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NEIGHBORING GROUP MIGRATION IN ENZYME-MEDIATED HALOHYDRIN FORMATION

Terry D. Lee, John Geigert*, Demetrios J. Dalietos and David S. Hirano
Cetus Corporation, 600 Bancroft Way, Berkeley, CA 94710

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Enzymatic halogenation of the double bond in allyl halides was influenced by intramolecular participation of the allylic halogen in the substrate molecule. Migration of the allylic halogen to the central carbon atom was observed in the enzymatic chlorination of allyl bromide, but not in the enzymatic bromination of allyl chloride. These results parallel the neighboring group effects observed for non-enzymatic halogenation of allyl halides.

Electrophilic addition to a double bond can be strongly influenced by intramolecular participation of other functionalities in the substrate molecule. Such stereodirecting effects are observed in the reaction of hypohalous acids with allyl halides (1, 2, 3). The reaction of hypochlorous acid (HOCl) with allyl bromide (**1**) yields three halohydrin positional isomers (**3**, **4**, and **5**), as shown in Scheme 1. This result is readily understood in terms of an initial chloronium ion intermediate I which can be displaced by the neighboring bromine to give bromonium ion intermediate II. Nucleophilic attack by H_2O takes place at each of the halonium ion carbon atoms of either intermediate to yield the three isomers. However, if allyl chloride (**2**) is reacted with hypobromous acid (HOBr), little or none of the halohydrin isomer (**3**) resulting from the migration of chlorine to the central atom is detected, as shown in Scheme 1. The neighboring chlorine is unable to compete with the initial bromonium ion intermediate and the isolated halohydrin isomers (**4** and **5**) are derived solely from intermediate II.

Previously, we demonstrated that the enzyme haloperoxidase catalyses the formation of halohydrins from alkenes in the presence of halide ion and hydrogen peroxide (4). We believe that the enzymatic reaction proceeds via enzyme generated hypohalous acid which then reacts chemically with the alkene substrate. With this proposed mechanism, the enzymatic halogenation of allyl halides should exhibit the same neighboring group effects as that observed for non-enzymatic halogenation. However, if the enzymatic reaction proceeds via an enzyme-bound electrophilic halogenating species as proposed by others (5), a major

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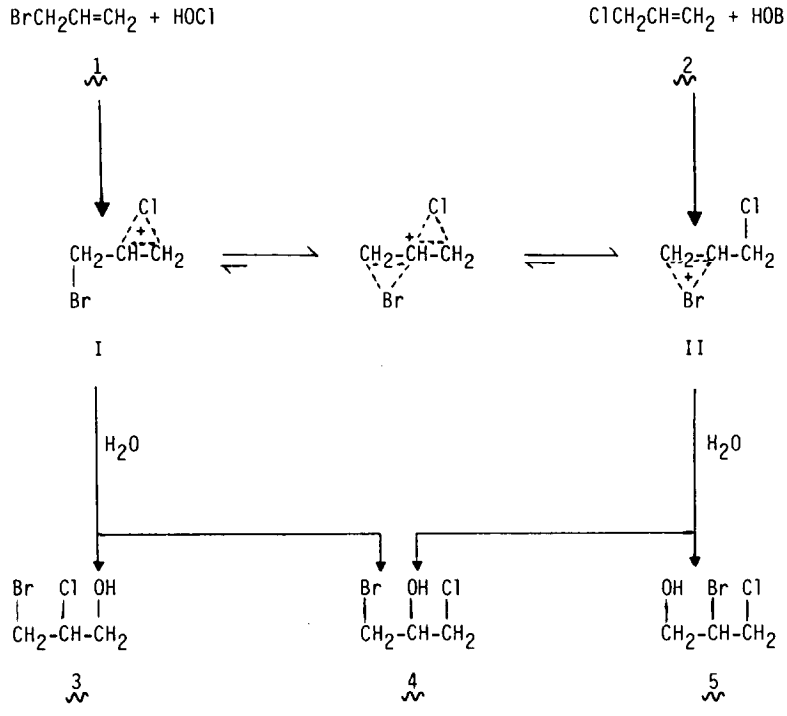

Scheme 1: Rearrangement Accompanying Addition of Hypohalous Acid to Allyl Halides

difference between enzymatic and non-enzymatic halogenation of allyl halides should be observed.

MATERIALS AND METHODS

Enzymatic Reactions. The halohydrin positional isomers were formed by incubating 200 µl of chloroperoxidase (Sigma Chemical Company; from Caldariomyces fumago; CPO; 2 mg protein/ml), potassium chloride or bromide (20 mM), allyl bromide or chloride (5 mM) and dilute hydrogen peroxide (30 mM) in 10 ml of 0.3 M potassium phosphate buffer (pH 3.0), for 15 min. at 25, C.

Assay. Product mixtures were isolated by extraction of the aqueous reaction mixture with diethyl ether. Product identification and ratios were determined by fused-silica capillary (SE-54, 30 m) gas chromatography-mass spectrometry (GCMS). The gas chromatographic technique, described previously (6) was temperature programmed from 60° to 200°C at 10°C/min. The capillary column was pushed through the mass spectrometer transfer line into the ion source to minimize resolution loss. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV, with a mass range from m/z 40 to 400 scanned every 2 seconds.

The distinct elution time and mass spectrum of each isomer permitted unambiguous product assignment. 3-Bromo-2-chloro-1-propanol (3): GC retention time of 5.42 min.; molecular mass ion at mass 172, 174 and 176 (3:4:1 in intensity) and base ion at mass 62 and 64 (3:1 in intensity; loss of CH_2BrOH from molecular ion). 1-Bromo-3-chloro-2-propanol (4): GC retention time of 4.26 min.; molecular mass ion at mass 172, 174 and 176 (3:4:1 in intensity) and base ion at mass 123 and 125 (1:1 in intensity; loss of CH_2Cl from molecular ion). 2-Bromo-3-chloro-1-propanol (5): GC retention time of 5.20 min.; molecular mass ion at mass 172, 174 and 176 (3:4:1 in intensity) and base ion at mass 106 and 108 (1:1 in intensity; loss of CH_2ClOH from molecular ion).

RESULTS AND DISCUSSION

Table 1 presents the results from enzymatic chlorination of allyl bromide and enzymatic bromination of allyl chloride using chloroperoxidase. The neighboring group participation as evidenced by the extent of migration of the original halogen to the central carbon (26% for allyl bromide, 0% for allyl chloride) is in good agreeement with results reported for non-enzymatic halohydrin formation.

The volatilization of radioactively-labelled halide salt (7), the absence of optically active halohydrin products from alkenes (8), and now neighboring group migration in enzyme-mediated halohydrin formation, all point to haloperoxidase generation of hypohalous acid as the mechanism for enzymatic halogenation.

Table 1: Halohydrin Positional Isomers Produced by Chlorination of Allyl Bromide and Bromination of Allyl Chloride

Reaction	Product Distribution (%) 3	4	5
$BrCH_2CH=CH_2$			
+ HOCl[a]	40	32	28
+ CPO/KCl/H_2O_2	44	30	26
$ClCH_2CH=CH_2$			
+ HOBr[a]	1	26	73
+ CPO/KBr/H_2O_2	0	20	80

[a] (De La Mare et al., 1962)

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