# Transformation of the Plant Growth Regulator Daminozide (Alar) and Structurally Related Compounds with Cu<sup>II</sup> Ions: Oxidation versus Hydrolysis

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As part of a study of metal ion effects on chemical transformations of nitrogen-containing agrochemicals, conversion of daminozide to succinate via cleavage of the hydrazide C–N bond was examined in the presence and absence of divalent metal ions. No conversion was observed in metal ion-free solutions or in the presence of 1.0 mM Ni<sup>II</sup>, Zn<sup>II</sup>, and Pb<sup>II</sup>. Cu<sup>II</sup>, in contrast, markedly increased rates of daminozide to succinate conversion. Halide ions (CI-, Br<sup>-</sup>) had no effect on daminozide conversion in the absence of metal ions but markedly increased conversion rates observed in the presence of Cu<sup>II</sup>. The nitrogen-donor ligands ethylenediamine, N-(2-hydroxyethyl)ethylenediamine, and 1,4,7,10-tetraazacyclododecane decreased rates of Cullfacilitated conversion, while 1,5,9-triazacyclododecane actually increased rates of conversion. H NMR and UV spectroscopy provide evidence for the formation of 1:1 Cu<sup>II</sup>daminozide complexes. Halide ion effects and nitrogendonor ligand effects point to an oxidative mechanism for Cullfacilitated daminozide breakdown, rather than hydrolysis. The structurally related compound butyric acid 2,2dimethylhydrazide (BH) is subject to the same Cullfacilitated breakdown via an oxidative mechanism. N,N-Dimethylsuccinamic acid (SA), in contrast, breaks down via a hydrolytic mechanism.

# Introduction

The plant growth regulator daminozide (succinic acid 2,2dimethylhydrazide, trade name alar) (Figure 1) and its hydrolysis product unsymmetrical dimethylhydrazine (UDMH or 1,1-dimethylhydrazine) are both toxic and potential carcinogens (1, 2). Prior use to improve the yield and quality of apples, grapes, cherries, and other fruits and vegetables has largely been curtailed because of perceived risks to children and other consumers (3, 4). Owing to its use in greenhouses, nurseries, and other applications, daminozide is still one of the most heavily used plant growth regulators in the U.S. (5). In addition to daminozide, the hydrazide moiety is found in other agrochemicals (e.g., maleic hydrazide and methoxyfenozide) and in pharmaceuticals (e.g., isoniazid and carbadox). The hydrazide moiety is also frequently



FIGURE 1. Structures of compounds investigated in this study.



FIGURE 2. Structures of N-donor ligands employed in this study: ethylenediamine (EN), *N*-(2-hydroxyethylethylenediamine (HEEN), 1,5,9-triazacyclododecane (12aneN<sub>3</sub>), and 1,4,7,10-tetraazacyclododecane (cyclen).

employed as a protective group in the synthesis of peptides and drugs (6, 7).

We selected daminozide as the representative hydrazide in a study of metal-catalyzed hydrolysis reactions of nitrogencontaining agrochemicals (8). As this report indicates, daminozide breakdown is significantly facilitated by the presence of Cu<sup>II</sup> ions but not by Ni<sup>II</sup>, Zn<sup>II</sup>, or Pb<sup>II</sup>. The Cu<sup>II</sup>facilitated breakdown is significantly enhanced by increasing concentrations of chloride or bromide ions. Halide ion effects have been observed in several reactions where  $\mathrm{Cu}^{\mathrm{II}}$  is reduced to Cu<sup>I</sup> (e.g., refs 9 and 10) but is not typically observed in metal ion-catalyzed hydrolysis reactions. With the help of Prof. Kenneth D. Karlin (Department of Chemistry, Johns Hopkins University), a series of N-donor ligands (EN, HEEN, 12aneN<sub>3</sub>, and cyclen in Figure 2) was selected to explore the details of the Cu<sup>II</sup>-facilitated reaction mechanism. HEEN, which possesses a pendant nucleophilic group, and EN, which does not, were part of our previous study of Cu<sup>II</sup>- and Zn<sup>II</sup>catalyzed dimetilan hydrolysis (11). The macrocyclic nature of  $12aneN_3$  and cyclen exerts strong geometric controls on the structure of complexes. Proton dissociation constants of coordinated water molecules (e.g., ref 12) and the relative stability of Cu<sup>II</sup> versus Cu<sup>I</sup> complexes (e.g., ref 13) are strongly influenced by imposed geometry.

Two structurally related compounds, butyric acid 2,2dimethylhydrazide (BH) and *N*,*N*-dimethylsuccinamic acid (SA) (Figure 1), were included in the study to discern differences between hydrazide and amide linkages. UV and H NMR spectroscopy was used to obtain evidence for the formation of  $Cu^{II}$ -daminozide complexes prior to daminozide breakdown.

## Materials and Methods

**Reagents.** Reagent grade water (18 M $\Omega$ -cm resistivity) was prepared using a glass distillation apparatus and a Milli-Q reagent-grade water system (Millipore). Unless otherwise

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specified, all chemical reagents were obtained from Aldrich at greater than 98% of purity and used without further purification. HEEN was obtained from Lancaster at greater than 99% of purity. Acetate buffer (5–10 mM acetic acid/ sodium acetate from J. T. Baker) was used to maintain pH in the range 3.6–5.7, while MOPS buffer (5–10 mM 4-morpholinepropanesulfonic acid and its sodium salt monohydrate) was used in the range 5.9–7.3.

Daminozide and SA were purchased from Chem Service at greater than 97% purity. BH was synthesized from butyryl chloride and UDMH. The synthesis procedure is described in the following: UDMH in dry methylene chloride was chilled in an ice/acetone bath and then mixed with 1 equiv of butyryl chloride and 1 equiv of triethylamine (serving as a proton sink). After the mixture was stirred overnight at room temperature, methylene chloride was added, and the solution was washed several times with ethyl acetate, sodium bicarbonate solution, and cold water. The methylene chloride layer was then collected and dried with sodium sulfate. Rotary evaporation was used to concentrate the mixture prior to separation on a 40-cm long silica-packed column. Three eluents were used to separate the synthesized BH from the reactants: (1) a 1:1 mixture of ethyl acetate and hexane; (2) pure ethyl acetate solution; and (3) a 4:1 mixture of ethyl acetate and methanol. The eluents were fractionally collected and examined by thin-layer chromatography (dyed with I2 for UV absorption). Most BH was recovered using the pure ethyl acetate eluent. The product BH was further concentrated by rotary evaporation and then characterized and confirmed using a 400-MHz NMR spectrometer.

**Reaction Setup.** All glassware was soaked in 6 N HNO<sub>3</sub> and rinsed several times with reagent-grade water prior to use. Screw-cap amber glass vials were autoclaved prior to use, and reaction solutions were filter-sterilized to inhibit biotic reactions. A Teflon-coated stir bar, reagent water, and appropriate amounts of stock solutions (electrolyte, buffer, metal salt, and ligand) were added to each vial, which was then covered with a silicone rubber/Teflon septum. No effort was made to exclude  $O_2$  or  $CO_2$ . After 3–4 h of stirring in a 25 °C water bath, an appropriate amount of daminozide, BH, or SA stock solution was added to initiate reaction. Aliquots were periodically collected for analysis.

**Chemical Analysis.** Reaction progress was followed using a Waters Quantum 4000 Capillary Electrophoresis system with a UV detector. Daminozide, SA, succinic acid, and butyric acid were analyzed using anion analysis mode and direct photometric detection (185 nm). A 60 cm  $\times$  75  $\mu$ m fusedsilica capillary column was used with 30–60 s hydrostatic injection and 18 kV applied voltage. The CE electrolyte consisted of 25 mM phosphate buffer (pH 7.4) and 0.50 mM tetradecyltrimethylammonium bromide (TTAB). BH was analyzed using cation analysis mode and direct photometric detection (185 nm). A 62 cm  $\times$  75  $\mu$ m hydrophobically coated capillary column was used (CElect-P175, Supelco) with 40 s hydrostatic injection and 22 kV applied voltage. The CE electrolyte consisted of 25 mM NaH<sub>2</sub>PO<sub>4</sub> and 3.7 mM H<sub>3</sub>PO<sub>4</sub> (pH approximately 3.0).

Indirect photometric detection (185 nm) was required for UDMH analysis. CE was run in cation mode using the CElect-P175 capillary column. The CE electrolyte consisted of 6.5 mM hydroxyisobutyric acid and 5 mM cationic chromophore 4-methylbenzylamine, which is displaced by the analyte during its electromigration, resulting in a negative peak. Quantification of UDMH was not successful since its concentration was near or below the detection limit (approximately  $20 \,\mu$ M) throughout most experiments performed with daminozide and BH. It was also suspected that UDMH may be oxidized by O<sub>2</sub> in the presence of Cu<sup>II</sup> ions (*14, 15*), thus further complicating detection. **Rate Constants and Mass Balance.** Conversion of daminozide to succinic acid, BH to butyric acid, and SA to succinic acid yielded good mass balance (90–110%) throughout the kinetic experiments. For this reason, reaction product concentration measurements could be used to back-calculate parent compound concentrations. Plots of the log of the parent compound concentration (direct measurements or back-calculated from product) versus time were linear, indicating pseudo-first-order kinetics:

$$-\frac{d[hydrazide \text{ or amide}]}{dt} = k_{obs}[hydrazide \text{ or amide}]$$
(1)

Slopes obtained by linear regression ( $r^2$  between 0.9800 and 0.9999) are reported as rate constants ( $k_{obs}$ ,  $h^{-1}$ ). Monitoring parent compound loss and product generation yielded comparable rate constant values. Values of  $k_{obs}$  below  $1.0 \times 10^{-4} h^{-1}$  (corresponding to half-lives longer than 290 days) could not be quantified, since actual parent compound loss cannot be distinguished from experimental error.

Cu<sup>II</sup>–Daminozide Complex Formation. An AMX 300-MHz NMR spectrometer (Bruker) was used to study complex formation between daminozide and Cu<sup>II</sup> ions in deuterium oxide (D<sub>2</sub>O). Samples contained 0–5 mM Cu(NO<sub>3</sub>)<sub>2</sub>, 5 mM daminozide, 5 mM deuterium acetate (CD<sub>3</sub>CO<sub>2</sub>D), and 0.25 mM NaOD, resulting in a pH of 5.0. Each sample was analyzed by H NMR immediately after Cu(NO<sub>3</sub>)<sub>2</sub> was added.

Complexation between daminozide and Cu<sup>II</sup> was also studied using a UV spectrophotometer (Shimadzu UV160U). The solutions were maintained at 25 °C and kept at pH 5.0 with 5 mM acetate buffer. Immediately after preparation, the absorbance of CuCl<sub>2</sub> solutions, daminozide solutions, and CuCl<sub>2</sub> plus daminozide solutions was measured at 240, 250, 260, 270, and 280 nm. Abs(X) represents the absorbance of a solution containing electrolyte, buffer, and reagent X. At a fixed wavelength, the increase in absorbance due to complexation (called  $\Delta$  absorbance) was calculated from the following equation:

 $\Delta$  absorbance = abs(Cu + daminozide) – abs(Cu only) – abs(daminozide only) (2)

In separate experiments,  $Cu^{II}$ -daminozide complexation was studied within pH 3.5–7.0. Solutions at pHs above 6.0 became cloudy due to  $Cu^{II}$  (hydr)oxide precipitation. The cloudy solutions were filtered by 0.22  $\mu$ m PTFE filters (Millipore) before UV absorbance measurement.

### Results

**SA Breakdown in the Presence and Absence of Metal Ions.** The first-order rate constant ( $k_{obs}$ ) for SA breakdown into succinic acid was found to increase as the solution pH was decreased and was unaffected by the presence of millimolar levels of Cu<sup>II</sup>, Zn<sup>II</sup> (Figure 3), Ni<sup>II</sup>, or Pb<sup>II</sup> ions. Furthermore, the rate of SA breakdown was also unaffected by addition of various concentrations of NaNO<sub>3</sub>, NaCl, or NaBr electrolyte regardless of whether metal ions were present.

Daminozide and BH Breakdown in the Presence and Absence of Metal Ions. Throughout the pHs examined (3.5  $^{\circ}$  pH  $^{\circ}$  7.5), negligible loss of daminozide or BH was observed in the absence of metal ions (Figure 4) or in the presence of millimolar levels of Ni<sup>II</sup>, Zn<sup>II</sup>, and Pb<sup>II</sup> ions. These conclusions were unaffected by whether additions of NaCl, NaBr, or NaNO<sub>3</sub> electrolyte were made.

In the presence of  $Cu^{II}$  ions, breakdown of daminozide into succinic acid and breakdown of BH into butyric acid occurred and followed pseudo-first-order kinetics. As shown in Figure 4, the log of first-order rate constants (log  $k_{obs}$ ) increased linearly as pH was increased (with slopes of 0.43)



FIGURE 3. Effect of pH on SA hydrolysis in the presence and absence of Cu<sup>II</sup> and Zn<sup>II</sup>. Reaction conditions: 1.31 mM SA, 1.5 mM CuCl<sub>2</sub> or ZnCl<sub>2</sub>, 5.0 mM acetate (below pH 5.5) or MOPS (above pH 6.0) buffer, 0.10 M NaCl, 25 °C.



FIGURE 4. Effect of pH on the Cu<sup>II</sup>-mediated breakdown of daminozide and BH. Reaction conditions: 1.01 mM daminozide or 1.08 mM BH, 1.5 mM CuCl<sub>2</sub>, 5.0 mM acetate (below pH 5.5) or MOPS (above pH 6.0) buffer, 0.05 M NaCl, 25 °C.

and 0.41 for daminozide and BH, respectively,  $r^2 > 0.96$ ) until a maximum was reached near pH 6. Above pH 6,  $k_{obs}$ values decreased sharply. This decrease of  $k_{obs}$  values coincided with a cloudy appearance in all Cu<sup>II</sup>-containing solutions, most likely caused by precipitation of Cu<sup>II</sup> (hydr)oxide solids. Below pHs where solid formation occurred, increasing Cu<sup>II</sup> concentration from 1 to 3 mM resulted in linear increases in  $k_{obs}$  for both daminozide and BH breakdown. The log–log plots for  $k_{obs}$  versus Cu<sup>II</sup> concentration yielded slopes of 0.85 and 0.72 for daminozide and BH, respectively ( $r^2 > 0.99$ , data not shown).

Speciation calculations for Cu using the equilibrium computer program HYDRAQL (*16*) and stability constants from Smith et al. (*17*) indicate that dissolved Cu<sup>II</sup>-hydroxo species reach their highest concentrations at pHs just below values required for (hydr)oxide solid precipitation, regardless of electrolyte identity (NaCl, NaBr, or NaNO<sub>3</sub>), buffer identity (acetate or MOPS), and ionic strength. At pH values above 6.2, Cu<sup>II</sup> (hydr)oxide starts to precipitate, and the concentrations of dissolved Cu<sup>II</sup> species decrease drastically. The effect of pH on Cu<sup>I</sup> speciation was also calculated. Equilibrium constants used to calculate concentrations of Cu<sup>I</sup>-chloride species are listed in Table S1 in the Supporting Information (*18*). Cu<sup>I</sup>-hydroxo species were not included in the calculation because equilibrium constants are not available. Under conditions comparable to Figure 4, 1.5 mM of Cu<sup>I</sup> is present predominantly as CuCl<sub>2</sub><sup>-</sup>, CuCl<sub>3</sub><sup>2-</sup>, and CuCl<sup>0</sup>(aq). Precipitation of Cu<sub>2</sub>O(s) does not occur until the pH exceeds 12.2, far above the pH values examined in this study.

Daminozide and BH Breakdown in the Presence of CuII: Halide Ion Effects. The rates of daminozide conversion to succinic acid and BH conversion to butyric acid were found to increase sharply as chloride and bromide concentration was increased (Table 1). Linear relationships between the  $k_{\rm obs}$  value and halide ion concentration were observed ( $r^2 >$ 0.99). Enhancement effects of chloride and bromide were similar, with the effects of bromide slightly greater. Compared to the absence of halide ions, the presence of 0.103 M Cl<sup>-</sup> or Br<sup>-</sup> increased the rate of Cu<sup>II</sup>-facilitated daminozide breakdown by more than 65 and 85 times, respectively. Compared to the absence of chloride ions, the presence of 0.103 M Cl- increased the rate of Cu<sup>II</sup>-facilitated BH breakdown by more than 14 times. Additions of comparable concentrations of NaNO<sub>3</sub>, on the contrary, had negligible effects (Table 1).

Speciation of  $Cu^{II}$  and  $Cu^{I}$  with varying  $Cl^{-}$  and  $Br^{-}$  concentration was calculated using HYDRAQL (*16*). Concentrations of  $Cu^{II}$ -halide species increase as halide ion concentration increases. The dependence of  $[CuCl^{+}]$  upon  $[Cl^{-}]$  is first-order, while the dependence of  $[CuCl_{2}^{0}(aq)]$  upon  $[Cl^{-}]$  is second-order. The speciation calculations indicate that in 0.10 M NaCl, concentrations of  $CuCl^{+}$  and  $CuCl_{2}^{0}(aq)$  are approximately 17 times and 175 times lower than the concentration of  $Cu^{2+}$ . In 0.10 M NaBr, the concentration of  $CuBr^{+}$  is approximately 29 times lower than the concentration of  $Cu^{2+}$ .

Compared to Cu<sup>II</sup>, Cu<sup>I</sup> forms much stronger complexes with Cl<sup>-</sup> and Br<sup>-</sup>. In the presence of 0.10 M NaCl, concentrations of CuCl<sup>0</sup>(aq), CuCl<sub>2</sub><sup>-</sup>, and CuCl<sub>3</sub><sup>2-</sup> are 75 times, 1732 times, and 53 times the concentration of Cu<sup>+</sup>(aq). In the presence of 0.10 M NaBr, CuBr<sup>0</sup>(aq), CuBr<sub>2</sub><sup>-</sup>, and CuBr<sub>3</sub><sup>2-</sup> are 202 times, 4222 times, and 2507 times the concentration of Cu<sup>+</sup>.

**Daminozide and BH Breakdown in the Presence of Cu<sup>II</sup>: Organic Ligand Effects.** Experiments with N-donor ligands were conducted at pH 5 with equal concentrations of Cu<sup>II</sup> and organic ligand (1 mM). The log $K_{CuL}$  values for 1:1 complex formation with each fully deprotonated ligand are 10.5 for EN, 10.02 for HEEN, 12.6 for 12aneN<sub>3</sub>, and 24.6 for cyclen (17). Equilibrium calculations indicate that 1:2 complexes comprise less than 1% of total dissolved copper at the pH and concentration conditions employed. Daminozide and BH did not break down when the organic ligand was present, but no metal ions were added. Hence, organic ligand effects arise solely from organic ligand–metal ion interactions.

HEEN contains a pendant alcohol group that is absent in EN. Experiments with both organic ligands were only performed in halide ion-containing solutions. HEEN and EN addition resulted in similar effects, causing a 44-62%decrease in Cu<sup>II</sup>-facilitated daminozide breakdown rate (Table 1). Regardless of the halide ion concentration employed, cyclen addition completely prevented daminozide breakdown from occurring (data not shown in Table 1). In halide ion-free solution, 12aneN<sub>3</sub> addition caused a 23% decrease in daminozide breakdown rate. In Cl<sup>-</sup>-containing solutions, 12aneN<sub>3</sub> addition caused an approximately 2-fold increase in daminozide breakdown rate. In Br<sup>-</sup>-containing solutions, 12aneN<sub>3</sub> addition caused a more modest (16–64%) increase

TABLE 1. Pseudo-First-Order Rate Constants for Daminozide and BH Breakdown in the Presence of 1.0 mM Cu<sup>II</sup> Alone or in the Presence of Both 1.0 mM Cu<sup>II</sup> and 1.0 mM Organic Ligand<sup>a</sup>

daminozide											
inorg salt	salt concn (M)	Cu <sup>II</sup> alone		Cu <sup>II</sup> plus 12aneN <sub>3</sub>		Cu <sup>II</sup> plus EN		Cu <sup>II</sup> plus HEEN		BH Cu <sup>II</sup> alone	
		<i>k</i> <sub>obs</sub> (h <sup>-1</sup> )	K/k <sub>o</sub>	<i>k</i> <sub>obs</sub> (h <sup>-1</sup> )	K∕/k₀	<i>k</i> <sub>obs</sub> (h <sup>-1</sup> )	K∕/k₀	<i>k</i> <sub>obs</sub> (h <sup>-1</sup> )	K∕/k₀	<i>k</i> <sub>obs</sub> (h <sup>-1</sup> )	K/k <sub>o</sub>
NaNO <sub>3</sub>	0.003 0.013 0.053 0.103	$\begin{array}{c} 2.06 \pm 0.02 \times 10^{-4} \\ 1.70 \times 10^{-4} \\ 1.67 \times 10^{-4} \\ 1.62 \times 10^{-4} \end{array}$	1.0 0.8 0.8	$1.58 \times 10^{-4}$	0.8					$1.01 \times 10^{-4}$	1.0
NaCl	0.103 0.003 0.013 0.053	$5.31 \pm 0.87 \times 10^{-4}$ $1.49 \pm 0.12 \times 10^{-3}$ $6.36 \pm 0.75 \times 10^{-3}$ $1.24 \pm 0.02 \times 10^{-2}$	2.6 7.2 30.8	$3.09 \times 10^{-3}$ $1.37 \times 10^{-2}$ $2.67 \times 10^{-2}$	15.0 66.5	$2.13 \times 10^{-4}$ $2.55 \times 10^{-3}$ $4.07 \times 10^{-3}$	1.0 12.4	$2.00 \times 10^{-4}$ $2.59 \times 10^{-3}$ $7.57 \times 10^{-3}$	1.0 12.6	$1.57 \times 10^{-4}$ $3.97 \times 10^{-4}$ $8.27 \times 10^{-4}$ $1.47 \times 10^{-3}$	1.6 3.9 8.2
NaBr	0.103 0.010 0.050 0.100	$\begin{array}{c} 1.36 \pm 0.02 \times 10^{-2} \\ 1.20 \pm 0.11 \times 10^{-3} \\ 8.02 \pm 0.26 \times 10^{-3} \\ 1.77 \pm 0.16 \times 10^{-2} \end{array}$	5.8 5.8 38.9 85.8	$\begin{array}{c} 2.07 \times 10^{-2} \\ 1.97 \times 10^{-3} \\ 1.11 \times 10^{-2} \\ 2.06 \times 10^{-2} \end{array}$	9.6 53.7 99.8	$\begin{array}{c} 8.07 \times 10^{-3} \\ 3.31 \times 10^{-4} \\ 8.02 \times 10^{-3} \\ 6.30 \times 10^{-3} \end{array}$	29.4 1.6 11.9 30.6	7.57 × 10 °	30.7	1.47 × 10 °	14.5

<sup>a</sup> The initial concentration of daminozide and BH was 1.0 mM. The rate constants were determined in various inorganic salt media at pH 5 (5–10 mM acetate buffer) and 25 °C.  $k_0$  is defined as the rate constant measured in halide ion-free and organic ligand-free medium (0.003 M NaNO<sub>3</sub>).  $K/k_0$  reveals the enhancement or inhibition in rate constant arising from addition of halide ion and organic ligand.

in daminozide breakdown rate (Table 1). Cyclen and 12ane $N_3$  were also added to  $Zn^{II}$ -containing solutions at pH 7.5; no daminozide breakdown was observed (data not shown in Table 1).

**Daminozide** – **Cu<sup>II</sup> Complex Formation.** D<sub>2</sub>O solutions of 5 mM daminozide were added with up to 5 mM of Cu(NO<sub>3</sub>)<sub>2</sub> at pH 5 and were immediately analyzed by H NMR. Daminozide breakdown was confirmed to be negligible during the acquisition time of H NMR spectra. Severe peak broadening occurred at low Cu<sup>II</sup> concentrations (10–40% of daminozide concentration) and increased as Cu<sup>II</sup> concentration was increased, indicating complexation between daminozide and Cu<sup>II</sup>. The H NMR spectra are provided in the Supporting Information (Figure S1.A-D).

Daminozide contains two identical methyl groups bound to the  $\beta$ -nitrogen of hydrazide and two linked methylene groups (one coordinated to the carboxylate group, and the other coordinated to the hydrazide linkage, Figure 1). Peak broadening was much more severe at the signals of the methylene groups than at the signal of the methyl groups, indicating close proximity of coordinated Cu<sup>II</sup>, most likely at the carboxylate or carbonyl oxygen donor groups. Signals from the two methylene groups could not, however, be readily distinguished from one another.

Differences in the UV spectrum of the metal ion–ligand complex in comparison to the spectrum of the free metal ion plus free ligand can be used to determine the stoichiometry of the complex and its associated stability constant (19). Absorbance measurements at 240–280 nm were used in this study, since inorganic Cu<sup>II</sup> complexes and daminozide-bound Cu<sup>II</sup> absorb at these wavelengths but the free daminozide species does not. At pH 5, addition of daminozide at a fixed CuCl<sub>2</sub> concentration ( $5.0 \times 10^{-4}$  M) resulted in significant changes in the Cu<sup>II</sup> spectrum as shown in Figure 5A. Separate experiments confirmed that Cu<sup>II</sup> dimerization was not significant under these conditions.

Job's method (20, 21) involves measuring the absorbance of a series of solutions in which the total ligand concentration ( $L_T$ ) and the total metal ion concentration ( $Cu_T$ ) are varied, but their sum is held constant. Using  $L_T + Cu_T = 1$  mM, 0.1 M NaCl, and pH 5, the  $\Delta$ (absorbance) was determined and plotted against the ratio of  $Cu_T/L_T$  (Figure 5B). Maximum  $\Delta$ (absorbance) occurred when  $L_T = Cu_T$ , indicating that 1:1  $Cu^{II}$ —daminozide complex is predominant. The same complex stoichiometry was also obtained when 0.10 M NaCl was replaced with either 0.10 M NaBr or 0.10 M NaClO<sub>4</sub>. Experiments were conducted at several different pH values.  $\Delta$ (absorbance) increased with increasing pH until the point

was reached when Cu<sup>II</sup> (hydr)oxide precipitation limited Cu<sup>II</sup> solubility (Figure 5C).

For 1:1 ML complex formation, the UV absorbance change from complexation typically exhibits a hyperbolic dependence on free ligand concentration (i.e., a complex formation isotherm)

$$\frac{\Delta(\text{absorbance})}{b} = \frac{\text{Me}_{\text{T}}K_{11}\Delta\epsilon_{11}[L]}{1+K_{11}[L]}$$
(3)

where *b* is the path length (1.0 cm in our experiments),  $K_{11}$  is the complex formation constant, and  $\Delta \epsilon_{11} = \epsilon_{11} - \epsilon_{Me} - \epsilon_L$  (the molar absorptivity of ML complex minus the absorptivities of free metal ion and free ligand) (*19*). When Me<sub>T</sub>  $\ll L_T$ , it can be assumed that  $[L] \simeq L_T$ .  $\Delta$ (absorbance) values as a function of total daminozide concentration ( $L_T$ ) were obtained using a complexometric titration at a fixed total metal ion concentration (Cu<sub>T</sub> = 0.5 mM), yielding the hyperbolic plot shown in Figure 5D.

The double-reciprocal of eq 3 is termed the Benesi-Hildebrand equation (*22*):

$$\frac{1}{\Delta(\text{absorbance})} = \frac{1}{\text{Me}_{\text{T}}K_{11}\Delta\epsilon_{11}} \times \frac{1}{[L]} + \frac{1}{\text{Me}_{\text{T}}\Delta\epsilon_{11}} \quad (4)$$

By plotting  $[L]^{-1}$  versus  $\Delta$ (absorbance)<sup>-1</sup>, the complex formation constant  $K_{11}$  can be determined from the slop and the *y*-axis intercept. Applying the Benesi-Hildebrand approach to the data in Figure 5D yielded  $K_{11}$  of 0.28 for the Cu<sup>II</sup>-daminozide complex.

#### Discussion

**SA Hydrolysis.** Unlike daminozide and BH, SA exhibits appreciable loss rates in metal ion-free solution. Loss rates are not affected by metal ion addition, ruling out oxidation by  $Cu^{II}$ . Hydrolysis is the only via mechanism for SA breakdown.

There are several reasons why SA should hydrolyze more rapidly than daminozide or BH. Back-bonding by the  $-NHNR_2$  moiety should yield a greater electron density on the carbonyl carbon of daminozide and BH than observed with the  $-NR_2$  moiety of SA. Greater electron density lowers electrophilicity and hence decreases hydrolysis rates. Deprotonation of the nitrogen adjacent to the carbonyl carbon is possible with daminozide and BH but not with SA. A deprotonation reaction of this kind increases electron density at the carbonyl carbon, which again lowers hydrolysis rates (23).



FIGURE 5. Complexation of Cu<sup>II</sup> and daminozide: (A) UV spectra of solutions containing CuCl<sub>2</sub> alone, daminozide alone, and CuCl<sub>2</sub> plus daminozide at pH 5.0. (B) Job's plot for the complexation of Cu<sup>II</sup> and daminozide at pH 5.0. The total concentration of daminozide and Cu<sup>II</sup> ions is held constant at 1.0 mM. (C) Effect of pH on the  $\Delta$ (absorbance) for daminozide and Cu<sup>II</sup> complex formation. (D)  $\Delta$ (absorbance) at increasing daminozide concentration with 0.5 mM CuCl<sub>2</sub> at pH 5.0.

Amides of succinic acid and other short-chain dicarboxylic acids are subject to intramolecular catalysis by the auxiliary carboxylate group (24-26). The increase in breakdown rate with decreasing pH is most readily explained by a general acid catalysis mechanism.

Although the extent of complex formation between  $Cu^{II}$  and SA was not investigated, it should be comparable in magnitude to values observed for the analogous daminozide complex ( $K \approx 0.28$ ). Using equimolar  $Cu^{II}$  and substrate concentrations and the medium conditions employed in our experiments, approximately 19% of substrate molecules should be complexed. This is a high enough percentage to reveal catalytic effects if present but not enough to reveal inhibitory effects of  $Cu^{II}$  coordination. Hence, we can conclude that  $Cu^{II}$  coordination does not catalyze SA hydrolysis but can make no statement regarding inhibition by coordinated  $Cu^{II}$ .

**Cu<sup>II</sup> Coordination and Hydrazide Breakdown.** Both H NMR and UV spectroscopy reveal the presence of a 1:1 Cu<sup>II</sup>– daminozide complex. As mentioned earlier, equimolar concentrations of Cu<sup>II</sup> and daminozide yield approximately 19% of daminozide molecules complexed. H NMR indicates that Cu<sup>II</sup> coordination to at least one of the carbonyl groups is taking place but provides no evidence regarding the relative importance of the auxiliary carboxylate group versus the carbonyl oxygen of the hydrazide group. The auxiliary carboxylate group has a p $K_a$  of 4.42 as estimated by the SPARC computer program (*27*). Because of its negative charge and greater basicity, the auxiliary carboxylate group forms a much stronger bond to Cu<sup>II</sup> than the carbonyl oxygen of the amide group.





The hydrazide linkage undergoes tautomerization as shown in Figure 6 (28). The basicity of the  $\alpha$ -nitrogen is considerably less than that of simple amines, owing to the electron withdrawing nature of the neighboring carbonyl group (29, 30). It is, however, greater than an amide nitrogen due to the neighboring  $\beta$ -nitrogen group. Simultaneous coordination of the carbonyl oxygen and the  $\alpha$ -nitrogen is unlikely, owing to the instability of four-membered chelate rings. Simultaneous coordination of the auxiliary carboxylate group and the  $\alpha$ -nitrogen, forming a seven-membered chelate ring, is expected to be weak. The  $\beta$ -nitrogen, in contrast, can coordinate a central metal ion along with the carbonyl oxygen to form an optimally sized, five-membered chelate ring. BH lacks the auxiliary carboxylate group but nevertheless is subject to Cu<sup>II</sup>-catalyzed breakdown. This observation lends support to the idea that coordination to atoms within the hydrazide linkage takes place.

Added ligand effects are strong, especially with Cl<sup>-</sup> and Br<sup>-</sup>. An expansion of  $k_{obs}$  can be written that acknowledges differences in reactivity between distinct Cu<sup>II</sup>-containing species, using a procedure similar to Millero et al. (*31*):

$$k_{\rm obs} = k_{\rm Cu}^{2+} [{\rm Cu}^{2+}] + k_{\rm CuOH^+} [{\rm CuOH^+}] + k_{\rm CuAc^+} [{\rm CuAc^+}] + k_{\rm CuX_+} [{\rm CuX^+}] + k_{\rm CuX_2} [{\rm CuX_2}^0] + \dots, \text{ etc.}$$
(5)



FIGURE 7. Plot of  $k_{obs}$ /[Cu<sup>2+</sup>] versus halide ion concentration for the Cu<sup>II</sup>-facilitated breakdown of daminozide and BH. Reactions contained 1.0 mM daminozide or BH, 1.0 mM Cu<sup>II</sup>, 5.0 mM pH 5 acetate buffer at 25 °C.

Using a preequilibrium assumption for  $Cu^{\rm II}$  speciation, this equation can be rewritten as

$$k_{\rm obs} = [{\rm Cu}^{2+}](k_{\rm Cu}^{2+} + k_{\rm CuOH^+}\beta_{\rm CuOH^+}[{\rm OH}^-] + k_{\rm CuAc^+}\beta_{\rm CuAc^+}[{\rm Ac}^-] + k_{\rm CuX^+}\beta_{\rm CuX^+}[{\rm X}^-] + k_{\rm CuX_2}\beta_{\rm CuX_2}[{\rm X}^-]^2 + ..., \text{ etc.})$$
(6)

where Ac<sup>-</sup> represents the acetate ion buffer and X<sup>-</sup> represents Cl<sup>-</sup> or Br<sup>-</sup>. For each species i,  $k_i$  is the corresponding rate constant and  $\beta_i$  is the complex formation constant.

Suppose that  $Cl^-$  or  $Br^-$  concentration is increased while other medium constituents (e.g. acetate concentration, pH) are fixed. Equation 6 can be simplified to the following equation

$$\frac{k_{\rm obs}}{[{\rm Cu}^{2+}]} = k_0 + k_{{\rm Cu}{\rm X}^+}\beta_{{\rm Cu}{\rm X}^+}[{\rm X}^-] + k_{{\rm Cu}{\rm X}_2}\beta_{{\rm Cu}{\rm X}_2}[{\rm X}^-]^2 \quad (7)$$

where  $k_0$  represents the aggregate effect of all halide ion-free Cu<sup>II</sup> species. For both daminozide and BH, plots of  $k_{obs}$ / [Cu<sup>2+</sup>] versus [X<sup>-</sup>] at pH 5.0 are linear (Figure 7), indicating that halide ion effects primarily reflect the 1:1 complexes Cu<sup>II</sup>Cl<sup>+</sup> and Cu<sup>II</sup>Br<sup>+</sup>; higher order terms can be ignored. Values for  $k_0$ , measured in halide ion-free solutions, are 0.27 M<sup>-1</sup> h<sup>-1</sup> for daminozide and 0.14 M<sup>-1</sup> h<sup>-1</sup> for BH. For daminozide, the rate constant for Cu<sup>II</sup>Cl<sup>+</sup> is equal to 132 M<sup>-1</sup> h<sup>-1</sup>, and the rate constant for Cu<sup>II</sup>Cl<sup>+</sup> is 307 M<sup>-1</sup> h<sup>-1</sup>. For BH, the rate constant for Cu<sup>II</sup>Cl<sup>+</sup> is 12.8 M<sup>-1</sup> h<sup>-1</sup>. Experiments with the other added ligands (EN, HEEN, 12aneN<sub>3</sub>, and cyclen) employed a single ligand concentration, and therefore rate constants corresponding to distinct Cu<sup>II</sup>–ligand complexes could not be calculated.

**Cu<sup>II</sup>-Facilitated Hydrazide Breakdown: Is It Hydrolysis?** Hydrolysis of RC(O)NHN(CH<sub>3</sub>)<sub>2</sub> yields RCOO<sup>-</sup> and NH<sub>2</sub>N-(CH<sub>3</sub>)<sub>2</sub> (UDMH). As far as RCOO<sup>-</sup> is concerned, stoichiometric conversion of daminozide into succinate and BH into butyrate was observed whenever Cu<sup>II</sup> was present. Our method for detecting UDMH was sensitive enough to detect stoichiometric production via daminozide and BH hydrolysis. The fact that detectable levels of UDMH were not observed indicates that either (i) another breakdown pathway is partially or completely responsible for daminozide breakdown or that (ii) UDMH is unstable under the experimental conditions employed (1.0 mM Cu<sup>II</sup>, saturation with respect to 0.21 atm  $O_2$ ).

Metal ion-catalyzed hydrolysis is believed to take place via (i) metal ion coordination of the substrate, (ii) metal ion coordination of the attacking nucleophile, (iii) metal ion coordination of the leaving group, or a combination of these three mechanisms (*11, 23, 32*). As far as Mechanisms i and iii are concerned, the greater propensity of Cu<sup>II</sup> to coordinate anionic oxygen- and neutral nitrogen-donor ligands should make it a better catalyst than the other three +II metal ions (Ni<sup>II</sup>, Zn<sup>II</sup>, and Pb<sup>II</sup>) examined.

Mechanism ii has been strongly tied to the ability of metal ions to induce deprotonation of coordinated water through the following equilibrium reaction:

$$Me(X)(H_2O)^{2+} = Me(X)(OH)^+ + H^+ *K_1 = \frac{[Me(X)(OH)^+][H^+]}{[Me(X)(H_2O)^{2+}]}$$
(8)

For the present discussion, X represents five coordinated water molecules.  $p^*K_1$  represents the pH where concentrations of  $Me(H_2O)_6^{2+}$  and  $Me(H_2O)_5(OH)^+$  are equal.  $Cu^{II}$  and  $Pb^{II}$  (possessing  $p^*K_1$  of 7.5 and 7.6, respectively) are far better at inducing the deprotonation of water than  $Ni^{II}$  and  $Zn^{II}$  (with  $p^*K_1$  values of 9.9 and 9.0, respectively) (*17*). Our observation that  $Cu^{II}$  yields far greater daminozide and BH breakdown rates than observed with  $Pb^{II}$  eliminates Mechanism ii as a significant mechanism but does not offer insight into the importance of Mechanisms i or iii.

In order for EN, HEEN, 12aneN<sub>3</sub>, and cyclen to raise or lower rates of hydrolysis via mechanisms i or iii, ternary metal ion-substrate-organic ligand complexes must be invoked. For reasons similar to those discussed in the preceding paragraph, Cu<sup>II</sup> should perform better than the other three +II metal ions if such ternary complexes are important. As far as Mechanism ii is concerned, the lowering of  $p^*K_1$  values by coordinated N-donor ligands has been carefully documented (e.g., refs 33 and 34).  $p^*K_1$  values for Cu<sup>II</sup>(12aneN<sub>3</sub>)- $(H_2O)_3^{2+}$  and  $Cu^{II}(cyclen)(H_2O)_2^{2+}$  (8.14 and 8.2, respectively (17)) are quite similar to one another and higher than reported earlier for  $Cu(H_2O)_6^{2+}$ . A decrease in breakdown rate upon 12aneN3 and cyclen addition would be expected if Mechanism ii is operative, and both ligands should yield a similar effect. Cyclen, however, has been observed to completely inhibit daminozide and BH breakdown under all conditions examined; 12aneN<sub>3</sub> slightly inhibits breakdown in halide ion-free solutions and actually accelerates breakdown in halide ioncontaining solutions. Hence, ligand effects on Cu<sup>II</sup> catalysis via Mechanism ii are not in accord with experimental observations.

 $p^*K_1$  values for  $Zn^{II}(12aneN_3)(H_2O)_3^{2+}$  and  $Zn^{II}(cyclen)-(H_2O)_2^{2+}$  (7.4 and 8.02, respectively) are significantly lower than for  $Zn(H_2O)_6^{2+}$  (9.0) (17). We would therefore expect that 12aneN<sub>3</sub> and cyclen addition would accelerate  $Zn^{II}$ -catalyzed hydrolysis via Mechanism ii. The lack of discernible daminozide and BH breakdown in  $Zn^{II}$  plus 12aneN<sub>3</sub> and  $Zn^{II}$  plus cyclen experiments is additional evidence that hydrolysis via Mechanism ii is not significant.

In earlier work with the pesticide dimetilan (11), millimolar concentrations of HEEN enhanced Cu<sup>II</sup>- and Zn<sup>II</sup>-catalyzed hydrolysis, while comparable concentrations of EN only inhibited the reaction. A modification of Mechanism ii is believed responsible; HEEN possesses a pendant alcohol group that undergoes metal ion-induced deprotonation, generating a nucleophile. As far as daminozide is concerned, EN and HEEN caused nearly equal decreases in breakdown



<sup>a</sup> Copper coordination geometry: oct = octahedral, tbp = trigonal bipyrimidal, sp = square pyramidal, and tet = tetrahedral.

TABLE 2. Experimentally Determined Rate Constant  $k_i$  for Copper Species and Standard One-Electron Reduction Potentials for Cu<sup>II</sup>/Cu<sup>II</sup> Redox Couples at 25 °C<sup>b</sup>

species	<i>k</i> i (daminozide)	<i>k</i> i (BH)	half reaction	E° (volts)	ref
Cu <sup>2+</sup> CuCl <sup>+</sup> CuBr <sup>+</sup>	0.27 M <sup>-1</sup> h <sup>-1</sup> 132 307	0.14 M <sup>-1</sup> h <sup>-1</sup> 12.8	$\begin{array}{l} Cu^{2+} + e^- \rightarrow Cu^+ \\ CuCl^+ + e^- \rightarrow CuCl^0(aq) \\ CuBr^+ + e^- \rightarrow CuBr^0(aq) \\ CuCl_2^0(aq) + e^- \rightarrow CuCl_2^- \\ 2CuO(s) + H_2O + 2e^- \rightarrow Cu_2O(s) + 2OH^- \end{array}$	0.153 0.330 0.386 0.567 -0.22	(36) (36) a (36) (37)

<sup>a</sup> Deduced in this study. <sup>b</sup> Ionic strength = 0.0 mole-equiv/L.

rates. Again, hydrolysis (or nucleophilic attack) via Mechanism ii appears to be inoperative.

Numerous points have been raised against metal ioncatalyzed hydrolysis via Mechanism ii, and evidence for catalysis via Mechanisms i and iii is at best inconclusive. One final point remains. Despite the fact that  $Cl^-$  and  $Br^$ do not significantly affect the logK for  $Cu^{II}$ -daminozide complex formation, breakdown reactions exhibit a strong, first-order dependence on halide ion concentrations ( $Cl^$ and  $Br^-$ ). We cannot think of a plausible explanation for such strong halide ion effects on hydrolysis reactions. Instead, daminozide and BH oxidation in the presence of  $Cu^{II}$  must be invoked.

**Cu<sup>II</sup>-Facilitated Hydrazide Oxidation: An Overview.** Since no efforts were taken to prevent contact with air, it can be presumed that all reaction solutions contained dissolved oxygen, a potential oxidant. Daminozide and BH breakdown were observed in copper-containing solutions but not in Ni<sup>II</sup>containing, Zn<sup>II</sup>-containing, or metal ion-free solutions. We can therefore assume that changes in copper oxidation state, or the relative accessibility of the Cu<sup>II</sup> LUMO orbital, are important aspects of the copper-facilitated reaction. Scheme 1 serves as a guide for this discussion. X refers to the contents of the inner coordination sphere of copper during electron transfer.

**Oxidation: Halide Ion Coordination and pH Effects.** The oxidation of ascorbic acid by Cu<sup>II</sup> is acutely sensitive to chloride ion concentrations (35). By analogy, the halide ion effects observed in our experiments involving daminozide and Cu<sup>II</sup> represent strong evidence for an oxidative mechanism. For the sake of discussion, the assumption that Cu<sup>2+</sup>-(aq) dominates Cu<sup>II</sup> speciation in halide ion-free solutions is a good one  $(k_0 \approx \hat{k}_{Cu}^{2+}; \text{ complexes with acetate have a})$ negligible effect). We can then compare relative rate constants calculated using eq 7 with standard reduction potentials listed in Table 2 (36, 37). With daminozide,  $\ln k_{CuX}^+$  increases linearly with increasing  $E^{\circ}$  ( $r^2 = 0.985$ ). As discussed earlier, halide ions coordinate Cu<sup>I</sup> far more strongly than Cu<sup>II</sup>. Partial Cu<sup>I</sup> character in the transition state would explain the emerging structure-reactivity relationship seen here. Only two points are available for reaction with BH, but the same relationship appears;  $Cu^{II}Cl^+(aq)$ , with the higher value of  $E^\circ$ , reacts more quickly than  $Cu^{2+}(aq)$ .

The preceding paragraph and Table 2 make it clear that each halide ion coordinated to  $Cu^{II}$  raises  $E^{\circ}$ , making  $Cu^{II}$  a stronger oxidant in a thermodynamic sense. In contrast,  $E^{\circ}$ for the  $Cu^{II}O(s)/Cu^{I}_{2}O(s)$  half-reaction is only -0.22 V (Table 2). Hence, coordination by oxo ions ( $O_{2}^{-}$ ) (and by inference hydroxide ions ( $OH^{-}$ )) makes  $Cu^{II}$  a weaker oxidant in a thermodynamic sense. This fact, along with the sequestration of  $Cu^{II}$  within precipitated particles, may be responsible for the drop off in daminozide and BH breakdown rates once the solubility with respect to (hydr)oxides is exceeded.

**Oxidation:** Nitrogen-Donor Ligand Effects. Our discussion of the effects of added EN, HEEN, cyclen, and 12aneN<sub>3</sub> is made difficult by the paucity of thermodynamic data for the Cu<sup>I</sup> complexes. EN and HEEN pose few constraints on the geometry of coordinated copper. If  $E^{\circ}$  for the Cu<sup>II</sup>(EN)<sub>2</sub><sup>2+/</sup> Cu<sup>I</sup>(EN)<sub>2</sub><sup>+</sup> half reaction (-0.014 V (*38*)) serves as any indication, coordination to aliphatic amine groups generally makes Cu<sup>II</sup> a weaker oxidant in a thermodynamic sense. It is reasonable, therefore, for coordination by EN and HEEN to decrease daminozide breakdown rates.

**Oxidation: Macrocyclic Ligand Effects.** Unlike the monoand bidentate ligands discussed so far, cyclen and 12aneN<sub>3</sub> raise issues regarding the coordination number and geometry of Cu<sup>II</sup> parent and Cu<sup>I</sup> product complexes. As summarized in Scheme 1, Cu<sup>II</sup> prefers six-coordinate (distorted octahedral) or five-coordinate (trigonal bipyramidal or square pyramidal) geometry, while Cu<sup>I</sup> prefers four-coordinate (tetrahedral) geometry (*39*). Cu<sup>I</sup> is a softer, more polarizable, and more hydrophobic metal ion than Cu<sup>II</sup>.

In metal ion-cyclen complexes, the four nitrogen donor atoms typically adopt a square planar configuration with the metal ion held slightly above this plane (40). This **meridional geometry** imposed by cyclen works well for  $Cu^{II}$ ; a coordination shell of five is achieved by placing the fifth Lewis Base axial relative to the plane defined by the cyclen ligand (12). Meridional geometry does not, however, allow  $Cu^{I}$  to adopt the tetrahedral configuration that it prefers.

The  $12aneN_3$  macrocycle is not large enough to wrap completely around a central metal ion. Instead,  $12aneN_3$ imposes a **facial geometry** on its complexes; the three nitrogen donor atoms all reside on one side of the central metal ion (12). With Cu<sup>II</sup>, either a coordination number of six (octahedral) or five (trigonal bipyramidal) are possible. In both configurations, two nitrogen donor groups reside in the equatorial plane, with the third in an axial position. 12aneN<sub>3</sub> readily coordinates Cu<sup>I</sup> via a tetrahedral geometry.

Zn<sup>II</sup>–12aneN<sub>3</sub> complexes have been extensively studied because of their resemblance to carbonic anhydrase and carboxypeptidase enzymes (*41*). It is interesting to note that coordination to 12aneN<sub>3</sub> changes the preference of Zn<sup>II</sup> for ligands in the fifth (and in some cases sixth) coordination position. Zn<sup>2+</sup>(aq) binds halide ions in the order F<sup>-</sup> > Cl<sup>-</sup> > Br<sup>-</sup> > I<sup>-</sup>, while Zn<sup>II</sup>(12aneN<sub>3</sub>)<sup>2+</sup> binds halide ions in the order I<sup>-</sup> > Br<sup>-</sup> > Cl<sup>-</sup> > F<sup>-</sup> (*42*). The somewhat hydrophobic 12aneN<sub>3</sub> ligand apparently causes Zn<sup>II</sup> to shift from "hard" to "soft" character, improving its ability to coordinate polarizable (soft) ligand donor atoms (*43*). If this same phenomenon applies to copper complexes, then the overall effect would be to shift the coordination properties of Cu<sup>II</sup> in a direction that more closely matches those of Cu<sup>I</sup>.

Preceding paragraphs have established that the meridional geometry of cyclen coordination is suitable for  $Cu^{II}$  but not for  $Cu^{I}$  and that the facial geometry of  $12aneN_3$  coordination is suitable for both oxidation states. Hence, the acceleratory effect arising from  $12aneN_3$  addition very likely arises from the **entatic state** of the coordinated  $Cu^{II}$  (*39, 44, 45*);  $12aneN_3$  forces  $Cu^{II}$  to adopt a coordination geometry that is similar to the transition state for the conversion of  $Cu^{II}$  to  $Cu^{I}$ .  $12aneN_3$  may also encourage coordination of halide ions  $(Br^- > Cl^-)$  in the fifth (and possibly sixth) coordination positions, forcing the geometry of the  $Cu^{II}$  complex to match the geometry of the transition state even more closely. Hence, all the synergistic and antagonistic ligand effects observed in this work make sense in terms of the known geometries of corresponding  $Cu^{II}$  and  $Cu^{I}$  complexes.

**Oxidation: Reactivity Comparisons.** Prior studies of the oxidation of daminozide by chloroperoxybenzoic acid, sodium hypochlorite, hydrogen peroxide, and singlet oxygen (46, 47) offers little insight into reactions with transition metal ion oxidants such as Cu<sup>II</sup>. Oxidation of N',N'-disubstituted hydrazides by Pb<sup>IV</sup> acetate and MnO<sub>2</sub> in aqueous acetic acid has been investigated for synthetic applications (6). Yields of the corresponding carboxylic acid were between 85 and 95% (6), in agreement with our own observation of sto-ichiometric conversion of daminozide to succinic acid and BH into butyric acid. Oxidations by Pb<sup>IV</sup> acetate in dry organic solvents yielded other products that presumably hydrolyze when water is present; these products suggest that the  $\beta$ -nitrogen serves as the initial electron donor in the degradative mechanism (6).

Hydrazine and 1,1-disubstituted hydrazines are readily oxidized by  $Cu^{II}$  salts in aqueous solutions, forming diazenes and tetrazenes, along with  $Cu^{I}$  (48). The electron-withdrawing nature of the carbonyl group within hydrazides is expected to substantially diminish reactivity. In dry organic solvents,  $Cu^{II}$  acetate and  $Cu^{II}$  acetylacetonate have been used to oxidize hydrazides to their corresponding carboxylic acids and dinitrogen (49).

 $\pi$ -Donor ligands that bind Cu<sup>I</sup> more strongly than Cu<sup>II</sup> substantially increase the oxidizing ability of Cu<sup>II</sup>. Substituted phenanthroline ligands, for example, can enable Cu<sup>II</sup> to oxidize even simple amines (*50*, *51*), including the Good buffer HEPES. Effects of the ligands employed in our study (EN, HEEN, 12aneN<sub>3</sub>, and cyclen) have not been previously explored.

**Oxidation:** The Role of Molecular Oxygen. Evidence already presented indicates that  $Cu^{II}$  is reduced to  $Cu^{I}$  as the oxidative degradation of daminozide and BH takes place.  $O_2(aq)$ , present in all our reaction solutions, may play one or more roles: (i) in accordance with Scheme 1,  $O_2(aq)$  may reoxidize  $Cu^{II}$  to  $Cu^{II}$ ; (ii)  $O_2(aq)$  may form adducts with copper such as  $Cu^{IO}2^+$  that react with substrate in unique ways; and

(iii) reactive oxygen intermediates  $O_2^{-}$ ,  $H_2O_2$ , and HO-(generated during Cu<sup>I</sup> oxidation by  $O_2(aq)$ ) may react with substrate or substrate degradation products, yielding new products (*52*).

The autoxidation of Cu<sup>I</sup> represents a mean of regenerating Cu<sup>II</sup> oxidant as substrate is being consumed, rendering a catalytic role of Cu<sup>II</sup> in the oxidation of daminozide and BH by oxygen. In our experiments, the initial Cu<sup>II</sup> concentration (1-3 mM) is always equal to or greater than the initial substrate concentration (1 mM), hence the first electron oxidation of substrate is not limited by the availability or regeneration of Cu<sup>II</sup> oxidant. The cycling of Cu<sup>II</sup>/Cu<sup>I</sup>, however, likely affects the formation of oxidation products. Prior work by Brown and Casida (1998) (46, 47) reported oxidation of daminozide by hydrogen peroxide and singlet oxygen. If the reactive oxygen intermediates (O2<sup>--</sup>, H2O2, and HO·) are involved in substrate oxidation, the complexity of reaction pathways and product formation is likely significantly increased. To discern the influence of oxygen, further studies should be conducted in O2-free conditions, where breakdown of daminozide and BH is expected to slow over time as Cu<sup>II</sup> is consumed and Cu<sup>I</sup> species are accumulated.

**Environmental Significance.** Copper salts (e.g., copper sulfate) are widely used fungicides in many agricultural applications (*53*) and are also commonly used in greenhouses and nurseries (*5*). Therefore the  $Cu^{II}$ -facilitated oxidation of daminozide is likely a viable transformation process for daminozide and will be sensitive to chloride or bromide concentration in the surroundings. Further investigations on the oxidation products of daminozide will benefit risk assessment since some of the potential products may be toxic. Similar reactions are likely important for other environmentally relevant hydrazide compounds and need to be further explored.

This study expands our earlier demonstration that complexing organic ligands yield synergistic or antagonistic effects with metal ions in facilitating hydrolysis reaction (11) to redox transformations of organic pollutants. This study illustrates that coordination with organic ligands influences the geometry and ligand preference of central metal ion and thus changes the susceptibility of central metal ion to electrontransfer reactions. Comparing the effects of different ligands provides a powerful tool for elucidating mechanisms of metalfacilitated organic pollutant degradation. Such an approach, although frequently used by bioinorganic chemists to elucidate mechanisms of metalloenzymes in facilitating chemical reactions, has only been used by a limited number of environmental scientists and merits further attention. This study also adds to the few studies (e.g., ref 54) that quantitatively link metal ion speciation and metal ion reactivity in the cases of organic pollutant transformation.

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#### Supporting Information Available

Stability constants for Cu(II/I) and halide complexes at 25  $^\circ C$  (Table S1) and H-NMR spectra of 5.0 mM daminozide

with increasing Cu(II) concentration at pH 5.0 (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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