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Evidence for the Formation of a *cis*-Dichlorovinyl Anion upon Reduction of *cis*-1,2-Dichlorovinyl(pyridine)cobaloxime

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The reduction of *cis*-1,2-dichlorovinyl(pyridine)cobaloxime, a model complex for the organometallic intermediate proposed in the dechlorination of trichloroethylene by cobalamin, was studied. Two mechanisms were considered for the Co–C bond cleavage following reduction. In the first, the Co–C bond cleaves to produce Co^{I} and a chlorovinyl radical, while the second pathway results in the formation of Co^{II} and a chlorovinyl anion. Four reducing agents, cobaltocene, decamethylcobaltocene, cob(I)alamin, and chromium(II), were used in the presence of H atom and proton donor species to identify the presence of chlorovinyl radical or chlorovinyl anion intermediates. Mechanistic conclusions were based on comparisons of the final product ratios of *cis*-dichloroethylene (cDCE) and chloroacetylene, which were found to have a direct relationship to the amount of proton donor available, with increased proton donor leading to increased cDCE production. The results support the intermediacy of a *cis*-1,2-dichlorovinyl anion.

Introduction

Trichloroethylene (TCE), a widely used industrial solvent, is a common environmental pollutant.[1] Cobalamin (vitamin B_{12}) has been found to catalyze the reductive dechlorination of TCE and may have applications in the remediation of contaminated sites.[2−8] Although the dechlorination of chlorinated ethylenes by cobalamin has been studied by many authors, the mechanism is not fully understood. We and others have found evidence for the formation of an organometallic intermediate in the dechlorination of TCE,[2−4,7−15] but how this intermediate is transformed to the observed products is still unclear.

Cobalamin's ability to dechlorinate alkyl halides has long been known, and its mechanism has been studied extensively,[16−18] while work with chlorinated ethylenes and the intermediates formed in their reduction has been more limited in scope. Two mechanisms have been proposed for Co−C

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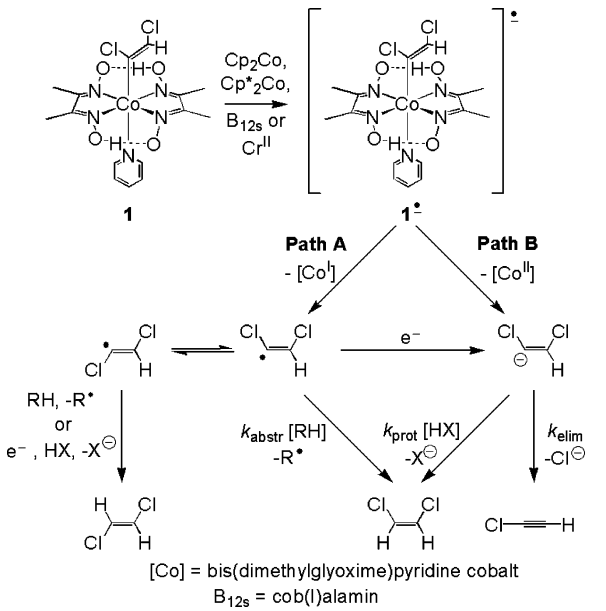
Scheme 1



bond cleavage of chlorovinyl organometallic intermediates; both involve a one-electron-reduced species (Scheme 1; *cis*-1,2-dichlorovinyl(pyridine)cobaloxime (**1**) is shown). In pathway A, the Co−C bond cleaves to produce Co^{I} and a chlorovinyl radical,[3] while pathway B results in the formation of Co^{II} and a chlorovinyl anion.[2,7,10,16,18−20] In the present study, the reduction of **1**, which is believed to be a good model for the intermediate formed in the dechlorination of TCE by cobalamin,[11−13,21] was examined with four reductants: cobaltocene (Cp_2Co), decamethylcobaltocene (Cp^*_2Co), cob(I)alamin, and chromium(II). With each of these reducing agents, experiments were performed with a H atom and a proton donor species to test for vinyl radical and vinyl anion intermediates.

Experimental Section

General Methods. All experiments were performed in a glovebox (nitrogen atmosphere) using dry reagents and solvents, unless otherwise noted. All reagents and solvents were purchased from commercial suppliers, unless otherwise stated. Dimethyl sulfoxide (DMSO) was distilled from CaH_2 under a nitrogen atmosphere and stored over activated 4-Å molecular sieves, *tert*-butyl alcohol (tBuOH) was distilled from CaH_2 under a nitrogen atmosphere and stored under an inert atmosphere, *cis*-dichloroethylene (cDCE) was predried over activated 4-Å molecular sieves, followed by drying over $CaCl_2$ before being vacuum transferred to a sealed container and stored in the dark over activated 4-Å molecular sieves. Decamethylcobaltocene (Cp^*_2Co) and cobaltocene (Cp_2Co) were sublimed at 135 and 60 °C, respectively, prior to use and stored under an inert atmosphere at −35 °C.[22] Cr^{II} was prepared through the reaction of $CrCl_3$ with zinc amalgam as previously described.[23]

Heptane and decane extractions were analyzed by GC−MS using an Agilent GC 6890 (Restek Rtx-1 Crossbond 100% dimethylpolysiloxane; 30 m × 0.32 mm × 5 μm film thickness) coupled to an Agilent MS 5973 mass selective detector operated in selective ion mode. Headspace samples (100 μL) were analyzed by GC−flame ionization detector (FID) using a Hewlett-Packard 5890 gas chromatograph (Supelco VOCOL fused silica capillary column; 30 m × 0.53 mm × 3 μm film thickness). Retention times of the products were confirmed through the injection of authentic standards. High-performance liquid chromatography (HPLC) chromatograms were acquired on an 1100 series Hewlett-Packard HPLC joined with a UV absorbance detector. Reaction mixtures were analyzed using a Supelcosil LC-18-DB (150 × 4.6 mm; 5-μm particle size) column with a mobile phase of 70:30 acetonitrile/pH 5 ammonium acetate buffered H_2O, with a flow rate of 1.0 mL/min, a 10-μL injection volume, and a 310-nm detection wavelength. UV−vis absorbance spectra were measured on a Jasco V-530 spectrophotometer. NMR spectra were obtained on a Varian Inova VI-600 spectrometer using an 8-mm broad-band probe tuned to the carbon nucleus as well a Varian Inova VI-500 spectrometer.

Amalgamated Zn. Approximately 5 g of dull metallic mossy Zn, of an average size of 3 mm × 10 mm, was combined in a flask with 100 mL of water and approximately 1.5 g of HgO. The bright-orange solid did not readily dissolve in the water and necessitated the addition of 10% HCl until HgO dissolved. Immediate reaction of HgO with Zn upon dissolution was observed. The resulting clear liquid solution and shiny Zn/Hg solid was then stirred for 30 min. The water solution was decanted off of the amalgamated Zn, and the solids were rinsed three times with water followed by drying under active vacuum at 100 °C for 1 h. The solids were stored under a nitrogen atmosphere. Prior to use, a single drop of liquid Hg was added, which immediately absorbed onto the surface of the Zn, leaving no free metallic Hg in the reaction vessel.

Synthesis of *cis*-1,2-Dichlorovinyl(pyridine)cobaloxime (1). The published method by Rich et al.[12] was followed as described with slight modifications to the purification steps to increase the yield of the reaction. Once the reaction was completed, the products were purified by column chromatography [silica gel, unstabilized tetrahydrofuran (THF)]. The large orange fraction was dried to a solid under vacuum, dissolved in chloroform, and washed with water (3×). After drying with $MgSO_4$, the product was recrystallized from chloroform/hexanes. The isolated yield of orange crystals was 78%.

Synthesis of *cis*-1,2-Dichlorovinyl(^{13}C)(pyridine)cobaloxime (1-$^{13}C_2$). The procedure outlined by Rich et al.[12] was modified to accommodate ^{13}C-labeled TCE. Cobalt(II) acetate tetrahydrate (0.102 g, 40.8 mmol), dimethylglyoxime (0.098 g, 84.0 mmol), and pyridine (90 μL, 119 mmol) were combined in a sealed flask with 5 mL of THF. Under a nitrogen flow, Zn powder (0.171 g, 262 mmol) was added to the solution. The flask was then sealed and frozen with liquid nitrogen before a vacuum (10^{-1} Torr) was

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established and the flask sealed. The reaction was warmed to room temperature and then heated to 65 °C for 15 min with stirring. The solution was then cooled to room temperature, and under a nitrogen flow the labeled TCE (34 μL, 37.3 mmol) was introduced. The flask was again sealed, frozen, and evacuated (10^{-1} Torr); the reaction mixture was then allowed to warm to room temperature, followed by heating at 65 °C for 1 h with stirring. The reaction solution was again cooled to room temperature. The resulting reddish-orange solution was filtered through Celite and rinsed well with THF. The filtrate was concentrated to 5−10 mL, then passed through a dry silica column (SiO_2, 5 cm, THF), and rinsed with THF until all of the yellow-orange color was removed from the silica gel. This yellow solution was then concentrated to a solid, followed by dissolution in chloroform. The solution was washed with H_2O (3×), the water layers were then back-extracted with chloroform (3×), and finally all of the chloroform layers were combined and dried over magnesium sulfate. After drying, the solution was concentrated to a solid and recrystallized from chloroform/hexanes. Yield: 0.130 g (27.9 mmol, 75 %). ^{1}H NMR (DMSO-d_6): δ 2.11 (12 H, s, CH_3), 5.53 (1 H, dd, 200.9, 17.0 Hz, C═CHCl), 7.52 (2 H, m, pyr), 7.91 (1 H, m, pyr), 8.28 (2 H, m, pyr). ^{13}C NMR (DMSO-d_6): δ 12.21 (CH_3), 105.18 (d, J = 62.6 Hz, CHCl), 126.13 (pyr), 139.15 (CoCCl and pyr), 149.16 (pyr), 151.34 (C═N).

Preparation of Stock Solutions. Fresh stock solutions of all reagents were prepared for each experiment. **(a) 1. 1** was dissolved in 5 mL of DMSO. Reaction injection volumes were adjusted to obtain a 2 mM final concentration of the substrate.

(b) Cobaltocenes. Stock solutions of the cobaltocenes were prepared by dissolving solid cobaltocene, Cp_2Co and Cp^*_2Co, respectively, in 10 mL of DMSO. Injection volumes were manipulated in order to obtain a reducing agent concentration of 0.5 mM.

(c) Cob(I)alamin. Cyanocobalamin was dissolved in 10 mL of DMSO with stirring. To this solution was added an excess of amalgamated Zn, and the reaction was stirred until the solution was a gray-green color, approximately 3 h at 2 mM. Complete conversion to the Co^I oxidation state was verified by UV−vis spectroscopy (see the Supporting Information).[10,24,25]

(d) Cob(II)alamin. For comparision purposes, an authentic sample of cob(II)alamin was prepared following the preparation method of Yamada et al.[26] Methylcobalamin (46 mg, 2.70×10^{-5} mol) was dissolved in 50 mL of isopropyl alcohol in a 100-mL Schlenk flask. The solution was degassed by bubbling nitrogen through the solution for 30 min. The solution was then cooled to 0 °C and irradiated using a ceramic metal halide lamp with slow N_2 bubbling. After 30 min of irradiation, the solution was concentrated to ~10 mL under vacuum at 40 °C. After concentration, 100 mL of degassed acetone was transferred by a cannula into the flask and allowed to sit under a nitrogen flow. The resulting brown solid (20 mg, 54%) was filtered and washed with acetone.

(e) Cr^{II}. $CrCl_3$ (16 mg, 1.01×10^{-4} mol) was dissolved in 10 mL of H_2O or D_2O. This solution was placed in a 50-mL Schlenk flask and sparged with nitrogen for 30 min. To this solution was added amalgamated zinc under a nitrogen flow, and the solution was again sparged with nitrogen for 30 min or until the green solution became pale blue in color. A total of 50 μL of this solution was then used in the reaction to give a Cr^{II} concentration of 0.5 mM.

(f) 2,2,6,6-Tetramethyl-1-piperidinyloxy (TEMPO). TEMPO (0.160 g, 1.02×10^{-3} mol) was dissolved in 5 mL of DMSO to give a stock solution concentration of 0.2 M. A total of 250 μL of this solution was used in the reaction; [TEMPO] = 0.05 M.

(g) 2,6-Lutidinium Tetraphenylborate ($LutH^+$). 2,6-Lutidinium chloride (0.140 g, 9.75×10^{-4} mol) was combined with sodium tetraphenylborate (0.693 g, 2.02×10^{-3} mol) in approximately 6 mL of DMSO and allowed to stir for 2 h. The resulting solution was filtered through glass fiber filter paper and brought to a volume of 10 mL with DMSO; [$LutH^+$] = 0.98 M. A total of 500 μL of this solution was used in the reaction; [$LutH^+$] = 0.05 M.

The solutions of TEMPO and $LutH^+$ were stored in a nitrogen atmosphere at −35 °C.

Reduction of 1 by Cp_2Co, Cp^*_2Co, and Cob(I)alamin. A stock solution containing 56 mM benzene (10 μL) or 43.4 mM pentane (10 μL), both used as internal standards, was prepared in 2 mL of DMSO. In a sealed reaction vessel, 20 μL of an internal standard solution was combined with **1** (2 mM) and DMSO. The reducing agent (0.5 mM) was then added dropwise, with stirring, to the reaction vessel (final volume of 1 mL). After the addition of the reducing agent, the vessel was sealed and the reaction was run to completion (Cp^*_2Co and Cp_2Co were stirred for 30 min at 25 °C; cob(I)alamin was stirred for 2−4 h at 25 °C). The reaction solution was then stirred at 30 °C for 5 min, at which point 250 μL of heptane or decane was added and the solution was again stirred at 30 °C for 5 min. The heptane or decane layer was then transferred to a GC vial containing a 300-μL glass insert, sealed with a septum, and analyzed by GC−MS.

Reduction of 1 in the Presence of Anion and Radical Traps. Reductions incorporating traps were run as previously described, with the trap added prior to the introduction of the reducing agent. tBuOH and CHD were used neat, while stock solutions of TEMPO and $LutH^+$ were syringed from stock solutions. All other conditions were held constant.

Reduction of 1 by Cr^{II}. Reaction vials were prepared in an inert-atmosphere glovebox (nitrogen) as described above with the exception of the addition of a reducing agent. The vials were removed from the glovebox, and the Cr^{II} solution was added by syringe under a nitrogen flow. The reactions were allowed to stir for 30 min at 25 °C prior to product extraction and analysis, both performed as described above.

When H_2O or D_2O were added to the reaction solution, the corresponding solution was degassed by sparging with nitrogen for 30 min in a septum-sealed vial, prior to the addition of the Cr^{II} solution.

Mass Balance Experiments Using HPLC. New stock solutions for **1** were prepared in DMSO at a concentration of 2.1 mM. This solution was used in the reduction experiments as described above and was also used to confirm the starting concentration of **1** by HPLC. This confirmation was conducted using 200 μL of the stock solution brought to 2 mL with methanol and analyzed. Similarly, at the completion of the reduction and the subsequent extraction, 200 μL of the DMSO reaction solution was diluted to 2 mL with methanol and analyzed to determine the concentration of **1** remaining after each respective reduction.

Stability of cDCE and Chloroacetylene (CA) to the Reducing Agent. A solution of cDCE and CA was prepared by combining an excess of cDCE (25 μL, 3.31×10^{-4} mol) with potassium bis-

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(trimethylsilyl)amide (11 mg, 5.51×10^{-5} mol) in 10 mL of DMSO in a 40-mL septum-sealed vial. The solution was stirred for 30 min at 30 °C. Headspace samples (100 μL) were withdrawn from the vial and analyzed by GC−FID. Aliquots of Cp_2Co, Cp^*_2Co, and Cr^{II} (2 mM) were added to the solution and allowed to stir for 30 min at 30 °C prior to analysis. Duplicate samples were removed from the vial and analyzed before further reducing agent was introduced. The products were found to be stable under these reaction conditions.

^{13}C NMR Experiments. Solutions. (a) 1-$^{13}C_2$. 1-$^{13}C_2$ (0.049 g, 10.6 mmol) was dissolved in a 5-mL solution of DMSO/DMSO-d_6 (approximately 60:40). Injection volumes were manipulated to keep the concentration of **1-$^{13}C_2$** greater than that of the respective reducing agent (700 μL; [**1-$^{13}C_2$**] = 14.8 mM) for the cobaltocenes (950 μL; [**1-$^{13}C_2$**] = 20.1 mM for Cr^{II}).

(b) Cp_2Co. Cp_2Co (0.017 g, 8.8 mmol) was dissolved in 2 mL of DMSO/THF (approximately 90:10). A total of 300 μL of this solution was used in the reduction experiment ([Cp_2Co = 13.2 mM).

(c) Cp^*_2Co. Cp^*_2Co (0.022 g, 6.7 mmol) was dissolved in 2 mL of THF. A total of 300 μL of this solution was introduced to the reduction (Cp^*_2Co = 10.1 mM).

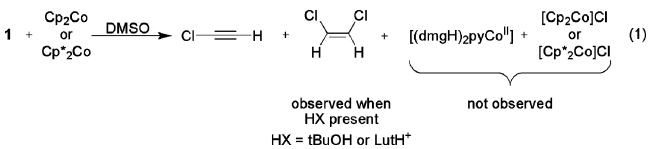
(d) Chromium(II). $CrCl_3$ (0.487 g, 308 mmol) were dissolved in 10 mL of H_2O. This solution was reduced as described above, and 50 μL was used in the reduction ([Cr^{II}] = 15.4 mM).

Reduction of 1-$^{13}C_2$ by Cp_2Co, Cp^*_2Co, and Cr^{II}. The reaction solutions were combined in an inert-atmosphere glovebox (nitrogen) and mixed thoroughly. The reactions were complete within minutes because of the high concentrations used in this experiment, and the samples were all analyzed using various NMR experiments.

Results and Discussion

Reduction experiments were performed in DMSO under a nitrogen atmosphere within sealed tubes to minimize product volatilization. A solution of the reducing agent was added dropwise to a solution of **1** (2 mM), yielding a final reducing agent concentration of 0.5 mM. The reactions were monitored by gas chromatography−mass spectrometry (GC−MS), high-performance liquid chromatography, and NMR. We also performed reduction reactions with ^{13}C-labeled **1** (**1-$^{13}C_2$**), in which the two vinyl carbons are labeled with ^{13}C, and monitored the reactions in sealed NMR tubes by $^{13}C\{^1H\}$ NMR. These reductions used reagents at concentrations 10-fold higher than those used for product quantification. Unfortunately, the concentrations necessary for this experiment were not obtainable with cobalamin.

The reductive cleavage of the Co−C bond in **1** was initially studied using two well-characterized outer-sphere electron-transfer agents: Cp_2Co ($E_{1/2}$ = −0.64 V)[27,28] and Cp^*_2Co ($E_{1/2}$ = −1.22 V)[28] (eq 1). Reactions with Cp_2Co and Cp^*_2Co generated CA as the only product detected by GC−MS in 34 ± 1% and 27 ± 5% yield according to eq 1.



(1)

Following the reduction by ^{13}C and ^{1}H NMR using **1-$^{13}C_2$**, a small amount of acetylene-$^{13}C_2$ is produced in the reduction with Cp_2Co, and a new peak appears in the chlorovinyl region of the ^{13}C NMR spectrum for both reductants but is at this time unidentified (**4-$^{13}C_2$**; Figure 1). Neither of these products accounts for a large percentage of the observed reactivity, and both are produced in amounts below the detection limit of the methods employed for product quantitation. In earlier studies involving the reduction of **1** with naphthalene and anthracene radical anions in THF, CA was also the only product detected.[12] Similarly, CA has been observed in the reduction of TCE by cobalamin and is also thought to be the source of acetylene when these reductions are performed in aqueous systems.[2,4] Production of CA as the only detected product of reduction provides evidence that the reaction is proceeding through a chlorovinyl anion as chlorine elimination from the radical species is calculated to be unfavorable.[29]

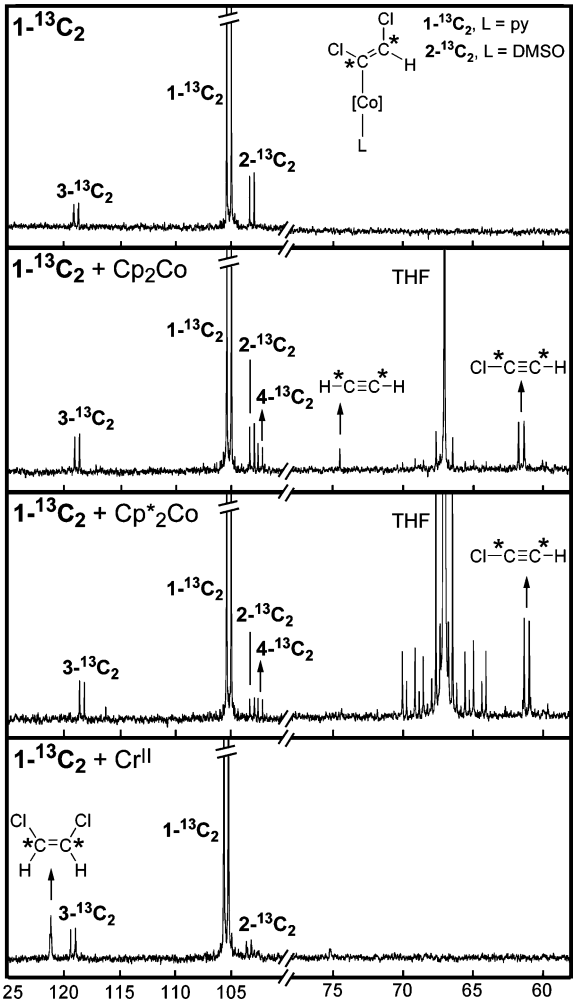


Figure 1. ^{13}C NMR of the reaction of **1-$^{13}C_2$** with Cp_2Co, Cp^*_2Co, and Cr^{II} in DMSO (and THF in the case of Cp_2Co and Cp^*_2Co). In the initial spectrum, ^{13}C-labeled *cis*-1,2-dichlorovinyl(pyridine)cobaloxime (**1-$^{13}C_2$**), *cis*-1,2-dichlorovinyl(DMSO)cobaloxime (**2-$^{13}C_2$**), and 1-chlorovinyl(pyridine)cobaloxime (**3-$^{13}C_2$**), a synthetic contaminant, are present. Upon reduction, an unidentified presumed chlorovinylcobalt complex (**4-$^{13}C_2$**), chloroacetylene-$^{13}C_2$, acetylene-$^{13}C_2$, and *cis*-dichloroethylene-$^{13}C_2$ are detected. Arrows indicate growth of products.

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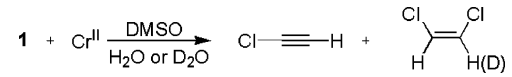
Reaction with Cp_2Co and Cp^*_2Co was further investigated through the addition of anion and radical traps. tBuOH (1 M) and 1,4-cyclohexadiene (CHD, 1 M) were used with both reducing agents, while TEMPO (0.05 M) and $LutH^+$ (0.05 M) were examined with Cp^*_2Co and Cp_2Co, respectively. The presence of radical traps (CHD and TEMPO) had no effect on the product distribution, but when the anion traps (tBuOH and $LutH^+$) were present, cDCE was also observed as a product of the reduction. The cDCE/CA ratio in the presence of tBuOH ($pK_a = 18$)[30] is quite small [(0.01 ± 0.005):1 and (0.2 ± 0.1):1 for Cp_2Co and Cp^*_2Co, respectively], but when $LutH^+$ ($pK_a = 6.8$)[28] was employed, the ratio was altered significantly [(1.2 ± 0.2):1]. Detection of cDCE illustrates the influence of proton donors on the product distribution and indicates the intermediacy of a chlorovinyl anion. Whether the anion species is produced directly via pathway B or indirectly by further reduction of the chlorovinyl radical produced via pathway A is unclear. If the two-step pathway is operative, there are severe kinetic constraints on its nature. Reduction by a 0.5 mM reducing agent can occur no faster than $\sim 10^6$ s^{-1} because of diffusion rates and therefore the radical cannot be trapped prior to inversion ($\sim 10^7$ s^{-1}).[29] The only reductant with a high enough local concentration to trap the *cis*-dichlorovinyl radical prior to inversion is the cob(I)aloxime formed at the same time as the vinyl radical. Thus, two viable options are direct production of *cis*-CHCl=CCl$^-$ and cob(II)aloxime or initial production of *cis*-CHCl=CCl$^\bullet$ and cob(I)aloxime followed by rapid in-cage electron transfer to yield the same products.

We examined the reduction of **1** by cob(I)alamin (B_{12s}) because it is thought that it is responsible for catalyzing the reduction of organocobalamin intermediates.[10,18,19] Unlike much of the work performed with cobalamin in which catalytic amounts are used in the presence of a bulk electron source such as titanium(III) citrate,[2−4,6,8] the reactive Co^I species was generated through reduction of cyanocobalamin by amalgamated zinc and then used as a stoichiometric electron donor.[24,32] There is some debate in the literature over the ability of amalgamated zinc to reduce cob(III)alamin to cob(I)alamin. It is thought that this reductant can only achieve a single-electron reduction to cob(II)alamin.[33] We have excluded the possibility that the active reductant in our experiments is cob(II)alamin. No reaction was observed between a pure sample of independently prepared cob(II)alamin[26] and **1**. Further evidence that cob(I)alamin was being produced by the amalgamated zinc reduction was provided by UV−vis spectroscopic data, which agrees with literature values (see the Supporting Information). Using cob(I)alamin as a stoichiometric electron donor allowed for the reaction conditions described above to be maintained while eliminating interference from a bulk electron source.[9,18]

Table 1. Reduction of **1** by Cr^{II} in the Presence of H_2O and D_2O



concn of H_2O/D_2O (M)	H_2O	D_2O	
	cDCE/CA	cDCE/CA	$C_2H_2Cl_2/C_2HDCl_2$
2.8	8 ± 1	4.2 ± 0.5	0.17 ± 0.03
6.9	12 ± 2	5 ± 2	0.09 ± 0.01
13.8	16 ± 2	9 ± 2	0.053 ± 0.002

The products observed in the reduction of **1** by cob(I)alamin include a mixture of cDCE ($6 \pm 1\%$) and CA ($27 \pm 3\%$) with a trace amount of tDCE ($0.13 \pm 0.02\%$) in an overall yield of $33 \pm 4\%$. The identity of these products is consistent with studies performed in aqueous solutions. However, in an aqueous system, the ratio of cDCE/CA has been observed to be 2.4:1,[8] while in our aprotic system, the ratio favors production of CA, (0.26 ± 0.04):1.

As with the Cp_2Co and Cp^*_2Co reductions, trapping experiments using CHD and tBuOH were again performed to test for the presence of reactive intermediates. In the case of CHD, no observable change in the cDCE/CA ratio was detected, whereas reduction in the presence of tBuOH increased the production of cDCE (for further experiments with tBuOH, see the Supporting Information). These trapping experiments make the production of tDCE an event that cannot be easily explained. If chlorovinyl radicals were solely active within this system, one would expect to observe a higher yield of tDCE based on the relative stabilities of the *cis*- and *trans*-dichlorovinyl radicals (cis:trans = 4−6:1) and their facile inversion, which has been found to be competitive with further reduction.[9,29] Studies aimed at explaining the production of tDCE when **1** is reduced by cob(I)alamin but not Cp_2Co or Cp^*_2Co are ongoing.

The role of proton donors on the resultant product ratio was studied further using the reduction of **1** in DMSO with aqueous Cr^{II} solutions. The products of this reduction included both cDCE and CA in a combined yield of $56 \pm 1\%$; both products are detected by GC−MS, but in the reduction of **1-$^{13}C_2$**, only cDCE-$^{13}C_2$ is observed by ^{13}C NMR (Figure 1). A systematic increase in the water concentration led to a corresponding increase in the protonated product, cDCE (Table 1). Substituting D_2O for H_2O resulted in an observed isotope effect of approximately two with significant deuterium incorporation into cDCE. The results observed with Cr^{II} are consistent with trapping of the chlorovinyl anion before elimination of chloride can proceed to produce CA.

As discussed above, the major products of the reductions were cDCE and CA, and throughout this study, these products were used as a diagnostic of the mechanism. However, care must be taken because in no case did the combined mass of these products account for more than half of the reducing equivalents. We have performed experiments

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designed to address the low mass balance and to identify other previously undetected products. First, we verified that the products, CA and cDCE, are stable to the reducing agents employed, Cp_2Co, Cp^*_2Co, and Cr^{II}. Additionally, the products expected from further reduction of CA and cDCE are not observed (in the case of ethylene and vinyl chloride) or are only observed in trace amounts (in the case of acetylene). Finally, as mentioned above, we performed reduction reactions using **1-$^{13}C_2$** monitoring by $^{13}C\{^1H\}$ NMR. Because this technique allows the reaction mixtures to be analyzed with minimal manipulation of the sample, product volatilization was minimized. Also, the large spectral width of ^{13}C NMR decreased the chances of overlapping signals and afforded us the ability to detect products resulting from the transformation of the two labeled chlorovinyl carbons. Using this method, cDCE and CA were observed without the other expected products of TCE dechlorination (tDCE, dichloroacetylene, vinyl chloride, or ethylene). Only two products in addition to cDCE and CA were detected, acetylene-$^{13}C_2$ and an unidentified presumed chlorovinyl product (**4-$^{13}C_2$**), and both products were minor contributors to the overall product distribution (Figure 1).

With no additional products contributing to the overall mass balance found by ^{13}C NMR, alternative sources of loss are proposed. In all of these reactions, cob(I)aloxime ($[Co^I]$) may be produced following the reduction of $[Co^{II}]$ and could reduce DMSO. With the high concentration of DMSO present in solution, this process would consume reducing agent equivalents. Alternatively, the reduction of **1** could produce products not detected by these methods (e.g., polymers).

Evidence for both carbon-centered radicals[8,10,11,15,19] and anions[2,7,10,16,18−20] has been found in the dechlorination of chlorinated ethylenes by cobalamin. The results of this study are consistent with the formation of chlorovinyl anions. What is unknown is whether these anions are produced directly or indirectly through further reduction of chlorovinyl radicals. For this latter pathway to be operative, the rate of further reduction would have to be competitive with both inversion and trapping of the chlorovinyl radical. At the concentrations used in this study, that reduction could only occur through a cage electron transfer from cob(I)aloxime, making the role of radicals, if formed, a transient one.

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Supporting Information Available: UV−vis spectra of the three oxidation states of cobalamin and tBuOH titration experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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