

## Reductive Cleavage of Hydroxylamine Derivatives by Dihydrolipoic Acid-Iron(II)

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The reduction of hydroxylamine derivatives was attempted with dihydrolipoic acid-iron(II) to obtain corresponding alcohols and amines in high yields under a mild condition (pH 9.1, 30 °C). Some dithiols other than dihydrolipoic acid which cyclize to form disulfides were also useful for the reduction, and ferrous ion worked as an effective catalyst. From the spectroscopic and kinetic studies, the reduction was found to proceed through 1:1:1 complex formation among dihydrolipoic acid, ferrous ion, and N-O compounds.

Lipoic acid (LA) is a well-known coenzyme important in the oxidative decarboxylation of  $\alpha$ -keto acids in living systems. As part of the investigation on the syntheses and reactions of LA derivatives, we have already reported the silver ion promoted acyl transfer of *S*-acyl or *S,S'*-diacyldihydrolipoamides to nucleophiles such as amines and alcohols and demonstrated the application to a polymer bearing lipoamide structure to provide polymeric acylating agents.<sup>1,2</sup>

Furthermore, the redox system of LA  $\rightleftharpoons$  dihydrolipoic acid (DHLA) is considered to be attractive as an effective redox reagent. It has been reported that dihydrolipoamide attached to polymer supports such as glass beads<sup>3</sup> and Sephadex<sup>4</sup> can be utilized in the regeneration of the SH group from oxidized SH enzymes such as papain.

Takahara and co-workers have reported<sup>5</sup> that canavaninosuccinic acid (1) might be cleaved to guanidinosuccinic acid (2) and homoserine (3) with DHLA-Fe(II) (Scheme I). This finding is extremely interesting in view of the reactivity of dithiol-Fe complexes which have some similarities with ferredoxins in biological systems and the applicability of the reaction to the reductive cleavage of N-O compounds such as hydroxylamine derivatives or oximes.

Though N-substituted hydroxylamines have been reduced to amines with Zn-HCl,<sup>6</sup> lithium aluminum hydride,<sup>7</sup> or diborane,<sup>8</sup> these reactions must be carried out under severe conditions or with nonselective reagents, leading to poor selectivities.

We report here the reductive cleavage of N-O compounds with the DHLA-Fe(II) system under mild conditions and with a high specificity, together with kinetic considerations.

### Results and Discussion

**Reduction of *O*-Benzylhydroxylamine by DHLA-Fe(II).** The reduction of *O*-benzylhydroxylamine (4) was carried out by DHLA in the presence of a catalytic amount

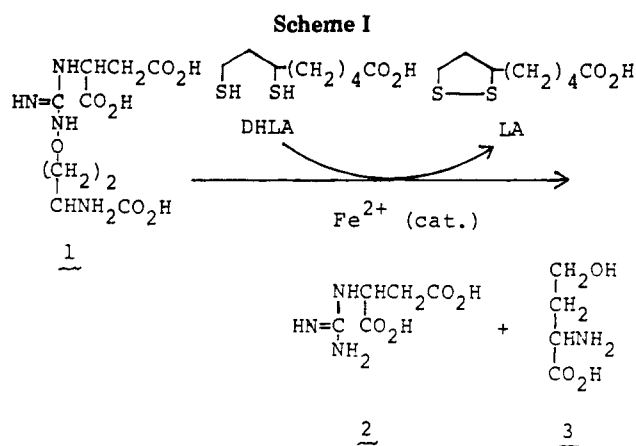
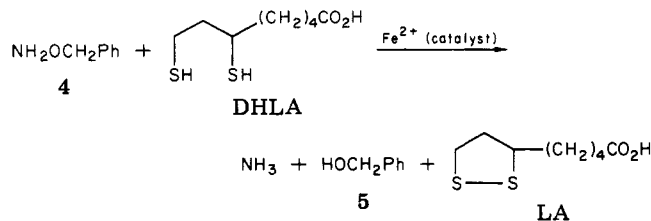


Table I. Reduction of *O*-Benzylhydroxylamine (4) by DHLA-Fe(II)<sup>a</sup>

reducing agent (concn, M)	yield of 5, %
DHLA ( $1.25 \times 10^{-2}$ ) + Fe <sup>2+</sup> ( $5.00 \times 10^{-5}$ )	95
DHLA ( $1.25 \times 10^{-2}$ )	0
Fe <sup>2+</sup> ( $5.00 \times 10^{-5}$ )	0
Fe <sup>2+</sup> ( $2.50 \times 10^{-2}$ )	4.3

<sup>a</sup> Conditions: [4] =  $1.00 \times 10^{-2}$  M, pH 9.10 borate buffer, 8 h, 30 °C.

of ferrous ammonium sulfate ( $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ ) in borate buffer (pH 9.1) at room temperature to give benzyl alcohol (5) in 95% yield (Table I). Ammonia gas was ascertained with Nessler reagent. LA produced by the oxidation of DHLA was also obtained in 95% yield. Therefore, the reaction was well represented by the following equation:



On the other hand, scarcely any 5 was obtained with either DHLA or ferrous ion alone, as shown in Table I. These results suggest that the complex formed from DHLA and ferrous ion plays an important role in the reduction of 4, as discussed later.

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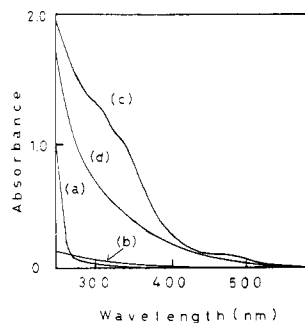
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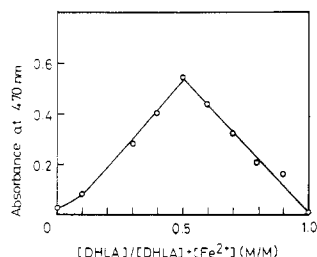
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**Figure 1.** Absorption spectra of DHLA-Fe(II) system: (a) DHLA, (b)  $\text{Fe}^{2+}$ , (c) DHLA- $\text{Fe}^{2+}$ , (d) DHLA- $\text{Fe}^{2+}$ -4,  $[\text{DHLA}] = [\text{Fe}^{2+}] = [4] = 5 \times 10^{-4} \text{ M}$ .



**Figure 2.** Continuous variation curve of DHLA and ferrous ion:  $[\text{DHLA}] + [\text{Fe}^{2+}] = 4.0 \times 10^{-3} \text{ M}$ , 0.1 M carbonate buffer, pH 9.8.

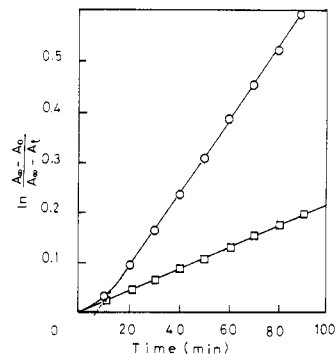
**Table II.** Reductive Cleavage of N-O Compounds by DHLA-Fe(II)<sup>a</sup>

substrate	conditions <sup>b</sup>	$10^4 k, \text{ s}^{-1}$
$\text{NH}_2\text{OCH}_2\text{Ph}$	A	1.20
$\text{NH}_2\text{OCH}_3$	A	0.35
$\text{NH}_2\text{C}(\text{NH})\text{NHOCH}_2\text{Ph}$	B	1.00
$\text{C}_6\text{H}_{11}\text{NHOH}$	A	5.40
$\text{PhCONHOCH}_2\text{Ph}$	B	0
$\text{PhCONHOH}$	A	0
$\text{Me}_2\text{C}=\text{NOCH}_2\text{Ph}$	B	0
$\text{Me}_2\text{C}=\text{NOH}$	A	0

<sup>a</sup>  $[\text{Substrate}] = 1.00 \times 10^{-2} \text{ M}$ ,  $[\text{DHLA}] = 1.25 \times 10^{-2} \text{ M}$ ,  $[\text{Fe}^{2+}] = 5.00 \times 10^{-5} \text{ M}$ . <sup>b</sup> Conditions A: 0.2 M borate buffer, pH 9.1. Conditions B: 0.2 M carbonate buffer-EtOH (1:1), pH 9.8, 30 °C.

To investigate the complex formation, the electronic spectra of the DHLA-Fe(II) system were measured. Neither DHLA nor ferrous ion showed absorption bands around 300–400 nm, but a new absorption band having shoulders at 330, 350, and 470 nm was observed by mixing the DHLA and ferrous ion solutions (carbonate buffer, pH 9.8) under an argon atmosphere and was attributable to the formation of a DHLA-Fe(II) complex (Figure 1). The composition of the complex was determined with the continuous variation method by plotting the absorbance of the mixture at 470 nm (Figure 2). As shown clearly in Figure 2, the complex (1:1 molar ratio) was formed from DHLA and ferrous ion. The stability constant ( $K_1 = 2.7 \times 10^4 \text{ M}^{-1}$ ) was obtained from the plot.<sup>9</sup>

**Reduction of Some N-O Compounds.** Reduction of some N-O compounds was attempted by the above-mentioned system. The progress of the reaction was monitored by the increase of the absorbance at  $\lambda_{\text{max}}$  of LA (= 333 nm), formed concurrently with the reduction. The changes of absorbance in the reduction of hydroxylamine derivatives followed first-order kinetics (Figure 3), and the first-order rate constants  $k$  were obtained from the slope of the plots



**Figure 3.** First-order plots of absorbance at 333 nm in the reductive cleavage of N-O compounds with DHLA-Fe(II) complex:  $[\text{N-O compound}] = 1.00 \times 10^{-2} \text{ M}$ ,  $[\text{DHLA}] = 1.25 \times 10^{-2} \text{ M}$ ,  $[\text{Fe}^{2+}] = 5.00 \times 10^{-5} \text{ M}$ , 0.2 M borate buffer, pH 9.1, 30 °C; O, 4; □,  $\text{NH}_2\text{OCH}_3$ .

**Table III.** Reductive Cleavage of O-Benzylhydroxylamine (4) by Thiol-Fe(II) Systems<sup>a</sup>

thiol	structure	yield of $\text{PhCH}_2\text{OH}$ , %
DHLA		93
dihydroliipoamide		100
bis(2-mercaptoethyl) ether		100
dithiothreitol		58
2,3-dimercapto-1-propanol		7
toluene-3,4-dithiol		0
ethanethiol	$\text{C}_2\text{H}_5\text{SH}$	4
thioglycolic acid	$\text{HSCH}_2\text{CO}_2\text{H}$	0
thiophenol	$\text{PhSH}$	5

<sup>a</sup> Conditions:  $[4] = 1.00 \times 10^{-2} \text{ M}$ ,  $[\text{dithiol}] = 2.00 \times 10^{-2} \text{ M}$  ( $[\text{monothiol}] = 4.00 \times 10^{-2} \text{ M}$ ),  $[\text{Fe}^{2+}] = 5.00 \times 10^{-5} \text{ M}$ , 0.2 M carbonate buffer-EtOH (1:1), pH 9.8, room temperature, 24 h.

(Table II). On the other hand, no reaction occurred in case of the derivatives of hydroxamic acids or oximes, where the basicities of the nitrogen ( $\text{p}K_a < 1$ ) were lower than those ( $\text{p}K_a = 4\text{--}6$ ) of hydroxylamine derivatives. These results suggest that the coordinating abilities of N-O compounds to the active Fe(II) complex, which might be controlled by the basicity of nitrogen in N-O bond, could be an important factor for the reaction.

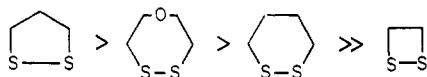
Some other substrates having C=O or C=C groups (benzaldehyde, furfural, cyclohexanone, methyl crotonate, cinnamic alcohol) could not be reduced with the DHLA-Fe(II) system, which indicated that the present system had a high specificity for the reduction of hydroxylamine derivatives.

**Reduction of O-Benzylhydroxylamine with Some Thiol-Fe(II) Systems.** In order to investigate the role of thiol group in this reducing system some monothiols and dithiols other than DHLA were examined in the reduction of O-benzylhydroxylamine in the presence of a catalytic

(9) S. E. Turner and R. C. Anderson, *J. Am. Chem. Soc.*, 71, 912 (1949).

amount of ferrous ion. The results are summarized in Table III.

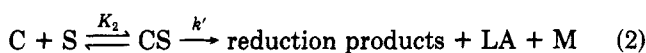
Monothiols showed little reactivity, while some dithiols gave reduction product in good yields presumably by chelate formation of the dithiols and ferrous ion. Contrary to the ready formation of five-membered metal chelates, 2,3-dimercapto-1-propanol and toluene-3,4-dithiol gave poor results, which suggested that the reactivity was controlled by not only the tendency of chelate formation but also the ease for the electron transfer from dithiols to substrate via ferrous ion. Affleck et al.<sup>10</sup> have reported that the tendency of the formation of cyclic disulfides in the oxidative cyclization of Bunte salts is as follows:



Furthermore, the cyclization of toluene-3,4-dithiol to a disulfide is difficult. These facts are well correlated with our results; that is, dithiols which advantageously form cyclic disulfides showed high reactivities.

**Reduction of *O*-Benzylhydroxylamine with DHLA-Metal Ions.** The effect of some metal ions such as Co(II), Mn(II), Cu(II), Zn(II), and Mo(VI) instead of ferrous ion on the reduction of 4 with DHLA was examined. Metal ions other than ferrous ion showed no ability to reduce 4, despite the formation of colored complexes with DHLA. This remarkable specificity is interesting in relation to ferredoxins in living systems which are formed mainly from Fe and thiol groups.

**Mechanistic Considerations.** Further, we now consider the mechanism of reductive cleavage of N-O compounds with the DHLA-Fe(II) system kinetically. From the results mentioned above, dithiols, which form chelates with ferrous ion and tend to cyclize oxidatively, have proven to be effective in the reduction. Substrates with high basicity on the nitrogen of an N-O bond are able to be reduced, presumably due to complexation with a dithiol-Fe(II) chelate as observed in Table II. Therefore, eq 1 and 2 are proposed in the case of the reduction of 4



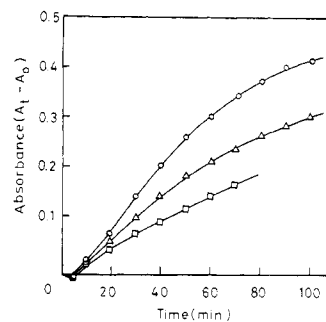
with the DHLA-Fe(II) complex, where L, M, C, S, and CS represent DHLA, ferrous ion, DHLA-Fe(II) complex, substrate 4, and DHLA-Fe(II)-substrate complex, respectively, and  $K_1$ ,  $K_2$  and  $k'$  represent equilibrium constants and a rate constant, respectively. Initially the concentration of C in the absence of substrate ( $=[\text{C}]_0$ ) is evaluated from eq 1, where the initial concentration of L ( $=[\text{L}]_0$ ) is much higher than that of M ( $=[\text{M}]_0$ ), and then the concentration of C in the absence of S ( $=[\text{C}]_0$ ) is represented by eq 3. The value of  $K_1[\text{L}]_0$  ( $=3.38 \times 10^2$ )

$$[\text{C}]_0 = K_1[\text{L}]_0[\text{M}]_0 / (1 + K_1[\text{L}]_0) \quad (3)$$

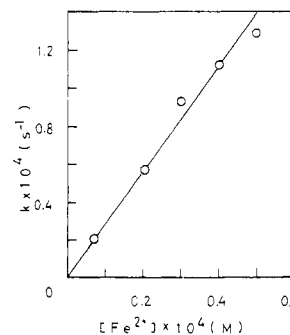
calculated from a  $K_1$  of  $2.7 \times 10^4 \text{ M}^{-1}$  and an  $[\text{L}]_0$  of  $1.25 \times 10^{-2} \text{ M}$  is much higher than 1; therefore eq 4 is obtained from eq 3.

$$[\text{C}]_0 = [\text{M}]_0 \quad (4)$$

In the presence of the substrate S, the complex CS is formed and is considered to be decomposed in the rate-



**Figure 4.** Dependence of the rate on the concentration of 4:  $[\text{DHLA}] = 1.25 \times 10^{-2} \text{ M}$ ,  $[\text{Fe}^{2+}] = 5.00 \times 10^{-5} \text{ M}$ , 0.2 M borate buffer, pH 9.1, 30 °C;  $[4] = 0.25 \times 10^{-2} \text{ M}$  ( $\square$ );  $[4] = 0.50 \times 10^{-2} \text{ M}$  ( $\Delta$ );  $[4] = 1.00 \times 10^{-2} \text{ M}$  ( $\circ$ ).



**Figure 5.** Dependence of  $k$  on the concentration of ferrous ion:  $[4] = 1.00 \times 10^{-2} \text{ M}$ ,  $[\text{DHLA}] = 1.25 \times 10^{-2} \text{ M}$ , 0.2 M borate buffer, pH 9.1, 30 °C.

determining step (eq 2). Therefore, the reduction rate is represented as follows by applying preequilibrium conditions.

$$\begin{aligned} \text{rate} &= k[\text{CS}] \\ &= \frac{kK_2[\text{C}]_0[\text{S}]}{1 + K_2[\text{S}]} \\ &= \frac{kK_2[\text{M}]_0[\text{S}]}{1 + K_2[\text{S}]} \end{aligned} \quad (5)$$

If  $K_2[\text{S}] \ll 1$

$$\text{rate} = kK_2[\text{M}]_0[\text{S}] \quad (6)$$

Here ferrous ion works as a catalyst, and thus the concentration of M is regarded to be constant, therefore

$$\text{rate} = k[\text{S}] \quad (7)$$

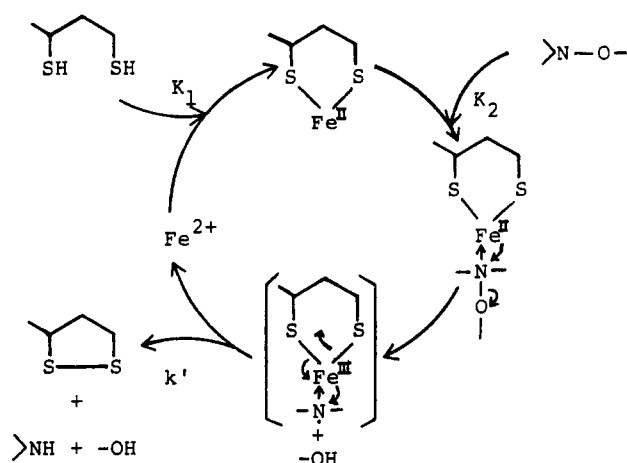
where  $k = kK_2[\text{M}]_0$ , which was well correlated with the results of Figure 3 (because  $[\text{LA}]_\infty - [\text{LA}] = [\text{S}]$ ). Furthermore, the initial concentration dependences of the rate were examined. The rate, followed spectrophotometrically by the appearance of LA, increased proportionally to the concentration of 4 (Figure 4), and the values of  $k$  obtained from the slopes of first-order plots were independent of the concentration of S. Further, the values of  $k$  were first order with respect to the concentration of M at lower than  $5 \times 10^{-5} \text{ M}$  (Figure 5). Thus eq 7 was well proved to hold for the present reaction.

Therefore, in the ferrous ion catalyzed reduction of 4 with DHLA, the reaction is presumed to proceed through eq 1 and 2 under the conditions of  $K_2[\text{S}] \ll 1$ , fast formation of complex CS, and slow decomposition of CS to the products.

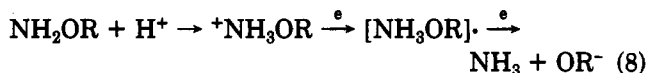
It has been suggested in the electrolytic reduction of aliphatic hydroxylamine derivatives that the N-O bond

(10) J. G. Affleck and G. Dougherty, *J. Org. Chem.*, 15, 865 (1950).

## Scheme II. Proposed Mechanism



is cleaved in low yields by way of the formation of free radical intermediates<sup>11</sup> (eq 8).



From the kinetic and spectroscopic considerations, the mechanism shown in Scheme II is proposed for the reduction of 4 by DHLA-Fe(II), where DHLA forms an active complex with ferrous ion and donates electrons to the N-O bond through the complex and where ferrous ion works as an effective catalyst ( $5 \times 10^{-3}$  equiv) by repeated formation of an active complex with free DHLA.

## Experimental Section

**General Procedures.** IR spectra were recorded on a Hitachi EPI-S2 spectrophotometer. <sup>1</sup>H NMR spectra were measured with a Varian EM 360 NMR spectrometer in deuteriochloroform with tetramethylsilane as an internal standard. UV absorption spectra were recorded on a Hitachi 200-10 spectrophotometer. GLC analyses were carried out on a JEOL JGC-1100 gas chromatograph (10% SE-30, stainless-steel column, 120 °C, N<sub>2</sub> carrier gas).

**Materials.** Dihydroliipoic acid (DHLA) was prepared by the procedure of Gunsalus et al.<sup>12</sup> Dihydroliipoamide was also prepared by the procedure of Reed et al.<sup>13</sup> and recrystallized from carbon tetrachloride, mp 62–64 °C (lit.<sup>13</sup> mp 64–66 °C). Other chemicals used in this study were reagent grade; the solvents were purified by the usual procedure.

**Preparation of N-(Benzyloxy)guanidine.** Cyanamide (1 g, 24 mmol), prepared from calcium cyanamide according to the procedure of Kurzer et al.,<sup>14</sup> was added to a solution of O-

benzyloxyamine (4; 3.24 g, 26 mmol) in ethanol (20 mL) and refluxed for 5 h. After the reaction the solvent was evaporated in vacuo to give crude oil (0.95 g, yield 24%) which was recrystallized from water as the tetraphenyl borate; mp 120–121 °C; NMR (CDCl<sub>3</sub>) δ 4.9 (s, 2 H, CH<sub>2</sub>Ph) 6.8–7.5 (m, 10 H, C<sub>6</sub>H<sub>5</sub>, NHC(=NH)NH<sub>3</sub><sup>+</sup>); IR (KBr) 1670 (NHC(=NH)NH<sub>3</sub><sup>+</sup>). Anal. Calcd for C<sub>32</sub>H<sub>33</sub>N<sub>3</sub>O<sub>3</sub>B: C, 76.34; H, 6.41; N, 8.35. Found: C, 76.34; H, 6.42; N, 8.52.

**Preparation of O-Benzylbenzohydroxamic Acid.** To a suspension of O-benzyloxyamine hydrochloride (0.16 g, 1 mmol) and triethylamine (0.24 mL, 2 mmol) was added benzoyl chloride (0.15 g, 1.1 mmol) dropwise at 0 °C, and the mixture was stirred for 24 h at room temperature. After the reaction, the solvent was evaporated, and the resulting residue was dissolved in ethyl acetate. The organic layer was washed with diluted hydrochloric acid, 10% aqueous sodium carbonate solution, and saturated aqueous NaCl solution and dried over anhydrous MgSO<sub>4</sub>. The solvent was evaporated, and the resulting residue was recrystallized from ethyl acetate-*n*-hexane (1:1) to yield O-benzylbenzohydroxamic acid: 0.12 g (yield 54%); mp 102–103 °C (lit.<sup>15</sup> mp 103–105 °C).

**Reductive Cleavage of N-O Compounds. (A) Kinetic Measurements.** DHLA was added to a 1-cm quartz cell and diluted with 0.2 M borate buffer (pH 9.2), prepared by mixing 0.2 M aqueous Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> solution (950 mL) and 0.1 N aqueous HCl solution (50 mL). To the solution was added 0.1 mM Fe<sup>2+</sup> solution, prepared by dissolving ferrous ammonium sulfate (FeSO<sub>4</sub>·(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>·6H<sub>2</sub>O, 0.00392 g) in distilled water (100 mL) previously bubbled with nitrogen gas, and N-O compound. The final concentrations in the solution were [DHLA] =  $1.25 \times 10^{-2}$  M, [substrate] =  $1.00 \times 10^{-2}$  M, and [Fe<sup>2+</sup>] =  $5.00 \times 10^{-6}$  M, and the pH was 9.1. After the solution was mixed, the appearance of lipoic acid (LA) was monitored at 333 nm (λ<sub>max</sub> of LA) as a function of time. The change of absorbance at 333 nm followed first-order kinetics up to at least 2 half-lives, and first-order rate constants *k* were obtained. All reaction systems were carefully degassed and replaced with nitrogen gas.

**(B) Determination of Benzyl Alcohol (5).** The reaction solution was prepared in the same way as for the kinetic measurement. After the reaction the solution was extracted with chloroform, and the organic layer was dried over anhydrous MgSO<sub>4</sub> and analyzed by way of GLC to determine the yield of 5.

**Registry No.** 4, 622-33-3; 5, 100-51-6; N-(benzyloxy)guanidine tetraphenyl borate, 81956-24-3; cyanamide, 420-04-2; O-benzyl hydroxylamine hydrochloride, 2687-43-6; benzoyl chloride, 98-88-4; O-methyl-hydroxylamine, 67-62-9; N-(benzyloxy)guanidine, 705-98-6; N-hydroxycyclohexanamine, 2211-64-5; O-benzylbenzohydroxamic acid, 3532-25-0; N-hydroxy benzamide, 495-18-1; 2-propanone O-(phenylmethyl) oxime, 3376-36-1; 2-propanone oxime, 127-06-0; dihydroliipoic acid, 462-20-4; dihydroliipoamide, 3884-47-7; bis(2-mercaptoethyl) ether, 2150-02-9; diithiothreitol, 3483-12-3; 2,3-dimercapto-1-propanol, 59-52-9; toluene-3,4-dithiol, 496-74-2; ethane-thiol, 75-08-1; thioglycolic acid, 68-11-1; thiophenol, 108-98-5; Fe<sup>2+</sup>, 15438-31-0.

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