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Enantioselective Epoxidation of Styrene Derivatives by Chloroperoxidase Catalysis

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ABSTRACT. Chloroperoxidase catalysed epoxidation of styrene derivatives by *t*-BuOOH preferentially gives (R) oxides with ee values between 28 and 68%. The data support the view of oxygen delivery from the ferryl oxygen directly to the substrate.

INTRODUCTION

Asymmetric epoxidation is of fundamental importance in biological processes such as the metabolism of xenobiotics having aromatic or olefinic double bonds and the cyclisation of squalene to triterpenoids and steroids. However, in contrast with chemical reagents such as chiral (salen) manganese complex derivatives[1] or chirally modified metalloporphyrins,[2] heme enzymes such as cytochrome c peroxidase,[3] afford epoxides with very low ee, whereas horseradish peroxidase is unable to catalyze the epoxidation of styrene.[4] Substantially higher enantioselectivities are observed in the oxidation of styrene, and *cis* and *trans*-ß-methyl styrene to the corresponding oxides[5] with cytochrome $P\text{-}450_{cam}$. Even simple aliphatic alkenes are epoxidized with medium ee by this enzyme .[6] Polycyclic aromatic epoxides are also produced with high enantioselectivity by cytochrome P-450 isozymes.[7] Just one case has been reported of stereoselective epoxidation of a simple, unfunctionalized alkene catalyzed by the fungal enzyme chloroperoxidase (E.C. 1.11.1.10 chloride/hydrogen peroxide oxidoreductase) from *Caldariomyces fumago*, namely, the epoxidation of *trans*-[1-^{2}H] styrene, that proceeds without detectable loss of stereochemistry.[4] Chloroperoxidase, like cytochrome c peroxidase is an anomalous peroxidase in that it catalyzes P-450 like monooxygenation reactions. We wanted to ascertain if this enzyme is able to transfer enantioselectively an oxygen atom to the double bond and to investigate the steric course of the reaction.

RESULTS AND DISCUSSION

Preliminary results with *p*-chlorostyrene at pH 6 in a phosphate buffer solution at 25° C, indicated that *t*-BuOOH is the oxidant of choice with respect to H_2O_2 in view of the higher chemical yield obtained, whereas the ee were practically the same (66-67%) with both oxidizing agents. The time course of *p*-chlorostyrene oxidation by *t*-BuOOH and H_2O_2 is shown in Table I.

Besides epoxide, the corresponding arylacetoaldehyde and diol were concomitantly produced. Without the enzyme, the olefin substrate was not oxygenated by the oxidants alone under identical reaction conditions.

Table I. Time course of chloroperoxidase catalysed oxidation of *p*-chlorostyrene as a function of the nature of the oxidant.[a]

Time (min)	Olefin(%)		Epoxide(%)		Aldehyde(%)		Diol (%)	
	t-BuOOH	H_2O_2	*t*-BuOOH	H_2O_2	*t*-BuOOH	H_2O_2	*t*-BuOOH	H_2O_2
0	100	100	0	0	0	0	0	0
10	98	98	2	1	-	-	-	-
30	80	95	10	2	5	-	-	-
60	63	91	21	3	8	-	-	-
120	50	89	28	4	10	1	-	-
240	42	70	33	9	12	3	2	1
360	38	53	35	11	12	4	6	2
960	34	-	36	-	13	-	8	-

[a] *p*-Chlorostyrene (7.2 μmol) was added to 1 ml of 0.05 M potassium phosphate buffer, pH 6, containing the oxidant (15 μmol) and chloroperoxidase (29 units). The mixture was shaken for the scheduled time, at 25°C, diluted with acetone (2 ml) and analyzed by chiral GLC.

In order to verify the generality of the reaction, we investigated the *t*-BuOOH oxidation of other substituted styrenes. Chemical yield, absolute configuration and ee of the obtained epoxides are reported in Table II. The data show that in the case of chloroderivatives the *para* and *meta* - substitution led to higher chemical yield than the *ortho*-substitution, without substantially affecting the enantioselectivity. This behaviour is in contrast with the results observed by us in the chloroperoxidase catalyzed sulfoxidation reaction, where *p*-substituted phenyl alkyl sulfides were oxidized in much higher enantioselectivity than *o*-substituted phenyl alkyl sulfides.[8] On the other hand, the epoxidation of *p*-nitrostyrene occured in poor chemical and optical yield. The ee of the epoxides did not change for the entire reaction period. The enzymatic asymmetric synthesis was not accompanied by kinetic resolution of the product since racemic *p*-chlorostyrene oxide was recovered unchanged under the usual reaction conditions.

Table II. Chemical yield, ee and absolute configuration of the epoxides obtained by chloroperoxidase catalysed oxidation of some arylalkenes by *t*-BuOOH.[a]

Substrate	Yield(%)	Ee(%)	Absolute configuration
Styrene	23	49	R
p-Chlorostyrene	35	66	R
m-Chlorostyrene	34	62	R
o-Chlorostyrene	3	64	(R)[b]
p-Bromostyrene	30	68	R
p-Nitrostyrene	5	28	(R)[b]

[a] For conditions see the Experimental section. [b] On the basis of the elution order on GLC.

The absolute configurations of the prevailing enantiomers of styrene epoxide (R) and diol (S) were determined by comparison with the authentic samples (Aldrich). In the case of *p*-chloro, *m*-chloro and *p*-bromostyrene epoxides the absolute configurations were assigned by comparison of their circular dichroism and ^1H NMR spectra, in the presence of $Eu(hfc)_3$ chiral shift reagent, with those of (R) styrene oxide. In the case of *o*-chloro and *p*-nitrostyrene epoxides the absolute configurations were deduced by the elution order on chiral GLC, the (R) enantiomer being eluted before the (S) enantiomer for all the epoxides reported in Table II. The (R) absolute configuration indicates that in all cases the approach by the oxidant to the *re* face of the double bond is the preferred one. The (S) configuration of 1-phenyl-1,2-ethanediol is a consequence of a S_N2 nucleophilic attack of H_2O on the benzylic carbon of (R) styrene oxide formed in the course of the enzymatic reaction, without appreciable racemisation (ee of diol, 48%). This stereochemical course was confirmed by performing the hydrolysis of a specimen of the (R) oxide under the usual reaction conditions in the absence of the enzyme.

Phenyl acetaldehydes produced as coproducts do not derive from the decomposition of the corresponding styrene oxides, as shown by Ortiz de Montellano [4] in the case of *trans*[1-^2H] styrene. These aldehydes probably derive from an hydrogen migration within an intermediate formed by reaction of the olefin with the oxygen-iron complex. This is the case also in the reaction catalysed by cytochrome P-450[9] or by chiral iron porphyrins.[10]

The influence of the addition of water-miscible cosolvents on the stereoselectivity of the oxidation of *p*-chloro styrene with *t*-BuOOH was also examined. Dioxane and acetone decreased both the optical and the chemical yield of the epoxide; for instance, at 10% concentration (v/v) ee values were 48% and 44%, and the rates were 30% and 40% lower than in buffer alone. On the other hand, acetonitrile only slightly affected the enantioselectiviy but, as a function of its

concentration, modified the products ratio by favouring the formation of *p*-chlorophenyl acetaldehyde and diol at the expenses of the epoxide (Table III).

Table III. Influence of acetonitrile concentration (v/v) on chloroperoxidase catalyzed oxidation of *p*-chlorostyrene by *t*-BuOOH.[a]

Time (min)	Conditions	Concentration(%)				Ee(%)
		Olefin	Epoxide	Aldehyde	Diol	Epoxide
0		100	0	0	0	
30	Buffer alone	82	11	6	-	66
	10% Acetonitrile	76	13	7	-	67
	20% Acetonitrile	71	10	11	-	60
	30% Acetonitrile	70	10	15	-	62
60	Buffer alone	66	22	8	-	67
	10% Acetonitrile	58	25	9	-	66
	20% Acetonitrile	56	12	16	-	58
	30% Acetonitrile	50	11	18	-	57
120	Buffer alone	46	30	11	-	66
	10% Acetonitrile	40	32	13	-	66
	20% Acetonitrile	38	15	18	-	59
	30% Acetonitrile	35	12	22	-	56
360	Buffer alone	35	38	13	7	65
	10% Acetonitrile	31	39	12	16	66
	20% Acetonitrile	30	18	23	20	58
	30% Acetonitrile	28	13	25	23	53

[a] For conditions see the legend to Table I.

The use of magnesium monoperoxyphtalate, an efficient oxygen donor for manganese porphyrin catalyzed epoxidation of alkenes,[11] gave unsatisfactory results. Indeed, starting from *p*-chlorostyrene the corresponding epoxide was obtained in 30% chemical yield and 30% ee, together with *p*-chlorophenyl acetaldehyde (10%), for a 300 min reaction time.

It is assumed that the mechanism of the epoxidation of olefin by chloroperoxidase[4] and cytochrome c peroxidase[3] with H_2O_2 involves a ferryl oxygen transfer from compound I. The substantial enantioselectivity observed by us for the first time in the chloroperoxidase oxidation of styrene derivatives with *t*-BuOOH (and H_2O_2) supports the view of oxygen delivery from the ferryl oxygen directly to the substrate. This also indicates that with the former enzyme the ferryl oxygen is more accessible to the olefins than with the latter one which gives low enantioselectivity.[3] As a matter of fact, in spite of the different steric and electronic requirements of the sulfoxidation and epoxidation reactions, chloroperoxidase affords almost enantiomerically pure sulfoxides[8] also in the oxidation of sulfides, whereas cytochrome c peroxidase leads to nearly racemic products.[4] Finally, it is worth mentioning that an iron-oxo intermediate has been postulated by Groves in the porphyrin-catalysed epoxidation of styrene[10] that leads preferentially, as in our case, to the (R) enantiomer of styrene oxide.

EXPERIMENTAL SECTION

Materials. Styrene, *o*-chlorostyrene, *m*-chlorostyrene, *p*-chlorostyrene and *p*-bromostyrene were commercial products from Aldrich. *p*-Nitrostyrene was prepared according to the literature.[12]

Styrene, *o*, *m*, and *p*-chlorostyrene, *p*-bromostyrene and *p*-nitrostyrene epoxides are known in the optically active form.[2,10,13] Chloroperoxidase from *Caldariomyces fumago* [RZ 0.6] was obtained from Sigma.

General Methods. The optical rotations were determined with a Perkin Elmer R241 polarimeter. The ^1H NMR spectra of the products were recorded in $CDCl_3$ on a Varian 390 instrument. The CD spectra were recorded on a Jasco J-500 dichrograph.

Enzymatic Oxidations: typical procedure. The alkene (1 mmol) and CPO (1000 units) were magnetically stirred in 140 mL of 0.05 M phosphate buffer, pH 6, at 25°C. The oxidant (2 mmol) in 7.5 mL of buffer solution pH 6, was added in 150 min in 30 aliquots at 5 min intervals and the reaction was carried out for 24 h. Extraction with 2 portions (200 mL each) of diethyl ether, followed by drying and evaporation of the organic solvent, gave the crude product, that was purified by column chromatography on florisil with mixtures of diethyl ether and light petroleum.

Determination of degree of conversion and enantiomeric excess. Both the degree of conversion of substrates into epoxides, aldehydes and diols, and the ee values of epoxides and diols were determined, in one run, by chiral GLC with a CP-Cyclodextrin-ß-2,3,6-M19 column (50 m, 0.25 mm ID, Chrompack) under the following conditions: oven temperature 110°C (initial time 20 min) to 180°C with a heating rate of 1°C/min; H_2 as carrier gas. Epoxide and diol enantiomers were base line separated.

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