APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1983, p. 366–374
0099-2240/83/020366-09$02.00/0

Vol. 45, No. 2

Haloperoxidases: Enzymatic Synthesis of α,β-Halohydrins from Gaseous Alkenes

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Received 6 July 1982/Accepted 24 September 1982

The enzymatic synthesis of α,β-halohydrins from gaseous alkenes is described. The enzymatic reaction required an alkene, a halide ion, dilute hydrogen peroxide, and a haloperoxidase enzyme. A wide range of gaseous alkenes were suitable for this reaction, including those containing isolated, conjugated, and cumulative carbon-carbon double bonds. Chlorohydrins, bromohydrins, and iodohydrins could be formed. The combining of this enzymatic synthesis with a previously described enzymatic synthesis of epoxides from α,β-halohydrins provides an alternate pathway, other than the well-known enzymatic direct epoxidation pathway, from alkene to an epoxide.

Reports of enzyme reactions on gaseous alkenes have focused primarily on the formation of epoxides from these substrates. Hou et al. (10) reported that a monooxygenase derived from several methylotrophic bacteria catalyzes the epoxidation of C_2 to C_4 *n*-alkenes, including butadiene. The monooxygenase derived from *Mycobacterium* sp. strain E20 by DeBont et al. (5) catalyzes all of the above reactions and includes the epoxidation of allene.

In the course of our studies on enzymatic reactions of industrially important alkenes, we discovered that a group of enzymes called haloperoxidases catalyze the formation of α,β-halohydrins from the gaseous alkenes. The enzymatic reaction occurs in a buffered, aqueous solution of alkene, halide ion (X^-), dilute hydrogen peroxide (H_2O_2), and biocatalyst (Fig. 1). Microbial sources of haloperoxidase include chloroperoxidase (CPO) from the fungus *Caldariomyces fumago* (14) and bromoperoxidase from over 50 algae (9).

MATERIALS AND METHODS

Biocatalysts. CPO (from *C. fumago*; 2 mg of protein per ml) and lactoperoxidase (LPO) (from milk; 5 mg of protein per ml) were purchased from Sigma Chemical Co. (St. Louis, Mo.). LPO is a bromoperoxidase.

Gaseous alkenes. Ethylene, propylene, butene-1, butene-2 (*cis* and *trans* mixture), isobutylene, butadiene, and allene were purchased from Matheson Gas Products (Lyndhurst, N.J.).

α,β-Halohydrin standards. 2-Chloroethanol, 2-bromoethanol, 2-iodoethanol, 1-chloro-2-propanol, and 1,4-dibromo-2,3-butanediol were purchased from Aldrich Chemical Co. (Milwaukee, Wis.). 1-Bromo-2-propanol (containing ~10% 2-bromo-1-propanol) and 1-bromo-3-buten-2-ol were purchased from Pfaltz and Bauer, Inc. (Stamford, Conn.). 2-Bromo-1-propanol was synthesized by the reduction of 2-bromopropionyl chloride (7). 1-Iodo-2-propanol was synthesized by the reaction of 1-bromo-2-propanol and iodide (15).

Enzymatic production of halohydrins. The enzymatic reaction mixtures were incubated in 100-ml Pyrex flasks equipped with a magnetic stir bar and stirrer. Each mixture contained 400 μl of haloperoxidase, 20 mM potassium halide, and 25 ml of 300 mM potassium phosphate buffer at various pH values. Because each haloperoxidase has specific requirements (13), the following conditions were used: CPO, pH 3.0 and Cl^-, Br^-, I^-; LPO, pH 6.0 and Br^-, I^-.

The gaseous alkenes were slowly (10 ml/min) and continuously bubbled through the reaction mixture during the reaction. Hydrogen peroxide was the last reagent added, 30 mM final concentration. Because hydrogen peroxide can oxidize certain critical sites on the enzyme molecule (e.g., sulfhydryl groups), thus damaging its activity, dilute levels of H_2O_2 must be used in the reaction mixture. After initiation, the reaction was allowed to proceed for 15 min. All reactions were run at room temperature and atmospheric pressure.

For each alkene tested, a control flask was included. This flask contained everything except haloperoxidase. Under the experimental conditions described, α,β-halohydrin product was not detected in the control flasks.

Reaction mixture analysis. Aliquots of reaction mixtures (10 μl) were injected into a Finnigan 4021 gas chromatograph-mass spectrometer equipped with a coiled, glass column (1.8 m by 4 mm) packed with Tenax-GC (80/100 mesh). The carrier gas was helium set at 25 ml/min. For rapid analysis of the many different reaction mixtures, the following temperatures were used: column temperature, programmed from 100 to 250°C at a rate of 10°C/min and then held at 250°C for 10 min; injector and jet separator temperatures, set at 260°C. The mass spectrometer was operated in the electron impact mode at 70 eV. The mass range from *m/z* 41 to 400 was scanned every 2 s. Confirmation of the identity of the reaction products

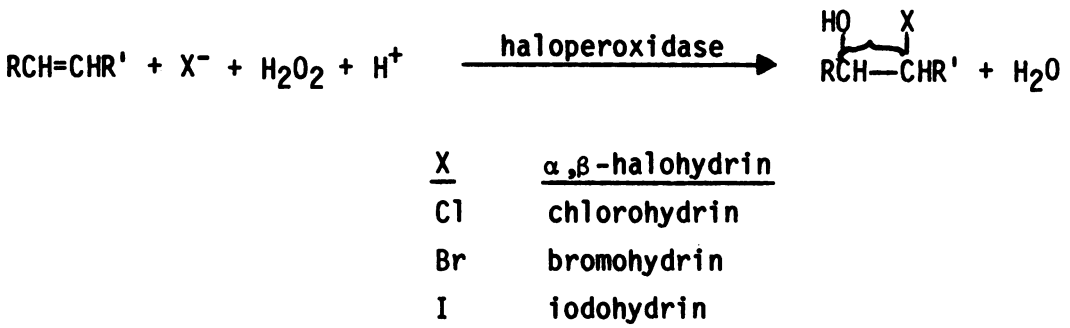


FIG. 1. General equation for haloperoxidase reaction on alkenes.

TABLE 1. CPO reaction on ethylene and propylene to yield α,β-halohydrins[a]

Reaction on:	X⁻	Product	GC_{rt} (min)[b]	Titer (μg/ml)
Ethylene	Cl	2-Chloroethanol	4.2	370
	Br	2-Bromoethanol	6.4	950
	I	2-Iodoethanol	10.7	230
Propylene	Cl	1-Chloro-2-propanol (a)	5.5	630
		2-Chloro-1-propanol (b)	6.0	60
	Br	1-Bromo-2-propanol (a)	8.1	1,910
		2-Bromo-1-propanol (b)	8.4	210
	I	1-Iodo-2-propanol (a)	10.3	420
		2-Iodo-1-propanol (b)	10.5	50

[a] Experimental conditions are described in the text. See Fig. 2.
[b] GC_{rt}, Retention time on gas chromatography.

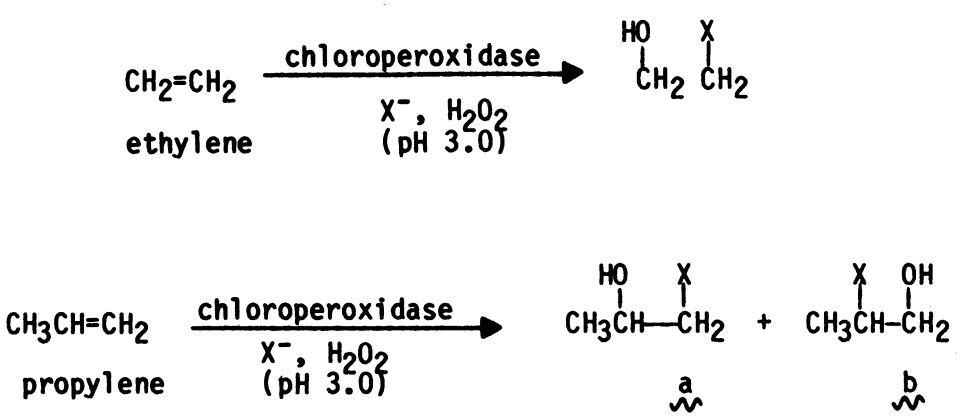


FIG. 2. CPO reaction on ethylene (top) and propylene (bottom) to yield α,β-halohydrins.

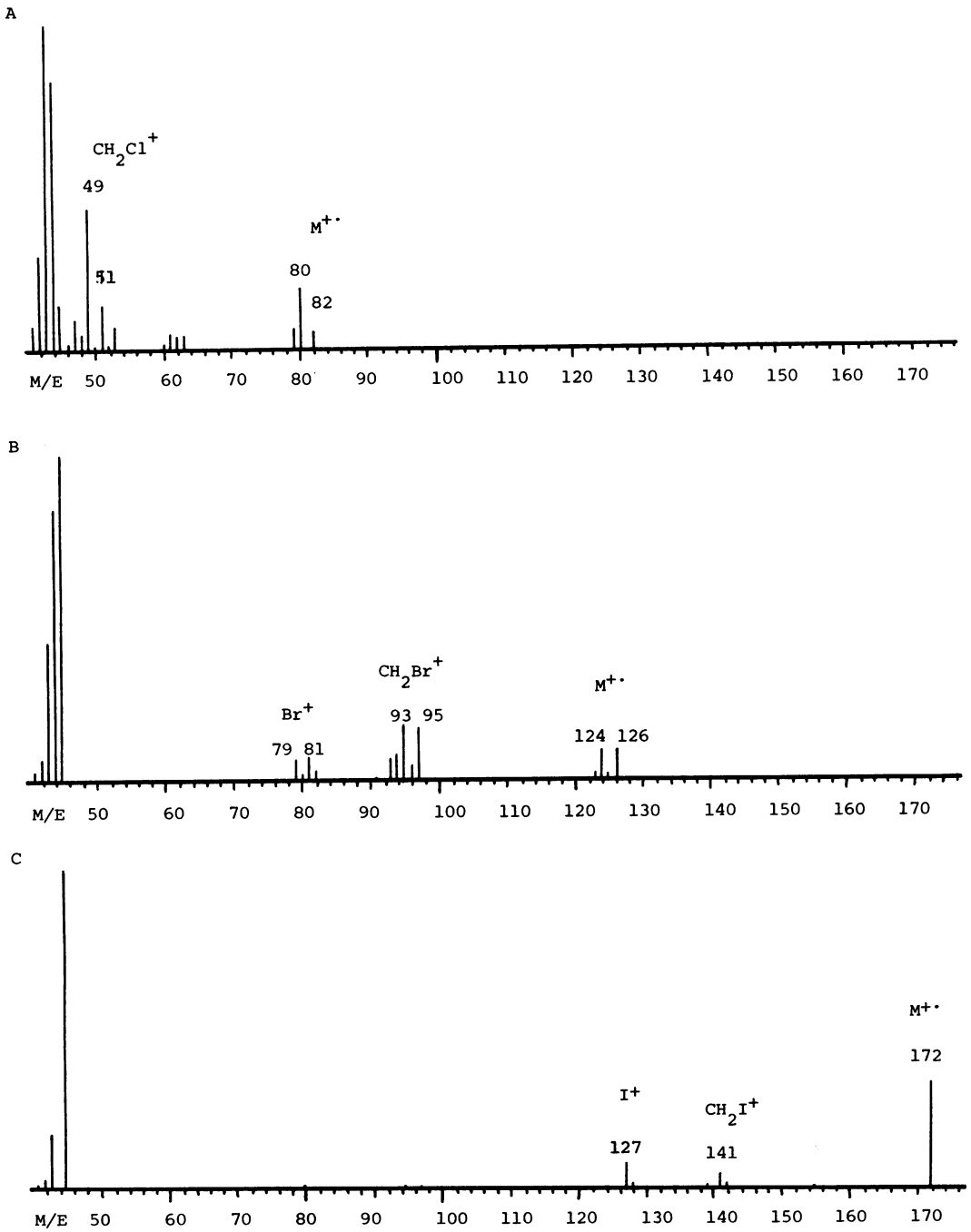


FIG. 3. Mass spectra of CPO-produced ethylene chlorohydrin (A), bromohydrin (B), and iodohydrin (C).

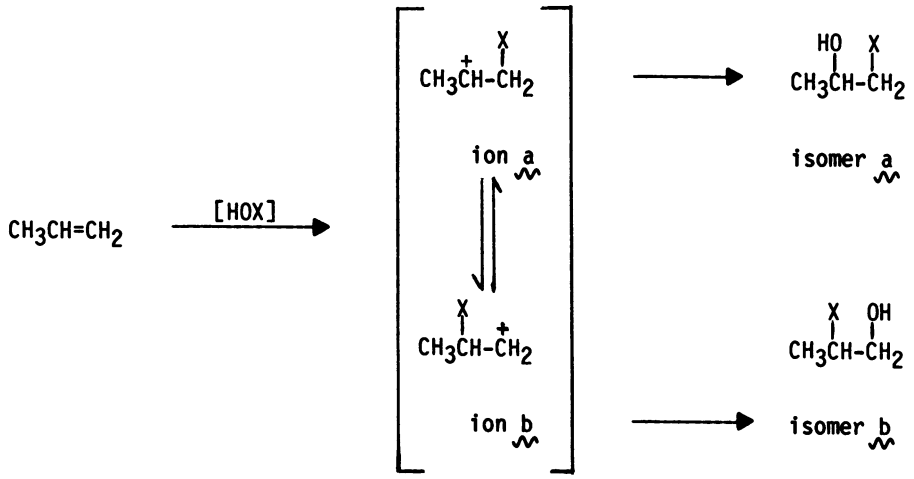


FIG. 4. Haloperoxidase reaction on propylene.

was made by gas chromatography retention time and mass spectral comparison with authentic standards whenever available. Not all reaction conditions were optimized nor was there complete conversion of substrate.

RESULTS AND DISCUSSION

A range of substrates are known to be halogenated by haloperoxidases, including β-keto acids, cyclic β-diketones, and phenols (14). However, the ability of haloperoxidases to yield α,β-halohydrins from carbon-carbon double bonds is not so well known. Previous studies with alkenes have included a few steroids and a vinyl phosphate (11, 14). In this report we describe the formation of α,β-halohydrins from ethylene, propylene, butene-1, butene-2 (*cis* and *trans*), isobutylene, butadiene, and allene. Since the formation of α,β-halohydrins from alkenes in the presence of hypohalous acid (HOX) is a well-known preparative and industrial procedure, the products formed in the enzymatic reactions were interpreted in terms of hypohalous acid addition across the carbon-carbon double bond.

Ethylene yielded a single α,β-halohydrin product with each halide ion used (Table 1, Fig. 2). Halogen on the product was confirmed by the isotopic abundances for chlorine (^{35}Cl-^{37}Cl, 3:1) and bromine (^{79}Br-^{81}Br, 1:1), whereas iodine was noted by the presence of the I^+ mass ion (*m/z* 127) in the mass spectra (Fig. 3). Detection of molecular ions in the mass spectra confirmed that halohydrin formation and not halogenation had indeed occurred.

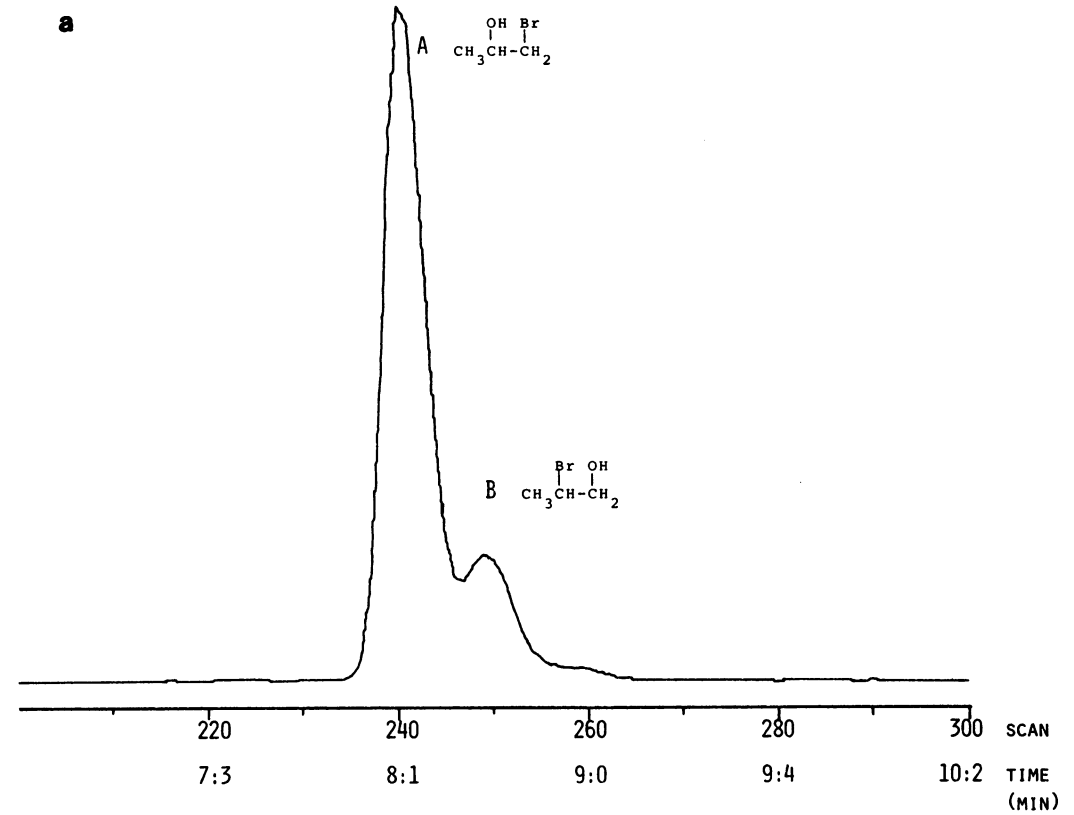
Propylene, being an asymmetric alkene, yielded two halohydrin positional isomers with each halide ion used (Table 1, Fig. 2). The ratio of positional isomers formed (~9:1, isomer **a**/isomer **b**) correlated with the stability of the postulated halonium ion intermediates (Fig. 4).

Ion **a** would be more stable than ion **b** due to the electron-donating ability of the methyl group. This ratio of positional isomers that was formed enzymatically matches the ratio that one would obtain by chemical addition of hypohalous acid to propylene (16). Although the gas chromatograph separation did not provide complete resolution of the positional isomers, this separation coupled with the diagnostic fragmentation of halohydrins in the mass spectrometer did permit unambiguous assignment of each positional isomer (Fig. 5 illustrates this for propylene bromohydrin).

Butadiene, being a conjugated alkene, could have yielded both 1,2- and 1,4-adduct products, due to the possible resonance of the intermediate allylic halonium ion (Fig. 6). However, only the 1,2-adducts were detected (Table 2, Fig. 7). Such exclusive formation of the 1,2-adduct has also been previously observed in the chemical addition of HOBr to butadiene (4).

The 1,2-adduct products could have further reacted to yield dihalohydrins (Fig. 8). However, under the conditions tested, only minor amounts of these dihalohydrins were produced.

Allene, being a cumulative alkene, could have yielded two halohydrin positional isomers (Fig. 9). However, none of the positional isomer that would have tautomerized to the α-haloketone was detected in the enzymatic reaction (Table 2, Fig. 7). Exclusive addition of the halogen on the central carbon and of the hydroxyl group on the terminal carbon has also been previously observed in the chemical addition of HOCl to



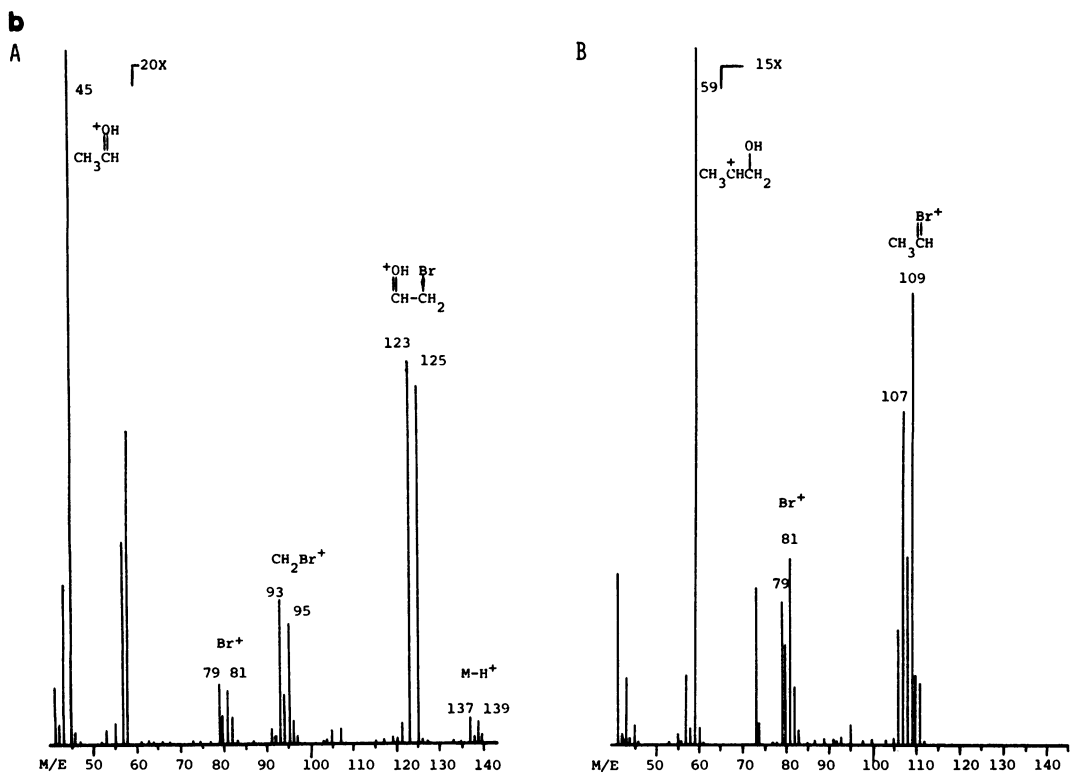


FIG. 5. Reconstructed ion chromatogram (a) and distinctive mass spectrum for each positional isomer of propylene bromohydrin (b). A, 1-Bromo-2-propanol; B, 2-bromo-1-propanol.

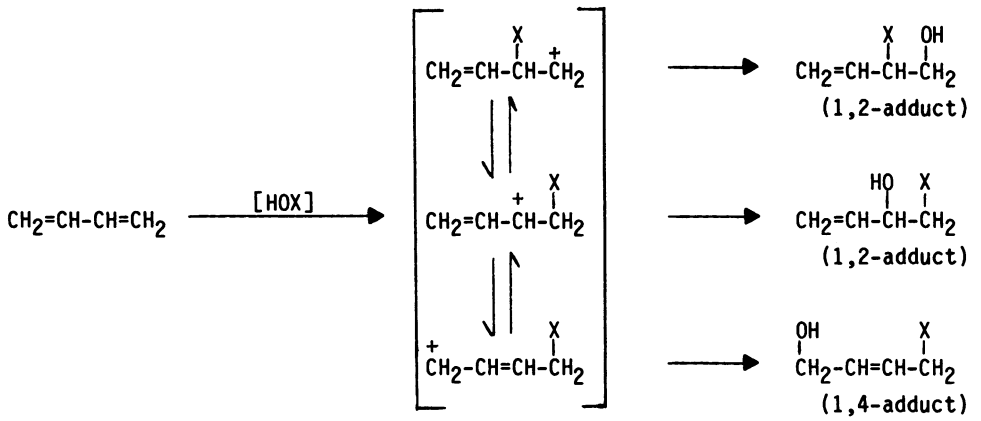


FIG. 6. Haloperoxidase reaction on 1,3-butadiene.

TABLE 2. Haloperoxidase reaction on butadiene and allene to yield α,β-bromohydrins[a]

Haloperoxidase reaction	Product	GC_{rt} (min)[b]	Titer (μg/ml)
LPO on butadiene	1-Bromo-3-buten-2-ol (a)	10.5	1,395
	2-Bromo-3-buten-1-ol (b)	11.2	160
	1,4-Dibromo-2,3-butanediol (c)	18.3	23
CPO on allene	2-Bromo-2-propen-1-ol (a)	8.1	420

[a] Experimental conditions are given in the text. See Fig. 7.
[b] GC_{rt}, Retention time on gas chromatography.

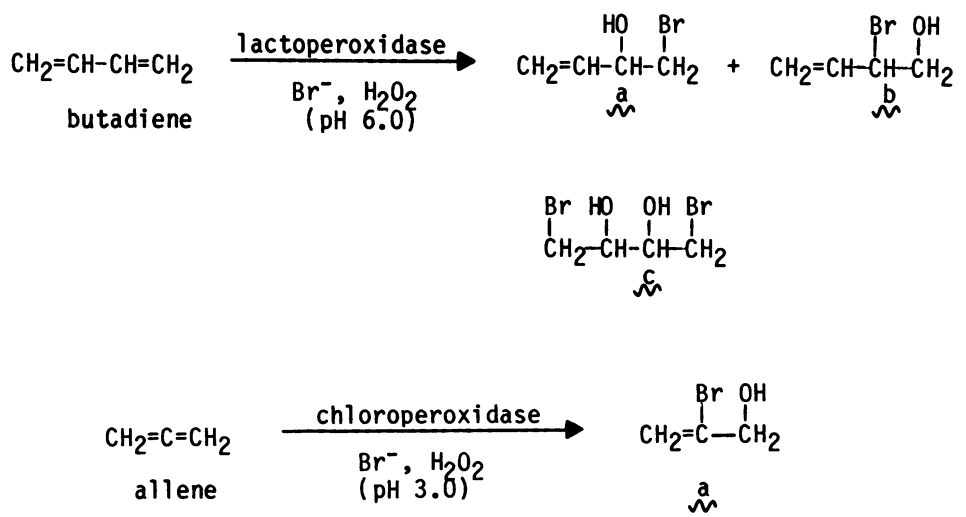


FIG. 7. LPO (top) and CPO (bottom) reactions on butadiene (top) and allene (bottom) to yield α,β-bromohydrins.

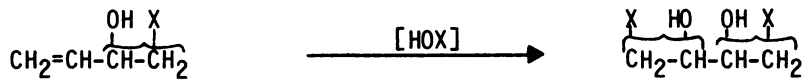


FIG. 8. Haloperoxidase conversion of 1,3-butadiene to monohalohydrins and dihalohydrins.

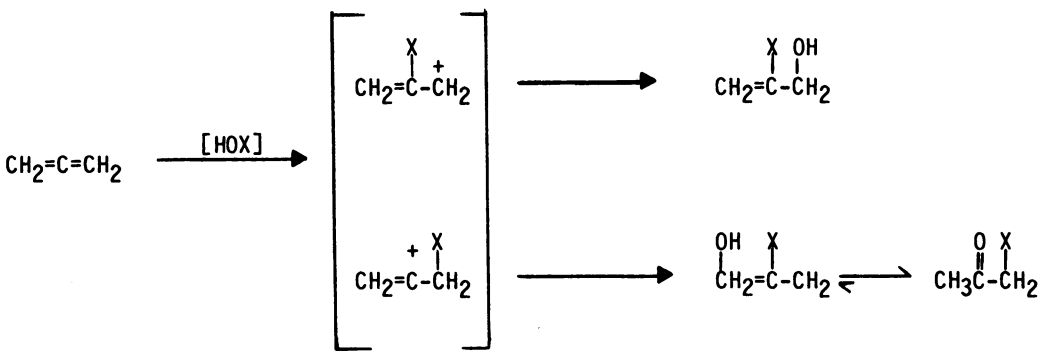


FIG. 9. Haloperoxidase reaction on allene.

allenic hydrocarbons (2). Under the conditions listed in Materials and Methods, formation of the dihalohydrin product was not observed (Fig. 10). The remaining gaseous alkenes also were shown to react with haloperoxidase to yield the expected halohydrin products (Fig. 11).

Therefore, although the mechanism of catalysis by the haloperoxidase enzymes is still a matter of discussion (8, 12), the products formed in these reactions are consistent with free hypohalous acid being generated by the enzyme. Regiospecific and stereospecific properties of the formed α,β-halohydrins were not investigated in the study. However, haloperoxidases are reported not to produce optically active halohydrin products (11).

Castro and Bartnicki (1, 3) observed an enzymatic route to epoxides that involved α,β-halohydrins. They isolated an organism, a *Flavobacterium* sp., that converted the chlorohydrin of allyl chloride and the bromohydrins of allyl alcohol, allyl bromide, propylene, and 2-butene to their respective epoxides. The enzyme responsible for this reaction was named halohydrin epoxidase.

The combining of halohydrin epoxidase with haloperoxidase provides an alternate pathway, other than the well-known direct epoxidation pathway, from an alkene to an epoxide (Fig. 12). Biological systems, especially those existing in an environment containing available halide salts (e.g., marine organisms), may use such an enzymatic strategy in various biosynthesis pathways. The numerous haloperoxidases, α,β-halohydrins, and epoxides found in such systems support this thought (6, 9).

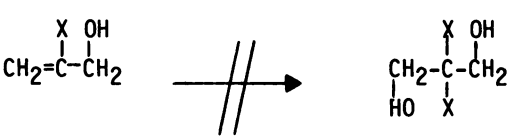


FIG. 10. Haloperoxidase does not convert allene to its dihalohydrin.

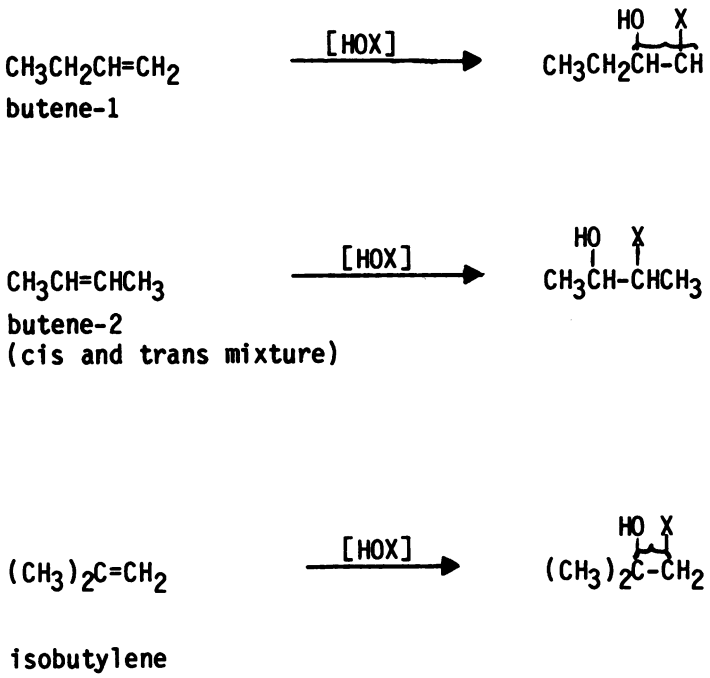


FIG. 11. Haloperoxidase reaction on butene-1, butene-2, and isobutylene.

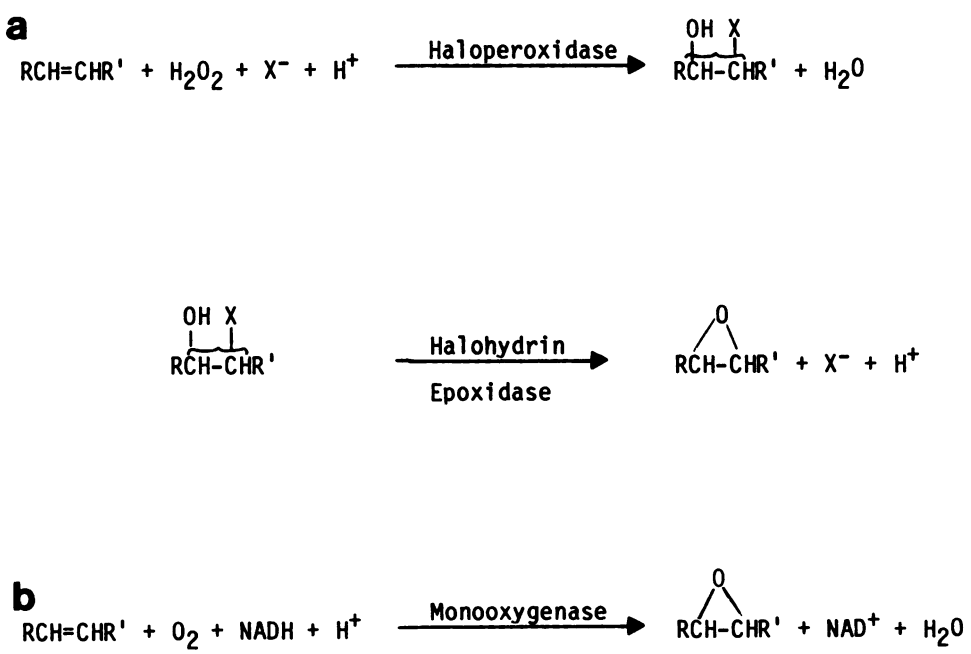


FIG. 12. Two enzymatic pathways to an epoxide from an alkene: (a) haloperoxidase coupled with halohydrin epoxidase and (b) direct epoxidation with a monooxygenase.

ACKNOWLEDGMENT

We thank M. Moreland for the synthesis of several α,β-halohydrin standards.

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