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Biodehalogenation

by C. E. Castro*

Haloorganic biocides are widely employed as soil fumigants to combat the destructive action of plant parasitic nematodes and fungi. These substances are dehalogenated by soil organisms, principally species of *Pseudomonas* and *Flavobacteria*, to nontoxic metabolites. The paths of metabolism of a variety of simply alkyl halides are described with emphasis upon the biodehalogenation step.

Teratogens, carcinogens
Terrible awful beasts
But O' the soil buggies
Eat them all at feasts

Simple alkyl halides are widely employed throughout California and elsewhere as soil fumigants to combat the destructive action of plant parasitic nematodes and fungi. Typical of this class of compounds are: methyl bromide, chloropicrin, ethylene dibromide, 1,2-dibromo-3-chloropropane, and *cis*- and *trans*-1,3-dichloropropene. In addition to their broad biocidal capacities, these compounds are volatile and possess good diffusion characteristics.

Analyses, in our laboratory, of the edible portion of crops grown in orchards and fields throughout the state that had been fumigated with these substances showed no organic halide to be present. On the other hand, an increase in inorganic bromide or chloride compared to that of untreated check crops could be detected. These results corroborated earlier industrial findings.

The lack of organic halide in any crop suggested that these substances may be metabolized or simply chemically degraded within the soil environment. Such is, indeed, the case, and I wish to present here some of the variety of biodehalogenation processes we have encountered. In many ways they resemble the transformations some of you have noted or expect in liver microsomes. Finally, I wish to comment on the reaction of iron porphyrins and hemeproteins with alkyl halides. This is a process we believe is fundamentally germane to the interaction of organic halides with living systems.

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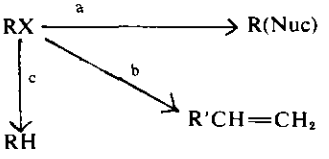
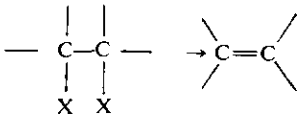
Soil and Microbial Dehalogenations

There are three general kinds of transformation a halide may undergo as shown in Eq. (1).



Nuc = SR, OH, –NHR, etc. (1)

Process (1a) can be considered a biological or biochemical nucleophilic substitution. It is a rather general process and we have observed several examples of it in which an external hydroxyl (water) or a neighboring one displaces halogen to result in an alcohol or epoxide, respectively. The simple elimination of HX [Eq.(1b)] represents a reaction that we have not observed with microorganisms. The reductive dehalogenation [Eq.(1c)] is reminiscent of the conversions of DDT to DDD by a variety of organisms (*1, 2*) or the exhalation of chloroform by dogs upon inhalation of carbon tetrachloride (*3*). If the halide bears a neighboring halogen, the reductive dehalogenation generally yields an olefin [Eq.(2)].

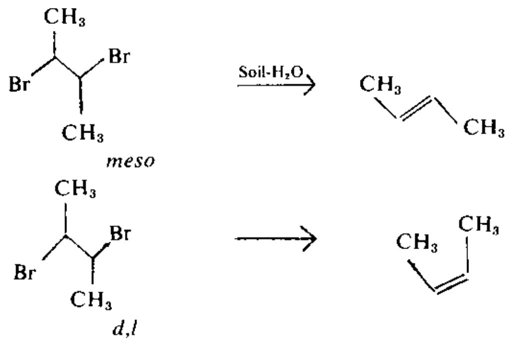


(2)

We have several examples of this with the substrates listed above. It, too, is a general transformation of the microbes.

Reductive Dehalogenation [Eq. (2)]

2,3-Dibromobutane. The *meso* and *d, l* isomers of 2,3-dibromobutane are converted (*4*) by a mixed microbial culture (primarily *Pseudomonas* and *Flavobacteria*) in the presence of soil to the 2-butenes in a stereospecific *trans* process [Eqs. (3)].



(3)

The dehalogenation does not occur in sterile soil under identical conditions.

Ethylene Dibromide. Similarly, in about two months, ethylene dibromide is converted almost completely and quantitatively (*4*) to ethylene by the microbes [Eq. (4)].

$$BrCH_2CH_2Br \xrightarrow[\text{pH above 7.0}]{\text{Soil-H}_2\text{O}} CH_2=CH_2 + 2Br^- \quad (4)$$

This transformation is also observed with the nematode *Aphelenchus avenae* (*5*). It is one of the initial reactions with the live nematode preceding death.

1,2-Dibromo-3-chloropropane. Rather amazingly, 1,2-dibromo-3-chloropropane is dehalogenated (*4*) by the soil bacteria in a related fashion to produce *n*-propanol [Eq. (5)].

$$Br\text{-}CH_2\text{-}CH(Br)\text{—}CH_2Cl \xrightarrow[\text{pH 7.5}]{\text{Soil-H}_2\text{O}} CH_3CH_2CH_2OH + 2Br^- + Cl^- \quad (5)$$

This transformation takes place in at least three discrete steps [Eqs. (6)–(8)].

$$Br\text{-}CH_2\text{-}CH(Br)\text{-}CH_2Cl \rightarrow CH_2=CHCH_2Cl + Br^- \quad (6)$$

$$CH_2=CH_2\text{-}CH_2\text{-}Cl \xrightarrow{H_2O} CH_2=CH_2CH_2OH + Cl^- \quad (7)$$

$$CH_2=CH_2\text{-}CH_2OH \rightarrow CH_3CH_2CH_2OH \quad (8)$$

The hydrolysis of allyl chloride does not require the presence of bacteria but the conversion of allyl alcohol does.

Biochemical Nucleophilic Substitution [Eq. (1a)].

Ethylene Dibromide. A study of the intoxication of the nematode *Aphelenchus avenae* by ethylene dibromide reveals the presence of two early metabolites just preceding death (*5*). The nematode represents the only organism we have found in which both a reductive dehalogenation and an apparent substitution process ensue [Eq. (9)], though the latter predominates. *O*-Acetylserine is the major product along with ethylene. In addition, *N*-acetylserine can be detected in the product mixture but it is the result of a rearrangement of the *O*-acetyl isomer [Eq. (10)] that occurs upon chromatography.

$$BrCH_2CH_2Br \xrightarrow{A.\ avenae} CH_2=CH_2 \quad (5\%)$$
$$BrCH_2CH_2Br \rightarrow CH_3\text{-}C(=O)\text{-}O\text{-}CH_2\text{-}CH(NH_2)\text{-}C(=O)\text{-}OH \quad (95\%) \quad (9)$$

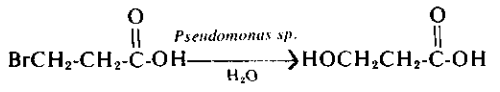
$$CH_3\text{-}C(=O)\text{-}O\text{-}CH_2\text{-}CH(NH_2)\text{-}C(=O)\text{-}OH \rightarrow HOCH_2\text{-}CH(NH\text{-}COH_3)\text{-}C(=O)\text{-}OH \quad (10)$$

3-Bromopropanol. 3-Bromophenol is readily metabolized (*6*) by resting cells of *Pseudomonas* sp. to carbon dioxide and acetate. The process entails the steps shown in Eq. (11).

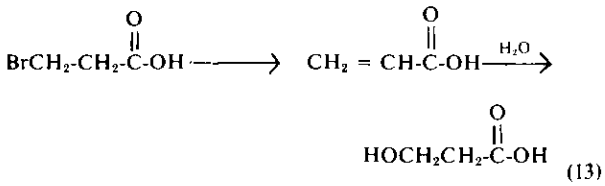
$$BrCH_2CH_2CH_2\text{-}OH \xrightarrow{Pseudomonas\ sp.} BrCH_2CH_2\text{-}C(=O)\text{-}OH \xrightarrow{k}$$
$$HOCH_2CH_2HC(=O)\text{-}OH \longrightarrow CO_2 + CH_3\text{-}C(=O)\text{-}OH \quad (11)$$

The slow step in the dehalogenation of β-bromopropionic acid to hydracrylic acid. The overall reaction has some additional significance in that hydracrylic acid (β-propiolactone) is a potent

carcinogen. The actual dehalogenation reaction [Eq. (12)] does not proceed via an elimination to acrylic acid followed by water addition. While the cells will take up acrylic acid, it is not metabolized further [Eq. (13)].

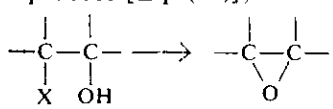


(12)



(13)

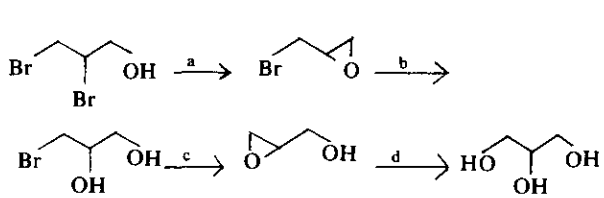
2,3-Dibromopropanol. In an effort to obtain more water-soluble vicinal dihalides for metabolic study, a series of dihalo alcohols was subjected to our soil screen (*7*). Pure cultures of flavobacteria could be isolated that readily metabolized these substances. However, instead of the reductive dehalogenation expected, an internal "nucleophilic substitution" to produce epoxides was observed. The process [Eq. (14)],



(14)

X = halogen

really a special case of Eq. (1a), is a general one for the flavobacteria, and the conversion of 2,3-dibromopropanol to glycerine [Eq. (15)] by resting cell suspensions is illustrative.

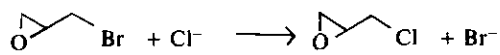


(15)

These reactions could also be effected by partially purified enzymes isolated from the bacteria.

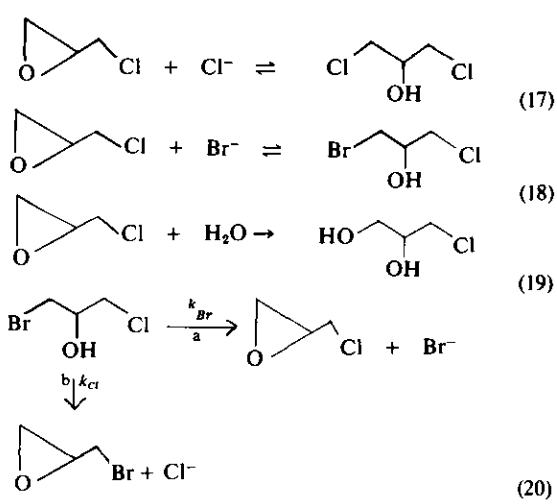
Epoxide Formation and Transhalogenation

In addition to the hydrolytic epoxide opening effected by these organisms, they are also capable of opening the oxirane with halide ion. The transhalogenation of epibromohydrin to epichlorohydrin [Eq. (1b)] was surprising.



(16)

Work with the partially purified enzymes (*8*) demonstrated the reaction was more complicated in that a series of reversible epoxide opening and forming reactions were occurring. The reactions of epichlorohydrin [Eqs. (17)–(20)] typify this complexity.



(17)

(18)

(19)

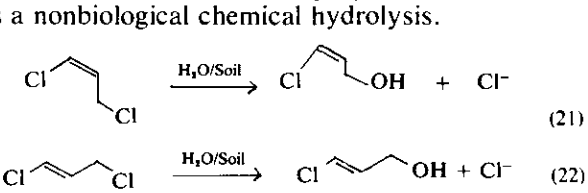
(20)

Both the epoxide opening and closing reactions are stereospecific trans processes.

Again, because one expects to encounter epoxide intermediates in studies of vinylic halides, these reactions take on added significance.

Cis- and *Trans*-1,3-Dichloropropene

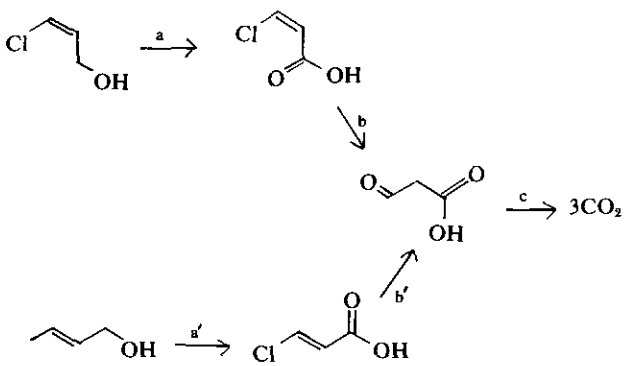
Of the potential substrates noted above as nematicides, only one is a vinylic halide. The first transformation of the dihalopropenes (*9*, *10*) in soil is a nonbiological chemical hydrolysis.



(21)

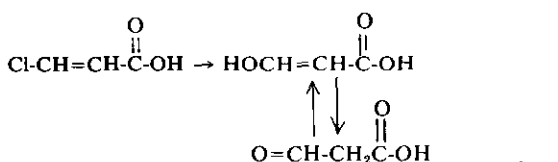
(22)

The rates here are only slightly faster than they are in aqueous buffer. On the other hand, the toxic chloroallyl alcohols resulting from the initial hydrolysis are nematicidal and they are readily metabolized to carbon dioxide water and chloride ion by resting cells of *Pseudomonas sp.* The sequence [Eq. (23)] is very similar to that observed for the metabolism of 3-bromopropanol by a different *Pseudomonas* (see above).



(23)

As with bromopropanol, the actual dehalogenation step [Eq. (24)] is a direct hydroxylation of the β-halo bond.



(24)

Propargylic acid is not an intermediate. The dehalogenation step is the one at which the stereochemistry is lost.

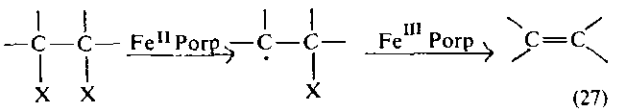
Oxidation of Hemeproteins by Alkyl Halides

Iron porphyrins are readily oxidized by a variety of alkyl halides at room temperature. The mechanism for the reductive dehalogenation or hydrogenolysis generally proceeds in two steps (*11, 12*), as shown in Eqs. (25) and (26).

$$RX + FE^{II}Porp \xrightarrow{k} R\cdot + XFe^{III}Porp \quad (25)$$

$$R\cdot + Fe^{II}Porp \xrightarrow{H+} RH + Fe^{III}Porp \quad (26)$$

The first step is rate-limiting and produces a free radical. The second step, the scavenging of the radical by the heme, is "exceedingly fast." These reactions have also been generally observed to occur with hemeproteins (*13, 14*). The reactivity encountered fits well with that expected by simple theory (*15*). If the radical generated in the first cleavage bears a neighboring halogen, the olefin is generated [Eq. (27)].



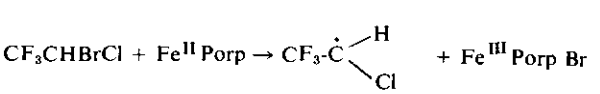
(27)

Examples here are (*1b*) given in Eqs. (28) and (29).

$$BrCH_2CH_2Br \rightarrow CH_2{=}CH_2 \quad (28)$$

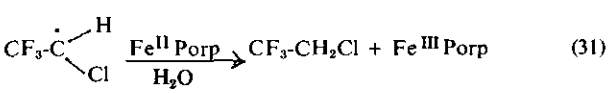
$$Cl_3CCCl_3 \rightarrow Cl_2C{=}CCl_2 \quad (29)$$

Considering the possible reactions of halothane with liver microsomes, a reductive metabolism with iron(II) cytochrome P-450 would certainly be reasonable and consistent with our results. Both Dr. Sipes and Dr. Brown have touched upon this, and the reductive path of intoxication suggested by Dr. Brown is particularly appealing. The first step in the reaction of halothane with the iron(II) porphyrin would be cleavage of the carbon bromine bond to generate a radical



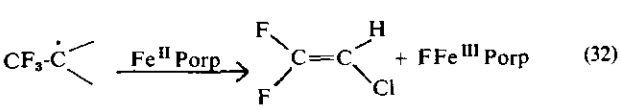
(30)

Three steps are now possible for the fate of the radical: hydrogenolysis to alkane [Eq. (31)],



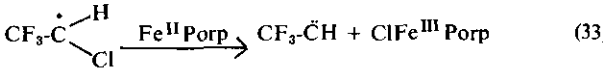
(31)

β-elimination to difluorochloroethane [Eq. (32)],



(32)

and α-elimination to trifluoromethylcarbene [Eq. (33)].



(33)

The carbene should rearrange to the olefin [Eq. (34)].

$$CF_3\text{-}\ddot{C}H \rightarrow CF_2{=}\ CHF \tag{34}$$

Thus, chlorotrifluoroethane, difluorochloroethylene, and trifluoroethylene are all possible initial metabolites. Of course, the free radical generated in the first step can of itself cause serious biological damage.

I wish to stop on this note and thank my colleagues, Eleanor Bartnicki and Nao Belser, for their patient and careful experimentation and their instruction. I also wish to pay tribute to the microorganism for their amazing capacity for organic synthesis with rather toxic substrates.

REFERENCES

1. Kallman, B. J., and Andrews, A. K. Reductive dechlorination of DDT to DDD by yeast. Science 141: 1050 (1963).
2. Bunyan, P. J., Page, M. M. J., and Taylor, A. *In vitro* metabolism of *p,p'*-DDT in pigeon liver. Nature 210: 1048 (1966).
3. Butler, T. C. Reduction of carbon tetrachloride *in vivo* and reduction of carbon tetrachloride and chloroform *in vitro* by tissues and tissue constituents. J. Pharmacol. Exptl. Therap. 134: 311 (1961).
4. Castro, C. E., and Belser, N. O. Biodehalogenation. Reductive dehalogenation of the biocides ethylene dibromide, 1,2-dibromo-3-chloropropane, and 2,3-dibromobutane in soil. Environ. Sci. Technol. 2: 779 (1968).
5. Castro, C. E., and Belser, N. O. Intoxication of *Aphelenchus avenae* by ethylene dibromide. Nematologica, in press.
6. Castro, C. E., and Bartnicki, E. W. Biological cleavage of carbonhalogen bonds. Metabolism of 3-bromopropanol by *Pseudomonas* sp. Biochim. Biophys. 100: 384 (1965).
7. Castro, C. E., and Bartnicki, E. W. Biodehalogenation. Epoxidation of halohydrins, epoxide opening, and transhalogenation by a *Flavobacterium sp.* Biochemistry 7: 3213 (1968).
8. Bartnicki, E. W., and Castro, C. E. Biodehalogenation. The pathway for transhalogenation and the stereochemistry of epoxide formation from halohydrins. Biochemistry 8: 4677 (1969).
9. Castro, C. E., and Belser, N. O. Hydrolysis of *cis-* and *trans*-1,3-dichloropropene in wet soil. J. Agr. Food Chem. 14: 69 (1966).
10. Belser, Nao, and Castro, C. E. Biodehalogenation—the metabolism of the nematocides *cis-* and *trans*-3-chloroallyl alcohol by a bacterium isolated from soil. J. Agr. Food Chem. 19: 23 (1971).
11. Wade, R. S., and Castro, C. E. The oxidation of iron(II) porphyrins by alkyl halides. J. Amer. Chem. Soc. 95: 226 (1973).
12. Wade, R. S., and Castro, C. E. The oxidation of hemeproteins by alkyl halides. J. Amer. Chem. Soc. 95: 231 (1973).
13. Castro, C. E., Robertson, C., and Davis, H. The reactions of octaethylporphyrin and its iron(II) and iron(III) complexes with free radicals. Bioorg. Chem. 3: 343 (1974).
14. Castro, C. E., and Bartnicki, E. W. Conformational isomerism and effective redox geometry in the oxidation of hemeproteins by alkyl halides. Cytochrome c and cytochrome oxidase. Biochemistry 14: 498 (1975).
15. Castro, C. E. Theory of hemeprotein reactivity. J. Theor. Biol. 33: 475 (1971).