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Novel Haloperoxidase Reaction: Synthesis of Dihalogenated Products

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The enzymatic synthesis of vicinal, dihalogenated products from alkenes and alkynes is described. The enzymatic reaction required an alkene or alkyne, dilute hydrogen peroxide, a haloperoxidase, and molar amounts of halide ions. Vicinal dichloro, dibromo, and diiodo products could be formed. A hydroxyl group on the carbon adjacent to the carbon-carbon double or triple bond lowered the halide ion concentration needed to produce the dihalo product. This reaction offers one explanation for the origin of natural, vicinal, dihalogenated products, such as those found frequently in marine microogranisms.

Haloperoxidase enzymes have been found in various sources, including the fungus *Caldariomyces fumago* (9) and numerous marine algae (6). In the course of our studies on novel enzymatic reactions of haloperoxidases, we discovered that these enyzmes catalyze the formation of α,β-halohydrins from alkenes and the formation of α-halogenated ketones from alkynes (5, 5a) (Fig. 1).

These and earlier haloperoxidase reactions were run in halide concentrations below 100 mM (3, 7, 10). It was not obvious that haloperoxidases produce different halogenated products in the presence of higher concentrations of halide ions. We now report the formation of vicinal, dihalogenated products from alkenes and alkynes in haloperoxidase reactions run in high (≤ 2,000 mM) concentrations of halide ions.

MATERIALS AND METHODS

Haloperoxidases. Chloroperoxidase (CPO; from *C. fumago*; 10^7 U/ml, as measured by the monochlorodimedon assay) and lactoperoxidase (LPO; from milk; 500 U/ml, as measured by the pyrogallol assay) were purchased from Sigma Chemical Co., St. Louis, Mo. LPO is a bromoperoxidase.

Substrates and product standards. Propylene and methyl acetylene were purchased from Matheson Gas Products, Lyndhurst, N.J.

Allyl alcohol, allyl chloride, 1-bromo-2-propanol, 3-buten-1-ol, 3-butyn-1-ol, 1-chloro-2-propanol, 1,2-dibromopropane, 2,3-dibromo-1-propanol, 1,2-dichloropropane, 2,3-dichloro-1-propanol, 4-penten-1-ol, and propargyl alcohol were purchased from Aldrich Chemical Co., Milwaukee, Wis., and Pfaltz and Bauer, Inc., Stamford, Conn.

Enzymatic reactions. The reaction mixtures were incubated at room temperature in 100-ml Pyrex flasks equipped with a magnetic stir bar and stirrer. Each mixture contained 400 μl of haloperoxidase, potassium halide, and 10 ml of 300 mM potassium phosphate buffer at pH 3.0 (for CPO) or 6.0 (for LPO). The concentration of potassium halide in the reaction mixture was varied from 20 to 2,000 mM.

The gaseous substrates were slowly (10 ml/min) and continuously bubbled through the mixture during the reaction. For the liquid substrates, the concentration was 20 mM at the start of the reaction. Hydrogen peroxide was the last reagent added (final concentration, 30 mM).

After initiation, the reaction was allowed to proceed for 15 min. All reactions were run at room temperature and atmospheric pressure. Reactions were not necessarily run under optimized conditions, nor were reactions run until completion of substrate conversion. No products were detected in controls (without added enzyme).

Reaction mixture analysis. Aliquots (10 μl) of reaction mixtures 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, which was set at 25 ml/min. For rapid analysis of the many different reaction mixtures, the column temperature was programmed from 100°C at a rate of 10°C/min and then held at 250°C for 20 min, and the injector and jet separator temperatures were set at 260°C. The mass spectrometer was operated in electron impact mode at 70 eV. The mass range from *m/z* 40 to 400 was scanned every 2 s.

Confirmation of the identity of the reaction products was made by determining gas chromatograph retention time and comparing mass spectra with those of available authentic standards. Halohydrin positional isomers were not individually quantitated.

RESULTS AND DISCUSSION

Although the mechanism of haloperoxidase reaction is under debate (8, 11), free hypohalous acid generated by the enzyme has been implicated in reactions on alkenes and alkynes. If this is true, then the first step in the reaction would be

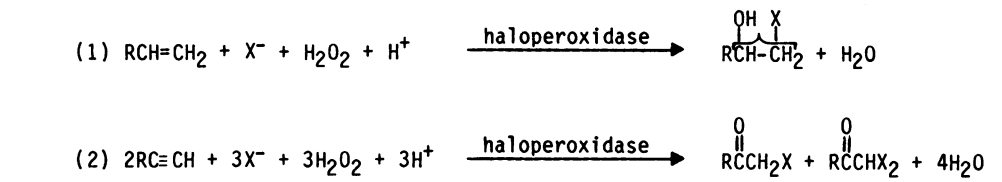


FIG. 1. Haloperoxidase formation of α,β-halohydrins from alkenes (1) and α-halogenated ketones from alkynes (2).

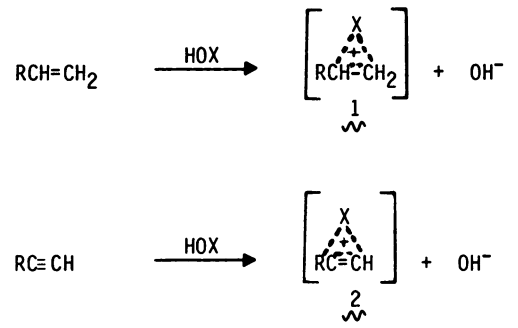


FIG. 2. Formation of halonium ion intermediates by addition of hypohalous acid (HOX) to alkenes and alkynes.

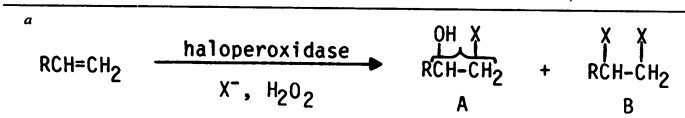
the formation of halonium ion intermediates (Fig. 2).

Under normal enzymatic reaction conditions, the nucleophile hydroxy ion (OH^-) reacts with the halonium ion intermediates to form α,β-halohydrins and α-haloketones (Fig. 1). However, other nucleophiles, placed in a competitive advantage, could also react with the halonium ion intermediates. One way of giving a halide ion (X^-) a competitive advantage is by flooding the reaction mixture with the nucleophile.

This idea was tested with a variety of alkenes (Table 1). The ratio of α,β-halohydrin product to vicinal, dihalogenated product was clearly a function of halide ion concentration in the reac-

TABLE 1. Yields of α,β-halohydrin product (A) and vicinal, dihalogenated product (B) formed by haloperoxidase reaction on alkenes, shown as a function of halide ion concentration in the reaction mixture[a]

Alkene substrate	Haloperoxidase	$[X^-]$ (mM)	Total product yield (μmol)	Approx B/A ratio
Propylene ($R = CH_3$)	CPO	Cl^-		
		20	82	<0.01
		2,000	31	1
	LPO	Br^-		
		20	103	<0.01
		200	88	0.07
		2,000	57	2
Allyl chloride ($R = CH_2Cl$)	LPO	Br^-		
		20	42	0.02
		2,000	11	3
Allyl alcohol ($R = CH_2OH$)	CPO	Cl^-		
		20	33	0.10
		2,000	82	8
		Br^-		
		20	37	0.13
		200	56	1
		2,000	94	15
		I^-		
		20	24	0.04
		2,000	54	4

[a] 

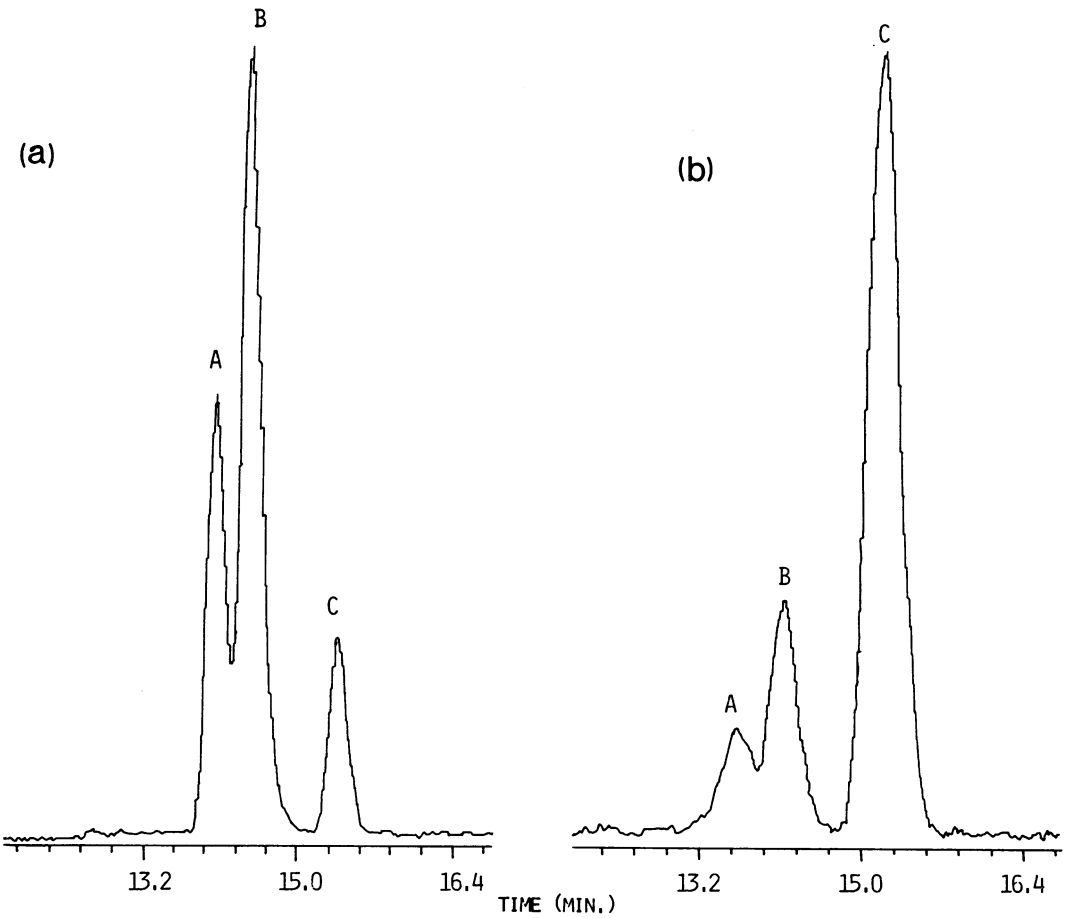


FIG. 3. Reconstructed ion chromatogram of the products formed in the enzymatic reaction of chloroperoxidase, allyl alcohol, bromide ions at 100 mM (a) and 1,000 mM (b), and H_2O_2.

tion mixture. The use of gas chromatography-mass spectrometry as the primary analytic tool permitted rapid and unambiguous assignment of the many products formed, as illustrated by the bromination of allyl alcohol by CPO (Fig. 3 through 6). Since the rate of product formation by haloperoxidase is a function of both substrate concentration and halide ion concentration (2), under high halide ion concentrations, some substrates yielded more product (e.g., allyl alcohol), and others yielded less (e.g., propylene).

A hydroxyl group on the carbon adjacent to the carbon-carbon double bond dramatically increased the proportion of vicinal, dihalogenated product formed (Table 1). This enhancement was lost as the hydroxyl group was moved further away from the double bond (Table 2).

A variety of alkynes were examined (Table 3), and the ratio of α-halogenated ketone product to vicinal, dihalogenated product was also found to be a function of halide ion concentration in the reaction mixture. The presence of a hydroxyl

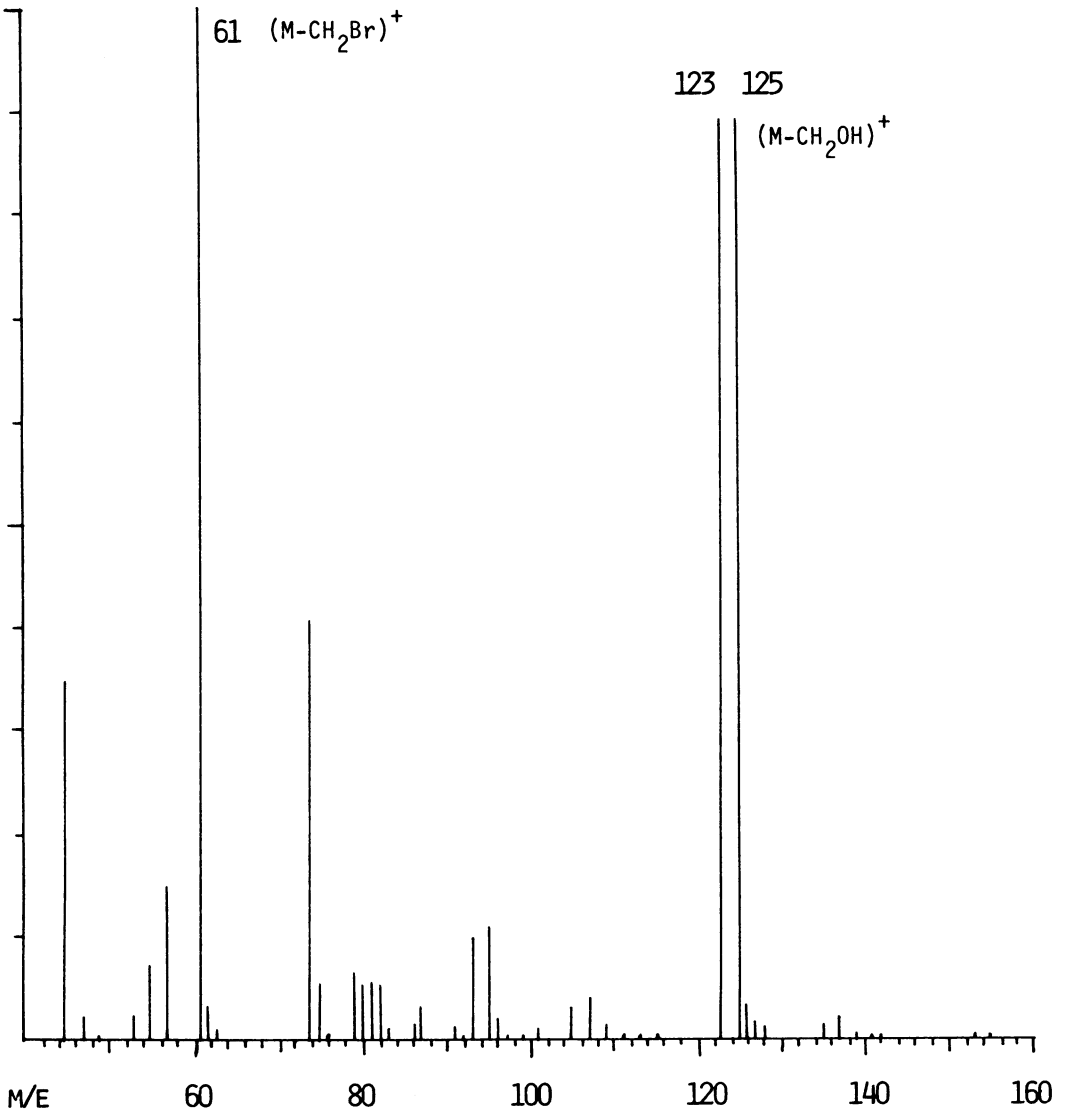


FIG. 4. Mass spectrum of product peak A formed in the enzymatic reaction of chloroperoxidase, allyl alcohol, H_2O_2, and bromide ions: 2-bromo-1,2-propanediol. Molecular weight of product, 154,156.

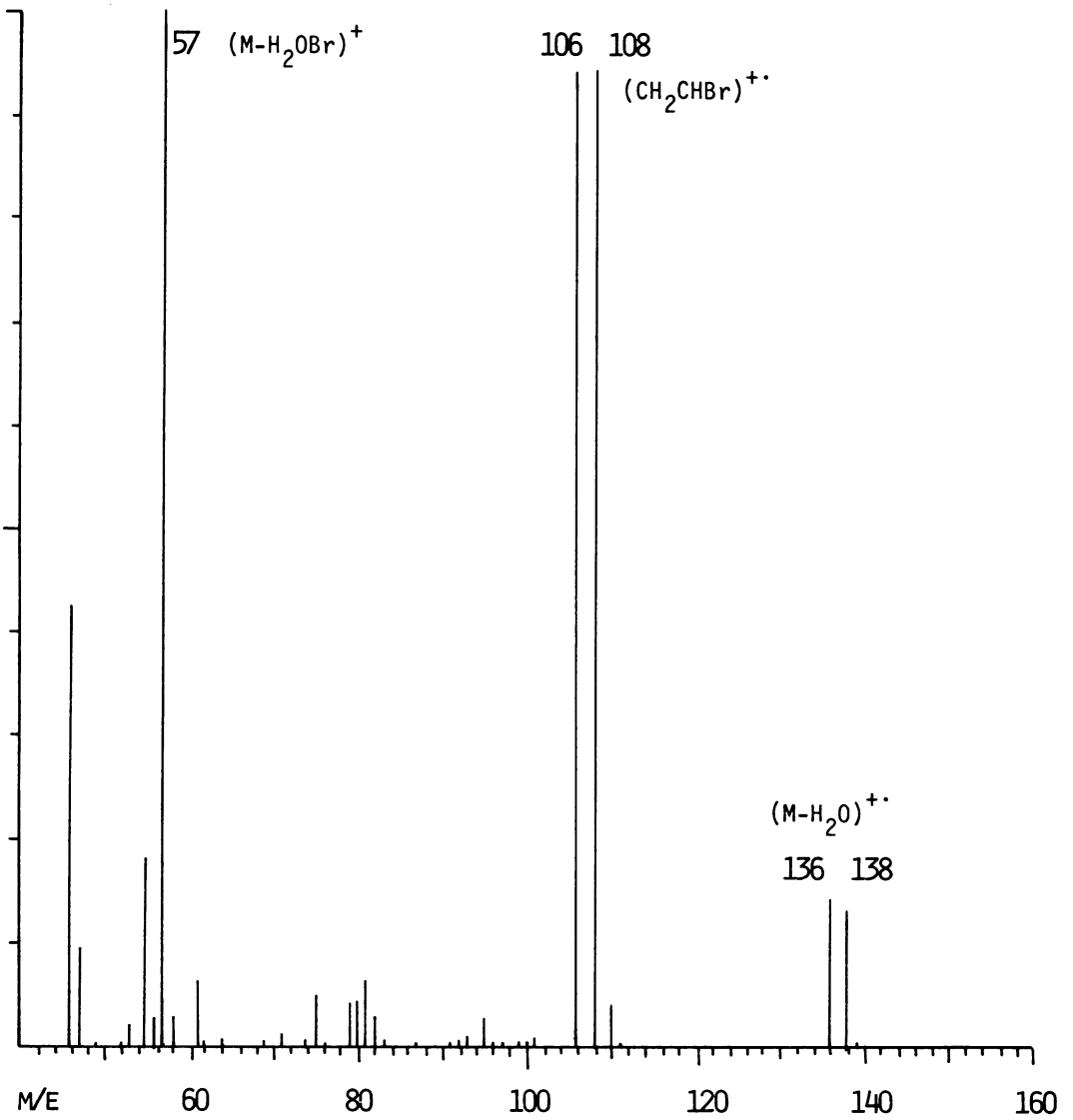


FIG. 5. Mass spectrum of product peak B formed in the enzymatic reaction of chloroperoxidase, allyl alcohol, H_2O_2, and bromide ions: 2-bromo-1,3-propanediol. Molecular weight of product, 154,156.

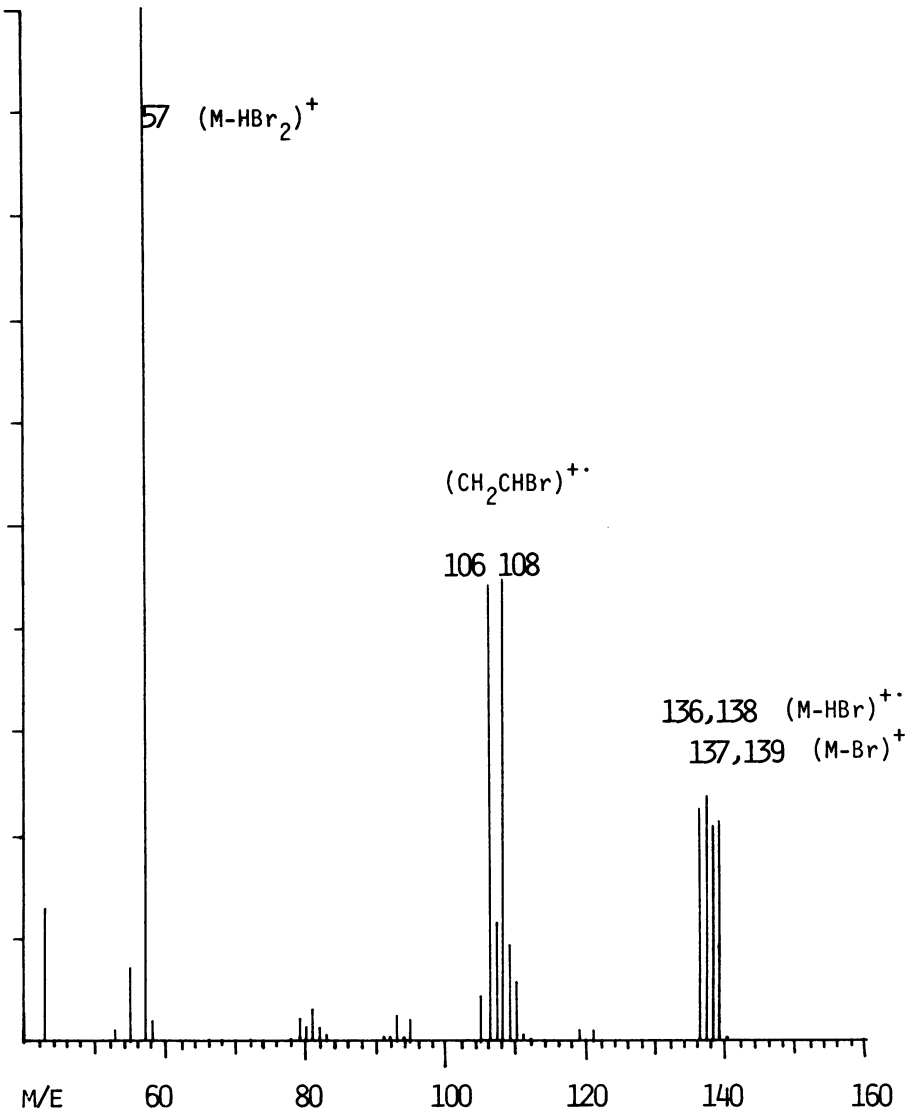


FIG. 6. Mass spectrum of product peak C formed in the enzymatic reaction of chloroperoxidase, allyl alcohol, H_2O_2, and bromide ions: 2,3-dibromo-1-propanol. Molecular weight of product, 216,218,220.

TABLE 2. Ratio of α,β-halohydrin product (A) to vicinal, dihalogenated product (B) formed by CPO reaction on alkenols, shown as a function of the position of the hydroxyl group relative to the double bond[a]

Alkenol substrate (n)	Approx B/A ratio
Allyl alcohol (0)	15
3-Buten-1-ol (1)	4
4-Penten-1-ol (2)	1

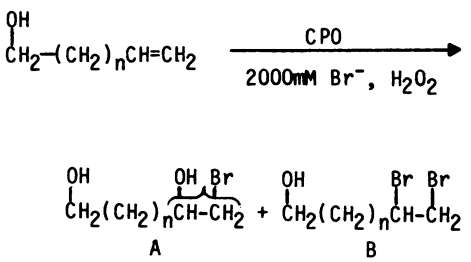
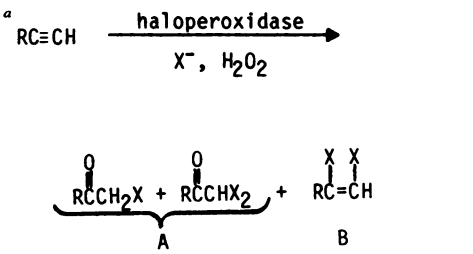
[a] 

TABLE 3. Ratio of α-halogenated ketone product (A) to vicinal, dihalogenated product (B) formed by haloperoxidase reaction on alkynes, shown as a function of halide ion concentration in the reaction mixture[a]

Alkyne substrate	Haloperoxidase	[X⁻] (mM)	Approx B/A ratio
Methyl acetylene (R = CH_3)	CPO	20	<0.01
		2,000	0.5
Propargyl alcohol (R = CH_2OH)	LPO	20	0.05
		2,000	9
3-Buten-1-ol (R = CH_2CH_2OH)	LPO	20	<0.01
		2,000	3

[a] 

group on the carbon adjacent to the triple bond again enhanced the proportion of vicinal, dihalogenated product formed.

Therefore, controlling the product in the haloperoxidase reaction by varying the halide ion concentration in the reaction mixture was demonstrated. These data on nucleophile competitiveness support the contention that halonium ion intermediates are involved in the reaction of haloperoxidase with alkenes and alkynes.

Vicinal, dihalogenated products have been found in many marine algae, and it has been postulated that halonium ion intermediates are involved in their biosynthesis (4). The reactions reported above support this. Haloperoxidases are found in marine algae (6), and localized high concentrations of various halide ions in algae can occur because of the ability of the algae to concentrate the ions from seawater (1). Seawater contains the necessary chloride ion concentration for production of dichlorinated metabolites: ~540 mM Cl^-.

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