acylhydroxylamines (e.g., **3**) might also complex metals, albeit less strongly. Jencks[6] noted that the decomposition of *O*-acylhydroxylamines in aqueous solution slowed when metal chelating agents such as EDTA were added. Novak[7] has studied the reductive cleavage of *N*-aryl-*O*-pivaloylhydroxylamines with reducing metals (Fe^{2+} and Cu^{+}). In this paper, we report the copper-catalyzed deacylation of *N*- and *O*-benzoylhydroxylamines, which we observe to occur via a coupled pair of redox reactions.

(1) Hay, R. W. *Reactions Coord. Ligands* **1989**, 223 and references therein.
(2) (a) Duerr, B. F.; Czarnik, A. W. *Tetrahedron Lett.* **1989**, *30*, 6051. (b) Duerr, B. F.; Czarnik, A. W. *J. Chem. Soc., Chem. Commun.* **1990**, 1707.
(3) Kroll, H. *J. Am. Chem. Soc.* **1952**, *74*, 2036.
(4) Mizukami, S.; Nagata, K. *Coord. Chem. Rev.* **1968**, *3*, 267.
(5) For an example of a benzohydroxamic acid–metal crystal structure, see: Harrison, P. G.; King, T. J. *J. Chem. Soc., Dalton Trans.* **1974**, 2298.
(6) Jencks, W. P. *J. Am. Chem. Soc.* **1958**, *80*, 4581.
(7) (a) Novak, M.; Lagerman, R. K. *J. Org. Chem.* **1988**, *53*, 4762. (b) Novak, M.; Lagerman, R. K. *Tetrahedron Lett.* **1989**, *30*, 1923.

Table I. Effect of Buffer Concentration on the Rate of Benzoic Acid Formation from Benzohydroxamic Acid in the Presence of 1 Equiv of Cu(II) at pH 7.0 and 50 °C

buffer concntrtn (M)	k_{app} (min^{-1})
0.05	8.9×10^{-5}
0.1	1.6×10^{-4}
0.2	2.2×10^{-4}

Results

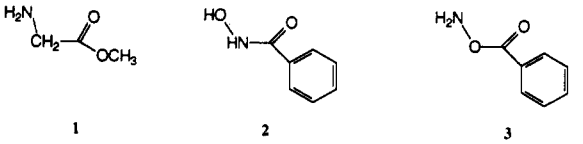
Reactions of Benzohydroxamic Acid. The hydrolysis of benzohydroxamic acid (**2**) was monitored using HPLC from pH 6.5 to 8.0 with three metal ions, Cu(II), Ni(II), and Zn(II), present in stoichiometric amount. The reactions were carried out in buffered solution at 50 °C and the formation of benzoic acid was followed using reversed-phase HPLC. To determine the rate of reaction in the absence of metal ions, control experiments were run in which 10 mol % of EDTA was added to complex trace metals.

The effects of pH and of metal ion on the rates of benzoic acid formation are summarized in Figure 2. Rate constants were determined by following the appearance of benzoic acid. Because of the very slow time frame of all but the Cu(II) reactions, rate constants are estimated on the basis of the time at which one-half of the expected benzoic acid has formed. Over the range studied, pH has only a slight influence in the absence of metal ions, with the reaction rate increasing at higher pH. Of the metal ions studied, only Cu(II) affords a significant increase in the rate of reaction. Given the slowness of the reaction and the accompanying uncertainty in accurately determining the rate of reaction, Zn(II) and Ni(II) give no measurable acceleration over the control reaction. In contrast, equimolar Cu(II) increases the rate from 17-fold at pH 6.5 to about 60-fold at pH 8.0.

To determine whether a stoichiometric or a catalytic amount of Cu(II) is necessary for complete conversion, the reaction was run using several ratios of Cu(II) to benzohydroxamic acid. All reactions were run at pH 7.0 as described previously and in all cases went to greater than 90% completion with apparent first-order behavior. As shown in Figure 3, Cu(II) is active as a catalyst in amounts as low as 0.1 equiv. The reaction was also carried out at three buffer concentrations using 1 equiv of Cu(II) at pH 7.0. As shown in Table I some catalysis by buffer is observed.

Reactions of *O*-Benzoylhydroxylamine. The hydrolysis of *O*-benzoylhydroxylamine (**3**) was studied from

0022-3263/92/1957-6129$03.00/0



1 2 3

Figure 1. Ligand similarity apparent in the structures of methyl glycinate (1), benzohydroxamic acid (2), and *O*-benzoylhydroxylamine (3).

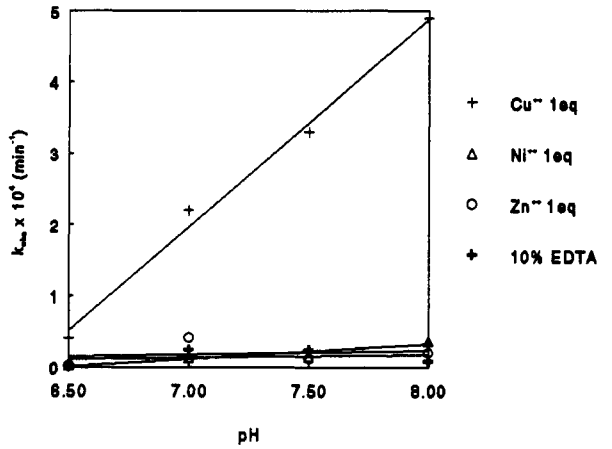


Figure 2. Effect of metal ions and pH on the rate constants for benzoic acid formation from benzohydroxamic acid (0.1 mM) at 50 °C. Reactions were carried out in 0.2 M MES buffer at pH 6.5 and 0.2 M HEPES buffer at pH 7.0, 7.5, and 8.0.

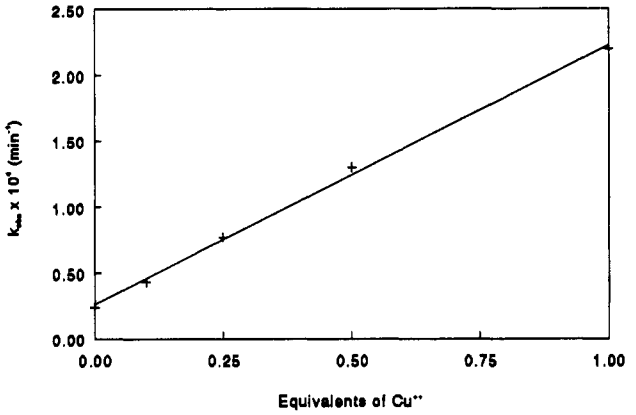


Figure 3. Effect of Cu(II) concentration (0–1 equiv) on the rate constants for benzoic acid formation from benzohydroxamic acid (0.1 mM) at pH 7.0 and 50 °C.

pH 6.5 to 8.0 using Cu(II), Ni(II), and Zn(II), present in stoichiometric amounts. The reactions were carried out in buffered solution at 50 °C and the formation of benzoic acid was followed using reversed-phase HPLC.

The effects of pH and of metal ions on the rate of benzoic acid formation are summarized in Figure 4. While Zn(II) and Ni(II) give no apparent acceleration over the control reaction, Cu(II) increases the rate by a much as 40-fold at pH 7.0.

To determine whether a stoichiometric or a catalytic amount of Cu(II) is necessary for complete conversion, the reaction was run at several ratios of Cu(II) to *O*-benzoylhydroxylamine. The reactions were run at pH 7.0 as described previously and in all cases went to greater than 90% completion. As shown in Figure 5, Cu(II) is active as a catalyst in amounts as low as 0.1 equiv. As shown in Table II, increasing the buffer concentration moderately slows the rate of the Cu(II)-catalyzed reaction.

In the presence of aqueous Cu(II), *O*-benzoyl-*N*-methylhydroxylamine (12) is converted to benzoic acid (6) and nitrosomethane dimer 11. The nitrosomethane dimer

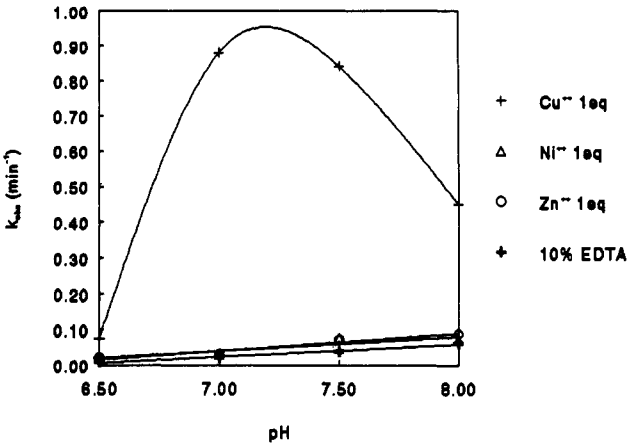


Figure 4. Effect of metal ions and pH on the rate constants for benzoic acid formation from *O*-benzoylhydroxylamine (0.1 mM) at 50 °C.

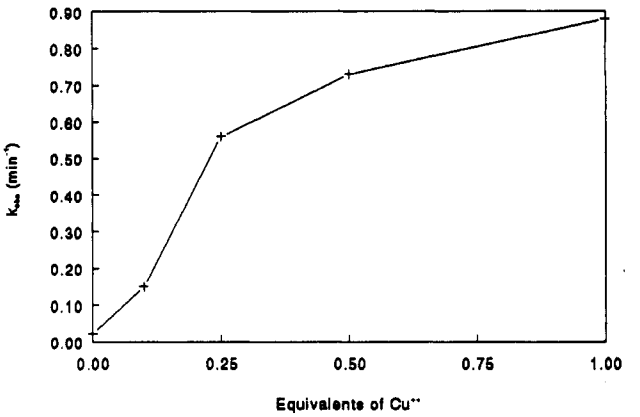


Figure 5. Effect of Cu(II) concentration on the rate constants for benzoic acid formation from *O*-benzoylhydroxylamine (0.1 mM) at pH 7.0 and 50 °C.

Table II. Effect of Buffer Concentration on the Rate of Benzoic Acid Formation from *O*-Benzoylhydroxylamine in the Presence of 1 Equiv of Cu(II) at pH 7.0 and 50 °C

buffer concntrtn (M)	k_{app} (min^{-1})
0.05	1.2
0.1	1.4
0.2	0.88

concentration was quantitated by measuring its absorbance at 264 nm. Oxidation of the hydroxylamine results in the reduction of Cu(II) to Cu(I). In deoxygenated solution, the product Cu(I) was precipitated as CuSCN[8] by the addition of NaSCN.

O-Benzoylhydroxylamine (3) is reduced by Cu(I), as predicted by the work of Novak.[7] On treatment with excess CuCl in deoxygenated solution, benzoic acid and ammonia are produced. The presence of ammonia was determined quantitatively by distilling the ammonia into distilled water that had been adjusted to pH 1 with concentrated HCl. This solution was treated with Nessler's reagent,[9] and the resulting yellow complex was measured spectrophotometrically at 400 nm. *O*-Benzoyl-*N*-methylhydroxylamine also reacts with Cu(I) in deoxygenated solution to give benzoic acid; no nitrosomethane dimer is observed.

Both oxidative and reductive pathways can be observed in one flask. When *O*-benzoyl-*N*-methylhydroxylamine

(8) Vogel, A. *Textbook of Quantitative Inorganic Chemistry*, 4th ed.; John Wiley and Sons: New York, 1978; p 462.
(9) Reference 8, p 730.

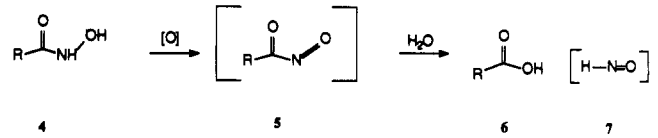


Figure 6. Oxidation of hydroxamic acids by periodic acid.[13]

(i.e., *N*-methyl-3) is treated with a less than stoichiometric amount of either Cu(II) or Cu(I) in deoxygenated solution, evidence of both oxidation and reduction is observed. HPLC analysis shows that all of the acylhydroxylamine is converted to benzoic acid, but only half of the hydroxylamine moiety is converted to the nitrosomethane dimer, as observed by UV spectroscopy. *O*-Benzoylhydroxylamine shows similar behavior.

Discussion

The hydroxamic acid functionality was chosen for this work because of its structural similarity to α-amino acids in forming metal complexes. Due to the slowness of the benzohydroxamate reactions, only the Cu(II)-catalyzed reactions were followed to completion. The Zn(II), Ni(II), and control reactions were monitored for 6 months, at which point hydrolysis had occured to about 50% completion. Because of the very long time frame involved, rate constants are estimated only and should not be used otherwise. Under the circumstances, the rate of reaction in the presence of Ni(II), Zn(II), and EDTA is probably identical within experimental error.

Of the three metal ions studied in this work, only Cu(II) showed significant catalytic properties. By analogy to α-amino esters, Cu(II) was expected to give the greatest acceleration. However, Zn(II) and Ni(II) were also anticipated to show some activity, given their effectiveness in the hydrolysis of α-amino esters and similar model systems. In addition, the ability of Zn(II) and Ni(II) to catalyze the formation of acetohydroxamic acid from acetic acid and hydroxylamine is known;[10] catalysis in the hydrolysis direction was thus anticipated. However, the effects of Zn(II) and Ni(II) on hydroxamic acid formation were observed at pH values below pH 6.0. Such catalysis may be slow above pH 6.5 where the present study was conducted. The lack of any acceleration of reaction by Zn(II) and Ni(II), and the redox chemistry of Cu(II), led us to consider an oxidative mechanism for the Cu(II)-catalyzed hydrolysis of benzohydroxamic acid. The ability of Cu(II) to act as an oxidant is well known.[11] Copper also mediates some biological oxidations and is an essential metal in galactose oxidase.[12] Cu(II) rapidly oxidizes hydroxylamine to nitrous oxide.[13]

In the Cu(II)-catalyzed hydrolysis of benzohydroxamic acid, there are two possible routes for oxidation to occur. One mechanism involves initial nonoxidative hydrolysis to give hydroxylamine and benzoic acid, followed by rapid oxidation of free hydroxylamine by Cu(II). The other possibility involves initial oxidation of the hydroxamate. Rowe and Ward have studied the oxidation of hydroxamic acids,[14] and they suggest that the initial product of oxidation is **5** (Figure 6). This species then reacts with nucleophiles with loss of HNO, which dimerizes rapidly to give N_2O and H_2O. Nitrous oxide has been detected as

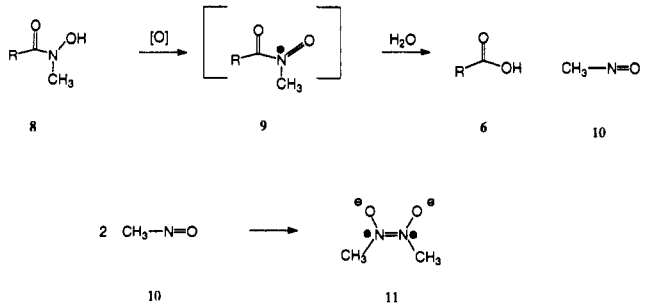


Figure 7. Oxidation of *N*-methylhydroxamic acids by periodic acid, and the dimerization of nitrosomethane.[14]

a product of the oxidation of hydroxylamine by periodic acid by IR spectroscopy,[15] and hydroxamic acids are expected to behave similarly. *N*-Methylhydroxamic acids (e.g., **8**) are oxidized in a similar fashion (Figure 7), yielding nitrosomethane dimer **11** as the final product.[15] Compound **11** is a water-soluble solid with a strong absorbance at 264 nm.

Due to the ease of detection of nitrosomethane dimer by UV–vis spectrocopy, *N*-methylbenzohydroxamic acid (**8**) was prepared to determine if the Cu(II)-catalyzed hydrolysis of this hydroxamic acid proceeds through an oxidative mechanism. When *N*-methylbenzohydroxamic acid was treated with Cu(II) at pH 7.0 and 50 °C, no hydrolysis was observed. Assuming that the oxidation of *N*-methylbenzohydroxamic acid follows a pathway similar to that proposed by Rowe and Ward, oxidation of *N*-methylbenzohydroxamic acid would produce **9** as the initial product. The difficulty of generating a positive charge adjacent to the already electropositive carbonyl carbon may explain the lack of Cu(II) reaction with *N*-methylbenzohydroxamic acid. Periodic acid easily oxidizes this compound, producing nitrosomethane dimer, but it is a much stronger oxidant than Cu(II): the standard reduction potential of periodate is 1.7 V, compared to 0.15 V for the Cu(II) to Cu(I) couple.[16]

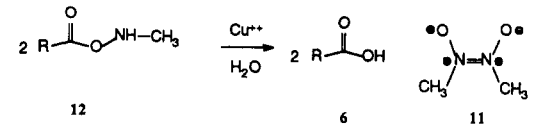
The Cu(II)-catalyzed hydrolysis appears to be an oxidative process. If the reaction were a simple hydrolytic cleavage, we would anticipate some acceleration of the reaction by Zn(II) and Ni(II). Furthermore, N-methylation (i.e., **8** vs **4**) should have if anything a favorable effect on such a hydrolysis (assuming metal ion complexation); thus, we conclude that oxidation occurs prior to hydrolysis.

The reaction of **2** was found to be catalytic in Cu(II), and in all cases apparent first-order kinetics were followed. Of course, first-order behavior is not required for a multistep mechanism. Since the reaction involves oxidation by Cu(II), the Cu(I) produced may be reoxidized by dissolved O_2, as in the Wacker process. This reoxidation is predicted to be much faster than the relatively slow Cu(II)-catalyzed reaction of benzohydroxamic acid. The reduction of benzohydroxamic acid by Cu(I) is not expected because the oxygen bears a formal charge; in any case, no benzamide is observed.

The hydrolysis of benzohydroxamic acid is very slow at neutral pH and 50 °C, having a half life of about 6 months. In the presence of Cu(II), the hydroxamic acid is hydrolyzed oxidatively at a much greater rate. The Cu(II) serves as a catalyst for this reaction and shows turn-over behavior at concentrations as low as 10 mol %.

Even more than hydroxamic acids, *O*-acylhydroxylamines were expected to mimic the behavior of α-amino

(10) (a) Lawlor, J. M. *J. Chem. Soc., Chem. Commun.* **1967**, 404. (b) Munson, J. W.; Connors, K. A. *J. Am. Chem. Soc.* **1972**, *94*, 1979.
(11) Gammp, H.; Zuberbuhler, A. D. *Metal Ions Biol. Systems* **1981**, *12*, 133.
(12) Hamilton, G. A.; Adolf, P. K.; de Jersey, J.; DuBois, G. C.; Dyrkacz, G. R.; Libby, R. D. *J. Am. Chem. Soc.* **1978**, *100*, 1899.
(13) Imamura, T.; Fujimoto, M. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2971.
(14) Rowe, J. E.; Ward, A. D. *Aust. J. Chem.* **1968**, *21*, 2761.
(15) Emery, T. F.; Neilands, J. B. *J. Org. Chem.* **1962**, *27*, 1075.
(16) Ramette, R. W. *Chemical Equilibrium and Analysis*; Addison-Wesley: Reading, MA, 1981.



12 6 11

Figure 8. Oxidation of *O*-benzoyl-*N*-methylhydroxylamine (12) by Cu(II).

esters. Unlike hydroxamic acids, which contain an amide linkage, *O*-acylhydroxylamines more closely resemble esters. That such compounds to react with low valent metal ions has precedent in Novak's work.[7]

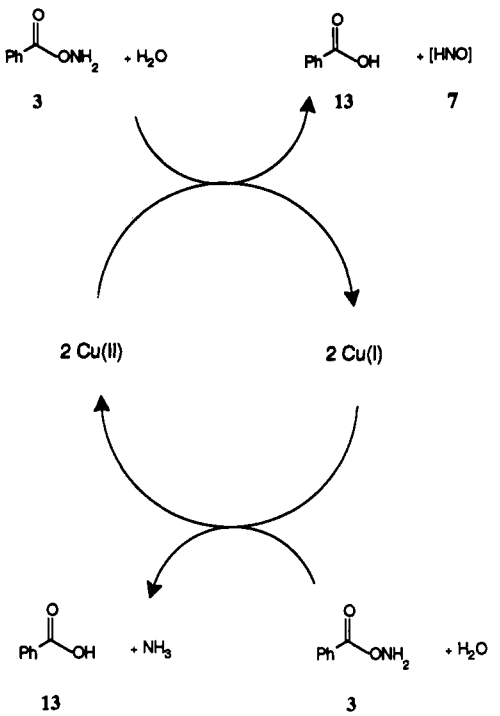
As with benzohydroxamic acid, Ni(II) and Zn(II) show no tendency to accelerate the rate of hydrolysis of *O*-benzoylhydroxylamine. Even with a 10-fold excess of Zn(II), no increase in rate was observed. In the presence of 1 equiv of Cu(II), an acceleration of 40-fold was obtained at pH 7. Given the lack of effect by the nonoxidative metals Zn(II) and Ni(II) and the ease of oxidation of hydroxylamine by Cu(II), this acceleration is probably due also to a redox mechanism.

Unlike the Cu(II)-catalyzed oxidative hydrolysis of benzohydroxamic acid, which increases in rate with pH, the oxidative hydrolysis of *O*-benzoylhydroxylamine has a rate maximum at about pH 7.2. Hydroxamic acids complex many metal ions tightly, giving an intense color in the presence of Fe(III). *O*-Benzoylhydroxylamine fails this ferric ion test and probably does not bind metal ions as strongly as benzohydroxamic acid. The observed pH profile is likely due to competitive complexation of the Cu(II) by the buffer. HEPES, while a weak Cu(II) binder, is present in large excess; both *O*-benzoylhydroxylamine and Cu(II) are present at 10^{-4} M, while the buffer is present at 0.2 M. As the pH rises, a greater proportion of the buffer will be unprotonated and will be a better ligand for Cu(II).

Further evidence of an oxidative pathway was seen in the study of the *N*-methyl compound. The oxidative hydrolysis of *O*-benzoyl-*N*-methylhydroxylamine (12) is also catalyzed by Cu(II), giving benzoic acid and nitrosomethane dimer as the products (Figure 8). The nitrosomethane dimer 11 was observed by its absorbance at 264 nm; its presence could not be measured by HPLC due to its extremely short retention time. Although simple hydrolysis followed by oxidation of hydroxylamine cannot be ruled out, we conclude that the Cu(II)-catalyzed hydrolysis of *O*-benzoylhydroxylamine occurs by an oxidative process.

The weak N–O bond also makes hydroxylamine prone to reduction, and Cu(I) will reduce it to ammonia in 0.5 M H_2SO_4.[17] At 25 °C, the half-life for reduction of hydroxylamine by Cu(I) in 0.5 M H_2SO_4 is greater than 1300 min. Under the conditions of the present study, Cu(II) oxidizes hydroxylamine to nitrous oxide. However, Cu(I) reduces hydroxylamine very slowly or not at all at pH 7.0.

Novak has shown that *N*-aryl-*O*-pivaloylhydroxylamines are reduced by Cu(I) under similar conditions.[7] Indeed, Cu(I) does catalyze the conversion of *O*-benzoylhydroxylamine to benzoic acid in our experiments. *O*-Benzoyl-*N*-methylhydroxylamine (12) also reacts in the presence of Cu(I), and no nitrosomethane dimer is observed by UV–vis. The ammonia formed on reduction of *O*-benzoylhydroxylamine has been detected by distillation, followed by treatment with Nessler's reagent. Since Cu(I) does not reduce hydroxylamine under these conditions and oxidation is not occurring, the Cu(I)-induced reaction of



3 13 7

2 Cu(II) 2 Cu(I)

13 3

Figure 9. Proposed redox cycle for the reaction of the Cu(II)/Cu(I) couple with *O*-benzoylhydroxylamine (3).

O-benzoylhydroxylamine must take place through a reductive mechanism.

Hydroxylamine is known to behave both as an oxidant and as a reductant, and its disproportionation reaction has been described.[18] Hydroxylamine itself reacts with Cu(II) under these reaction conditions, but Cu(I) does not. In contrast, *O*-benzoylhydroxylamine reacts with both oxidation states of copper under the conditions of this work.

If *O*-benzoylhydroxylamine is allowed to react with a subequivalent amount of either Cu(II) or Cu(I) in deoxygenated solution, then both reaction pathways can occur. In this way, copper cycles between Cu(II) and Cu(I) while *O*-benzoylhydroxylamine is alternately oxidized and reduced (Figure 9). This can be observed in the reaction of *O*-benzoyl-*N*-methylhydroxylamine. With a catalytic amount of either Cu(I) or Cu(II), the reaction goes to completion, with formation of both 11 and ammonia.

Experimental Section

General. HPLC was carried out on an IBM LC/9533 system using an IBM C-18 reversed-phase column and a 20-μL injection loop. Elution was carried out at a flow rate of 1 mL/min with continuous UV detection of the eluant at 254 nm. The solvent system used was a solution of buffer/methanol (4:7); the buffer used was pH 2.0 aqueous potassium phosphate (0.2 M) prepared from phosphoric acid and KOH in HPLC grade water. HPLC grade solvents were purchased from Fisher Scientific Company, Pittsburgh, PA.

Benzohydroxamic acid was purchased from the Aldrich Chemical Company, Milwaukee, WI. All metal ions used were purchased as the hydrated perchlorate salts from GFS Chemical Company, Columbus, OH. *N*-Methylbenzohydroxamic acid was prepared as described by Plapinger.[19]

The buffer solutions used were 0.2 M 2-morpholinoethanesulfonic acid (MES) for pH 6.5 and 0.2 M *N*-(2-hydroxyethyl)-piperazine-*N*'-2-ethanesulfonic acid (HEPES) for pHs 7.0, 7.5, and 8.0. pH measurements were made with a Fisher Accumet pH meter, Model 810.

(17) Tomat, R.; Rigo, A. *Electroanal. Chem. Interfacial Electrochem.* **1975**, *59*, 191.

(18) The disproportionation of NH_2OH was first reported by W. Lossen in 1865, as cited in: *Wiss. Z., Karl-Marx-Univ., Leipzig, Math-Naturwiss Reihe* **1960**, *9*, 17.

(19) Plapinger, R. E. *J. Org. Chem.* **1959**, *24*, 802.

***O*-Benzoylhydroxylamine Hydrochloride.**[6] *p*-Nitrophenyl benzoate (500 mg, 2.1 mmol)) was dissolved in ethanol (50 mL) at 65 °C. A solution of hydroxylamine hydrochloride (215 mg, 3.1 mmol) in 1.0 M aqueous NaOH (2.5 mL) was added, and the resulting mixture was stirred at room temperature for 15 min. The solvent was removed by rotary evaporation and the residue was dissolved in ethanol/water 1:1 (20 mL) and extracted with $CHCl_3$ (3 × 10 mL). The combined organic portions were washed with 0.2 M aqueous Na_2CO_3 (3 × 10 mL) followed by saturated aqueous NaCl (10 mL). After drying over $MgSO_4$ and subsequent filtration, 4 drops of concentrated HCl were added and the solution was cooled in an ice bath. Filtration gave *O*-benzoylhydroxylamine hydrochloride as a colorless solid (113 mg, 32%): mp 70–105 °C dec (lit.[20] mp 70–100 °C).

***O*-Benzoyl-*N*-methylhydroxylamine Hydrochloride.** *p*-Nitrophenyl benzoate (500 mg, 2.06 mmol) was dissolved in 95% ethanol (50 mL) at 65 °C. A solution of *N*-methylhydroxylamine hydrochloride (259 mg, 3.1 mmol) in 1.0 M aqueous NaOH (2.5 mL) was added, and the resulting mixture was stirred for 25 min. The volume was reduced by half using rotary evaporation, water (25 mL) was added, and the resulting solution was extracted with $CHCl_3$ (3 × 20 mL). The combined organic portions were washed with 0.2 M aqueous Na_2CO_3 (4 × 20 mL). The organic portions were combined, and the volume was again reduced by half using rotary evaporation. Concentrated HCl (4 drops) was added and the solution was cooled in an ice bath. *O*-Benzoyl-*N*-methylhydroxylamine hydrochloride crystallized as colorless needles (123 mg, 32%): mp 129–130 °C dec; ^1H NMR (DMSO-d_6) δ 2.89 (s, 3, CH_3), 7.55 (t, 2, Ar), 7.70 (t, 1, Ar), 7.92 (d, 2, Ar); ^{13}C NMR (DMSO-d_6) δ 37.6 (CH_3), 127.3 (Ar), 129.07 (Ar), 134.1 (Ar), 164.3 (C=O).

Nessler Test for Ammonia. The Nessler reagent solution was prepared as described in Vogel.[9] The solution to be tested (50 mL) was placed in a 100-mL round bottom flask attached to a distilling head and condenser. The receiving flask contained distilled water (50 mL) adjusted to pH 2 with concentrated aqueous HCl. Concentrated aqueous NaOH (1 mL) was added to the test solution, which was then heated at reflux for 30 min. The solution in the receiving flask was made basic with concentrated aqueous NaOH, and following the addition of Nessler reagent solution (1 mL) the absorbance at 400 nm was measured.

Treatment of *O*-benzoylhydroxylamine (480 μM) in pH 7 HEPES buffer with $Cu(ClO_4)_2$ (54 μM) at room temperature for 1 day yielded complete conversion to benzoic acid and 220 μM ammonia, as determined by the Nessler test.

Precipitation of CuSCN.[8] A solution of $Cu(ClO_4)_2$ (11 mg, 3.0×10^{-5} mol) in pH 7.0 HEPES buffer (0.2 M, 100 mL) was degassed with N_2 for 2 h. *O*-Benzoylhydroxylamine hydrochloride (6 mg, 3.3×10^{-5} mol) was added with stirring. After 30 min, the reaction had gone to completion as determined by HPLC. Concentrated HCl (0.25 mL) was added and the solution was heated to 90 °C. NaSCN (3.2 mg, 3.9×10^{-5} mol) was added and the solution was allowed to stand at room temperature. The solution first became cloudy; then CuSCN precipitated as a white powder. In the absence of *O*-benzoylhydroxylamine, no precipitate formed.

Tests for the Presence of Hydroxylamine. 2,4-Dinitrofluorobenzene Method.[21] A 25-mL portion of the solution to be tested was made basic with concentrated NaOH and shaken in a separatory funnel with 1 mL of 2,4-dinitrofluorobenzene solution (1% w/v in acetone) for 2 min. Petroleum ether (10 mL) and 2.5% aqueous NaOH (25 mL) were added, and the solution was shaken for 1 min. After separation, the organic layer was evaporated to dryness; the residue was dissolved in 25 mL of the HPLC solvent. HPLC analysis showed 2,4-dinitrophenol with a retention time of 5 min and two peaks corresponding to the presence of hydroxylamine with retention times of 9 min (small) and 15 min.

***p*-Nitrobenzaldehyde Method.**[22] *p*-Nitrobenzaldehyde (20 mg, 0.13 mmol) was dissolved in 20 mL of 1:1 ethanol/1.2 M aqueous HCl. To 1 mL of this solution was added 1 mL of the solution to be tested. This mixture was heated at 90 °C for 30 min. After cooling, 0.6 M NaOH (1.5 mL) was added and the solution was diluted to 8 mL with ethanol. The absorbance of this solution was measured at 368 nm.

Kinetic Measurements. The hydrolysis reactions were monitored by HPLC as described in the General Experimental Section. Typical retention times are benzohydroxamic acid, 3.15 min; benzoic acid, 4.7 min; *N*-methylbenzohydroxamic acid, 3.6 min. A solution of benzohydroxamic acid (1.09×10^{-4} M) and metal perchlorate (1.09×10^{-4} M) in a round bottom flask was sealed with a rubber septum and heated at 50 °C in a water bath. Aliquots were withdrawn periodically for HPLC analysis. The control reactions were identical except for the absence of metal ion and the addition of EDTA (0.5×10^{-5} M).

Acknowledgment. The financial support of the National Science Foundation is acknowledged with gratitude. FT-NMR spectra were obtained by using equipment funded in part by NIH Grant No. 1 S10 RR01458-01A1. A.W.C. thanks the A. P. Sloan and Dreyfus Foundations for support in the form of Fellowships and Eli Lilly and Company and Merck for support in the form of Granteeships.

(20) (a) Carpino, L. A.; Giza, L. A.; Carpino, B. A. *J. Am. Chem. Soc.* **1959**, *81*, 955. (b) Carpino, L. A. *J. Am. Chem. Soc.* **1960**, *82*, 3133.

(21) Cohen, I. C.; Norcup, J.; Ruzicka, J. H. A.; Wheals, B. B. *J. Chromatogr.* **1969**, *44*, 251.

(22) Johnson, D. P. *Anal. Chem.* **1968**, *40*, 646.