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Asymmetric oxidation of 1,3-cyclohexadiene catalysed by chloroperoxidase from *Caldariomyces fumago*

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Abstract

Chloroperoxidase from *Caldariomyces fumago* catalyses the oxidation of prochiral 1,3-cyclohexadiene using TBHP as terminal oxidant. The process occurs enantioselectively and furnishes the non-racemic *trans* diols 1,2- and 1,4-dihydroxycyclohexene, (−)-**3** and (+)-**4**, in good *ee* and yield. © 2000 Elsevier Science Ltd. All rights reserved.

The preparation of chiral compounds in non-racemic form is a goal of great interest in organic synthesis, due to the large application that these compounds have in several fields, such as in medicinal chemistry.[1] Recently, our interest in this field has been directed toward the use of biocatalysis to realise regio- and stereoselective discrimination of alcohol functions so as to achieve polyhydroxylated compounds in enantiopure form.[2] The enantioselective direct introduction of oxygen onto olefins could be a valid alternative to access these compounds and biocatalysis of haloperoxidases, realising oxygenase-type reactions, is very useful and effective for this purpose.[3] In particular the use of *Caldariomyces fumago* chloroperoxidase (CPO) is especially advantageous, since this usually involves peroxides (H_2O_2 or ROOH), without requiring expensive cofactors. Moreover, due to its broad substrate acceptance, this chloroperoxidase has great synthetic potential and has allowed the stereoselective epoxidation and the hydroxylation of a wide range of olefins in satisfactory yield and with high enantiomeric excess.[4] The enantioselelective oxidation of allylic alcohols to aldeydes has also recently been reported.[5] However, the behaviour of this enzyme on prochiral conjugate dienes has not been investigated to date.

With the intention of investigating the enantioselective course of the oxygen addition to these unsaturated systems we have taken cyclohexadiene **1** as an example. The presence in this molecule of a symmetry axis C_2 limits the number of possible stereoisomers that can be

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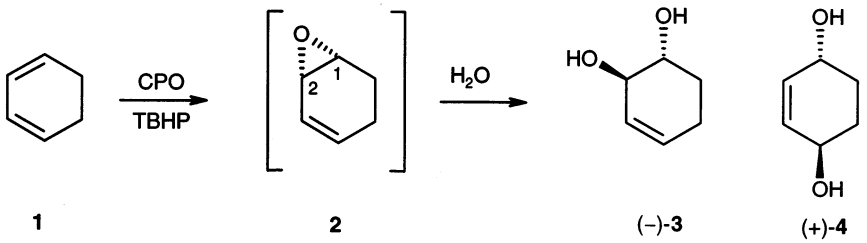
obtained, and the stereochemical course of the reaction can be described in terms of enantiotoposelectivity with regard to the double bonds on the cyclohexane framework. In this communication we report the preliminary results of this investigation.

The oxidation of cyclohexadiene **1** was carried out in citrate buffer (0.1 M, pH 5.0) using commercial *C. fumago* chloroperoxidase as the catalyst. To prevent irreversible inactivation of CPO the TBHP (*tert*-butyl hydroperoxide), chosen as terminal oxidant, was added in two aliquots thus maintaining its low concentration throughout 48 hours.



1 **2** (−)-**3** (+)-**4**

The monitoring of the reaction by GC–MS revealed the absence in the reaction mixture of the expected cyclohexadiene oxide and the formation of two products in a 1.3:1 ratio that, at complete conversion of the substrate, were recovered in 50 and 34% yield by chromatographic purification, and subjected to physiochemical investigation.[6] The NMR inspection and the observed optical activity allowed us to assign to them the structures of 1,2- and 1,4-dihydroxycyclohexene and indicated a *trans*-dihydroxyl relationship. The GC analysis on a chiral column indicated the same enantiomeric excess of 70% for both the alcohols.[7] Although in the reaction mixture the cyclohexadiene oxide **2** has not been detected, it is plausible that in the first step of CPO action it is the intermediate product to suffer fast nucleophilic attack by water, with partial rearrangement, giving the *trans*-diols (−)-**3** and (+)-**4**, respectively. This chemical behaviour of cyclohexadiene oxide hydrolysis has been widely investigated in the literature,[8] and has in the past already been proposed in the transformation of **1** under the oxidative action of the mono-oxygenase Cytochrome P-450.[9]

The absolute configuration of the newly generated stereogenic centres in (−)-**3** was determined as 1*R*/2*R* by comparing its specific rotation to the literature value ($[\alpha]_D = -50$, $c = 0.9$, H_2O; lit.[10] $[\alpha]_D$ approximately +76 in H_2O for the 1*S*/2*S* enantiomer), consequently compound (+)-**4** was assigned the configuration (1*R*,4*R*).

It should be emphasised that the unknown enantiomeric excess of epoxide **2** does not allow the assertion if the enantiomeric ratio observed in the formation of (−)-**3** is the exclusive consequence of the enantiotopic discrimination due to the CPO action, or the result of different addition rates of the water at positions C-1 and C-2 during the chemical hydrolysis mentioned above. Nevertheless taking into account that in the case of the 1,4-isomer, (+)-**4**, the same enantiomeric excess value was detected, it is true to state that the epoxide-ring opening is regioselective. Conversely the enzymatic addition of oxygen to the olefin occurs with partial enantiotoposelectivity (Fig. 1).

In conclusion chloroperoxidase from *C. fumago* is able to catalyse the oxidation of prochiral cyclohexadiene using TBHP as terminal oxidant. The process occurs with enantioselectivity and furnishes the non-racemic *trans*-diols (−)-**3** and (+)-**4** in good *ee* and yield. The potentiality of this enzyme in the hydroxylation of other prochiral cycloalkene derivatives is under investigation in our laboratory.

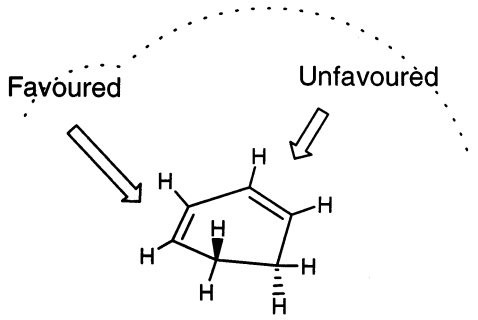


Figure 1. CPO enantiotoposelective recognition in cyclohexadiene

Acknowledgements

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6. *Preparation of (1*R,*2*R*)*-trans-*3-cyclohexene-1,2-diol (–)*-**3** *and (1*R,*4*R*)*-trans-*2-cyclohexene-1,4-diol* (+)-**4**: A solution containing **1** (300 mg, 3.7 mmol) in citrate buffer (18 ml, 0.1 M, pH 5) was stirred vigorously at room temperature for 5 min and then a suspension of CPO (1850 U) was mixed. TBHP (4.2 mmol) was added in two aliquots at 24 h intervals. The emulsion was stirred at room temperature for 48 h and then the product mixture was recovered by repeated extractions with diethyl ether and then with ethyl acetate. The organic phase was dried over anhydrous Na_2SO_4 and taken to dryness. The residue was purified on silica gel column (ethyl acetate:hexane 7:3) to afford (–)-**3** and (+)-**4** in a 50 and 34% yield, respectively. The ratio of 1,2-/1,4-diols and their *ee* values were determined by GC analysis on Megadex DMP β-dimethylpentyl βCDX OV1701 capillary column. Data for (–)-**3**: *ee* = 70% (determined by chiral GC analysis after exhaustive chemical acetylation). ^{1}H NMR ($CDCl_3$/TMS, 250.13 MHz): δ (ppm) 1.62 (dddd, 1H, J = 12.5, 11.5, 8.5, 8.0 Hz), 1.93 (ddt, 1H, J = 12.5, 4.0, 3.7 Hz), 2.15 (m, 2H), 3.60 (ddd, 1H, J = 11.5, 7.5, 4.0 Hz), 4.10 (m, 1H), 5.54 (dd, 1H, J = 10.0, 2.5 Hz), 5.69 (dd, 1H, J = 10.0, 2.0 Hz); ^{13}C NMR ($CDCl_3$/TMS, 62.9 MHz): δ (ppm) 24.8, 28.5, 73.2, 73.3, 128.2, 128.8. Data for (+)-**4**; *ee* = 70%. ^{1}H NMR ($CDCl_3$/TMS, 250.13 MHz): δ (ppm) 1.50 (ddd, 2H, J = 10.2, 8.2, 2.2 Hz), 2.13 (m, 2H), 4.26 (bt, 2H), 5.80 (s, 2H); ^{13}C NMR ($CDCl_3$/TMS, 62.9 MHz): δ (ppm) 30.2, 66.1, 132.5.
7. Improvement of enantiomeric excesses of products was achieved by crystallisation. Diol (+)-**4** was obtained from ethyl acetate with *ee* 94%, $[\alpha]_D$ = +144.7 (c = 0.25, $CHCl_3$), mp 86–87°C; the 1,4-isomer in diethyl ether/chloroform gave crystallisation of racemate and (–)-**3** with *ee* 89%, $[\alpha]_D$ = 18.2 (c = 1.8, $CHCl_3$) was recovered from the solution.

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