Mechanism of Aqueous Decomposition of Trichloroethylene Oxide

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Abstract: The aqueous decomposition of trichloroethylene (TCE) oxide is shown to involve both pH-independent and hydronium ion-dependent regions. C−C bond scission is a major reaction at all pH values. Disappearance of TCE oxide is the rate-determining step for the formation of CO under the conditions studied. The product distribution of CO and three carboxylic acids (HCO_2H, Cl_2CHCO_2H, and glyoxylic acid) did not change considerably over the pH range of −1.5−14, in general, even though the hydrolysis mechanism changes from hydronium ion-dependent to pH-independent. Mechanisms for the hydronium ion-dependent and pH-independent hydrolysis of TCE oxide were elucidated on the basis of the results of $H_2{}^{18}O$ and H incorporation and identification of products of the reaction of TCE oxide with lysine in both $H_2{}^{16}O$ and $H_2{}^{18}O$. In the pH-independent hydrolysis, a zwitterionic intermediate could be formed and undergo an intramolecular rearrangement (Cl^- shift) to generate dichloroacetyl chloride, which would subsequently decompose to Cl_2CHCO_2H. The zwitterionic intermediate could also hydrolyze at the less sterically hindered methylene to give a glycol anion, which would dehydrohalogenate to form an oxoacetyl chloride intermediate. The oxoacetyl chloride could hydrolyze to generate either glyoxylic acid, as a final product, or an anionic intermediate, which could go through a concerted mechanism to generate CO, HCO_2H, and chloride. A mechanism proposed for the hydronium ion-dependent hydrolysis is very similar to that for the pH-independent hydrolysis except for the first step, which involves hydronium ion attack on TCE oxide to form a TCE-oxide cation intermediate. The lysine amide adducts were characterized by HPLC and mass spectrometry as those resulting from reaction with the postulated acyl chlorides.

Introduction

Oxiranes, also called "epoxides," are versatile intermediates in organic synthesis.[1−11] They are easily prepared from a variety of starting materials, and the inherent polarity and strain of the three-membered ring makes them reactive toward a large number of reagents, such as electrophiles, nucleophiles, acids, bases, reducing agents, and some oxidizing agents.

Halogenated epoxides are rather rare but nonetheless an important part of the diverse oxirane family. The mechanisms of their thermal decomposition have been studied in organic solvents and shown to be dominated by halide migration.[12,13] However, the hydrolytic reactions are more complex and, with polyhalogenated alkene epoxides, involve extensive C−C scission, which is poorly understood but may be highly relevant to issues of the associated biology and toxicology. Trichloroethylene (TCE) is an extensively used industrial solvent and common water pollutant.[14] The compound is of biological interest because oxidative metabolism of TCE leads to the covalent binding of TCE to proteins and possibly DNA[15−20] as well as to inactivation of cytochrome P450.[21] Furthermore, human liver microsomes oxidize TCE to products that alkylate protein and, to a much lesser extent, DNA.[21] Studies of the microsomal oxidation of TCE suggest that a number of potentially reactive electrophiles are formed during oxidation metabolism of TCE. TCE oxide (**1**) is one of the products[13,21] and has often been postulated to be responsible for the covalent binding of TCE to proteins and possibly DNA.[17,18] Currently, it is not known whether TCE oxide reacts directly with nucleophiles, or whether decomposition products of TCE oxide or other P450 oxidation products react.

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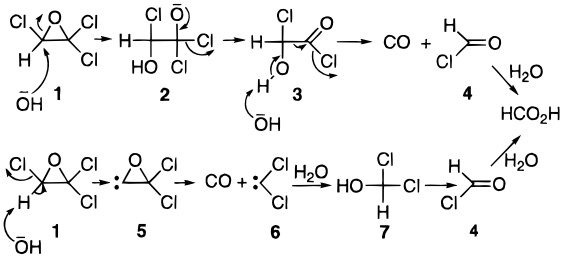
Scheme 1. Two Proposed Mechanisms (This Laboratory[24]) for the Formation of CO and HCO_2H under Basic Conditions



In 1978 Van Duuren et al. synthesized TCE oxide and reported its hydrolysis and thermolysis rates.[22] Cl_2CHCO_2H was the only product reported to be formed in the hydrolysis and thermolysis of TCE oxide. The mechanism for the thermal rearrangement of TCE oxide was proposed to proceed through an α-carbonyl carbonium ion intermediate.[22] However, a mechanism for hydrolysis of TCE oxide was not considered. Henschler et al. later reported that TCE oxide rearranged to Cl_2CHCO_2H or hydrolyzed to CO, HCO_2H, and to a much lesser extent, glyoxylic acid.[23] Mechanisms were also proposed to account for the formation of glyoxylic acid, CO, and HCO_2H in aqueous solutions. Our laboratory subsequently examined the rearrangement of TCE oxide under a variety of conditions.[24] In the absence or presence of cytochrome P450, TCE oxide did not rearrange to form chloral,[24] in contrast to the proposals of Byington and Leibman[25] and Henschler et al.[23] Hydrolysis of TCE oxide yielded primarily glyoxylic acid and Cl_2CHCO_2H at low pH or CO and HCO_2H at neutral or high pH.[24] Mechanisms were proposed to account for the hydrolysis and rearrangement of TCE oxide under acidic and basic conditions, with two possible mechanisms for the observed C−C scission postulated for neutral and basic conditions (Scheme 1). The first involves nucleophilic attack by hydroxide ion and opening of the oxirane ring; the second involves formation of a carbene intermediate **5**. Other work with the analogue vinylidene chloride epoxide also indicated extensive C−C bond scission.[26] This behavior is not observed with the more stable monochloro epoxide, 2-chlorooxirane.[27]

To elucidate the mechanism of aqueous decomposition of TCE oxide, we carried out an extensive study on this epoxide, including pH profiles of its disappearance and the formation of CO, product analyses over a wide pH range, $H_2{}^{18}O$ incorporation, H incorporation, and reaction of **1** with the model nucleophile lysine in both $H_2{}^{16}O$, $H_2{}^{18}O$, and ^2H_2O.

Experimental Section

Chemicals. Solvents for HPLC and HPLC/MS were HPLC grade (EM Science, Gibbstown, NJ). Solvents for GC/MS were nanograde (Mallinckrodt Baker, Paris, KY). TCE was distilled to remove inhibitors prior to epoxide synthesis. Reagents for synthesis, kinetics, and product analysis were ACS grade or better, unless otherwise specified.

Spectroscopy. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM 400 spectrometer at 400.13 MHz and 27 °C (Bruker, Billerica, MA). Samples were either prepared in D_2O, $CDCl_3$, or CD_3OD (100.0 atom % D grade, Aldrich Chemical Co., Milwaukee, WI). Mass spectrometry was done with a HP 5890A gas chromatograph (Hewlett-Packard Company, Wilmington, DE) and a Finnigan MAT INCOS 50 mass spectrometer (Finnigan Mat, San Jose, CA) for CO (electron impact mode) or performed with a Waters 2690 separations module (Alliance) (Waters Corporation, Milford, MA) and a Finnigan MAT TSQ/SSQ 7000 series mass spectrometer for carboxylic acids (electrospray mode unless noted otherwise).

Syntheses. TCE oxide (1). The epoxide was synthesized by using a general two-phase method for acid-sensitive epoxides.[28] *m*-Chloroperbenzoic acid (*m*CPBA, 25 g) was dissolved in 200 mL of CH_2Cl_2. The CH_2Cl_2 solution was washed with 0.10 M sodium phosphate buffer (pH 7.4, 3 × 200 mL) and 200 mL of the same buffer was added. TCE (5 g) was added dropwise, and the mixture was stirred at room temperature for 2 h. The organic layer was washed with 0.5 M $Na_2S_2O_3$ (3 × 200 mL), washed with ice-cold 6 N NaOH (3 × 100 mL), and dried over anhydrous Na_2SO_4. The dried solution was carefully concentrated under a stream of N_2 to ~10 mL. The product, TCE oxide (yield 10% based on TCE), was purified by distillation (~40 °C, 130 mmHg). The ^1H NMR spectrum showed two singlets at δ 6.52 and 5.31, corresponding to TCE and TCE oxide, respectively.[22,24] TCE oxide accounted for 20% of the volume. The decoupled ^{13}C NMR spectrum showed two singlets for TCE oxide at 73.7 (*C*HCl) and 85.7 ppm (CCl_2), in addition to the two singlets for TCE at 116.6 (*C*HCl) and 123.9 ppm (CCl_2).[24]

The same procedure was used to prepare [^{2}H]TCE oxide from ^{2}H-TCE (Cambridge Isotopes, Cambridge, MA). The ^{13}C NMR spectrum showed two peaks for [^{2}H]TCE oxide at 73.7 (t, C^2HCl) and 85.7 ppm (s, CCl_2) in addition to the two peaks for [^{2}H]TCE at 116.6 (t, C^2HCl) and 123.9 ppm (s, CCl_2).

***N*$^\alpha$-Formyl-*N*$^\epsilon$-CBZ-L-lysine.** Acetic anhydride (1.5 mL) was added dropwise to a mixture of 88% HCO_2H (7 mL) and *N*$^\epsilon$-CBZ-L-lysine (1.5 g). After 4 h (room temperature), the reaction mixture was evaporated to dryness in vacuo. The product was further purified by recrystallization from EtOH[29] (yield 65%): ^1H NMR (CD_3OD) δ 1.34 (m, 2H, γ-CH_2), 1.45 (m, 2H, β-CH_2), 1.60−1.90 (m, 2H, δ-CH_2), 3.07 (t, 2H, ϵ-CH_2), 4.57 (t, 1H, α-C*H*), 4.99 (s, 2H, PhCH_2), 7.20−7.30 (m, 5H, Ph), 8.13 (s, 1H, C*H*O).

***N*$^\alpha$-Formyl-L-lysine.** *N*$^\alpha$-Formyl-*N*$^\epsilon$-CBZ-L-lysine (vide supra, 0.8 g) was hydrogenated over Pd-charcoal (0.15 g) in 8 mL of CH_3OH at room temperature for 5 h. After removal of the catalyst (filtration) and solvent (in vacuo), the product was purified by recrystallization from EtOH,[29] yield 55%: ^1H NMR (D_2O) δ 1.40 (m, 2H, γ-CH_2), 1.67 (m, 2H, β-CH_2), 1.83 (m, 2H, δ-CH_2), 2.95 (t, 2H, ϵ-CH_2), 3.10 (t, 1H, α-C*H*), 8.06 (s, 1H, C*H*O); MS, *m*/*z* 175.22 (MH^+).

***N*$^\epsilon$-Dichloroacetyl-L-lysine.** Cl_2CHCO_2H (0.11 mL) was added dropwise to a solution of *N*$^\alpha$-*tert* BOC-L-lysine in CH_3OH with 0.15 g NaOH at room temperature. After 2.5 h, concentrated HCl (80 μL) was added to the reaction and the mixture was taken to dryness in vacuo. The residue was dissolved in a mixture of 1 mL of H_2O and 2 mL of CF_3CO_2H and evaporated to dryness again after 30 min. The product was purified by recrystallization from EtOH,[30,31] yield 60%: ^1H NMR (D_2O) δ 1.38 (m, 2H, γ-CH_2), 1.59 (m, 2H, β-CH_2), 1.88 (m, 2H, δ-CH_2), 3.22 (t, 2H, ϵ-CH_2), 3.70 (t, 1H, α-C*H*), 6.26 (s, 1H, Cl_2CH); MS, *m*/*z* 258.22 (MH^+).

***N*$^\epsilon$-Acryloyl-L-lysine.** Acryloyl chloride (0.12 mL) was added dropwise to a solution of 0.3 g *N*$^\alpha$-*tert* BOC-L-lysine in a mixture of 3 mL of aqueous 1 N NaOH and 3 mL CH_3CN. Two hours later, the mixture was acidified to pH with 1 N HCl, and the mixture was extracted with EtAc (3 × 3 mL). The EtAc extracts were combined, dried over anhydrous Na_2SO_4, and evaporated to dryness in vacuo. The residue was dissolved in H_2O/CF_3CO_2H (1:1, v−v). After 30 min, the mixture was taken to dryness, first under N_2 and then in vacuo (yield

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78%):[30,31] ^{1}H NMR (CD_3OD) δ 1.12 (m, 4H, γ-CH_2 and β-CH_2), 1.47 (m, 2H, δ-CH_2), 2.80 (t, 2H, ϵ-CH_2), 3.50 (t, 1H, α-C*H*), 5.17 (dd, 1H, CH_2═C*H*), 5.75 (d, 2H, CH_2═CH).

***N*$^\epsilon$-Glyoxylyl-L-lysine.** A solution of *N*$^\epsilon$-acryloyl-L-lysine (vide supra, 0.2 g) in CH_3OH was treated with a stream of O_3 for 45 min at −78 °C. The reaction was purged with Ar for 5 min to remove dissolved O_3 and 5 mL of $(CH_3)_2S$ was added, and the reaction mixture was stirred at −78 °C for 2 h and then at room temperature for 12 h.[32,33] The reaction mixture was taken to dryness in vacuo and the product was purified by recrystallization from EtOH (yield 30%): ^{1}H NMR (D_2O) δ 1.45 (m, 2H, γ-CH_2), 1.68 (m, 2H, β-CH_2), 1.87 (m, 2H, δ-CH_2), 2.98 (t, 2H, ϵ-CH_2), 3.76 (t, 2H, α-C*H*), 5.12 (s, 1H, COC*H*O); ^{13}C NMR (D_2O) 22.0 ppm (γ-*C*H$_2$), 26.6 (δ-*C*H$_2$), 30.5 (β-*C*H$_2$), 53.9 (ϵ-*C*H$_2$), 55.3 (α-*C*H), 169.0 (*C*OCHO), 174.7 (*C*OCHO), 175.7 (*C*OOH); MS, *m*/*z* 203.22 (MH^+).

Kinetics. TCE oxide was diluted into dry CH_3CN, and the concentration was estimated using a colorimetric assay with 4-(4-nitrobenzyl)pyridine, using $\epsilon_{560} = 3.0 \times 10^4\ M^{-1}\ cm^{-1}$.[34] The kinetics of the decomposition of TCE oxide were observed by diluting TCE oxide stock solution into 1.0 mL of buffer (final concentration ∼1.0 mM) at 0 °C. Aliquots of 50 μL were withdrawn at various times, and the concentration of residual TCE oxide was measured colorimetrically at 520 nm following reaction with 4-(4-nitrobenzyl)pyridine.[24]

The kinetics of formation of CO were determined with an electrochemical sensor (ERMF-0503; Draeger, Pittsburgh, PA). The detector was placed in a circuit containing the reaction vial and a peristaltic pump.[35] TCE oxide stock solution was injected into a vial containing 1.0 mL of buffer (final concentration ∼1.0 mM) at 0 °C. The readings of the sensor were recorded at various times.

Product Analysis. CO was measured using the electrochemical detector system described above. The gas volume of the circuit was pumped through the system until it reached an equilibrium between the gas and water phase. Subsequently, the reading of the sensor was recorded. The amount of CO formed was calculated based on a standard curve generated using measured volumes of pure CO gas.

HCO_2H and Cl_2CHCO_2H were measured by HPLC. TCE oxide stock solution was injected into 1.0 mL of buffer (final concentration ∼1.0 mM). After 5 min, the reaction solution was acidified with conc H_2SO_4 (to pH ∼1), and the mixture was extracted with Et_2O (3 × 1.0 mL). The Et_2O extracts were combined and extracted with 1.0 mL of 10 mM tetrabutylammonium hydrogen sulfate (TBAS) (pH 6.0) in H_2O. The final aqueous solution was subjected to HPLC analysis. For HPLC analysis, a stock solution (200 mM) of the ion-pair reagent TBAS was prepared and the pH was adjusted to 6.0 with solid Na_2HPO_4. The isocratic reverse phase solvent was prepared by diluting the 200 mM stock TBAS to give a final concentration of 10 mM in H_2O.[36] HPLC conditions (Econosphere C18, 4.6 × 150 mm) were: flow rate 1.5 mL min^{-1}, solvent: 10 mM TBAS in H_2O (pH 6.0), λ 210 nm.

Glyoxylic acid formation (from TCE oxide) was measured in reactions of the type described above. After 5 min, the products were derivatized with 2,4-dinitrophenylhydrazine and analyzed for glyoxylic acid by isocratic reversed-phase HPLC.[24] HPLC conditions (Zorbax C18, 6.2 × 80 mm) were: flow rate 1.0 mL min^{-1}, solvent A: CH_3CN/H_2O, 10/90, v−v, solvent B: CH_3CN/H_2O, 45/55, v−v, t =0 min: 100% A, t = 3 min: 50% A, 50% B, t = 5 min: 100% B, t = 9 min: 100% B, t = 12 min: 100% A, λ 360 nm.

^{18}O Incorporation into TCE Oxide Products. Decomposition of TCE oxide was carried out at room temperature in $H_2{}^{18}O$ (95% atom excess, Aldrich). The reaction was allowed to proceed for a certain amount of time, depending on which products were analyzed, and Et_3N was added to neutralize the sample.

Incorporation of $H_2{}^{18}O$ into glyoxylic acid derived from TCE was measured in the following manner. TCE oxide (4 μL of a mixture of TCE oxide/TCE, 1:5, v−v) was injected into a tightly capped vial (Teflon liner) containing 100 μL $H_2{}^{18}O$ (95% atom excess) and 6 μL of diglyme. After 30 s of the reaction, 2 mg of $NaBH_4$ was added to the reaction mixture to reduce the glyoxylic acid to glycolic acid and minimize carbonyl exchange. In an alternate experiment, the reaction was diluted 10-fold with unlabeled H_2O ($H_2{}^{16}O$) to exchange the aldehyde oxygen to ^{16}O, and then $NaBH_4$ was added. The reaction solutions were subjected to HPLC/MS analysis. The relative amounts of ^{18}O and ^{16}O in glyoxylic acid were determined by monitoring the signals at *m*/*z* 75, 77, 79, and 81. HPLC/MS conditions (Zorbax RX-C18, 2.1 × 150 mm) were: flow 0.5 mL min^{-1}, 50 mM NH_4HCO_3 (pH 6.0), atmospheric pressure chemical ionization source, mass range *m*/*z* 20−180. All results are presented as means ± SD of triplicate determinations.

HCO_2H and Cl_2CHCO_2H analyses were done in the same general manner as that described above with the same HPLC/MS conditions. After 120 s reaction time, the reaction mixture was made basic by the addition of 4 μL Et_3N and used for HPLC/MS analysis. In the case of HCO_2H, the relative amounts of ^{18}O and ^{16}O were determined by monitoring the signals at *m*/*z* 45, 47, and 49. For Cl_2CHCO_2H, the relative amounts of ^{18}O and ^{16}O were quantitated using the signals at *m*/*z* 127, 129, and 131.

CO analyses used the same general experimental procedure. After a certain amount of time, 10 μL of the headspace of the reaction vial was injected into the GC/electron impact (EI) MS system for analysis. The relative amounts of ^{18}O and ^{16}O in CO were determined by monitoring the signals at *m*/*z* 28 and 30. The GC/EI MS conditions (Alltech AT-Mole Sieve column, 0.53 mm i.d. × 30 m) were: isothermal temperature 40 °C, injector temperature 150 °C, septum purge 4.9 mL min^{-1}, total flow 49 mL min^{-1}, purge-off time 0 s, purge-on time 30 s, mass range *m*/*z* 20−50, multiplier voltage 1200 V.

Control experiments for ^{18}O incorporation were done with the organic acids. HCO_2H (1 μL, 88% in H_2O), glyoxylic acid (0.1 mg), or glycolic acid (0.1 mg) was added to 50 μL of $H_2{}^{18}O$ (95% atom excess). In the case of glyoxylic acid, $NaBH_4$ (1 mg) was added to the reaction mixture after 30 s. For both HCO_2H and glycolic acid, 4 μL of Et_3N was added after 2 min reaction time. The amounts of ^{18}O incorporation into those acids were determined by direct injection of the diluted reaction mixtures (CH_3OH) into MS. The amount of ^{18}O remaining with each of those acids after HPLC column separations was also quantified by HPLC/MS, using the conditions described earlier. ^{18}O-enriched Cl_2CHCO_2H was synthesized by reacting $Cl_2CHCOCl$ with $H_2{}^{18}O$ (95% atom excess). The amount of ^{18}O incorporation into Cl_2CHCO_2H was determined by direct injection of the diluted reaction mixture (CH_3OH) into the mass spectrometer (direct loop injection). The amount of ^{18}O remaining with Cl_2CHCO_2H after HPLC column separations was also quantified by HPLC/MS.

^{2}H Incorporation into TCE Oxide Products. ^{2}H incorporation into TCE oxide products (HCO_2H, Cl_2CHCO_2H, and glyoxylic acid) was done in the same general manner as that described above. After 3 min of the reaction, 4 μL of Et_3N was added to the mixture. The relative amounts of ^{2}H and ^{1}H were determined by monitoring the signals at *m*/*z* 45 and 46 for HCO_2H, 73 and 74 for glyoxylic acid, and 127 and 128 for Cl_2CHCO_2H.

Control experiments for ^{2}H incorporation were done with the organic acids as described for the ^{18}O incorporation experiments.

^{1}H Incorporation into ^{2}H-TCE Oxide Products. ^{1}H incorporation into ^{2}H-TCE oxide products was performed in the same general manner as that described above for the opposite experiment (vide supra).

Reaction of TCE Oxide with Lysine. TCE oxide (2 μmol), in CH_3CN, was added to a solution of L-lysine (2.6 μmol) in H_2O (0.10 mL). After 30 min, the reaction mixture was evaporated to dryness. A coupling buffer (CH_3CN/pyridine/Et_3N/H_2O, 10/5/2/3, v−v−v−v, 0.10 mL) was used to dissolve the residue, and then phenylisothiocyanate (PITC) (5 μL, 42 μmol) was added. After 15 min, the reaction mixture was taken to dryness in vacuo. The residue was dissolved in 0.5 mL of a solution of 0.1 M $NH_4CH_3CO_2$ (pH 6.4)/CH_3CN/CH_3OH (5/4/1,

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v−v−v) for both HPLC/UV(at 254 nm) and HPLC/MS analyses.[37] HPLC conditions (Zorbax XDB-C8, 2.1 × 150 mm) were: flow rate 0.3 mL min^{-1}, solvent A: 20 mM $NH_4CH_3CO_2$ in H_2O (pH 6.0), solvent B: 40 mM $NH_4CH_3CO_2/CH_3CN/CH_3OH$, pH 8.4, 5/4/1, v−v−v, t = 0 min: 95% A, 5% B, t = 5 min: 85% A, 15% B, t = 12.5 min: 50% A, 50% B, t = 16 min: 50% A, 50% B, t = 17.5 min: 0% A, 100% B, t = 19 min: 0% A, 100% B, t = 21.5 min: 95% A, 5% B, t = 28 min: 95% A, 5% B. In control experiments, TCE oxide (2 μmol) was added to 0.10 mL of H_2O. After 30 min, lysine (2.6 μmol) was added to the reaction mixture. Analysis of the products was done as described for the direct reaction with TCE oxide.

^{18}O Incorporation into Reaction Products of TCE Oxide with Lysine. ^{18}O incorporation into reaction products of TCE oxide with lysine was carried out in the same manner as that described above except that $H_2{}^{18}O$ (95% atom excess) was used instead of unlabeled H_2O ($H_2{}^{16}O$). After derivatization with PITC, the residue was dissolved in 0.5 mL of a solution of 10 mM $NH_4CH_3CO_2$ in H_2O/ CH_3CN (3/7, v−v) for HPLC/MS analyses.[37] The relative amounts of ^{16}O and ^{18}O were determined by monitoring the signals at *m*/*z* 310 and 312 for *N*-formyllysine, 396 and 398 for *N*-dichloroacetyllysine, and 338 and 340 for *N*-glyoxylyllysine, as well as by collecting full spectra. HPLC conditions (Zorbax RX-C18, 2.1 × 150 mm) were: flow rate 0.2 mL min^{-1}, solvent A: 10 mM $NH_4CH_3CO_2$ in H_2O (pH 4.0), B: 10 mM $NH_4CH_3CO_2$ in H_2O/CH_3CN, 3/7, v−v, t = 0 min: 95% A, 5% B, t = 10 min: 85% A, 15% B, t = 25 min: 50% A, 50% B, t = 32 min: 50% A, 50% B, t = 35 min: 0% A, 100% B, t = 40 min: 0% A, 100% B, t = 41.5: min 95% A, 5% B, t = 45 min: 95% A, 5% B.

^{2}H Incorporation into Reaction Products of TCE Oxide with Lysine. ^{2}H incorporation into reaction products of TCE oxide with lysine was performed in the same manner as described above, using $^{2}H_2O$ instead of $H_2{}^{18}O$. The relative amounts of ^{1}H and ^{2}H were determined by monitoring the signals at *m*/*z* 310 and 311 for *N*-formyllysine.

Results

pH Dependence of Decomposition of TCE Oxide (1). The decomposition of TCE oxide and the formation of CO both showed clear first-order kinetics. At 23 °C the $t_{1/2}$ of TCE oxide is 12 s. To make careful comparisons, we did all kinetic assays at 0 °C, where the $t_{1/2}$ is ∼100 s. Most of the determinations were in the pH range of −2−14 at 0 °C (ionic strength 0.10 M). The change in k_{obsd} as a function of pH for the decomposition of TCE oxide is shown in Figure 1A with $k_H = 3.3 \times 10^{-2}$ M^{-1} s^{-1} and $k_{H_2O} = 6.9 \times 10^{-3}$ s^{-1}. The k_{obsd} (k_{H_2O}) for the formation of CO (Figure 1 B) was 8×10^{-3} s^{-1}, identical to that for TCE oxide hydrolysis.

Products of TCE Oxide Hydrolysis. Four products (CO, HCO_2H, Cl_2CHCO_2H, and glyoxylic acid) were observed, as before,[24] and quantitated (Figure 2). The formation of HCHO could not be detected by HPLC (as the 2,4-dinitrophenylhydrazone)[38] or ^{1}H NMR product analyses. The sum of the four products was in the range of 70−95% theoretically expected from TCE oxide at all pH values. For instance, at neutral pH (7.3) 1.0 mM TCE oxide yielded 0.36 mM Cl_2CHCO_2H, 0.20 mM glyoxylic acid, 0.42 mM HCO_2H, and 0.21 mM CO. The amount of Cl_2CHCO_2H formed was higher at pH < 0 and almost constant in the pH range 0−14. In the case of glyoxylic acid, the amount formed was similar at all pH values (with two low values probably due to buffer artifacts). CO formation was less at low pH (−2 to 0) and constant in the pH range 0−9.5. Between pH ∼9.5−11.5 the amount of CO increased (∼2-fold) with increasing pH and was rather constant from pH 11.5 to 14. The amount of HCO_2H formed remained constant from pH ≈ −1 to 11 and increased at pH > 12.

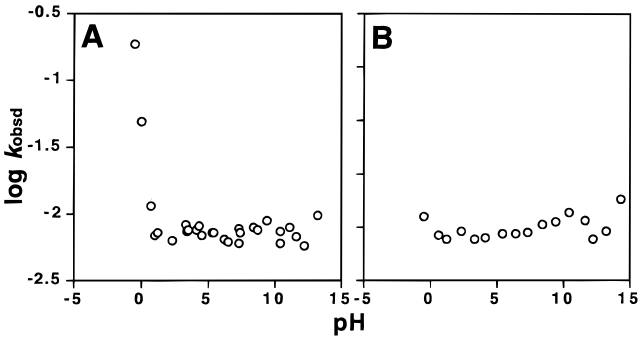

Figure 1. pH profiles for the hydrolysis of TCE oxide (**1**) and the formation of CO in aqueous solutions at 0 °C. (A) TCE oxide disappearance, (B) CO formation. Buffers used include $HClO_4$/NaOH (pH −0.49, −0.04, 0.63, and 0.99), H_3PO_4/NaOH (pH 1.17, 2.34, 3.25, and 3.30), EDTA/HCl (pH 4.14, 5.41, 6.15, 7.34, 8.38, 9.37, and 10.36), EDTA/NaOH (pH 11.62), NaH_2PO_4/NaOH (pH 6.50, 7.25, and 7.40), CH_3CO_2H/NaOH (pH 3.37, 3.50, 4.30, and 4.51), 2-(*N*-morpholino)-ethanesulfonic acid (MES)/NaOH (pH 5.30), 2-(*N*-cyclohexylamino)-ethanesulfonic acid (CHES)/NaOH (pH 8.70), 3-(*N*-cyclohexylamino)-1-propanesulfonic acid (CAPS)/NaOH (pH 10.39 and 11.13), and $HClO_4$/NaOH (pH 12.20 and 13.24). The ionic strength was 0.10 M ($NaClO_4$) in all cases.

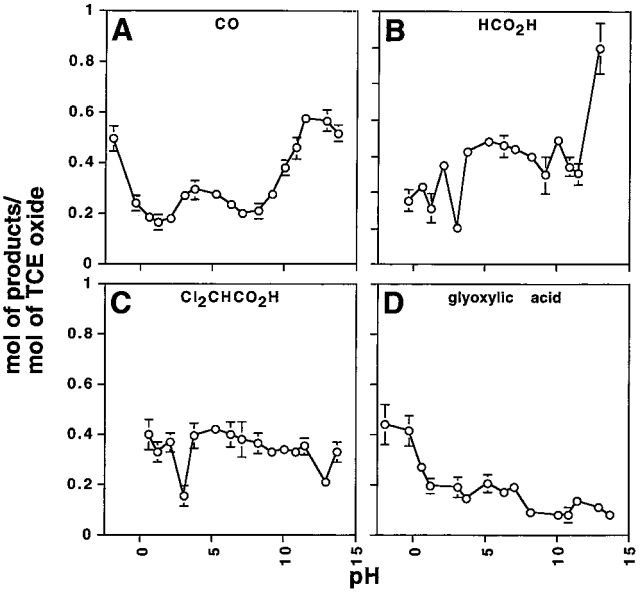

Figure 2. Products formed in the hydrolysis of TCE oxide (**1**) at varying pH in aqueous solutions at 0 °C. (A) CO, (B) HCO_2H (C) Cl_2CHCO_2H, (D) glyoxylic acid. Buffers are listed under Figure 1. Results are presented as means ± range of duplicate experiments in each case. When range values are not indicated, the range was within the limits shown by the mean point.

$H_2{}^{18}O$ Incorporation into TCE Oxide Products. ^{18}O incorporation into TCE oxide degradation products was quantitated either by HPLC/MS, in the case of these acids, or by GC/MS in the case of CO (Table 1). CO was formed without any ^{18}O incorporation. About 90% of the HCO_2H formed had two ^{18}O atoms incorporated into the product. In the case of Cl_2CHCO_2H, 91% contained one ^{18}O atom. The carbonyl group of the glyoxylic acid can readily exchange with $H_2{}^{18}O$. To reduce the amount of carbonyl exchange, $NaBH_4$ was added to the reaction mixture of TCE oxide in $H_2{}^{18}O$ after 30 s. An $H_2{}^{18}O$ control experiment indicated that about 50% of standard glyoxylic acid contained one ^{18}O atom after 30 s (carbonyl exchange). Control experiments for ^{18}O incorporation done under the same conditions indicated that there was no ^{18}O incorporation into HCO_2H, Cl_2CHCO_2H, or glycolic acid (the

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Table 1. $H_2{}^{18}O$ Incorporation into CO, HCO_2H, Cl_2CHCO_2H, and Glyoxylic Acid Formed in the Decomposition of TCE Oxide

product	method	% with no ^{18}O	% with one ^{18}O	% with two ^{18}O	% with three ^{18}O
CO	GC/MS[a]	99.4 ± 0.2	0.6 ± 0.2		
	GC/MS[b]	99.2	0.8		
	GC/MS[c]	99.5 ± 0.1	0.5 ± 0.1		
HCO_2H	HPLC/MS[d]	2 ± 1	10 ± 1	88 ± 1	
Cl_2CHCO_2H	HPLC/MS[d]	9 ± 1	91 ± 1		
$HOCH_2CO_2H$	HPLC/MS[e]	1 ± 1	4 ± 1	90 ± 2	5 ± 1
	HPLC/MS[f]		91 ± 2	9 ± 2	

[a] $H_2{}^{18}O$ (95% atom excess, 0.10 mL) was used. The data were obtained by the selected ion monitoring (SIM) method. The reaction time was 45 min. [b] The reaction solvent for TCE oxide hydrolysis was 0.055 N KOH (in 95% atom excess $H_2{}^{18}O$) (SIM method, reaction time 45 min). [c] Conditions are in footnote *a* except that the reaction time was 2 min and total ion current data was collected for the analysis. [d] Et_3N was added to the reaction mixture after 2 min reaction time. [e] $NaBH_4$ was added to the reaction mixture after 30 s. [f] The sample was diluted with unlabeled H_2O (i.e., $H_2{}^{16}O$, 10 vol) after 2 min, allowed to stand for 10 min, and then $NaBH_4$ was added.

reduced product of glyoxylic acid by $NaBH_4$) after 2 min reaction time. Glyoxylic acid, recovered as glycolic acid, mostly contained two (90%) ^{18}O atoms. In a separate experiment, TCE oxide was mixed with $H_2{}^{18}O$ for 2 min and then diluted into a 10-fold excess of unlabeled H_2O ($H_2{}^{16}O$) prior to the addition of $NaBH_4$ and mass spectral analysis. In this experiment, the extent of labeling at the carboxylic acid oxygens can be determined, since any ^{18}O incorporated into the aldehyde is diluted out. One ^{18}O atom (91%) was found to be incorporated into the carboxylic oxygen in this experiment.

H Incorporation into TCE Oxide Products. When analyses similar to those described above were done with ^2H_2O and TCE oxide, <5% of each product showed incorporation of ^2H. An inverse experiment was done with [^{2}H]TCE oxide and ^1H_2O and the same results were obtained (i.e., no incorporation of ^1H).

The amounts of Cl_2CHCO_2H formed in the decomposition of TCE oxide and [^{2}H]TCE oxide were compared to determine if a hydrogen kinetic isotope effect existed. The differences in yield were within ±5% in the pH range 0.5−5.

Reaction of TCE Oxide with Lysine. Five lysine adducts were detected by both HPLC/UV and HPLC/MS (Figure 3). N^{α}-Formyllysine, N^{ϵ}-dichloroacetyllysine, and N^{ϵ}-glyoxylyllysine were synthesized, and N^{ϵ}-formyllysine was purchased as standards for the reaction. The four standards eluted from the column with similar t_R values as the peaks (t_R 9:18, 10:58, 11:19, and 16:45) displayed in Figure 3. The control experiment for the reaction indicated no formation of these five lysine adducts, under the same conditions, when TCE oxide hydrolysis preceded the addition of lysine (Figure 3).

The mass spectra of the products derived form the reaction match those expected and experimentally determined for the standard compounds. The MH^+ multiplet for N^{ϵ}-dichloroacetyllysine showed a ratio of m:m + 2:m + 4 peaks near the theoretical ratio of 9:4:1 for a dichloro compound (with correction for ^{13}C natural abundance). The unknown compound at t_R 7:40 is probably N^{α}-glyoxylyllysine, as judged by its mass spectrum. The possibility that N^{α}-dichloroacetyllysine is formed and eluted with the N^{ϵ} isomer cannot be excluded on the basis of the available evidence.

The relative amounts of lysine adducts formed in the reaction were estimated based on the standard curves obtained using the four authentic lysine adducts mentioned previously. N^{α}-Formyllysine, N^{ϵ}-formyllysine, N^{ϵ}-dichloroacetyllysine, and N^{ϵ}-glyoxylyllysine accounted for 4, 12, 5, and 9% of the starting material (TCE oxide), respectively. The relative amount of the unknown compound at t_R 7:40 (which is probably N^{α}-glyoxylyllysine) was estimated at 3%, based on the standard curve for N^{ϵ}-glyoxylyllysine.

^{18}O Incorporation into Reaction Products of TCE Oxide with Lysine. In the reaction of **1** with lysine in $H_2{}^{18}O$, ~90% of the formyllysine formed had one ^{18}O atom incorporated into the product (Table 2). In the case of dichloroacetyllysine only 3% contained one ^{18}O atom. Most of the glyoxylyllysine (95%) had no ^{18}O incorporated. Control experiments done with the synthetic lysine adducts (formyllysine, dichloroacetyllysine, and glyoxylyllysine) under the same conditions indicated that there was no ^{18}O incorporation into any of the lysine adducts.

^{2}H Incorporation into Reaction Products of TCE Oxide with Lysine. In the reaction of **1** with lysine in ^2H_2O (99% enriched), ~5% of the *N*-formyllysine contained ^2H.

Discussion

pH Profiles. The pH rate profile in Figure 1 A indicated that, in the pH range studied, the rate law for the hydrolysis of TCE oxide (**1**) contains two terms: a pH-independent term, k_{H_2O} (6.9×10^{-3} s^{-1}) and a hydronium ion-dependent term, k_H (3.3×10^{-2} M^{-1} s^{-1}).[39,40]

$$k_{obsd} = k_{H_2O} + k_H[H^+] \quad (1)$$

A hydroxide ion-dependent region can also be involved in the decomposition of epoxides.[39,41,42] In the case of TCE oxide, none was observed, possibly because of assay limitations. The rate law for the formation of CO (Figure 1 B) contained only a pH-independent term, k_{H_2O} (8×10^{-3} s^{-1}), in the pH range 0−14. The rate constants for pH < −1 or pH > 15 were not amenable to measurement because of the response time (10−25 s) of the electrochemical CO sensor.

Comparison of the pH-independent rate constant for the formation of CO with that for the disappearance of TCE oxide indicates that the disappearance of TCE oxide is the rate-limiting step for CO formation under the conditions examined.

Product Analyses of TCE Oxide. Four major products (CO, HCO_2H, Cl_2CHCO_2H, and glyoxylic acid) are formed in the hydrolysis of TCE oxide.[21,23,24,43,44] Their amounts were not dramatically affected by pH except at pH > 14 (Figure 2). HCHO was not formed. The small breaks seen at certain pH values (e.g., pH 3 in the case of Figure 2 B and C) are possibly related to the buffer and not considered to be unusual. The lack of a major pH effect on product distribution indicated that the mechanism leading to the formation of each of the four final products is not affected dramatically (<3×) by changing the pH in the range of −1.5 to 14, even though the hydrolysis of TCE oxide **1** is changed from hydronium ion-dependent to pH-independent. At higher pH (pH > 14), the amount of HCO_2H increased.

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(44) Fox, B. G.; Borneman, J. G.; Wackett, L. P.; Lipscomb, J. D. *Biochemistry* **1990**, *29*, 6419−6427.

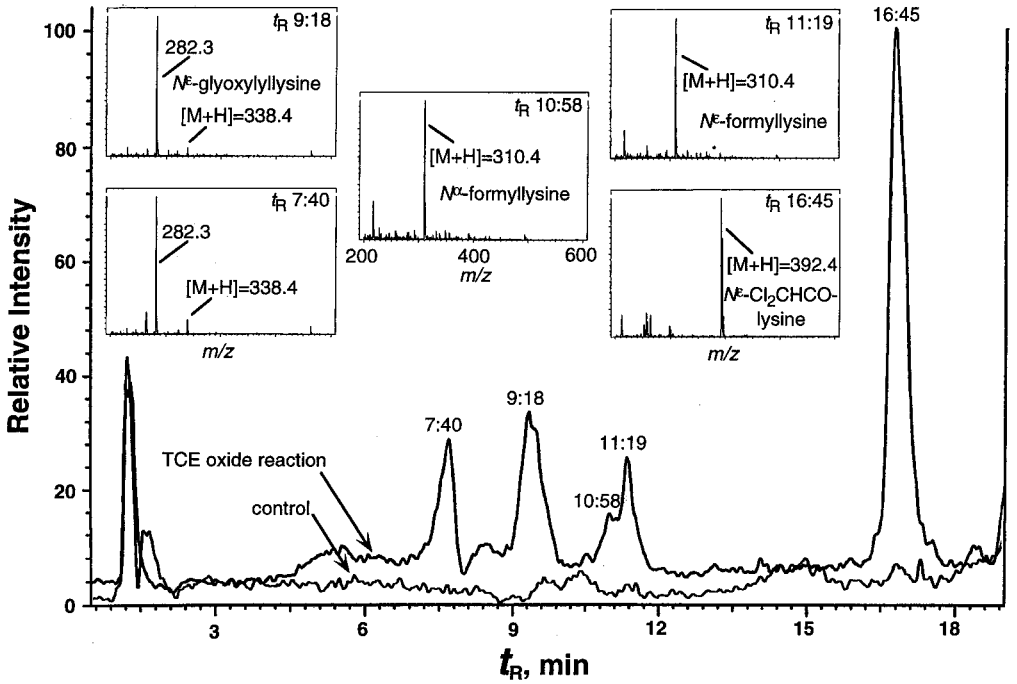


Figure 3. HPLC/MS traces and mass spectra of the PITC derivatives of the reaction of lysine with TCE oxide (**1**). The control experiment for this reaction is also shown. The structure assignments for the PITC derivatives are indicated in the inserts showing the mass spectra. As indicated in the text, the material eluting at t_R 7:40 is probably N^{α}-glyoxylyllysine.

Table 2. $H_2{}^{18}O$ Incorporation into Lysine Conjugates Derived from Trichloroethylene Oxide

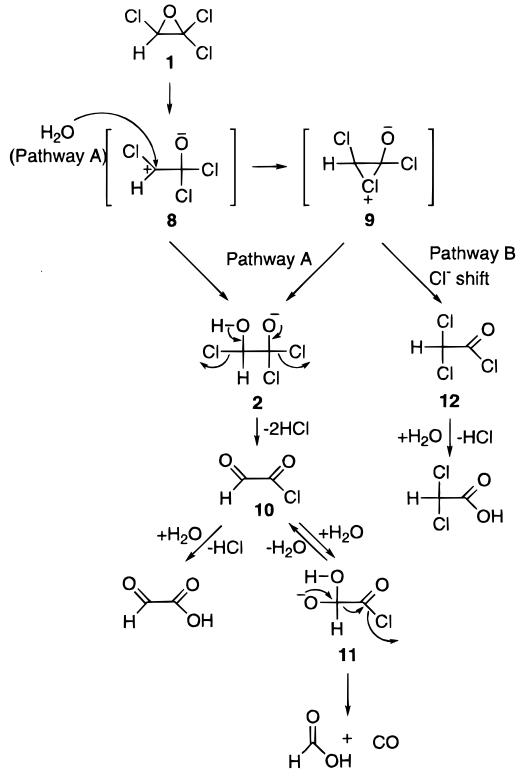
product[a]	% with no ^{18}O	% with one ^{18}O
N-formyllysine	10 ± 2	90 ± 2
N-dichloroacetyllysine	97 ± 1	3 ± 1
N-glyoxylyllysine	95 ± 2	5 ± 2

[a] Isotopic content was determined by HPLC/MS in all cases.

Mechanism of pH-Independent Hydrolysis of TCE Oxide. The dominant mechanism for hydrolysis of TCE oxide (**1**) is displayed in Scheme 2. In the formation of Cl_2CHCO_2H (Scheme 2), a zwitterionic intermediate **9** could be formed (from **8** which could be formed by cleavage of the C−O bond of TCE oxide) and undergo an intramolecular rearrangement (Cl^- shift) to generate dichloroacetyl chloride **12** which would subsequently decompose to Cl_2CHCO_2H.[12,13,22−24,41,45] The zwitterion intermediate **9** could also hydrolyze at the less sterically hindered and more electronically deficient carbon of the zwitterion to give a glycol anion **2**, which would dehydrohalogenate to form a reactive oxoacetyl chloride (glyoxyl chloride) intermediate **10**. The oxoacetyl chloride **10** could hydrolyze to generate either glyoxylic acid[23,24] or an anionic intermediate **11** which could generate HCO_2H, CO, and chloride through a concerted mechanism.[46] The decomposition of the anionic intermediate **11** could also go through stepwise mechanisms to form HCO_2H and CO as shown in Scheme 3, which cannot be ruled out by the available evidence. In Scheme 3, as demonstrated in pathway A, the anionic intermediate **11** could go through a C−C bond cleavage to form HCO_2H and formyl chloride anion, the latter of which would lose chloride to afford CO. In pathway B the anionic intermediate **11** could eliminate chloride first to generate an intermediate **13**, then with the loss of CO, the intermediate **13** would generate HCO_2H.

The mechanism postulated in Scheme 2 is supported by the following results.

Scheme 2. Mechanism of pH-Independent Hydrolysis of TCE Oxide (**1**) in Aqueous Solution at 0 °C



$H_2{}^{18}O$ Incorporation. Without any further ^{18}O exchange the Cl_2CHCO_2H, HCO_2H, CO, and glyoxylic acid formed in the hydrolysis of TCE oxide in the presence of $H_2{}^{18}O$ should theoretically contain one, two, none, and two ^{18}O atoms, respectively. (Less than 5% ^{18}O was found incorporated into the other standard carboxylic acids in control experiments.) The results from the $H_2{}^{18}O$ experiment (Table 1) showed that Cl_2CHCO_2H, HCO_2H, CO, and glyoxylic acid contained ~91%

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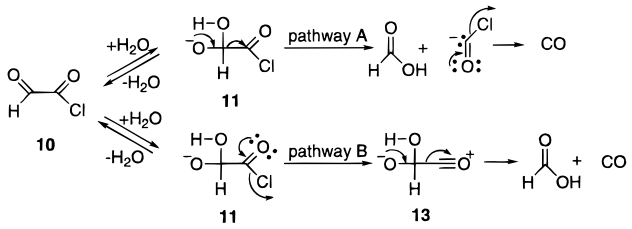
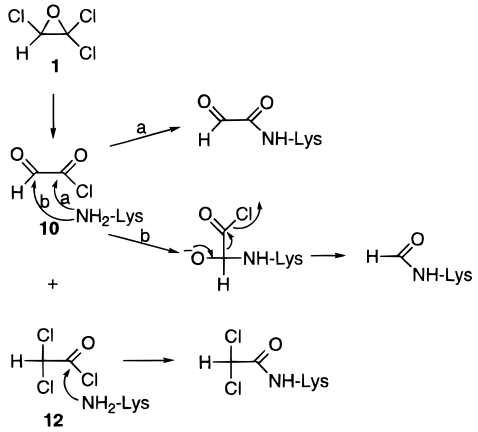
Scheme 3. Possible Mechanism for the Formation of HCO_2H and CO from Oxoacetyl Chloride (**10**) through Stepwise Pathways in Aqueous Solution at 0 °C



Scheme 4. Mechanism of Reaction of TCE Oxide (**1**) with Lysine in Aqueous Solution



one, ~90% two, <1%, and 90% two ^{18}O atom(s), respectively, consistent with the mechanism proposed in Scheme 2. The labeling pattern for glyoxylic acid indicates that one oxygen from H_2O is incorporated into the carboxylate. A second oxygen (from H_2O) appears to be incorporated into aldehyde. Thus, only one of the two carboxylic acid oxygen atoms originated in TCE oxide.

H Incorporation Experiments. No deuterium incorporation from ^2H_2O into Cl_2CHCO_2H, HCO_2H, or glyoxylic acid was found to occur during the hydrolysis of TCE oxide. Further, no protium from H_2O was incorporated into the products derived from [^{2}H]TCE oxide, consistent with Scheme 2 in which the H of TCE oxide is found in the carboxylic acid products.

Reaction of TCE Oxide with Lysine. The reactive acyl halides **10** and **12** should be potentially reactive with nucleophiles such as lysine (Scheme 4). N^{ϵ}-Dichloroacetyllysine (and most probably N^{α}-dichloroacetyllysine) (total 5% yield), N^{α}- and N^{ϵ}-glyoxylyllysine (total yield 12%, under the assumption that the unknown compound at t_R 7:40 is probably N^{α}-glyoxylyllysine), and N^{α}- and N^{ϵ}-formyllysine (total yield16%) were detected by both HPLC/UV and HPLC/MS measurements. It was recently reported that an intermediate species, dichloroacetyl chloride **12**, was identified in the photooxidation of TCE on TiO_2 using infrared spectroscopy.[47] This reported observation also suggests the possible existence of dichloroacetyl chloride **12** in our system, which was trapped by lysine, in the reaction of TCE oxide, to form N^{ϵ}-dichloroacetyllysine and probably N^{α}-dichloroacetyllysine.

^{18}O Incorporation into Reaction Products of TCE Oxide with Lysine. The results of ^{18}O incorporation experiments showed that ~90% of the *N*-formyllysine contained one ^{18}O atom (Table 2). The *N*-dichloroacetyllysine and *N*-glyoxylyllysine contained <5% ^{18}O. These results are consistent with

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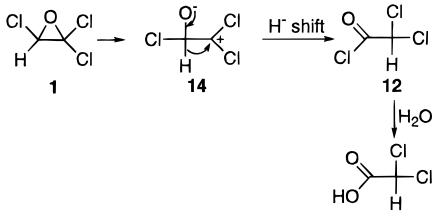
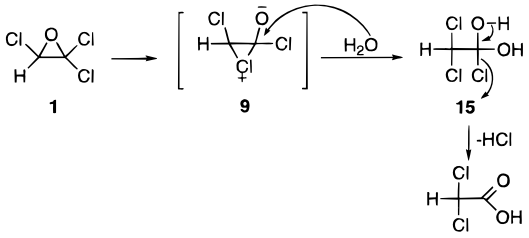
Scheme 5. Possible Mechanism for the Formation of Cl_2CHCO_2H Involving a Hydride Shift



Scheme 6. Possible Mechanism for the Formation of Cl_2CHCO_2H, Involving Hydrolysis of the Zwitterionic Intermediate **9** at the More Substituted Carbon Site



Schemes 2 and 4. The results also argue against formyl chloride **4** as an intermediate in the formation of *N*-formyllysine. Formyl chloride yields CO at pH <13.[35] However, the hydrolysis of TCE oxide in $H_2{}^{18}O$ yielded CO without ^{18}O incorporation (Table 1) and *N*-formyllysine with complete ^{18}O incorporation. Control experiments done with the synthetic lysine adducts (N^{ϵ}-formyllysine, dichloroacetyllysine, and glyoxylyllysine) under the same conditions indicated that there was no ^{18}O incorporation into any of the lysine adducts.

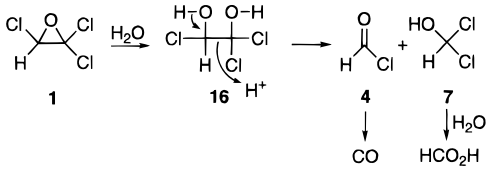
Hydronium Ion-Dependent Hydrolysis of TCE Oxide. The mechanism proposed for hydronium ion-dependent hydrolysis of TCE oxide is very similar to that for the pH-independent hydrolysis except for the first step, which involves hydronium ion attacking TCE oxide to form a TCE oxide cation intermediate. The mechanism appears to be very similar to that of Scheme 2, the pH-independent reaction, in that the product distribution is not dramatically changed (Figure 2).

Other Possible Mechanisms for Hydrolysis of TCE Oxide. One possible mechanism proposed for the formation of Cl_2CHCO_2H involves a hydride shift (Scheme 5). In general, α-chloroepoxide rearrangements involve halogen migration.[12] Product analysis showed that the amount of Cl_2CHCO_2H formed in the hydrolysis of [^{2}H]TCE oxide was within ±5% of the Cl_2CHCO_2H formed from TCE oxide. A significant kinetic deuterium isotope effect on a hydride shift might have been expected if a hydride shift were involved, which would lower the yield of Cl_2CHCO_2H in favor of other products.

Another possible mechanism proposed for the formation of Cl_2CHCO_2H is displayed in Scheme 6. The zwitterion intermediate **9** could hydrolyze at the more sterically hindered carbon site to give a dichloroacetyl chloride hydrate anion **15** which would dehydrohalogenate to form Cl_2CHCO_2H. However, if this pathway is dominant, dichloroacetyllysine would contain ≥50% one ^{18}O atom in the ^{18}O incorporation study with lysine. Therefore Scheme 6 is inconsistent with the experimental results presented previously and could not be the dominant pathway for the formation of Cl_2CHCO_2H.

One possible mechanism for the formation of CO and HCO_2H could involve initial cleavage of C−C bond of TCE oxide. In this mechanism, hydrolysis of TCE oxide could give an ether intermediate. This unstable ether intermediate could react with H_2O to generate formyl chloride **4** and chlorodihydroxymethane,

Scheme 7. Mechanism Proposed by Henschler et al.[23] to Account for Formation of CO and HCO_2H



which could form CO[35] and HCO_2H. However, in ab initio molecular orbital studies on the structure and stability of ethylene oxide cation radical, it has been concluded that the C−O bonds are relatively weak and easily broken.[48,49] The fission of the C−C bond requires more energy (105−120 kJ mol^{-1}). In the case of TCE oxide, the scission of the C−C bond will most likely require more energy than that of C−O bond, and we do not favor this pathway.

Several proposed mechanisms for the formation of CO and HCO_2H in the previous literature[23,24] are inconsistent with our experiment results. A mechanism proposed by Henschler et al.[23] (Scheme 7) predicts 100% ^2H incorporation from ^2H_2O into HCO_2H in the hydrolysis of TCE oxide carried out in the presence of ^2H_2O. This possibility is excluded by the results presented here. Another problem with the results presented in this earlier paper is that the reported product distribution differs considerably from our own[24] (Table 1). Further, the different patterns of ^{18}O incorporation into CO (Table 1) and *N*-formyllysine (vide supra) argue against a role of formyl chloride **4**.

In 1982 this laboratory proposed a mechanism similar to that of Scheme 2 to account for formation of Cl_2CHCO_2H and glyoxylic acid and two possible mechanisms to account for the formation of CO and HCO_2H under basic conditions, based on evidence available at that time (Scheme 1). Three pieces of new evidence are inconsistent with Scheme 1. First, one would expect base-catalyzed decomposition of TCE oxide at high pH. However, only pH-independent hydrolysis was observed up to pH ∼15. Second, formyl chloride **4** predominantly decays into CO except at very high pH (∼14), at which HCO_2H is formed.[35] Third, 50% ^2H incorporation from ^2H_2O into HCO_2H would be expected in ^2H_2O in the carbene mechanism presented in Scheme 1. However, no ^2H incorporation was observed in this study.

Conclusions

The decomposition of TCE oxide (**1**) is considered as a paradigm for multi-halogenated epoxide hydrolysis and involves pH-independent and hydronium ion-dependent regions (Figure 1 A). C−C bond scission in epoxides is associated with one carbon being at the oxidation state equivalent to that bearing two leaving groups (Cl). The formation of CO mainly involves a pH-independent region (Figure 1 B). Comparison of the rates and pH profile for the formation of CO (Figure 1 B) with the disappearance of TCE oxide (Figure 1 A) implies that the disappearance of TCE oxide is the rate-determining step for the formation of CO under the conditions studied. The similar product distribution of CO and these carboxylic acids (HCO_2H, Cl_2CHCO_2H, and glyoxylic acid) over the pH range of −1.5 to 14 (Figure 2) indicates that the mechanism leading to the formation of each of the four major products is not dramatically affected by pH even though the hydrolysis of TCE oxide is changed from being hydronium ion-dependent to pH-independent. The mechanisms for the hydronium ion-dependent and pH-independent hydrolysis (Scheme 2) of TCE oxide were developed based on three experimental results: $H_2{}^{18}O$ incorporation (Table 1), H incorporation, and reaction of TCE oxide with lysine in $H_2{}^{16}O$ (Figure 3) and in $H_2{}^{18}O$. In the pH-independent hydrolysis (Scheme 2), a zwitterionic intermediate **9** could be formed (from TCE oxide) and undergo an intramolecular rearrangement (Cl^- shift) to generate dichloroacetyl chloride **12** which would subsequently decompose to Cl_2CHCO_2H.[12,13,22−24,41,45] The zwitterionic intermediate **9** could also hydrolyze at the less sterically hindered carbon to give glycol anion **2** which would dehydrohalogenate to form an oxoacetyl chloride intermediate **10**. The oxoacetyl chloride **10** could hydrolyze to generate either glyoxylic acid[23,24] or an anionic intermediate **11** which could go through a concerted mechansim to afford CO and HCO_2H.[46] Our mechanism proposed for the hydronium ion-dependent hydrolysis is very similar to that for the pH-independent hydrolysis except for the first step, which involves hydronium ion attacking TCE oxide to form a TCE oxide cation intermediate. Several mechanisms (Schemes 1 and 5−7) (either possible or proposed in previous literature) are discussed but ruled out on the basis of evidence presented in this study.

The hydrolytic mechanism is of interest in that it yields acyl halides **10** and **12**, which were shown to react with lysine. The direct reaction of TCE oxide with nucleophiles would not be expected to yield those adducts, in that all should contain linkages of the type $N-CH_2-Cl$, $S-CH_2-Cl$, etc. Whether lysine adducts are prominent in protein modification is currently unknown and the subject of further investigation.

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