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Letter

Epoxidation of indene by chloroperoxidase

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Abstract

The stereochemistry of the chloroperoxidase-catalyzed epoxidation of indene has been elucidated. In aqueous solution the intial epoxide product is not stable and opens to form the *cis-trans* diols. When the reaction was carried out in the absence of water, the epoxide enantiomers could be isolated. Under these conditions in 1*R*2*S* enantiomer was formed in approximately 30% ee.

Keywords: Epoxidation; Indene; Chloroperoxidase

1. Introduction

Chloroperoxidase (CPO) from *Caldariomyces fumago* catalyzes the asymmetric epoxidation of a variety of olefins at high enantiomeric excess (ee) and yields [1–4]. CPO can be isolated with comparative ease at low cost and exhibits higher turnovers in comparison to synthetic asymmetric epoxidation catalysts of the manganosalen group [5,6]. Therefore, CPO holds much promise for industrial applications [7].

We have undertaken the epoxidation of indene (**A**), to obtain enriched chiral precursors of indene diol (**C**), which is readily derived by in situ hydrolytic cleavage of the original epoxide (**B**) (Scheme 1). The potential applications of these chiral synthons and their immediate derivatives have been discussed elsewhere [8,9]. Our results shed light on the stereochemistry of CPO-catalyzed epoxidations and reports the first non-aqueous reaction medium for CPO.

2. Materials and methods

Indene, indan-2-one and indene bromohydrin, *tert*-butyl hydrogen peroxide (*t*BHP), *tert*-butanol (*t*BuOH), glycerol and Jacobsen's catalyst were purchased from Aldrich. Racemic indene oxide was synthesized by two routes — (a) epoxidation of indene with *m*-chloroperoxybenzoic acid [10] and (b) treatment of indene bromohydrin with base [11]. Racemic *cis*-indene diol was synthesized from *cis*-2-for-

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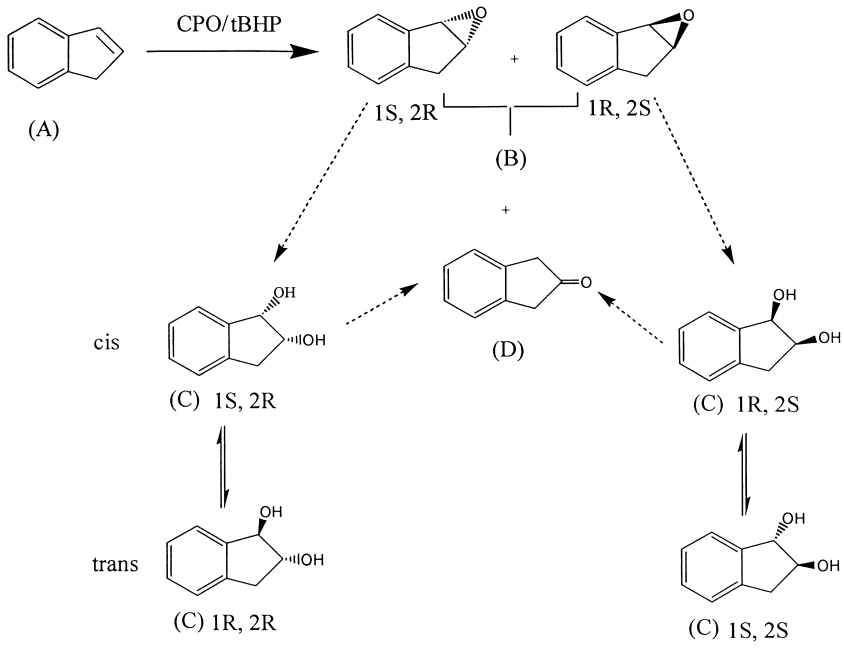

Scheme 1.

myloxyhydroxyindane [12] and racemic *trans*-indene diol was synthesized from indene bromohydrin [12]. The resolution of epoxide and diols were done with an HPLC instrument using Pirkle concept Whelk-O-1 SS column (Regis Technologies) using hexane-isopropyl alcohol (98.2:1.8, 1 ml/min, UV detector) mixture as the solvent front. Identification of the different stereoisomers was done by comparison with known standards.

3. Results and discussion

Indene is an air- and light-sensitive olefin with a very low solubility in water. The progress of the reaction is monitored by the disappearance of indene by GC (Fig. 1). Under the given reaction conditions, the turnovers (moles of substrate converted per mole of enzyme) is calculated to be around 1000 and more than 90% indene was converted at an unoptimized 0.07 mol% of CPO, compared to the optimized 0.4 mol% Jacobsen's manganosalen-catalyzed process [13]. From the normal aqueous reaction mixtures, the epoxide was not isolated as a product because it spontaneously hydrolyzes in water to give a mixture of *cis* and *trans* diols (at overall ~40% yield).

In order to determine the stereochemistry of the original epoxide formed, a novel reaction medium devoid of water was devised. To a solution of 10 mg of lyophilized CPO (at pH 5, 0.1 M sodium phosphate buffer) in 500-μl glycerol, 15 μl of indene and 20 μl of 70% *t*BHP were added. The reaction mixture was flushed with nitrogen and stirred at room temperature for 40 min. The epoxide formed was ether-extracted, concentrated and injected on HPLC. The chromatogram was poorly resolved, owing to the elution of indan-2-one (**D**) (a side-product of the reaction), between the two epoxide enantiomers. In comparison with the chromatogram

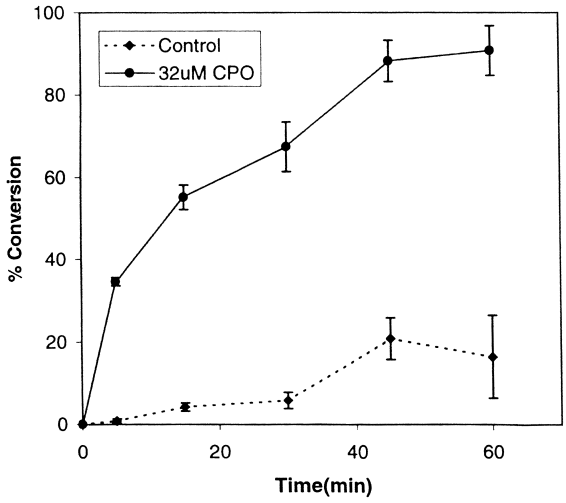

Fig. 1. A progress curve for epoxidation of indene by CPO in presence of tBHP. Initial conditions — to 850 μl of 100 mM phosphate buffer (pH 6), 50 μl of substrate mix (700 μl DMF + 200 μl *t*BHP + 100 μl indene) was added and 100 μl of ~300 μM CPO solution was added to commence the reaction. The vial was stirred at room temperature and samples were analyzed after extraction into isooctane (containing undecane as internal standard), and this was injected on GC. The control vial had no enzyme.

of the standard stereoisomers (made using Jacobsen's manganosalen catalyst), the ee was noted to be in favor of the 1*R*2*S* enantiomer. This is the first report of a successful employment of an essentially non-aqueous reaction environment for CPO. The problem posed by the high viscosity of glycerol (which makes it a poor candidate for routine usage) is alleviated to a certain extent by employing *t*BuOH as a thinning co-solvent at $< 30\%$ (v/v).

The CPO reaction products had to be analyzed with respect to the resolvable *trans*-diols to determine the exact ee. The *cis*:*trans* values obtained with GC (FID) was lower than the values obtained with HPLC (UV absorption at 220 nm) and NMR (C2 proton integral at 3.85 ppm of *cis*-diol and 4.26 ppm of *trans*-diol [14]) for a given composition of diols. We prefer to use the normal phase HPLC rather than GC method to quantify the *cis*:*trans* mixture as it is milder and more reproducible (Fig. 2).

The individual 1*S*2*R* and 1*R*2*S* enantiomers of indene oxide were subjected to hydrolysis. The *cis* and *trans* diols retained the same ee as their respective epoxides and this was not seen to vary, unlike the *cis*:*trans* diol ratio, which did change with time. This is observed because the nucleophilic hydroxyl attack occurs on the benzylic carbon, leading to the inversion of configuration at C1. In the literature, much data

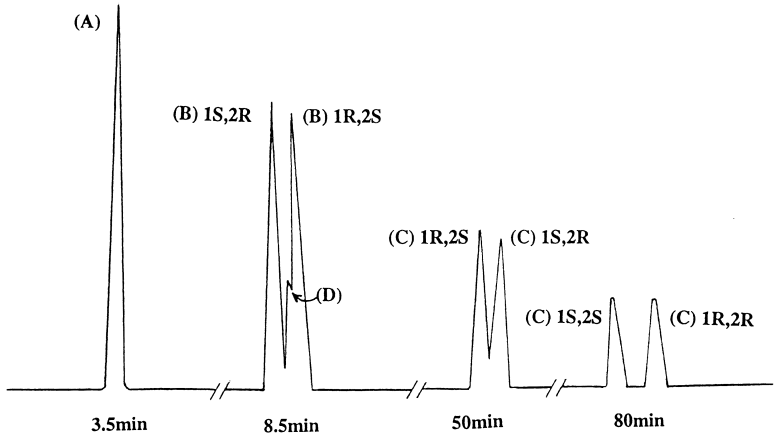

Fig. 2. Schematic representation (not to scale) of the elution pattern of reaction components on Pirkle Concept Whelk-O-1-SS column.

and speculations have been presented [15–17] on the peculiar oxirane opening of indene oxide, which yielded different ratios of *cis* and *trans* diols and indan-2-one, under different conditions. The transition of *cis*-diol to *trans*-diol or vice versa (in water), is now understood to be an equilibration process, the rate of which depends on conditions like temperature and pH. In our study, we have been able to enhance the yield up to 6:1 in favor of *cis*-diol by the incorporation of low levels of ceric ammonium sulfate (CAS) in the reaction medium. However, CAS in dimethyl sulfoxide preferentially gave *trans*-diol. Also, incubation of the epoxide at alkaline pH and in the presence of cosolvents like *t*BOH and dimethyl formamide yielded more *trans*-diol. These results show that the solvent environment plays a crucial role in the fate of oxirane ring opening by nucleophiles presumably by (a) stabilizing the transient reaction intermediates and (b) determining the directional collapse of charged adducts formed on the cyclopentenyl frame.

We have reported the epoxidation of phenyl ring derived olefins like *cis*-β-methylstyrene and 1,2-dihydronaphthalene [1]. Both give the 1*S*2*R* epoxide at an ee of greater than 95%. The hydrolytic product of CPO catalyzed indene oxide yields 1*S*2*S* *trans*-diols at 33% ee and 1*R*2*S* *cis*-diol implying the formation of 1*R*2*S* epoxide at 33% ee. This is similar to the epoxidation of styrene by CPO where the 1*R* epoxide is formed at 49% ee [28].

The active site (distal pocket) of CPO is comparatively small and the molecular dimensions of the substrate must play a very crucial role in the reaction stereochemistry. In the case of *cis*-β-methyl styrene, Sundaramoorthy et al. [18] showed that the orientation of the C3 methyl carbon and its interaction with Glu 183 is very crucial in establishing the 1*S*2*R* stereochemistry of the reaction. In indene, the puckering of the cyclopentenyl ring is envisaged to make the 1*R*2*S* prochiral indene molecule marginally more energetically feasible for closer orientation to the oxoferryl CPO catalytic intermediate. In case of styrene, the C3 carbon is altogether absent, therefore, the forces driving the 1*S* stereochemistry is absent.

Recent success in producing directed evolution mutants of CPO resistant to terminal olefin inactivation [19] indicate that it should be quite feasible to produce CPO mutants that can catalyze indene epoxidation at higher enantioselectivity. Also, in our preliminary kinetic experiments, we have seen that we can significantly enhance CPO turnovers. Thus, future efforts are directed to enhancing the enantioselectivity of CPO and improving the process parameters for obtaining greater product yields.

Acknowledgements

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