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Mechanistic Studies on the Vitamin B_{12}-Catalyzed Dechlorination of Chlorinated Alkenes

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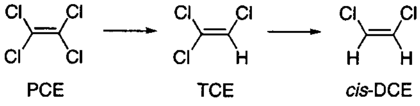
Perchloroethylene (PCE) is an abundant pollutant that ranks high on the priority list of the US Environmental Protection Agency. PCE causes liver and kidney tumors in animal studies,[1] and has been classified as a probable human carcinogen. Vitamin B_{12} is a promising catalyst for the decontamination of polluted environments via a reductive dechlorination process.[2] At present, the mechanistic details of this transformation are unclear. We report here studies that provide support for an electron-transfer mechanism.

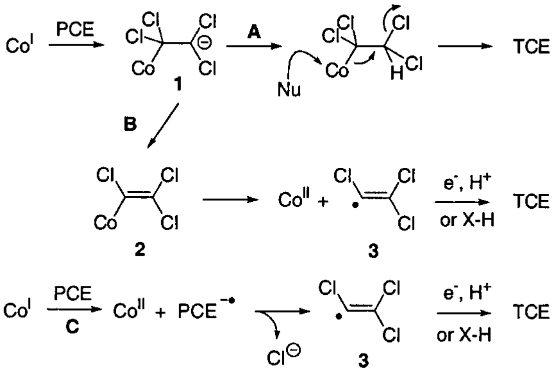
PCE has a long environmental half-life,[3] due in part to slow oxidative breakdown under aerobic conditions. Several strains of anaerobic microbes *reductively* dechlorinate PCE to trichloroethylene (TCE), and subsequently to *cis*-1,2-dichloroethylene (DCE) (eq 1).[4] The enzymes involved utilize a vitamin B_{12} derivative,[5] a novel and unprecedented role for this versatile cofactor. Interestingly, an earlier report by Gantzer and Wackett indicated that vitamin B_{12} can catalyze the reaction in eq 1 in the absence of enzymes.[2a] This finding has spurred studies on the feasibility of using B_{12} as a remediation catalyst.[2b−f]



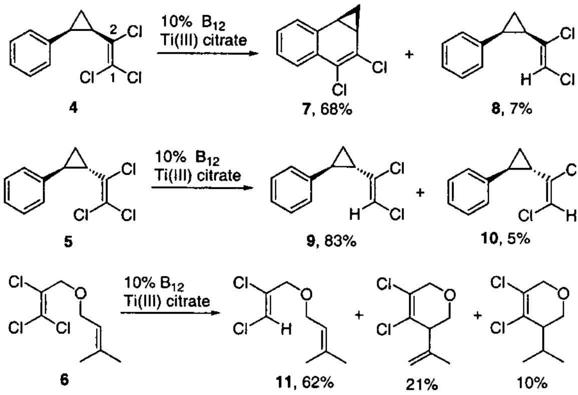
(1)

The extensive literature on vitamin B_{12} provides a basis for the formulation of at least three possible mechanisms for the dechlorination of PCE as shown in Scheme 1. Only strong reductants such as Ti(III) citrate support catalytic turnover, implying that Co(I) is the active form of the catalyst.[6] Cob(I)-alamins are well-known for their high nucleophilicity (Pearson constant of 14[7]) suggesting a nucleophilic attack of the catalyst on the electron-deficient olefin.[8] At least two different pathways can provide the observed product from intermediate **1** (corrin ligand not shown). Electrofugal Co−C bond cleavage (pathway A), possibly assisted by an external nucleophile, has precedent in organocobalamins with good leaving groups in the β-position.[9] An alternative addition−elimination route (pathway B) would lead to intermediate **2**. In fact, Lesage and co-workers[2f] have detected mono- and dichlorinated vinylcobalamins in dechlorination reactions by mass spectrometry. The authors proposed a homolytic cleavage of the Co−C bond of **2** leading to Co(II) and a trichlorovinyl radical **3**. This same species has been suggested as an intermediate in a nonnucleophilic mechanism involving a one-electron transfer from the reduced corrinoid to PCE (pathway C).[2c] This trichlorovinyl radical could be reduced and protonated, or could abstract a hydrogen atom from a suitable donor. We have employed radical traps, labeling studies, and stopped-flow spectroscopy to distinguish between these mechanisms. Our results are taken to support pathway C.

Scheme 1



Scheme 2



Intramolecular radical traps have been used extensively to probe for the possible intermediacy of free radicals in chemical reactions.[10] Phenyl-substituted cyclopropanes have recently been reported as probes for ketyl radical anions, and ring opening with rate constants of $10^5\ s^{-1}$ was observed.[11] Vinyl radicals are known to cyclize readily with appropriately positioned alkenyl[12] or aryl[13] groups. On the basis of these considerations, compounds **4**−**6** were designed to probe the involvement of radical anions[14] and/

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[1] (a) *Bioassay of Tetrachloroethylene for Possible Carcinogenicity*; NCI Technical Report No. 13; National Cancer Institute: Bethesda, MD, 1977. (b) *Toxicology and Carcinogenesis Studies of Tetrachloroethylene*; NTP Technical Report No. 311; National Toxicology Program: Research Triangle Park, NC, 1986.

[2] (a) Gantzer, C. J.; Wackett, L. P. *Environ. Sci. Technol.* **1991**, *25*, 715−722. (b) Lesage, S.; Brown, S.; Millar, K. *Groundwater Monit. Rem.* **1996**, *16*, 76−85 (c) Glod, G.; Angst, W.; Holliger, C.; Schwarzenbach, R. P. *Environ. Sci. Technol.* **1997**, *31*, 253−260. (d) Burris, D. R.; Delcomyn, C. A.; Smith, M. H.; Roberts, A. L. *Environ. Sci. Technol.* **1996**, *30*, 3047−3052. (e) Burris, D. R.; Delcomyn, C. A.; Deng, B. L.; Buck, L. E.; Hatfield, K. *Environ. Toxic. Chem.* **1998**, *17*, 1681−1688. (f) Lesage, S.; Brown, S.; Millar, K. *Environ. Sci. Technol.* **1998**, *32*, 2264−2272.

[3] Vogel, T. M.; Criddle, C. S.; McCarty, P. L. *Environ. Sci. Technol.* **1987**, *21*, 722−736.

[4] For reviews see: (a) Mohn, W. W.; Tiedje, J. M. *Microbiol. Rev.* **1992**, *56*, 482−507. (b) Fetzner, S.; Lingens, F. *Microbiol. Rev.* **1994**, *58*, 641−85. (c) Wohlfarth, G.; Diekert, G. *Curr. Opin. Biotechnol.* **1997**, *8*, 290−295.

[5] (a) Neumann, A.; Wohlfarth, G.; Diekert, G. *J. Biol. Chem.* **1996**, *271*, 16515−16519. (b) Schumacher, W.; Holliger, C.; Zehnder, A. J.; Hagen, W. R. *FEBS Lett.* **1997**, *409*, 421−425.

[6] At pH 8, the one-electron redox potentials of Ti(III) citrate and Cob-(I/II)alamin are −630 and −610 mV, respectively. See: Zehnder, A. J. B.; Wuhrmann, K. *Science* **1976**, *194*, 1165−1166. Lexa, D.; Savéant, J.-M. *Acc. Chem. Res.* **1983**, *16*, 235−243.

[7] Schrauzer, G. N.; Deutsch, E.; Windgassen, R. J. *J. Am. Chem. Soc.* **1968**, *90*, 2441−2442.

[8] Compound **1** could be formed either via a classical two-electron nucleophilic addition or in a two-step electron transfer-radical combination process providing either **1** or **2**.

[9] Hogenkamp, H. P. C. Reactions of Alkyl Ligands Coordinated to Cobalamins and Cobaloximes. In *B_{12}*; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 1, Chapter 9.

[10] (a) Griller, D.; Ingold, K. U. *Acc. Chem. Res.* **1980**, *13*, 317−323. (b) Newcomb, M.; Curran, D. P. *Acc. Chem. Res.* **1988**, *21*, 206−214. (c) Newcomb, M. *Tetrahedron*, **1993**, *49*, 1151.

[11] (a) Tanko, J. M.; Phillips, J. P. *J. Am. Chem. Soc.* **1999**, *121*, 6078−6079. (b) Tanner, D. D.; Chen, J. J.; Luelo, C.; Peters, P. M. *J. Am. Chem. Soc.* **1992**, *114*, 713−717.

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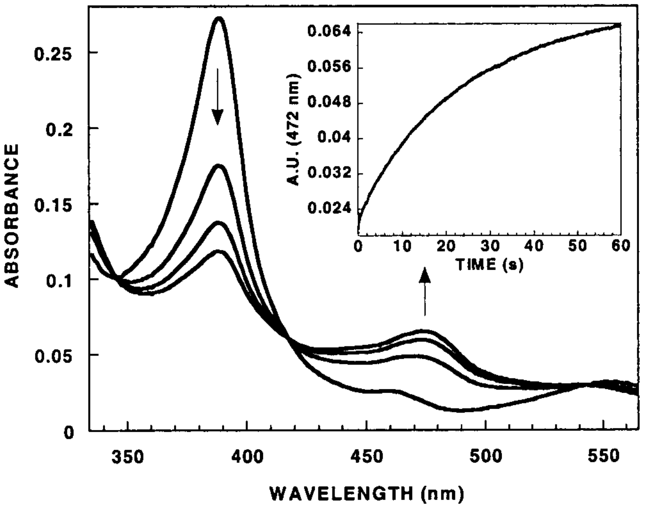

Figure 1. Stopped-flow rapid scanning spectra taken 0.16, 19, 39, and 58 s after mixing Co(I) with PCE; final concentrations were 10 μM and 1 mM, respectively. The inset shows Co(II) formation.[26]

or vinyl radicals. The probes were reacted with catalytic vitamin B_{12} in the presence of excess Ti(III) citrate as reducing agent. *cis*-Cyclopropane **4** yielded **7** as the major product along with a small amount of reduction product **8**.[15] The trans isomer **5** on the other hand provided only reduced materials. In both reactions a small amount of allene (2−3%) was detected,[16] that may have arisen from ring opening of a radical anion or a vinyl radical formed at carbon 2.[17] B_{12}-catalyzed dechlorination of **6** provided a mixture of ring-closed and reduced products. Cyclization occurred exclusively in 6-(π-endo)exo fashion[18] and no conjugated products were observed.[19] Collectively, these experiments provide support for radical intermediates consistent with mechanisms B and C.[20]

The generation of reduced products **8**−**11** prompted us to investigate the source of the vinyl hydrogen. These products may be formed by reduction of the radicals to their respective anions followed by protonation or by hydrogen atom abstraction from a suitable donor. Two complementary experiments were carried out in which Ti(III) citrate and vitamin B_{12} were reacted with **5** in a 1:1 mixture of either H_2O and $(CH_3)_2CDOH$ or D_2O and $(CH_3)_2CHOD$. A significant fraction of the products resulted from deuterium atom (eq 2) or hydrogen atom (eq 3) transfer from C2 of 2-propanol, a good hydrogen atom donor.[21] The higher percentage of hydrogen atom transfer from 2-propanol (eq 3) compared to deuterium atom transfer from 2-d_1-*i*-PrOH (eq 2) likely reflects a primary kinetic isotope effect on the abstraction step.[22]

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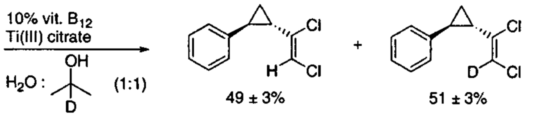

(2)

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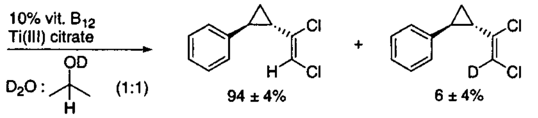

(3)

We next turned our attention to the catalyst itself. The oxidation state of the metal in mechanisms A−C varies, providing an avenue for differentiation using UV−visible spectroscopy. Vitamin B_{12} was prereduced with 7 equiv of Ti(III) citrate in a 1:9 solution of EtOH and 50 mM Tris buffer, pH 8.0. A solution of the reduced cobalamin (Co^I) was mixed in a stopped-flow system with PCE at 25 °C. The Co^I absorbance at 390 nm disappeared concomitant with the emergence of a band with a λ_{max} at 475 nm corresponding to Co^{II} (Figure 1).[23] Two isosbestic points at 418 and 543 nm suggest Co^I transfers an electron to PCE to form Co^{II} without the formation of an intermediate.[24] No evidence could be found for Co(III) intermediates such as a trichloroethenyl cobalamin **2**.[25]

In summary, this work supports a one-electron transfer from cob(I)alamin to PCE as the first step in the catalytic dechlorination of this ubiquitous pollutant. A detailed kinetic investigation of the electron transfer step is currently in progress.

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Supporting Information Available: Procedures for the preparation of **4**−**6** and full characterization of the products of dechlorination (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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[12] (a) Stork, G.; Baine, N. H. *J. Am. Chem. Soc.* **1982**, *104*, 2321−2323. (b) Beckwith, A. L. J.; O'Shea, D. M. *Tetrahedron Lett.* **1986**, *27*, 4525−4528. (c) Montevecchi, P. C.; Navacchia, M. L. *J. Org. Chem.* **1997**, *62*, 5600−5607. (d) Curran, D. P. Radical Addition Reactions. In *Comprehensive Organic Synthesis*; Trost, B. M., Ed.; Pergamon Press: Oxford, U.K., 1991; Vol. 4, Chapter 4.1.

[13] (a) Snider, B. B.; Zhang, Q.; Dombroski, M. A. *J. Org. Chem.* **1992**, *57*, 4195−4205. (b) Citterio, A.; Sebastiano, R.; Maronati, A.; Santi, R.; Bergamini, F. *J. Chem. Soc., Chem. Commun.* **1994**, 1517−1518. (c) Nanni, D.; Pareschi, P.; Tundo, A. *Tetrahedron Lett.* **1996**, *37*, 9337−9340. (d) Montevecchi, P. C.; Navacchia, M. L. *J. Org. Chem.* **1998**, *63*, 537−542.

[14] A PCE radical anion will likely have a very different electronic structure than a ketyl radical anion and may not even exist as a discrete intermediate in the case of a concerted dissociative electron transfer. Theoretical studies to address these questions are in progress.

[15] Product **7** may result from a 6-(π-endo) cyclization or 5-(π-endo) cyclization to give an ipso-substituted cyclohexadienyl radical that can undergo ring expansion, see refs 13d and 12b.

[16] A small amount (2−7%) of fully dechlorinated alkene was also detected in the reactions of **4**−**6**. No acetylene products (<1%) were detected.

[17] Relatively little is known about the rate of cyclopropane ring opening when located α to a vinyl radical.

[18] 6-(π-Endo)exo cyclization of vinyl radicals in preference to 1,5-hydrogen transfer has been reported previously, see: Curran, D. P.; Shen, W. *J. Am. Chem. Soc.* **1993**, *115*, 6051−6059 and ref 12c.

[19] A similar observation was made in another cobaloxime mediated cyclization reaction: Baldwin, J. E.; Li, C.-S. *J. Chem. Soc., Chem. Commun.* **1987**, 166−168.

[20] For other support for the intermediacy of vinyl radicals, see: Lesage, S.; Brown, S. *Abstracts of Papers*; 208th National Meeting of the American Chemical Society; American Chemical Society: Washington, DC, 1994; ENV 652−656.

[21] Deuterium transfer from 2-propanol to a trichloroethenyl radical has been previously reported. See ref 2c.

[22] The vinyl radical may partition between hydrogen/deuterium atom abstraction from *i*-PrOD/2-d_1-*i*-PrOH and an isotope-insensitive reduction to the vinyl anion, which will be protonated by H_2O/*i*-PrOH (eq 2) or deuterated by D_2O/*i*-PrOD (eq 3). The species responsible for reducing the vinyl radical is not known and could be Co(I) ($E°$ −0.61 V),[6] Ti(III) ($E°$ −0.63 V),[6] or the ketyl radical formed in the hydrogen atom abstraction from *i*-PrOH ($E°$ −1.04 V: Benderskii, V. V.; Krivenkov, A. G. *Russ. Chem. Rev. Engl. Transl.* **1990**, *59*, 1). At present we cannot rule out that the highly reducing ketyl radical may also contribute to PCE reduction as suggested by one of the referees.

[23] EPR analysis of the reaction mixture supports the formation of Co(II) in quantities similar to those observed by UV−vis spectroscopy.

[24] We cannot unequivocally rule out slow formation of an alkylcobalamin that is converted to Co(II) with a much faster rate.

[25] Similarly, the reactions of compounds **4**−**6** with Co(I) produced Co(II).

[26] Co(I) consumption and Co(II) formation in Figure 1 displayed biphasic kinetics, and was fitted to a double exponential. $k_1 = 0.175 \pm 0.006$ s^{-1}; $k_2 = 0.0366 \pm 0.0002$ s^{-1}. The origin of the biphasic kinetics is under investigation.