Environ. Sci. Technol. **1999**, *33,* 1015−1020

Iron Porphyrin and Cysteine Mediated Reduction of Ten Polyhalogenated Methanes in Homogeneous Aqueous Solution: Product Analyses and Mechanistic Considerations

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Reductive dehalogenation reactions of polyhalogenated C_1- and C_2-compounds are presently of particular interest because of the potential applicability of such processes in the treatment of wastes as well as in remediation approaches to removing such compounds from contaminated soils and aquifers. In this context, it is not only important to know the reaction kinetics of a specific compound with relevant reductants but also the type of product(s) formed under given conditions. In this study we have identified reaction intermediates as well as the final products of the reduction of 10 polyhalogenated methanes (PHMs) by an iron porphyrin in the presence of cysteine. Cysteine was chosen for two reasons: (i) as bulk electron donor and (ii) as an aqueous organic compound exhibiting functional groups (i.e., $-NH_2$, $-SH$) that may undergo reactions other than just hydrogen abstraction with reactive intermediates such as radicals and carbenes. The data obtained support our hypothesis postulated in an earlier kinetic study that the initial and rate-determining step in the reduction of PHMs by the iron porphyrin is a dissociative one-electron transfer. Furthermore, it is shown that in fast consecutive reactions involving primarily the mercapto group of cysteine, all compounds were completely dehalogenated. Except for the fluorine containing compounds, the carbon of a given PHM was quantitatively recovered as *N*-formylcysteine. In the case of the fluorinated compounds, carbene intermediates could be trapped, which reacted further to some unidentified product(s), possibly including carbon monoxide. Finally, it is shown that the reduction of tetrahalomethanes by cysteine as sole reductant leads predominantly to the formation of the corresponding haloforms, suggesting that, in this case, the reaction occurred primarily by an X-philic dissociative two-electron transfer. The results of this study offer an interesting perspective for a fast complete dehalogenation of PHMs by using a very reactive one-electron donor (i.e., a reactive iron species) in the presence of organic matter exhibiting reduced sulfur groups.

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Introduction

Polyhalogenated methanes (PHMs) are used in large quantities as solvents, refrigerants, foaming agents, pesticides, propellants, and for several other purposes. It is, therefore, not surprising that such compounds are found to be not only ubiquitous pollutants in the atmosphere *(1*−*4*), but also in the subsurface, which they may enter due to accidents, leakages from tanks, or from leaching waste disposal sites *(5*−*7*). In anoxic natural environments, PHMs may undergo abiotic and/or microbially catalyzed reductive dehalogenation reactions leading to a variety of products including less halogenated methanes *(8, 9*), carbon monoxide *(10*−*12*), and a variety of other usually unknown products that may be formed by reaction of reactive radical or carbene intermediates with aqueous organic compounds. From an environmental engineering point of view, reductive dehalogenation reactions of PHMs are interesting because of their potential applicability in the treatment of polyhalogenated alkanes (PHAs) wastes as well as in remediation approaches to removing such compounds from contaminated soils and aquifers. In this context, not only is it important to know the factors that determine the reaction rates of PHMs with potential electron donors (e.g., transition metal complexes *(13*−*15*), reduced iron and sulfur species including zerovalent iron *(16*)), but one also has to be aware of possible reaction mechanisms and pathways, to anticipate the formation of hazardous compounds, and/or to promote reaction pathways that lead to harmless products.

In earlier work *(17*), we have investigated the kinetics of the reduction of a series of polyhalogenated methanes and ethanes by two electron-transfer mediators, namely, an iron porphyrin (in the presence of cysteine as bulk electron donor) and mercaptojuglone, the latter being a representative of an organic compound formed under sulfate reducing conditions. From the analysis of the relative reaction rates of the compounds in the different model systems and from comparison with other rate data from the literature, we have postulated that the initial and rate-determining step in the reduction of the polyhalogenated alkanes by the iron porphyrin is an outer sphere, dissociative one-electron transfer, while the initial reaction with the mercaptojuglone may also occur by a nucleophilic attack at a halogen atom, which is equivalent to a dissociative two-electron transfer.

The major goals of this study were (i) to provide additional evidence for the postulated initial reaction step(s) and (ii) to gain insight into the reaction pathways of reactive intermediates (i.e., radicals, anions, carbenes) that may be formed after the initial transfer of electrons. Of particular interest was the type of products formed in the presence of an aqueous organic compound (i.e., cysteine) exhibiting functional groups (i.e., $-NH_2$, $-SH$), that may undergo reactions other than just hydrogen abstraction with the reactive intermediates.

In the study presented in this paper, we have identified intermediates and final products of the reduction of 10 polyhalogenated methanes (see Table 1) by an iron porphyrin in the presence of cysteine. In addition, for some compounds, the products formed from the reaction with cysteine as sole reductant (blank reaction) have also been determined.

Experimental Section

Chemicals. The following chemicals were purchased from Fluka AG (Buchs, Switzerland) and used without further purification: iron(II) chloride tetrahydrate, sodium acetate, sodium perchlorate, acetylacetone (>99,5%), Amberlite IRA-

10.1021/es980553y CCC: $18.00
Published on Web 02/13/1999

TABLE 1. Products and Intermediates Found in the Iron Porphyrin System

compd	products [a]			intermediates
	halides	halogenated methanes	*N*-formylcysteine	
CCl_4	420 ± 60% Cl^- [b]	<1% $CHCl_3$	125 ± 20%	no free carbene CCl_3−radical trapped
CBr_4	460 ± 70% Br^-	3 ± 1% $CHBr_3$	95 ± 10%	no free carbene
$CBrCl_3$	320 ± 40% Cl^-; 120 ± 20% Br^-	<1% $CHCl_3$	115 ± 15%	no free carbene
CBr_2Cl_2	210 ± 30% Cl^-; 230 ± 30% Br^-	5 ± 2% $CHBrCl_2$	110 ± 15%	no free carbene
$CHBr_3$	330 ± 50% Br^-	<1% CH_2Br_2	130 ± 20%	no free carbene
$CHBrCl_2$	160 ± 30% Cl^-; 115 ± 20% Br^-		130 ± 20%	no free carbene
$CHBr_2Cl$	100 ± 20% Cl^-; 215 ± 30% Br^-		85 ± 20%	no free carbene
$CFBr_3$	110 ± 30% F^-; 330 ± 50% Br^-		30 ± 15%	fluorobromo−and dibromocarbene trapped
$CFCl_3$	100 ± 40% F^-; 360 ± 60% Cl^-		40 ± 15%	fluorochlorocarbene trapped
CF_2Br_2	200 ± 70% F^-; 280 ± 50% Br^-		20 ± 15%	difluorocarbene trapped

[a] The time for complete dehalogenation depends on the substrate and varies from several minutes (CBr_4) to several weeks ($CFCl_3$). [b] Amount of starting material = 100%.

401, ethanol (puriss), *n*-propanol, dipotassium hydrogen phosphate, tetrachloromethane (>98%, IR spectroscopy grade), tetrabromomethane (purum, >98%), chloroform (IR spectroscopy grade; stabilized with 0.5% ethanol), bromoform (>99%), dibromomethane (>99%), fluorotribromomethane (>99%), dibromodifluoromethane (>98%), fluorotrichloromethane (>99.5%), bromodichloromethane (>98%), dibromochloromethane (purum, >97%), cystine, formic acid (>98%), zinc dust, ethyl acetate, 3-pentenoic acid, methanol, magnesium, magnesium sulfate, diethyl ether, diisopropyl ether, β-methallyl chloride, tetrahydrofuran, benzyltriethylammoniumchloride, cysteine, ethyleneglycol-monoethyl ether, *N*-nitrosotoluene-4-sulfomethylamid, 2-morpholinoethanesulfonic acid monohydrate (MES), 4-(2-hydroxyethyl) piperazine-1-propanesulfonic acid (HEPES), 4-(2-hydroxyethyl)piperazine-1-propanesulfonic acid (HEPPS), *N*-[tris(hydroxymethyl)methyl]-3-amino-propanesulfonic acid (TAPS), and 2-(cyclohexylamino)ethanesulfonic acid (CHES). Hexane and pentane were from Burdick & Jackson. Dibromodichloromethane (95%), bromotrichloromethane (99%), and 2-iodobenzotrifluoride were from Aldrich Chemie, Switzerland. ^{13}C-CCl_4 was purchased from Chemical Isotopes Laboratories CIL (>99%). ^{14}C-CCl_4 was from NEN Products Du Pont, Boston, with a specific activity of 0.17 GBq/mmol (4.6 mCi/ mmol; (radiochemical purity: 99%, determined by gas liquid chromatography: Porapak QS Durapak Porasil C column at 180 °C). Insta-Gel, a universal liquid scintillation cocktail for aqueous and nonaqueous samples, was purchased from Packard (reorder no. 6013009). Isopropyl-d_7 alcohol (99,5%) was purchased from Armar AG (Döttingen, Switzerland). Deuteriochloroform ($CDCl_3$) was from Dr. Glaser AG (Basel, Switzerland). The following chemicals were synthesized (see Supporting Information): *meso*-tetrakis-(*N*-methylpyridyl)iron porphyrin, *N*-formylcysteine, 3-methyl-3-butenoic acid, diazomethane, 2-(2,2-dichloro-1-methylcyclopropyl)ethanoic acid, 2-(2,2-dibromo-1-methylcyclopropyl)ethanoic acid, and 2-(2-bromo-2-chloro-1-methylcyclopropyl)ethanoic acid.

Stock Solutions and Buffers. Stock solutions of about 10 mM iron porphyrin were prepared in 50 mM phosphate buffer or in 2 mM phosphate buffer, pH = 7, depending on the experiment. The exact concentration of the iron porphyrin was determined by UV−vis spectrophotometry at 598 nm (ϵ = 9600 M^{-1} cm^{-1}).

Stock solutions of halogenated methanes were prepared in oxygen-free methanol in the glovebox. Concentrations were 0.1 M. Further dilution for the preparation of spike solutions (1 mM) was carried out in the glovebox.

Buffers were prepared by using HCl or NaOH to get the desired pH after dissolving the corresponding salt in Nanopure water. Buffers as well as glassware were autoclaved at 121 °C for 30 min. The solutions were purged with nitrogen gas (99.995%) for 1 h/100 mL and immediately transferred into the glovebox.

Experimental Procedures. The ^{14}C-CCl_4 experiment was conducted in a 59-mL serum flask sealed with a Viton stopper (Maagtechnik, Dübendorf, Switzerland) and an aluminum crimp cap. The reaction mixture was prepared in an anaerobic glovebox: 50 mM phosphate buffer (pH = 7) (see Stock Solutions and Buffers), 5 mM cysteine, and 30 μM iron porphyrin were used. Fifty-seven microliters of an anaerobic methanolic 1 mM ^{14}C-CCl_4 solution containing 1 mM 2-iodobenzotrifluoride as an internal standard was introduced by a gastight Hamilton syringe through the Viton stopper. The flask was incubated at 25 °C in the dark for 48 h. After injection of an equal volume of nitrogen gas (purity 99.995%) 100 μL of the solution were withdrawn, extracted with 1 mL of pentane, and analyzed with GC-ECD (see Analytical Procedures). Several procedures were used to prepare samples for analysis of radioactivity-distribution: (1) One milliliter samples were extracted with 1 mL of either pentane, hexane, or ethyl acetate, and 500 μL of the organic phase were mixed with 4.5 mL of the scintillation cocktail. (2) Five hundred microliters of the reaction mixture plus 150 μL of a 1 M sodium hydroxide solution was transferred directly to 4.5 mL of the scintillation cocktail, and the activity was measured with a scintillation counter (see Analytical Procedures). Blank samples (water spiked with the methanolic CCl_4 solution) were mixed with the scintillation cocktail and served as reference (3). Five hundred microliter samples were either acidified with 150 μL of 1 M sulfuric acid or made alkaline with 150 μL of 1 M sodium hydroxide and flushed with nitrogen gas for 1 h. Activity measurements were performed by mixing the solution with 4.5 mL of the scintillation cocktail. (4) One hundred microliter samples were injected in an ion chromatographic system (see Analytical Procedures). Fractions of each peak in the ionic chromatogram were collected and mixed with 5 mL of the scintillation cocktail. Each experiment was carried out in triplicate.

^{13}C-CCl_4 Experiment. The same conditions as in the ^{14}C-CCl_4 experiment were used. Fifty-seven microliters of a 1 mM anaerobic methanolic ^{13}C-CCl_4 solution containing 1 mM 2-iodobenzotrifluoride was added to start the reaction. One hundred microliter samples were analyzed with an ion chromatographic system. Forty fractions of the peak showing activity in the previous ^{14}C-experiment were collected and lyophylized (gentle evaporation of water). Then the sample was redissolved in D_2O, and ^{13}C- and ^1H-NMR spectra were recorded.

$D^\bullet$ abstraction experiments were conducted in 12 mL serum flasks sealed with Viton stopper and aluminum crimp caps. The reaction mixture contained 2 mM phosphate buffer, pH = 7 (see Stock Solutions and Buffers), 5 mM cysteine, and 30 μM iron porpyhrin. Blank samples were prepared without

iron porphyrin. Additionally, either 1% (v/v), 0.5%, or 0.1% of isopropyl-d_7 alcohol was added to the samples. The experiments were started by adding 60 μL of an anaerobic methanolic solution containing 1 mM $CX_aY_{(4-a)}$ (X, Y = Cl, Br) and 0.1 mM 2-iodobenzotrifluoride. The concentration was 5 μM for $CX_aY_{(4-a)}$ (X, Y = Cl, Br) and 0.5 μM for the internal standard.

The flasks were incubated at 25 °C in the dark, except for the blank system of CCl_4 which was incubated at 60 °C. One hundred microliter samples were extracted with 1 mL of pentane and analyzed with GC-MS in the single ion recording (SIR)-mode (see Analytical Procedures). The experiment was done in duplicate, and each sample was measured three times.

Carbene trap experiments were performed in two series with two different carbene traps at slightly different conditions. Series 1 was conducted in 59-mL serum flasks under the following conditions: 2 mM phosphate buffer (pH = 7) (see Stock Solutions and Buffers), 2.5 mM cysteine, 250 μM iron porphyrin (in the blank system no iron porphyrin), 200 μM of $CX_aY_{(4-a)}$ (X, Y = F, Cl, Br) or $CHX_aY_{(3-a)}$ (X, Y = Cl, Br), respectively, 10 μM of 2-iodobenzotrifluoride, and 4 mM of 3-pentenoic acid (each replicate was prepared once with and once without 3-pentenoic acid, also the blank system). The flasks were stored at 25 °C in the dark. After complete degradation (checked by extraction in pentane and analysis with GC-ECD), two drops of 10% sulfuric acid were added to 2 mL samples which were then extracted with 1 mL of diethyl ether. Three hundred microliters of the ethereal phase was dried over magnesium sulfate and then filtered through a pasteur pipet containing a small amount of wad. One hundred microliters of this dried ethereal phase was transferred to a GC-MS vial, and 40 μL of a 0.4 mol/L ethereal diazomethane solution was added. The slightly yellow sample was analyzed with GC-MS (see Analytical Procedures).

Series 2 contained 2 mM phosphate buffer, pH = 7 (see Stock Solutions and Buffers), 5 mM cysteine, 500 μM iron porphyrin (in the blank system no iron porphyrin), 100 μM of $CX_aY_{(4-a)}$ (X, Y = F, Cl, Br) or $CHX_aY_{(3-a)}$ (X, Y = Cl, Br) respectively, 10 μM of 2-iodobenzotrifluoride, and 4 mM of 3-methyl-3-butenoic acid. Each experiment was done with and without 3-methyl-3-butenoic acid. The flasks were stored at 40 °C in the dark. The same procedure for GC-MS analysis was used as described for series 1.

The product studies were done with the reaction solutions of series 2 (see carbene trap experiments). Halogenated compounds were identified and quantified by taking 100 μL samples and extracting them with 1, 10, and 20 mL of pentane depending on the concentrations of the products, and the linear range of the response factor of each compound was analyzed. Analysis was done with GC-ECD (see Analytical Procedures). Halide ions, formate, and *N*-formylcysteine were analyzed by transferring 0.8 mL samples to autosampler vials and injecting 10 μL into an ion chromatographic system (see Analytical Procedures).

Analytical Procedures. The pentane extracts were analyzed by GC-ECD on a Carlo Erba HRGC 5160 equipped with an autosampler AS 200 and a Cryo 520 CO_2 cooling system. One and a half microliters was injected in the splitless mode. A 20 m DB-624 column (i.d. 320 μm, 0.25 μm film thickness) was used. The injector and detector temperatures were 200 and 300 °C, respectively. Hydrogen gas at a flow of 2 mL/min and a mixture of argon (90%) and methane (10%) were used as carrier and makeup gas, respectively. The detector was an electron capture detector (Carlo Erba ECD 400 with a Ni-63 source), with the following temperature program: 40 °C (4 min); 8 °C/ min to 130 °C (0 min); 35 °C/ min to 200 °C (5 min).

The pentane extracts of the $D^{\bullet}$ abstraction experiments were analyzed by GC-MS on a Fisons Instruments GC 8165 equipped with a Fisons Instruments MD 800 mass spectrometer. Two microliters was injected using an on-column injection. The column was a 30 m FS 68 DB-624 (i.d. 250 μm, 2 μm film thickness). Single ion recording modus was used for quantification, i.e., the ratio of m/z 118, 120, 122 to 119, 121, 123 for $CHCl_3$ to $CDCl_3$ and also the fragment masses 83, 85, 87 to 84, 86, 88 ($CHCl_2$ to $CDCl_2$) in their characteristic isotopic distribution. Helium at a flow of 2 mL/min was used as carrier gas. The temperature program was 40 °C (4 min); 8 °C/ min to 130 °C (0 min); 35 °C/ min to 200 °C (5 min).

The methyl esters of the dihalocyclopropylethanoic acids (prepared by reaction of the acids with ethereal diazomethane) were analyzed by GC-MS on a Fisons Instruments GC 8165 equipped with a Fisons Instruments MD 800 mass spectrometer. One microliter was injected using an on-column injection. A glass capillary column (25 m × 250 μm i.d., coated with PS-089, 0.23 μm) was used. Helium at a flow of 4.4 mL/min was used as carrier gas. Masses from m/z 30 to 300 were analyzed (Full Scan Modus). The solvent delay was 3 min, and the ionization method was EI^+. The temperature program was 35 °C (2 min); 10 °C/min to 200 °C (2 min).

Ionic species were analyzed on a DX-300 chromatographic system (Dionex) equipped with a standard AGP pump, a PED in the conductivity detection mode, and an AI-450 software version 3.31. The anion separation column (200 × 4 mm) IonPac AS11 with the corresponding guard column (50 × 4 mm) AG11 were used. The eluent (1 mL/min) conductivity was chemically suppressed by a self-regenerating suppressor (ASRS, current setting 3) that uses the column effluent to supply the proton generated by water electrolysis. An anion trap column (ATC-1, 9 × 24 mm) was installed in front of the injection valve to minimize interferences from anionic impurities in the eluent during gradient elution. The gradient program was 0−1.2 min: 5 mM NaOH, 1.2−9 min linear increase to 25 mM NaOH, 9−9.5 min: back to 5 mM NaOH, 9.5−13.8 min: 5 mM NaOH. Samples were injected directly after withdrawing them from the serum flasks.

Radioactivity measurements were carried out with a liquid scintillation counter (Kontron, Switzerland). Samples were measured in plastic vials (20-ACB from Chromacol Ltd. with plastic screwtops).

^{1}H- and ^{13}C-NMR spectra were recorded on an ASX-400 NMR spectrometer from Bruker.

Results and Discussion

Reaction with the Iron Porphyrin: Products and Product Identification. Table 1 summarizes the data obtained from the product analyses of the reduction of the 10 compounds investigated in the iron porphyrin system (after complete degradation of the PHAs). As can be concluded from the halogen mass balances and from the fact that no significant amounts of volatile products (i.e., less halogenated methanes) could be detected, all compounds were completely dehalogenated. In addition, except for the fluorinated compounds, only one main product, i.e., *N*-formylcysteine (for structure see Figure 1) was found (see below). Speculations about the unknown additional product(s) accounting for the incomplete mass balance of the fluorinated methanes will be made later when discussing the reaction mechanisms.

For the initial identification of the major product(s) and for assessing the carbon mass balance, experiments with ^{13}C-and ^{14}C-tetrachloromethane were conducted. In the ^{14}C-experiments it was found that the total activity of the product remained in the aqueous phase after extraction with organic solvents as well as after purging the solution with nitrogen at pH 0 and 14. Furthermore, in an ion chromatogram of the reaction solution, the total activity was found to be confined to one single peak, indicating only one major product. This product was isolated in an experiment using ^{13}C-tetrachloromethane and subjected to 2D-NMR spectroscopy. Figure

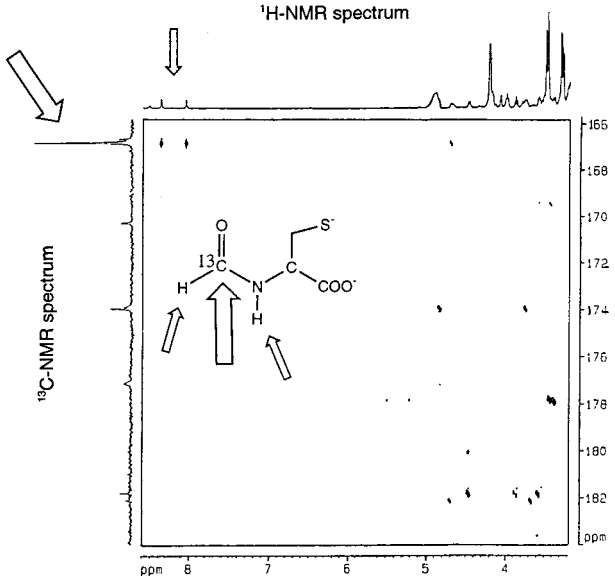

FIGURE 1. 2D-NMR spectrum of the ^{13}C-labeled isolated product. The ^{13}C-spectrum consists mainly of one signal (large arrow). The corresponding carbon atom couples with two protons which are indicated by the small arrows in the ^{1}H NMR-spectrum and in the formula.

TABLE 2. Reduction of Tetrachloromethane: Portion of Deuterated Chloroform in the Iron Porphyrin System as a Function of the Amount of Isopropyl-d_7 Alcohol Present in Solution

amount of isopropyl-d_7 alcohol (in % of total vol)	% of deuterated chloroform
1	12.62 ± 0.91
0.5	7.97 ± 0.26
0.1	2.40 ± 0.31

1 shows the 2D (^{1}H, ^{13}C)-spectrum of the ^{13}C-enriched product. Note that only part of the ^{13}C-NMR spectrum is shown because the artificially enriched ^{13}C gave only one predominant signal at 166.8 ppm. As is indicated in Figure 1, the ^{13}C nucleus corresponding to this signal is part of a spin−spin coupling system involving two protons with chemical shifts of 8.0 and 8.4 ppm in the ^{1}H-NMR spectrum. This result is consistent with an *N*-formyl group *(18)*. The proposed structure was confirmed by synthesis of *N*-formylcysteine (see complementary material *(19)*) which yielded a 2D-NMR spectrum identical to the one obtained from the product of CCl_4 reduction.

Mechanistic Considerations. To get hints on the reaction mechanism(s), experiments for detecting possible intermediates (i.e., radicals, carbenes) were carried out using 2-propanol-d_7 as D$^{\bullet}$ donor (Table 2) and two water soluble olefins (3-pentenoic acid and 3-methylbutenoic acid) as carbene traps (see Experimental Section). The results summarized in Table 1 show that no carbenes were trapped except for the fluorinated compounds (group 3, see Figure 2). These findings indicate that the *N*-formylcysteine was not primarily formed by the (expected) well-known reaction of the amino group with a given (di)halocarbene *(20, 21)* and that, therefore, an alternative reaction mechanism has to be considered. Furthermore, the results from experiments conducted with CCl_4 in the presence of various amounts of 2-propanol-d_7 (Table 2) suggest that the reaction occurred via a radical intermediate (i.e., $^{\bullet}CCl_3$) as $CDCl_3$ was found. $CDCl_3$ is formed by D atom abstraction from 2-propanol-d_7. It should be noted that in the case of the trihalomethanes, the oxidation state of the carbon was the same in the *N*-formylcysteine as compared to the starting material (i.e., + II), while for the other compounds a net transfer of two electrons occurred. Thus, when postulating an initial one-electron transfer for all compounds, a reoxidation step has to be included at least for the trihalomethanes.

Figure 2 shows the proposed reaction mechanism scheme for the reduction of the 10 compounds in the iron porphyrin/cysteine system. Note that because no volatile halogenated products were detected in significant amounts, the formation of anion intermediates ($:CX_3^-$, $:CHX_2^-$) is not postulated to be a major pathway. Anionic intermediates are protonated and form less halogenated methanes compared to the parent compound *(22)*. Hence, except for the possible direct formation of carbenes from the trihalomethane radicals in the case of fluorinated compounds (group 3: pathway on the left in Figure 2), the key reaction is postulated to be the coupling of the tri- or dihalomethane radical with cystine (the oxidation product of cysteine) or cysteine-S$^{\bullet}$, which is produced by the rereduction of the iron porphyrin. Note that this reaction leads to a reoxidation of the carbon atom of the halogenated parent methane. In the case of the trihalomethanes (group 2), R−S−CHX_2 is directly formed (see pathway on the right in Figure 2), which then hydrolyses to yield *S*-formylcysteine. Finally, because the *N*-formyl group is thermodynamically favored over the *S*-formyl group *(23)*, an intramolecular group transfer is proposed to give the identified product *N*-formylcysteine. As an alternative, this intramolecular transfer could occur before hydrolysis of the R−S−CHX_2 group (see Figure 2).

In the case of the tetrahalomethanes (group 1), the situation seems somewhat more complicated. To yield the final product, the R−S−CX_3 formed has to be reduced in subsequent reactions. As indicated in Figure 2, this can be achieved by forming a R−S−CX_2^- which is then protonated to give R−S−CHX_2. The formation of R−S−CX_2^- from R−S−CX_3 can occur in two ways, either by transfer of two electrons (reactions 1a and 1b) or by an X-philic attack of RS^- (reaction 2). An alternative mechanism would be the formation of a R−S−$CX_2^{\bullet}$ radical by nucleophilic attack of RS^- at the $^{\bullet}CX_3$ radical (reaction 3a, *(24)*) with subsequent transfer of an electron (reaction 3b). Finally, as an alternative to the formation of a R−S−CX_2^- species, the R−S−$CX_2^{\bullet}$ radical could abstract a hydrogen from the cysteine to yield RS−CHX_2 (reactions 1c and 3c). More information about which of these pathways is/are dominant could be obtained by conducting experiments in D_2O.

The different behavior of the fluorinated compounds is not too surprising since, compared to chlorine and bromine, fluorine is a much better π-donor and, therefore, can stabilize the carbene more effectively *(22)*. Furthermore, in a study on the reductive transformation of a series of halogenated methanes by cytochrome $P450_{CAM}$ under anaerobic conditions *(11)*, it was found that $CFCl_3$ was reduced to carbon monoxide, while other polyhalogenated nonfluorinated methanes were transformed primarily to the corresponding less halogenated compounds. The authors of that study suggested that the reaction of $CFCl_3$ occurred via the formation of a :CFCl carbene binding to the iron center where it was subequently transformed to CO. Considering their results, it is, therefore, not unlikely that the missing product in the reduction of the three fluorinated methanes investigated in our study is carbon monoxide (see Figure 2), and it would explain why no formate (hydrolysis product of the carbene) was found.

Reaction with Cysteine (Blank Reaction). Table 3 gives an overview of the products found for the reduction of some of the halomethanes with cysteine as the sole reductant. This "blank" reaction was used to get hints on the reaction pathway(s) of the compounds with an electron donor exhibiting strong nucleophilic properties. Because these

Group 1: CBr_4, CBr_2Cl_2, $CBrCl_3$, CCl_4 Group 2: $CHBr_3$, $CHBr_2Cl$, $CHBrCl_2$

Group 3: $CFBr_3$, $CFCl_3$, CF_2Br_2

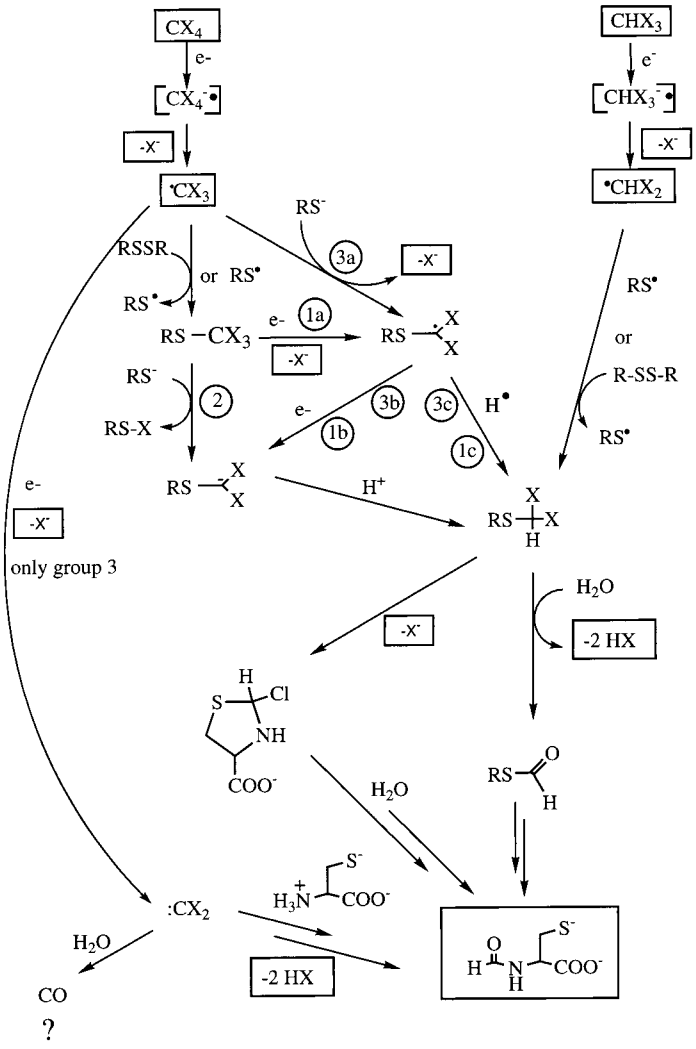

FIGURE 2. Proposed reaction pathway(s) for the degradation of 10 polyhalogenated methanes in the iron porphyrin/ cysteine system. Detected or trapped intermediates and isolated products are framed. Reaction numbers are encircled. Note that the reaction sequence 1a + 1b leads to the same products as the alternative sequence 3a + 3b or the direct X-philic attack at a halogen atom (reaction 2). The sequence 3a + 3c leads to the same product as 1a + 1c.

TABLE 3. Products and Intermediates Found in the Reaction with Cysteine (Blank Reaction)

	products[c]			
compd	**halides and other ions detected**	**halogenated methanes**	***N*-formylcysteine**	**intermediates**
CCl_4[a]	210 ± 30% Cl^-; 5 ± 2% $HCOO^-$	52 ± 5% $CHCl_3$	53 ± 10%	dichlorocarbene trapped[b]
CBr_4	150 ± 20% Br^-; 3 ± 2% $HCOO^-$	91 ± 10% $CHBr_3$	12 ± 10%	dibromocarbene trapped
$CBrCl_3$	80 ± 10% Cl^-; 115 ± 20% Br^-	80 ± 5% $CHCl_3$	5 ± 4%	dichloro- and bromochlorocarbene trapped
CBr_2Cl_2	50 ± 20% Cl^-; 120 ± 20% Br^-	61 ± 22% $CHBrCl_2$; 1 ± 1% $CHBr_2Cl$	14 ± 10%	dichlorocarbene trapped

[a] Results from measurements carried out at $T = 55$ °C. A temperature different from room temperature was chosen because at 25 °C no reaction products were observed within two weeks. [b] Results obtained with 3-pentenoic acid as carbene trap (series 1); all other trapping experiments were performed with 3-methyl-3-butenoic acid (series 2). [c] Amount of starting material = 100%.

reactions occurred at a much slower rate as compared to the reactions with the iron porphyrin, a reasonable product analysis was only possible for the most reactive tetrahalomethanes.

As is evident from a comparison of Tables 1 and 3, with cysteine as the sole reductant, in contrast to the iron porphyrin/cysteine system, a major fraction of the tetrahalomethanes was converted to the corresponding haloforms, and carbene species could be trapped. Hence, for the reaction with cysteine, a different mechanism has to be considered than in the iron porphyrin/cysteine system. Since $R-S^-$ species have been shown to undergo nucleophilic substitu-

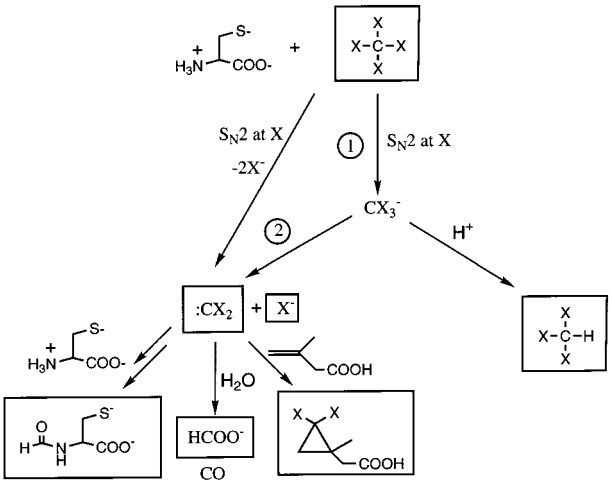

FIGURE 3. Proposed reaction pathway(s) for the degradation of polyhalogenated methanes in the blank system (cysteine only). Detected or trapped intermediates and isolated products are framed. Reaction 1 is an X-philic attack at halogen leading to an anion which can either take up a proton or eliminate a halide anion forming a carbene.

tion reactions at halogen atoms (i.e., X-philic reactions, *(25*−*28)*), a possible initial reaction step could involve a two electron transfer from the cysteine to the tetrahalomethane with concurrent or subsequent protonation (pathway 1 in Figure 3) or formation of a carbene (pathway 2 in Figure 3). The carbene can then react further to yield *N*-formylcysteine or hydrolyze to formate, which, in the case of CCl_4 and CBr_4, was also found in small amounts (see Table 3). In this case the absence of an iron center apparently makes the formation of CO a less likely process.

Significance of the Results. The results of this study are considered important from both a basic scientific as well as from a very practical point of view. First, the data obtained support our conclusions drawn in an earlier kinetic study *(17)* that the initial and rate-determining step in the reduction of PHMs by the iron porphyrin is a (outer sphere) dissociative one-electron transfer (radical intermediates were trapped). The data also corroborate our hypothesis, that in reactions involving PHMs and organic reductants exhibiting mercapto groups (e.g., mercaptojuglone as a model compound for natural organic matter in the presence of hydrogen sulfide *(29)*), an alternative initial reaction step may be an X-philic dissociative two-electron transfer as postulated in Figure 3 for cysteine. Second, the proposed reaction mechanism(s), particularly the proposed involvement of R−SH or R−S−S−R groups in the complete dehalogenation of PHMs, may be helpful in the (re)interpretation of microbially mediated dehalogenation reactions of such compounds *(30)*. In addition, reduction of PHMs in organisms could lead to the formylation of NH_2- or SH-groups present in biologically important molecules. Therefore these reactions should be taken into account when evaluating the toxicity of PHMs *(31)*. Finally, from a practical engineering point of view, the results of this study offer an interesting perspective for a fast complete dehalogenation of PHMs, by using a very reactive one-electron donor (i.e., a reactive iron species) in the presence of organic compounds exhibiting reduced sulfur and nitrogen groups. Using such an approach, the formation of the less halogenated volatile compounds, that are orders of magnitudes less reactive that the parent compounds *(17)*, can be prevented. Consequently, further treatment of such products is not necessary.

Acknowledgments

We thank Michael Berg for help with GC-MS measurements, Martin Schwarz for synthesizing cyclopropane standards and 3-methyl-3-butenoic acid, Etienne Michel for performing a number of IC measurements, and Roland Hany for the NMR measurements. Jim Anderson, Rick Devlin, Stefan Haderlein, Thomas Hofstetter, and Jörg Klausen are kindly acknowledged for reviewing the manuscript.

Supporting Information Available

Syntheses of *meso*-tetrakis(*N*-methylpyridyl)iron porphyrin, *N*-formylcysteine, 3-methyl-3-butenoic acid, diazomethane, **1**, 2-(2,2-dibromo-1-methylcyclopropyl)ethanoic acid, and 2-(2-bromo-2-chloro-1-methylcyclopropyl)ethanoic acid. This material is available free of charge via the Internet at http://pubs.acs.org.

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Received for review May 29, 1998. Revised manuscript received December 14, 1998. Accepted January 5, 1999.

ES980553Y