Biodehalogenation. Reductive Reactivities of Microbial and Mammalian Cytochromes P-450 Compared with Heme and Whole-Cell Models

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The products stoichiometries and kinetics of the reduction of trichloronitromethane, bromotrichloromethane, carbon tetrachloride, ethylene dibromide, and 1,2-dibromo-3-chloropropane by iron(II) deuteroporphyrin IX and rat liver P-450$_{PB}$ and P-450$_{cam}$ have been compared with the reactions of reduced liver microsomes and the whole-cell model *Pseudomonas putida* (PpG-786). The polyhalomethanes in all cases undergo quantitative reductive hydrogenolysis: $RCX_n + 2PFe^{II} + H^+ \rightarrow RCX_{n-1} + 2PFe^{III} + X^-$. The vicinal halides are quantitatively converted by all P-450 components to the corresponding olefins: $>C(X)—C(X)< + 2PFe^{II} \rightarrow >C{=}C< + 2PFe^{III} + 2X^-$. In contrast to the steric retardation of the rates exhibited by P-450$_{cam}$, rate constants for the mammalian enzyme were the same as those obtained for the heme in homogeneous solution. Microsomal reactions followed the same general reactivity patterns but with a compressed scale. The whole-cell conversions are controlled by permeation.

Relatively simple alkyl halides are effective agents for the control of plant parasitic nematodes and fungi. Their presence in the environment in trace quantities has been the basis of considerable concern because the general biocidal properties of these materials render them potential threats to man.

In our studies of biodehalogenation processes, we have found biochemical hydroxylation and reduction by microorganisms (Castro, 1977; Castro et al., 1983) can be major means of detoxifying these substances. Reductive dehalogenation was particularly efficient with *Pseudomonas putida* (PpG-786) (Castro et al., 1983). The responsible enzyme in this organism was identified as the heme protein P-450$_{cam}$ (Castro et al., 1985). However P-450 enzymes are also present in a range of mammalian tissue (Burke and Orrenius, 1982). Consequently, it was of interest to compare the chemistry and reactivity of a mammalian enzyme with its' bacterial counterpart and active site.

Cytochrome P-450 mediated dehalogenation in mammalian liver may represent the major pathway of dehalogenation in vivo. The reactions of reduced liver microsomes with halomethanes (Mansuy et al., 1974; Ullrich, 1977; Nastainczyk et al., 1978, 1982; Ahr et al., 1982; Fujii et al., 1984), carbon tetrachloride (Uehleke et al., 1973; Bini et al., 1975; Wolf et al., 1977), bromotrichloromethane (Bini et al., 1975; Wolf et al., 1977), and other polyhalo C_1 compounds (Wolf et al., 1975, 1977a,b) have received considerable attention.

The reduction of trichloroethane and tetrachloroethane (Thompson and Mastovich, 1985) has also been noted. These reactions may in some cases represent a detoxification but in others, for example, the conversion of *vic*-dihalides to haloolefins (Mansuy et al., 1974; Ullrich, 1977; Nastainczyk et al., 1978, 1982; Ahr et al., 1982; Fujii et al., 1984; Thompson and Mastovich, 1985) may represent a reductive activation of the substrate. Thus, a subsequent oxygen insertion by P-450 may lead to the in vivo generation of carcinogenic epoxides (Henschler, 1977; Guengerich and MacDonald, 1984).

While the reactions of the halides observed to date are consistent with the chemistry of heme and other heme proteins in the G conformation (Castro, 1964; Wade and Castro, 1973a,b; Castro and Bartnicki, 1975; Castro et al., 1978) detailed kinetics and the direct comparison of products and reactivity with the enzyme in various environments have been lacking.

In this work we extend our studies to encompass a mammalian P-450 and liver microsomes. We compare the products and rates of heme oxidation by five alkyl halides (including EDB and DBCP) in five environments: homogeneous solution, P-450$_{cam}$, phenobarbital-induced P-450 from rat liver, reduced rat liver microsomes, and the whole-cell PpG-786. While the heme environment is different in each of these entities, it was of interest to learn to what extent the chemistry paralleled that observed with active-site models in homogeneous solution. A major finding is that the mammalian enzyme reacts as rapidly as a heme in homogeneous solution.

EXPERIMENTAL SECTION

Materials. Deuteroheme, P-450$_{cam}$, and *P. putida* (PpG-786) were obtained as previously described (Castro et al., 1985). The mammalian enzyme and liver microsomes were isolated from phenobarbital-induced Sprague–Dawley rats (Imai and Sato, 1974b; West et al., 1979) following literature procedures (Imai and Sato, 1974a; Omura and Sato, 1964; Ortize de Montellano et al., 1981). Final chromatography for the purified enzyme was on hydroxyapatite. This yielded a fraction having 10–12 nmol of P-450/mg of protein. The subsequent Sephadex step was omitted because the purification derived from it was marginal.

The organic substrates were purified as previously described (Wade and Castro, 1973a; Castro et al., 1985). Carbon tetrachloride 90 atom % ^{13}C was purchased from MSD Isotopes and used directly.

Methods. *GC–MS.* Products were quantitated by gas chromatography and qualitatively confirmed by GC–MS (Wade and Castro, 1973a; Castro et al., 1985) as described earlier. The previously unreported GC–MS procedures for ethylene and allyl chloride utilized a 15-m methyl silicone column directly fitted to a VG-ZAB mass spectrometer. Parent ions at 28 (ethylene) and 76 and 78 (allyl chloride) were observed.

Gas Chromatography. The following columns and conditions were employed: 4.5 ft, $^1/_8$ in. Porapak P at 150 °C ($BrCCl_3$, CCl_4, $CHCl_3$); 4.5 ft, $^1/_8$ in. 30% SE30 on Chromasorb W at 140 °C (EDB, DBCP); 2.5 ft, $^1/_8$ in. Porapak Q at 140 °C (Cl_3CNO_2, $CHCl_2NO_2$, CH_2ClNO_2,

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CH_3NO_2, allyl chloride, ethylene). In all cases quantitation was accomplished by spiking with an internal standard.

Reactions and kinetics with deuteroheme, P-450$_{cam}$, and resting-cell suspensions of PpG-786 were conducted and monitored in the manner recently described (Castro et al., 1985). However, the new whole-cell kinetic runs in this work with carbon tetrachloride, ethylene dibromide, and 1,2-dibromo-3-chloropropane were followed by direct potentiometric analysis for halide ion (Castro et al., 1983). Also these substrate levels were lower (10^{-5} M) than we usually employ. At higher levels (10^{-3} M) no reactions ensued with whole cells. Reactions with whole cells were conducted in air. Reactions with the heme and the enzymes were run under argon as described earlier.

As an illustration of procedures the reaction of 1,2-dibromo-3-chloropropane with each of the biological components is detailed.

Reaction with Heme. *1,2-Dibromo-3-chloropropane.* A 50-mL three-neck flask was equipped with a Teflon-coated magnetic stirring bar a serum-capped inlet stopcock, an outlet stopcock connected to a mercury trap, and a sealed glass drooping T-shaped tube that contained a clean stirring bar in one leg. After being purged with argon, the flask was charged with 0.18 g (3×10^{-4} mol) of chloroiron(III) deuteroporphyrin IX in 30 mL of 2:1 *N*-methylpyrrolidone–acetic acid and 0.1 g of acetic acid washed Matheson electrolytically reduced iron powder. After the mixture was purged for 20 min, stirring was commenced under a slight argon purge and continued for 30 min. At this time the stirring bar and excess iron powder were removed from the mixture by an external magnet and deposited in the vacant leg of the T-tube. The fresh stirring bar was added to the homogeneous red 10^{-2} M heme solution. [For spectra of the iron(II) and iron(III) adducts, cf. Wade and Castro (1973a).] Stirring was recommenced, and 17 μL (1.5×10^{-4} mol) of 1,2-dibromo-3-chloropropane was added via a hypodermic syringe during an argon purge. The system was sealed under argon and stirred for 3 h. During this time the red heme solution changed to the red-brown color of iron(III) deuteroporphyrin. The flask was opened to air, and the contents were analyzed by direct FID gas chromatography on the SE-30 column. The product peak coemerged with allyl chloride. The nature of the product was qualitatively confirmed by GC–MS as noted above. The yield was determined by spiking with authentic chloride to be 1.45×10^{-4} mol (97 ± 5%). It should be noted that, with higher concentrations of heme and longer reaction times, the allyl chloride can be converted to coupling products (Wade and Castro, 1973a).

For ethylene dibromide a similar reaction setup was employed except the flask was fitted with a manometer. Direct GC analysis of the gas and solution phases established an ethylene yield of 103 ± 5%. The qualitative nature of the product was confirmed by GC–MS as indicated above.

Reactions of the other substrates with hemes were conducted in similar fashion, and they have been described in detail (Castro et al., 1985).

Kinetics with Hemes. Rates were monitored under argon by visible spectroscopy at 627 nm (PFe^{III} appearance) in the manner previously described (Wade and Castro, 1973a; Castro et al., 1985). Reproducibility of runs was <±10%.

Reactions with Whole Cells. General reaction and workup conditions followed those previously described (Castro et al., 1983, 1985). A cell suspension (0.1 g/mL) of *P. putida* 0.1 M phosphate buffer, pH 7.4, was incubated at room temperature in air with 1,2-dibromo-3-chloropropane at a starting concentration of 1.0×10^{-5} M. Note at higher concentrations no reaction was observed. Usually 50 mL portions of cell suspension in 250-mL Erlenmeyer flasks fitted with serum-capped stopcocks were incubated and shaken at room temperature. After 2 h, the bromide ion concentration was 2.0×10^{-5} M. Direct gas chromatographic analysis of the mixture showed only allyl chloride and no DBCP. The product was confirmed by GC–MS.

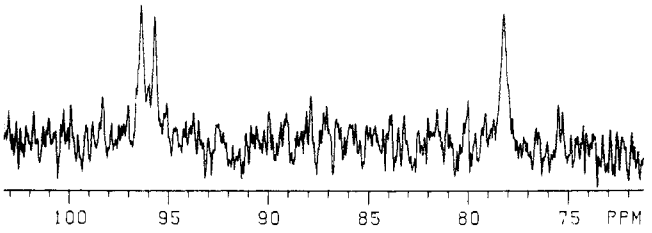

Figure 1. ^{13}C NMR spectrum of a resting-cell suspension of PpG-786, 0.1 g/mL, charged with 10^{-2} mol of $^{13}CCl_4$/L of mixture.

Kinetics. Three-milliliter aliquots were removed, treated with 0.1 mL of concentrated HNO_3, and centrifuged. The clear supernatant solution was diluted with an equal volume of a solution containing 0.75 M HNO_3 and 1.25 M KNO_3. Bromide ion was analyzed via direct potentiometry with an Orion specific ion electrode and a double-jacketed calomel reference electrode that contained KNO_3 in the outer chamber. A typical rate plot is shown in Figure 2. Reproducibility of repeat runs was ±10%.

Ethylene dibromide was reacted in similar fashion. The reaction proceeded at a much slower rate (Table II). At 3 h 84% of the starting EDB remained. Ethylene in the gas phase corresponded to 16 ± 1% of the product mixture. The ethylene was confirmed by the GC–MS procedure outlined above.

In addition, a reaction with 90 atom % $^{13}CCl_4$ and a cell suspension of *P. putida* was followed qualitatively by ^{13}C NMR. For this purpose 5 mL of a standard cell suspension (Castro et al., 1985), 0.1 g/mL, in 0.1 M phosphate buffer, pH 7.4, was placed in a 20-mL wide-bore (11-mm i.d.) NMR tube. To the suspension was added 5 μL of $^{13}CCl_4$ (5×10^{-5} mol). This exceeds the solubility of CCl_4 in water. After 5 h spectra were gathered on a 300-MHz Nicolet instrument using a 12-mm broad-band probe over an 8-h period. A two-level decoupling experiment was conducted. The observed pulse was 12.5 μs, which is equal to 22.5° with an interpulse delay of 3.5 s. The total number of scans was 7200 and required 16K of computer memory. The spectrum was scanned from −50 to +220 ppm with a resolution of 2.5 Hz/peak. No resonances other than those shown in Figure 1 were detected, sensitivity <1 mol %.

Reactions with liver microsomes at pH 7.4 were conducted under two sets of conditions under argon. For product characterization, the initial concentration of reactants was $[NADPH]_0 = 2.0 \times 10^{-2}$ M, $[substrate]_0 = 2.0 \times 10^{-3}$M, and $[P\text{-}450]_0$ (RLM) $= 4.0 \times 10^{-5}$ M. The latter was determined from known extinction coefficients for the purified CO adduct (Ortize de Montellano et al., 1981). Approximately 2.5-mL scale reactions were conducted under argon in argon-purged reactivials and analyzed by gas chromatography after 24 h. The concentration of substrate was critical. As observed with whole cells (Castro et al., 1985), the higher concentrations destroy the integrity of the microsomes. Thus, with chloropicrin at 10^{-2} M, the microsomal suspensions changed from pink to yellow within 10 min and no nitromethane was produced. At 5×10^{-3} M the microsomal destruction was evident in less than 1 h. With DBCP at 24 h, analysis on the Porapak

Table I. Products, Yields, and Enzyme Turnover

substrate[a]	product	conversion[b]	turnover[c]
Cl_3CNO_2	CH_3NO_2	72	216
$CBrCl_3$	$CHCl_3$	40	40
CCl_4	$CHCl_3$	40	40
$BrCH_2CH_2Br$	$CH_2=CH_2$	16	16
$BrCH_2BrCHCH_2Cl$	$CH_2=CHCH_2Cl$	14	14

[a] Conditions: $[substrate]_0 = 2.0 \times 10^{-3}$ M, [P-450 in RLM]$_0 = 4.0 \times 10^{-6}$, $[NADPH]_0 = 2.0 \times 10^{-2}$. [b] Moles of product (100)/mole of substrate charged. [c] Moles of product/P-450 equivalent.

P column indicated 86% of the starting halide remained. The yield of allyl chloride was $(0.6 \pm 0.1) \times 10^{-6}$ mol (86 ± 14%).

Kinetics. For kinetics an excess of substrate was employed: $[substrate]_0 = 2.0 \times 10^{-3}$ M, $[NADPH]_0 = 1.5 \times 10^{-4}$ M, and $[P\text{-}450]_0 = 7.1 \times 10^{-7}$ M. NADPH oxidation was followed spectrophotometrically at 340 nm in 1-cm cuvettes.

Both reactions and kinetics with P-450$_{cam}$ and the mammalian enzyme were conducted under argon using 1-cm serum-capped cuvettes. Reactions were initiated by injection of the substrate under argon. With P-450$_{cam}$ initial rates were monitored following the decrease in absorption (iron(II)) at 542 nm. Products were analyzed by direct GC analysis.

Kinetics with the purified mammalian enzyme followed generally the procedures used for P-450$_{cam}$ (Castro et al., 1985). In this case the enzyme was reduced directly in the spectrophotometric cuvettes by sodium dithionite under argon. The reduction was conducted usually to the 70–90% level (to be sure no excess dithionite was present, λ_{max} 316). Rates were monitored by following the decrease in absorbance at 550 nm. Rate constants were determined by initial slopes.

RESULTS

Salient features of the reactions of rat liver microsomes with the halides are given in Table I. Except for chloropicrin the reduced product shown is produced exclusively in all cases. As with the bacterial enzyme, the heme and whole cells, chloropicrin is reduced stepwise to nitromethane (Castro et al., 1983, 1985). Dichloro- and chloronitromethane are intermediates and were detected in these runs (eq 1). The overall conversion requires six

$$Cl_3CNO_2 \rightarrow Cl_2CHNO_2 \rightarrow ClCH_2NO_2 \rightarrow CH_3NO_2 \quad (1)$$

hemes. Thus, the enzyme turnover in this case is $6(0.72)(2.0 \times 10^{-3})/(4.0 \times 10^{-5}) = 216$. For bromotrichloromethane and carbon tetrachloride the reductive hydrogenolysis (Castro et al., 1985) (eq 2, P = P-450, X

$$CX_4 + 2PFe^{II} + H^+ \rightarrow HCX_3 + 2PFe^{III} + X^- \quad (2)$$

= halogen) requires two hemes. Elimination of halogen from the vicinal dihalides (eq 3), ethylene dibromide, 1,2-dibromo-3-chloropropane (DBCP) also requires two heme equivalents (Wade and Castro, 1973a). Because of the molar ratio of substrate to P-450 (50 in these latter cases), the number of turnovers equals the percent conversion.

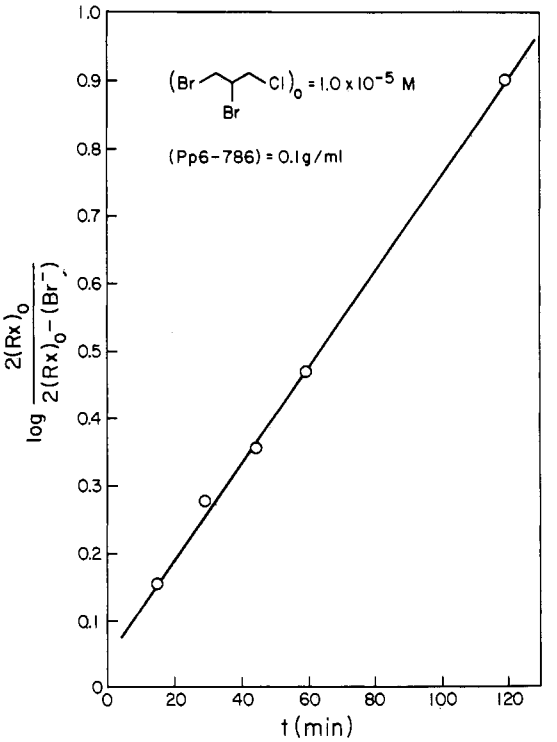

Figure 2. Rate plot of the conversion of DBCP to allyl chloride (eq 3).

$$>C(X)—C(X)< + 2PFe^{II} \rightarrow >C=C< + 2PFe^{III} + 2X^- \quad (3)$$

The ^{13}C NMR spectrum of the conversion of carbon tetrachloride to chloroform by a resting-cell suspension of *P. putida* is shown in Figure 1. The spectra were gathered over an 8-h period beginning 5 h after mixing. It will be noted that two distinct resonances are observed for CCl_4, a broader one at 96.3 ppm and a narrower one at 95.6 ppm. The $CHCl_3$ shows as a broad singlet at 78.1 ppm. We assign the broader resonances to the substances within or occluded to the cells. The narrower CCl_4 signal represents the substance in solution. No other resonances were detected over the range −50 to +220 ppm. These results are in accord with earlier observations (Castro et al., 1985) that this transformation proceeds quantitatively according to eq 2. No carbon monoxide was detected. The ease of the methodology also suggests that biodehalogenation reactions may be monitored more readily by the ^{13}C technique rather than the more conventional ^{14}C procedures we have employed in the past.

The rate constants for the oxidation of the hemes in these various environments are given in Table II. The substrates are listed in decreasing order of reactivity toward iron(II) deuteroporphyrin IX in homogeneous 1:1 *N*-methylpyrrolidone–acetic acid solution. A typical rate

Table II. Rates of Oxidation of Deuteroheme and the P-450 Enzymes

substrate	PFE^{II},[a] L/mol s^{-1}	P-450 (rat), L/mol s^{-1}	P-450$_{cam}$, L/mol s^{-1}	RLM, mol NAD/ mol P-450 s^{-1}	PpG-786,[e] s^{-1}
trichloronitromethane	$\geq 8 \times 10^4$ [d]	$\geq 8 \times 10^4$	160[d]	161	5.4[d]
bromotrichloromethane	310[d]	290	5.4[d]	22	0.3[d]
carbon tetrachloride	34[d]	31	2.3[d]	13	6.7×10^{-4}
ethylene dibromide	0.012	≤0.03[b]	≤0.012[b]	13	5.0×10^{-5}
1,2-dibromo-3-chloropropane	0.016	≤0.01[b]	≤0.07[b]	13[c]	2.8×10^{-4}

[a] Reproducibility ±10%. [b] Slow rates with the enzymes taken from maximal initial slopes. [c] This number approaches the background levels for NADPH oxidation in the absence of substrate. [d] Values taken from Wade and Castro (1973a). [e] For second-order rate constants, rate = k[RX][number of organism]/10^{14}. Thus, for chloropicrin $k = 5.4 \times 10^{-14}$ L/organism s^{-1}.

plot for the reduction of DBCP by whole cells is shown in Figure 2. The units for the oxidation of the heme and the P-450 enzymes in homogeneous solution are the same ($L/mol\ s^{-1}$), and these constants are directly comparable.

$$d[PFe^{III}]/dt = k[substrate][PFe^{II}]$$

It will be noted that for all substances, within experimental error, the rate constants for the mammalian enzyme are the same as that for deuteroheme in homogeneous solution.

The relative order of halide reactivity toward the P-450 microsomal preparation and with P-450_{cam} in the bacterium follow the general trend exhibited for the heme and the enzymes, but the relative rates and their units are different. In these systems the rate-limiting step is not the intrinsic rate of reaction with the heme. We presume the whole-cell rates reflect the rate of permeation to the active site. This may also be a factor with the microsomes.

As with other relatively slow substrates, the rate constants with P-450_{cam} and EDB and DBCP parallel those of the heme active site.

DISCUSSION

The mechanisms for the oxidation of hemes by alkyl halides proceeds in three discrete steps (Wade and Castro, 1973a; Castro et al., 1985):

(i) Ligation of iron (Wade and Castro, 1985).

$$PFe^{II} + RX \rightleftarrows PFe(XR)$$

(ii) Cleavage of the C–X bond to generate a radical (Wade and Castro, 1973a).

$$PFe(XR) \rightleftarrows [PFe\cdots X\cdots R] \rightleftarrows PFe^{III} + R^{\bullet}$$

(iii) Rapid scavenging of the radical by the heme (Castro et al., 1974) (reverse of ii also noted).

$$PFe^{II} + R^{\bullet} \rightarrow [PFeR] \xrightarrow{H_2O} PFeOH + RH$$

Alternatively, when the radical contains a halogen on the adjacent carbon, conversion to alkene can ensue:

$$>C(X)—\dot{C}< + PFe^{II} \rightarrow PFe^{III}X + C{=}C$$

With hemes in homogeneous solution the bond-breaking step (ii) is rate limiting. With heme proteins, because of steric constraints imposed upon the heme active site by the conformation of the enzyme, the ligation step (i) can be rate limiting. P-450_{cam} exhibits this steric inhibition of rate (Castro et al., 1985) when the enzyme is exposed to halides that will undergo the bond-cleavage step (ii) quickly. On the other hand, when the rate of (ii) is slow, the rate constants for the enzyme approach those found for the heme in homogeneous solution as they do in this work with EDB and DBCP.

The scavenging of the radical by the heme represents the combination of two paramagnetic centers. Consequently the reaction competes with radical–radical combination (Wade and Castro, 1973a; Castro et al., 1974), and its rate is essentially diffusion controlled ($\sim 10^9\ L/mol\ s^{-1}$) (Brault and Netta, 1983).

A surprising observation in this work is that mammalian rat liver P-450 exhibits no steric inhibition at all. Indeed with five substrates of widely differing activity the solubilized enzyme exhibits the same rate constants as those of the heme in homogeneous solution. Clearly the heme is far more accessible in the rat liver enzyme than it is in the microbial enzyme. Moreover, the consistency in rates over such a wide range emphasizes that the mechanism operating with hemes in homogeneous solution is operating with the mammalian enzyme. In addition, steric effects are not important. This raises a question as to whether or not the solubilized enzyme can be taken as "native". Despite its correct properties we presume this membrane-bound cytochrome may have a more tightly drawn conformation about the heme in its native biological environment. However, comparable rate measurements under these conditions cannot be made.

All of the substrates in Table II may have a deleterious effect upon man. Chloropicrin, ethylene dibromide, and 1,2-dibromo-3-chloropropane are or have been employed as soil fumigants for the control of fungi and nematodes, and the latter two have been detected in the soil water environment. Two points may be inferred from the data in Table II. First, bacterial degradation of all of these substances is possible. Note even for a slow-reacting substrate, DBCP, the $t_{1/2}$ from the resting-cell experiment with *P. putida* is 40 min. Second, all of the substances are capable of biotransformation in mammalian liver. Whether or not the substances produced (Table I) are more or less toxic and whether they may be bioactivated to even more toxic substances in the same organ (perhaps by the same enzyme) remain to be investigated.

Taken together, the results in Tables I and II establish that the reductase capacity with widely divergent P-450's is consistent with the chemistry and mechanism established for hemes in homogeneous solution.

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Registry No. P-450, 9035-51-2; Cl_3CNO_2, 76-06-2; $CBrCl_3$, 75-62-7; CCl_4, 56-23-5; $Br(CH_2)_2Br$, 106-93-4; $BrCH_2BrCHCH_2Cl$, 96-12-8; iron(II) deuteroporphyrin IX, 18922-88-8.

LITERATURE CITED

Ahr, H. J.; King, L. J.; Nastainczyk, W.; Ullrich, V. "The Mechanism of Reductive Dehalogenation of Halothane by Liver Cytochrome P-450". *Biochem. Pharmacol.* **1982**, *31*, 383–390.

Bini, A.; Vecchi, G.; Vivoli, G.; Vannini, V.; Cessi, C. "Detection of Early Metabolites in Rat Liver After Administration of Carbon Tetrachloride and Bromodichloromethane". *Pharmacol. Res. Commun.* **1975**, *7*(2), 143–149.

Brault, D.; Netta, P. "Pulse Radiolysis of Porphyrin and Ferriporphyrin Solutions in 2-Propanol–Carbon Tetrachloride Systems. Protonation and Ligand Exchange Kinetics". *J. Phys. Chem.* **1983**, *87*, 3320–3327 and references therein.

Burke, M. D.; Orrenius, S. "Isolation and Comparison of Endoplasmic Reticular Membranes and Their Mixed Function Oxidase Activities from Mammalian Extrahepatic Tissues". In *International Encyclopedia of Pharmacology and Therapeutics*; Schenkman, J. B., Kupfer, D., Eds.; Section 108, Hepatic Cytochrome P-450 Monooxygenase System; Pergammon: New York, 1982.

Castro, C. E. "The Rapid Oxidation Of Iron(II) Porphyrins by Alkyl Halides. A Possible Mode of Intoxification of Organisms by Alkyl Halides". *J. Am. Chem. Soc.* **1964**, *80*, 2310.

Castro, C. E. "Biodehalogenation". *Environ. Health Perspect.* **1977**, *21*, 279–283 and references therein.

Castro, C. E.; Bartnicki, E. W. "Conformational Isomerism and Effective Redox Geometry in the Oxidation of Hemeproteins by Alkyl Halides. Cytochrome *c* and Cytochrome Oxidase". *Biochemistry* **1975**, *14*, 498–503.

Castro, C. E.; Robertson, C.; Davis, H. "The Reactions of Octaethylporphyrin and its Iron(II) and Iron(III) Complexes with Free Radicals". *Bioorg. Chem.* **1974**, *3*, 343–360.

Castro, C. E.; Bartnicki, E. W.; Belser, N. O. "The Oxidation of Heme Proteins by Alkyl Halides. A Probe for Axial Inner Sphere Redox Capacity in Solution and in Whole Cells". *Biochemistry* **1978**, *17*, 5582–5586.

Castro, C. E.; Wade, R. S.; Belser, N. O. "Biodehalogenation: The Metabolism of Chloropicrin by *Pseudomonas sp*". *J. Agric. Food Chem.* **1983**, *31*, 1184–1187.

Castro, C. E.; Wade, R. S.; Belser, N. O. "Biodehalogenation. Reactions of Cytochrome P-450 with Polyhalomethanes".

Biochemistry **1985**, *24*, 204–210.
Fujii, K.; Morio, M.; Kikuchi, H.; Ishihara, S.; Okida, M.; Ficor, F. "*In vivo* Spring-trap Study in Anaerobic Dehalogenation of Halothane". *Life Sci.* **1984**, *35*, 463–468.
Guengerich, F. P.; MacDonald, T. L. "Chemical Mechanisms of Catalysis by Cytochromes P-450: A Unified View". *Acc. Chem. Res.* **1984**, *17*, 9–16.
Henschler, D. "Metabolism and Carcinogenicity of Halogenated Olefins—A Comparison of Structure and Activity". *EHP, Environ. Health Perspect.* **1977**, *21*, 61–65.
Imai, Y.; Sato, R. "A Gel Electropholetically Homogeneous Preparation of Cytochrome P-450 From Liver Microsomes of Phenobarbital Pretreated Rats". *Biochem. Biophys. Res. Commun.* **1974a**, *60*, 8–14.
Imai, Y.; Sato, R. "An Affinity Column Method for Partial Purification of Cytochrome P-450 from Phenobarbital-Induced Rabbit Liver Microsomes". *Biochemistry* **1974b**, *75*, 689–697 and references therein.
Mansuy, D.; Nastainczyk, W.; Ullrich, V. "The Mechanism of Halothane Binding to Microsomal Cytochrome P-450". *Naunym-Schmiedeberg's Arch. Pharmacol.* **1974**, *285*, 315–324.
Nastainczyk, N.; Ullrich, V.; Sies, H. "Effect of Oxygen Concentration on the Reaction of Halothane with Cytochrome P-450 in Liver Microscomes and Isolated Perfused Rat Liver". *Biochem. Pharmacol.* **1978**, *27*, 387–392.
Nastainczyk, N. W.; Ahr, H. J.; Ullrich, V. "The Reductive Metabolism of Halogenated Alkanes by Liver Microsomal Cytochrome P-450". *Biochem. Pharmacol.* **1982**, *31*, 391–396.
Omura, T.; Sato, R. "The Carbon Monoxide Binding Pigment of Liver Microsomes". *J. Biol. Chem.* **1964**, *239*, 2370–2378.
Ortize de Montellano, P. R.; Mico, B. A.; Matthew, J. M.; Kunze, K. L.; Miwa, G. T.; Lu, A. Y. H. "Selective Inactivation of Cytochrome P-450 Isozymes by Suicide Substrates". *Arch. Biochem. Biophys.* **1981**, *210*, 717–728.
Thompson, J. A.; Mastovich, S. L. "Dynamic Headspace Analysis of Volatile Metabolites from Reductive Dehalogenation of Trichloro and Tetrachloroethanes by Hepatic Microsomes". *Anal. Biochem.* **1985**, *145*, 376–384.
Uehleke, H.; Hellmer, K. H.; Tabatelli, D. "Binding of Carbon-14 Labelled-Carbon Tetrachloride to Microsomal Proteins *in vivo* and Formation of Chloroform by Reduced Liver Microsomes". *Xenobiotica* **1973**, *3*, 1–11.
Ullrich, V. "The Mechanism of Cytochrome P-450 Action". In *Micromes and Drug Oxidation*; Ullrich, V., Root, I., Hildebrandt, A., Estabrook, R. W., Conney, A. H., Eds.; Pergamon: New York, 1977.
Wade, R. S.; Castro, C. E. "Oxidation of Iron(II) Porphyrins by Alkyl Halides". *J. Am. Chem. Soc.* **1973a**, *95*, 226–230.
Wade, R. S.; Castro, C. E. "The Oxidation of Hemeproteins by Alkyl Halides". *J. Am. Chem. Soc.* **1973b**, *95*, 231–234.
Wade, R. S.; Castro, C. E. "Halomethane Adducts of Iron(II) Porphyrins". *Inorg. Chem.* **1985**, *24*, 2862–2863.
West, S. B.; Huang, M. T.; Miwa, G. T.; Lu, A. Y. H. "A Simple Rapid Procedure for the Purification of Phenobarbital Inducible Cytochrome P-450 From Rat Liver Microsomes". *Arch. Biochem. Biophys.* **1979**, *193*, 42–50.
Wolf, C. R.; King, L. J.; Parke, D. V. "Anaerobic Dechlorination of Trichlorofluoromethane by Liver Microsomal Preparations *in vivo*". *Biochem. Soc. Trans.* **1975**, *3*, 175–177.
Wolf, C. R.; Mansuy, D.; Nastainczyk, W.; Ullrich, V. "The Interaction of Polyhalogenated Methanes with Ferrous Cytochrome P-450". In *Microsomes and Drug Oxidations*; Ullrich, V., Roots, I., Hildebrandt, A., Estabrook, R. W., Conney, A. H., Eds.; Pergamon: New York, 1977a.
Wolf, C. R.; Mansuy, D.; Nastainczyk, W.; Deutschmann, G.; Ullrich, V. "The Reduction of Polyhalogenated Methanes by Liver Microsomal Cytochrome P-450". *Mol. Pharmacol.* **1977b**, *13*, 698–705.

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Effect of Soil Applications of Sodium Molybdate on the Quality of Potatoes: Polyphenol Oxidase Activity, Enzymatic Discoloration, Phenols, and Ascorbic Acid

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The effect of soil applications of molybdenum on polyphenol oxidase activity, enzymatic discoloration, and total phenolic, chlorogenic acid, tyrosine, and ascorbic acid contents of Katahdin potatoes was investigated. Sodium molybdate was applied at rates of 0.0, 2.8, 6.7, and 10.1 kg/ha. Ascorbic acid increased significantly ($p < 0.01$) with increasing levels of molybdenum fertilization. No significant changes in polyphenol oxidase activity, enzymatic discoloration, or total phenolic, chlorogenic acid, and tyrosine contents were observed at 2.8 and 6.7 kg/ha applications of sodium molybdate. However, there was a highly significant ($p < 0.01$) decrease in polyphenol oxidase activity, enzymatic discoloration, and total phenolic, chlorogenic acid, and tyrosine contents in tubers from plants receiving 10.1 kg/ha of sodium molybdate.

Molybdenum, the only period 5 transition element of consequence to plants, is an essential nutrient for normal plant growth, metabolism, and reproduction. Although it is a mineral, usually occurring as Mo(IV), in aqueous solutions it is predominantly present as molybdate oxyanion, MoO_4^{2-} [Mo(VI)], the form available to plants. This form is the highest oxidative state of the metal. The functions of molybdenum as a plant nutrient are due to the valency changes it undergoes as a prosthetic moiety of certain enzymes, which are few in number and include nitrogenase, nitrate reductase, xanthine dehydrogenase, aldehyde oxidase and sulfate oxidase. Nicholas et al. (1962) showed that the protein component of all these enzymes was similar and speculated that molybdenum may have similar catalytic properties in all these enzymes. However, among these enzymes, only nitrogenase and nitrate reductase have been extensively studied and their functions in nitrogen metabolism are well documented. Agarwala et al. (1979),

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