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Chiral synthons via chloroperoxidase catalysis

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Abstract

Recent experiments from several laboratories have shown that chloroperoxidase is able to catalyze a broad spectrum of stereoselective hydroxylation, sulfoxidation and epoxidation reactions. Our laboratory has investigated the substrate specificity of chloroperoxidase for chiral epoxidation of mono- and disubstituted alkenes. Chloroperoxidase is able to efficiently utilize alkenes having chain lengths of nine or fewer carbon atoms except for monosubstituted olefins which often function as reversible suicide inhibitors of the enzyme. Excellent substrates are created by *cis*-1-methyl and 2-methyl substitutions on the olefinic double bond. Kinetic, enantioselectivity and epoxide yield data have been obtained on a series of brominated and unbrominated methallyl alkenes and substituted styrenes. The results indicate that methallyl alkenes and styrenes can function as good substrates. K_m values for the methallyl substrates are in the millimolar range and the V_{max}'s reach turnovers of 200 per min. Hammett plot data are consistent with the formation of a radical as opposed to a carbocation intermediate in the rate determining step in the epoxidation of substituted styrenes. Many of the epoxide products derived from chloroperoxidase catalysis can serve as chiral synthons for drug and natural product synthesis.

Keywords: Chloroperoxidase; Asymmetric epoxidation; Chiral epoxides

1. Introduction

Since the first description of chloroperoxidase (CPO, from *Caldariomyces fumago*) 32 years ago [1], considerable energy has been spent elucidating the enzyme's structure and functions. Structural investigations of the native enzyme have recently culminated in an X-ray crystal determination [2]. As predicted from spectral evidence, the enzyme possesses an active site pocket consisting of a cysteine thiolate-bound heme. The distal side of this heme is substantially polar in nature and allows access to the Fe^{3+} center by oxidants and substrates. Hydrogen peroxide, acyl peroxides, and alkyl peroxides rapidly oxidize the Fe^{3+} ion to Fe^{5+}, or more exactly a porphyrin cation radical-ligated Fe^{4+} species known as compound I, thus setting the stage for substrate oxidation.

2. Results and discussion

Halide ion (with the exception of F^-) is readily oxidized by CPO compound I and will result in the halogenation of suitable substrates [3–8]. The natural product caldariomycin is biosynthesized in this way. To date, however, this

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Table 1
The asymmetric epoxidation reactions catalyzed by chloroperoxidase [24]

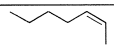
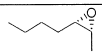
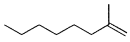
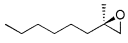
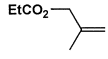
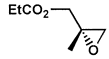
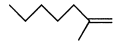
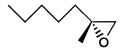
entry	alkene	major epoxide	ee (%)	conversion (%)	yield (%)
1			96	100	78
2			97	17	12
3			74	10	-
4			96	73	67

Table 2
The enantioselective epoxidation of alkenes catalyzed by chloroperoxidase [25]

entry	alkene	major epoxide	ee (%)	yield (%)	turnovers
1			49	89	900
2			89	55	440
3			70	41	3400
4			89	22	1700
5			94	34	4200
6			95	23	1700

reaction has not been described to occur enantioselectively with prochiral compounds. This is odd in light of recent evidence pertaining to an enzyme-bound chlorinating intermediate [9]. CPO catalyzed halide-independent reactions are numerous and include sulfoxidations [10–14], dealkylations of alkylamines [15,16], dimerization of phenols [17], and the oxidations of alcohols to aldehydes [5,18,19], aldehydes to acids [20], amines to nitroso compounds [21,22], and alkenes to epoxides [20,23–28]. Epoxidations were eventually discovered to occur enantioselectively and with reasonably high conversions [24] (Table 1). This finding has stimulated us to investigate the phenomenon further, since enantiopure epoxides serve as excellent intermediates for the synthesis of optically active fine chemicals. The synthons that we have been concentrating on are listed in Fig. 1 with achieved or potential targets. Progress on each front will be discussed in turn.

Initially it appeared as though the epoxide

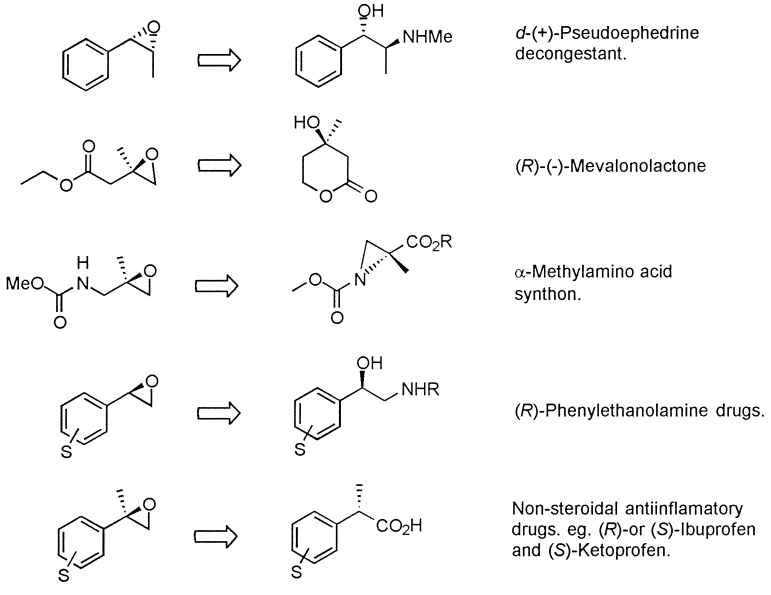


Fig. 1. The CPO-generated epoxide synthons and their target compounds.

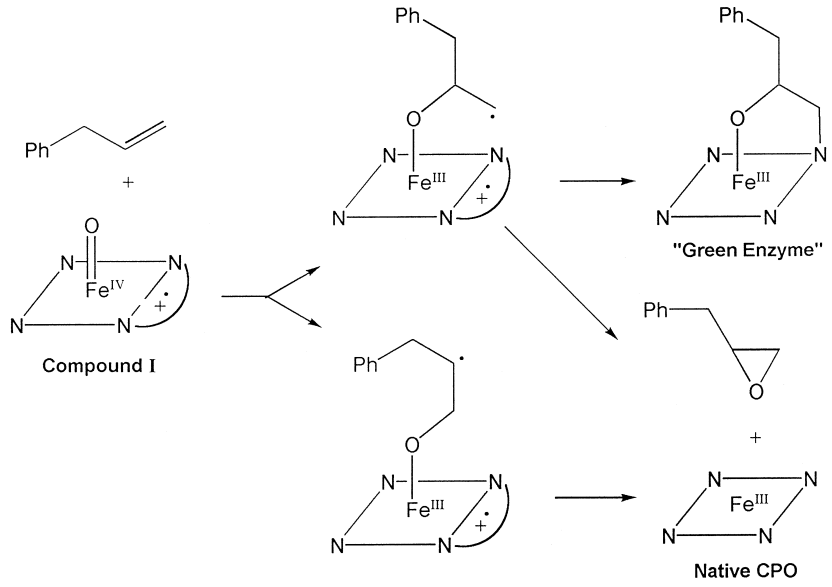
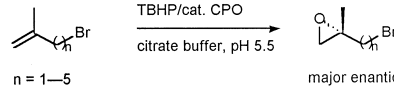


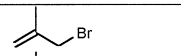
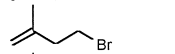
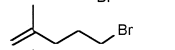
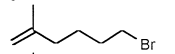
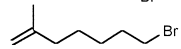
Fig. 2. CPO inactivation caused by allylbenzene *N*-alkylation of the heme.

derived from *cis*-*β*-methylstyrene (Table 1, entry 4 and Fig. 1) could easily be converted to the decongestant D-(+)-pseudoephedrine. During the epoxidation process, however, with some batches of alkene the reaction mixture quickly turned green and reaction ceased. It was subsequently discovered that these samples of *cis*-*β*-methylstyrene were contaminated with tiny amounts of allylbenzene. This monosubstituted alkene was the suicidal culprit, as a green CPO species was produced involving *N*-alkylation of the heme [29,30] (Fig. 2). Amazingly, native CPO possessing most of its original activity was regenerated after three days sitting at room temperature. A resurrection had occurred.

Another of the substrates that had caught our attention was 2-methyl-1-octene (Table 1, entry 3). We wished to elaborate on this theme in search of functionally interesting olefins while continuing to examine substrate specificity and corresponding mechanistic implications. Results with 2-methyl-1-alkenes were promising (Table 2). These substrates proved to be rapidly oxidized giving epoxides with high to excellent enantioselectivities and usually requiring less than one mequiv. of enzyme [25]. CPO is, however, sensitive to the steric requirements of the olefin substrates. This fact was ascertained quantitatively using *ω*-bromo-2-methyl-1-alkenes as probes [27]. Table 3 illustrates the effect of chain length on several reaction parameters. The smallest olefin, methallyl bromide (entry 1), reacts with complete conversion but moderate enantioselectivity. The trend is toward higher ee as the chain increases in length to an

Table 3
The chloroperoxidase-mediated epoxidation of *ω*-bromo-2-methyl-1-alkenes: conversions, kinetic parameters and enantioselectivities as a function of chain length [27]

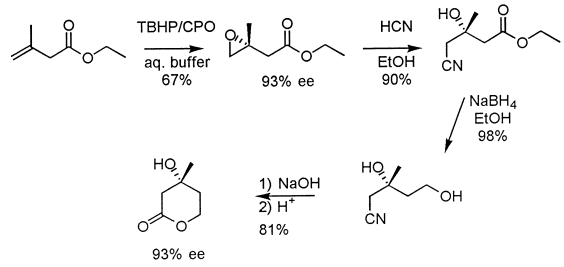


entry	substrate	ee (%)	conv (%)	yield (%)	V_{max} (μmol/min /mg CPO)	K_m (mM)
1		62	100	61	—	—
2		88	100	93	46.7	3.7
3		95	65	89	8.3	0.86
4		87	30	33	2.8	0.42
5		50	20	42	—	—

optimum and then ee declines. Rate, measured as V_{max}, slows with larger substrates resulting in decreased conversions—the enzyme is inactivated before olefin can be consumed.

2,2-Disubstituted oxiranes such as those in Tables 2 and 3 are synthetically interesting intermediates. Addition reactions should occur with very high regioselectivity at the unsubstituted position giving rise to optically active tertiary alcohols. A paucity of reliable methods exists for construction of such compounds. A good example was found in the synthesis of (*R*)-(−)-mevalonolactone: a survey of the literature revealed that all methods provided the lactone in many steps, low overall yield, moderate enantiomeric excess, required expensive starting materials, or various combinations thereof. Meanwhile, a retrosynthetic analysis starting with an appropriately functionalized epoxide provided confidence that CPO could rescue the situation if used in the key stereogenic step. A number of candidate alkenes were screened and an ester proved to be the winner (Table 4). Thus, a quick and efficient preparation of (*R*)-(−)-mevalonolactone [26] was worked out as illustrated in Scheme 1.

Another more recently completed synthesis is depicted in Scheme 2. Again the epoxide is generated in high yield and ee. While unreported in the literature, this compound can be



Scheme 1. The synthesis of (*R*)-(−)-mevalonolactone.

very useful. We have demonstrated its conversion to an aziridinecarboxylate which may serve as a synthon for α-methylamino acids [31].

Clearly what we should like to find for synthetic purposes are substrates that react quickly and with high enantioselectivity. Already we are equipped with clues and the general sensation that if we operate on intuition alone, we will miss some pleasant surprises. For instance, styrene itself is epoxidized without considerable stereoselectivity, but what of ring-substituted styrenes or α-methylstyrenes? Epoxides derived from these species may serve as such useful synthons for elaboration into important medicinal products that it would be worthwhile to investigate.

As mentioned, allylbenzene and many other monosubstituted olefins are actually suicide inhibitors toward CPO. However, if the ethenyl moiety is attached directly to a group of some steric bulk, as is the case for styrene, such

Table 4
The potential mevalonolactone precursors screened for enantioselectivity [26]

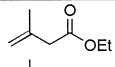
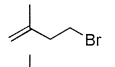
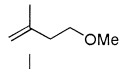
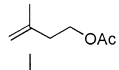
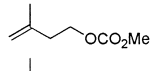
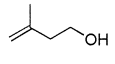
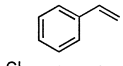
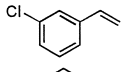
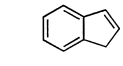
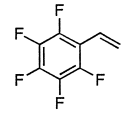
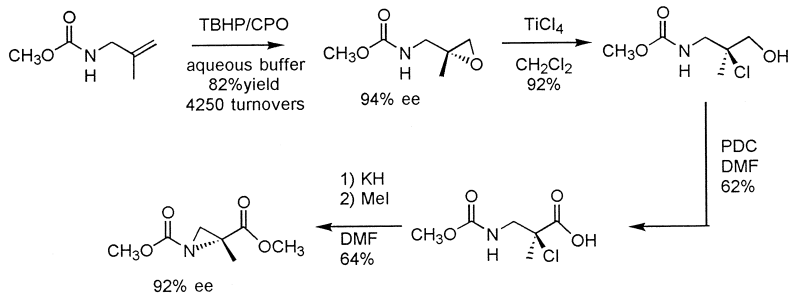
entry	substrate	epoxide ee (%)
1		93 (*R*)
3		85
3		76
4		44
5		43
6		—[a]

Table 5
The epoxidation of styrenes by chloroperoxidase

entry	alkene	results
1		49% ee
2		75% ee
3		mostly diol: cis/trans = 3.7:1 cis = 14% ee
4		No Reaction



Scheme 2. The synthesis of (*R*)-dimethyl 2-methylaziridine-1,2-dicarboxylate.

olefins may serve as reasonably good substrates. Styrene itself is epoxidized with only 49% ee (Table 5). But we were intrigued by the fact that *m*-chlorostyrene is epoxidized by CPO with 75% ee—a substantial increase. Ring substitution effects enantioselectivity in ways that are not currently predictable. Since styrene oxides may be easily aminated to phenylethanolamines and many of these serve as drugs that are currently drawing interest in their optically pure (*R*)-configurations (Fig. 3), it may be worthwhile to explore ring substitution as a parameter of epoxidation efficiency and selectivity. This is an ongoing endeavor. So far we have noticed that pentafluorostyrene is not a substrate (Table 5), presumably for electronic reasons. Indene, a rather special type of styrene, is epoxidized but hydrolyzes rapidly in even slightly acidic aqueous solutions. This is a general problem for styrenes possessing electron-donating substituents. To make matters worse, indene appears not to be oxidized with considerable enantioselectivity as the *cis*-diol is only 14% optically pure.

α-Methylstyrene is a CPO substrate somewhat slower than styrene but with a respectable 89% ee for the corresponding epoxide. Overoxidation to acetophenone predominates unless O_2 is removed. The reaction mixture may be purged with N_2 and sealed with good results. We envision converting a suitably substituted α-methylstyrene to non-steroidal anti-inflammatory arylpropionic acids (Scheme 3). Unfortunately, bulky substituents suppress reaction with CPO even to the point of complete cessation. Electron-donating substituents are to be avoided because of their tendency to promote solvolysis and rearrangement reactions. The cyano group

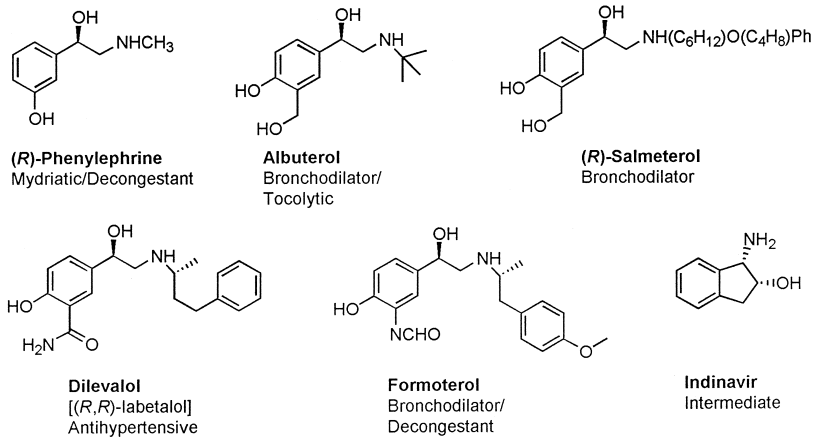
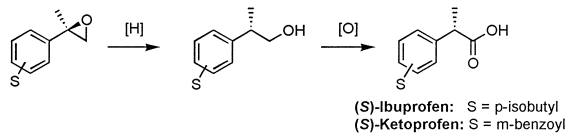


Fig. 3. Phenethylamine-type target drugs possibly obtainable from substituted styrenes.

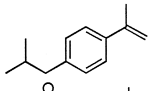
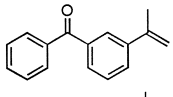
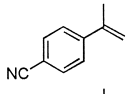
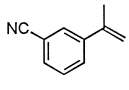


Scheme 3. The possible synthesis of arylpropionic acid analgesic/anti-inflammatories.

is sterically and electronically admissible, though we have discovered (Table 6) that the resulting epoxide possesses moderate (74% for *p*-cyano) to low (20% for *m*-cyano) ee.

In related developments, a Hammett study is in progress to illuminate the nature of CPO compound I. Preliminary results for styrene and three *para*-substituted styrenes (Fig. 4) show a good correlation ($r^2 = 0.972$) between relative rate ratios and Hammett sigma values. The *p*-CF_3 styrene value was not used in the linear least-squares calculation. Its rate was too slow to measure accurately under these conditions. But once the olefin achieves a threshold electron density, the Hammett rho value ($\rho = -0.25$) suggests a low dependence upon electron donation and essentially no carbocationic character in the transition state of the rate-limiting step. This is in contrast to other model metalloporphyrin oxidation reactions which generally show large negative rho values ($\rho^+ = -1.9$) [32,33] that correlate with sigma-plus. Single turnover conditions are planned that will eliminate any rate contribution arising from compound I formation.

Table 6
The epoxidations of α-methylstyrenes by chloroperoxidase

entry	alkene	results
1		89% ee, mostly acetophenone unless O_2 is excluded.
2		Very low conversion
3		No Reaction
4		74% ee
5		20% ee

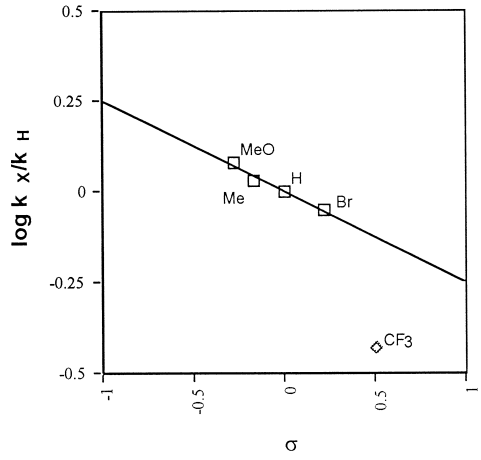


Fig. 4. Hammett plot for the epoxidation of *para*-substituted styrenes catalyzed by CPO. The rates (k_x, where x = MeO, Me, H, Br, or CF_3) for our studies are V_{max} and the conditions are: styrene substrates 0.1–2 mM, CPO 3.2 μM, *t*-BuOOH 10 mM, in 10 mM Na citrate buffer, pH 5.5 containing 25% *t*-butanol at 22°C.

3. Conclusion

CPO is a readily available and considerably stable enzyme that is well suited for important and valuable synthetic strategies. Herein we have described a few successful applications and some work in progress. Without doubt, intuition can lead us to significant discoveries in CPO-catalyzed epoxidations and other reactions. In addition, surprises await the adventurous investigator.

Acknowledgements

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