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Catalytic behaviour of chloroperoxidase from *Caldariomyces fumago* in the oxidation of cyclic conjugated dienes

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Abstract—Chloroperoxidase from *Caldariomyces fumago* has been investigated as a catalyst for the oxidation of cyclic conjugated dienes. The nature of the substituents and the size of the carbocycle affect the enantioselectivity of the enzyme. An unexpected course of the CPO-catalyzed oxidation has been observed in the reaction of *cis*,*cis*-1,3-cyclooctadiene. © 2002 Published by Elsevier Science Ltd.

1. Introduction

Chloroperoxidase (CPO) from *Caldariomyces fumago* is a promising biocatalyst for asymmetric oxidation.[1,2] The epoxidation of olefins with hydroperoxides is the most studied reaction catalyzed by CPO, since chiral epoxides are important intermediates in asymmetric syntheses of biologically or pharmaceutically active compounds.

The CPO-catalyzed oxygen transfer to carbon–carbon double bonds has been investigated on a broad range of alicyclic mono-olefins, and the effects of chain length, double bond position and *cis*/*trans*-stereochemistry on the enantioselectivity and reactivity of the enzyme were observed.[3–6]

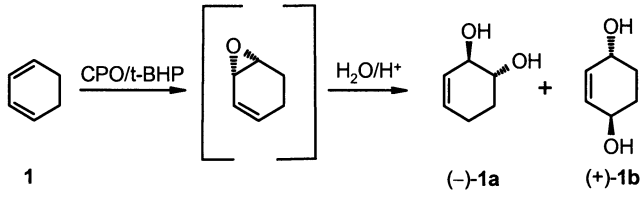
In our previous work we described the CPO-catalyzed epoxidation to 1,3-cyclohexadiene, the first example of a biocatalytic oxidation of a cyclic conjugated diene.[7] The satisfactory results we obtained in terms of the chemical yields and ee of the products prompted us to extend the methodology to substituted cyclohexadienes and larger cyclic conjugated systems.

2. Results and discussion

2.1. The role of substituents in the oxidation of 1,3-cyclohexadiene

CPO-catalyzed oxidation with *tert*-butyl hydroperoxide (*t*-BHP) of 1,3-cyclohexadiene **1**, having pro-enantiomeric faces and enantiotopic double bonds, was complete after 48 h and two diols, (−)-**1a** and (+)-**1b** (ratio 1.3:1), were isolated in satisfactory yield and 70% ee.[7] The chiral monoepoxide was not detected, possibly due to its rapid hydrolysis under the reaction conditions (Scheme 1).

Taking this result into consideration, we decided to investigate the behavior of different conjugated cyclic dienes. We first considered diol **2** (Table 1), in which the presence of the two *cis*-substituents reduces the molecular symmetry but retains the enantiotopic nature of the double bonds. The CPO-catalyzed oxidation of **2** proceeded with a lower reaction rate with respect to that of **1**. The expected epoxide **2a** was detected in traces, and after 72 h a single product was isolated in 25% yield.[8] NMR analysis and the specific rotation value allowed us to define this product as 5-cyclohexen-1α,2β,3α,4β-tetrol, (+)-**2b**, indicating CPO-attack only to the double bond *anti* to the original hydroxyl groups. The 78% ee determined for (+)-**2b** revealed good enantiotopic CPO-recognition regarding the two double bonds of the substrate.



Scheme 1. Oxidation of 1,3-cyclohexadiene catalyzed by CPO.

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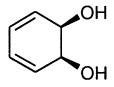
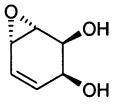
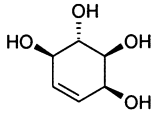
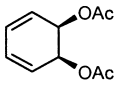
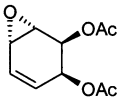
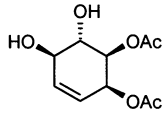
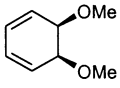
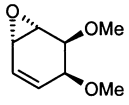
Table 1. Oxidation of 1,3-cyclohexadienes catalyzed by CPO[a]

Substrate	Time (h)	Conv (%)	Epoxide	Hydrolysis product
 **2**	72	45	 **2a** traces	 **(+)-2b** 25%, ee 78%
 **3**	48	100	 **3a** *racemic*	 **3b** *racemic*
 **4**	48	9	 **4a** *racemic*	no hydrolysis product was detected

[a] Reaction conditions: 0.1 mmol of substrate; 400 U CPO; 2 ml citrate buffer (0.1 M, pH 5); 0.1 mmol *t*-BHP.

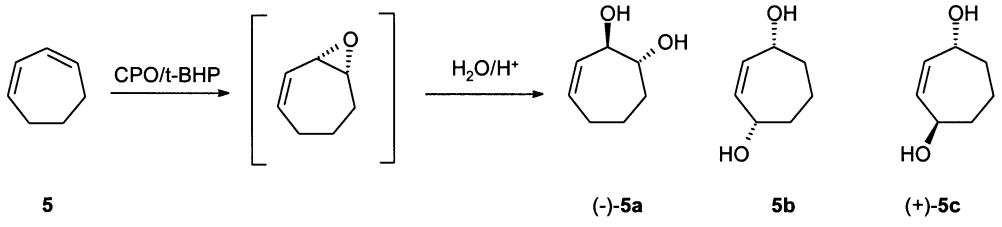
In a subsequent experiment, the diacetate **3** was tested as a substrate. After 48 h, complete conversion was observed with formation of two reaction products, **3a** and **3b**. In this case the intermediate epoxide, **3a** (10%), was evidenced in solution and its *anti* configuration determined by comparison of the spectroscopic properties with those of an authentic sample (prepared by oxidation of **3** with *m*-CPBA).[9] Formation of a single epoxide again demonstrated the high facial-selectivity in the oxygen transfer from enzyme to substrate. Chiral GC and NMR analyses evidenced that both **3a** and **3b** were racemic mixtures, thus indicating the detrimental effect of the acetyl groups on the enantiotopic recognition of the two double bonds of **3** by the enzyme. With the aim of better evaluating this effect, we considered the methoxy derivative **4** as a substrate. The oxidation reaction gave low conversion (9%) with formation of a single epoxide, **4a**. Its structure was assigned by comparison with a synthetic sample obtained by epoxidation of **4** with *m*-CPBA. In this case the chiral GC analysis indicated that a racemic mixture had also formed. No hydrolysis product was detected in the reaction mixture.

2.2. Enzymatic oxidation of larger cycles

The study of the CPO-catalyzed oxidation was then extended to larger cyclic dienes, namely 1,3-cycloheptadiene **5** and *cis*,*cis*-1,3-cyclooctadiene **6**.

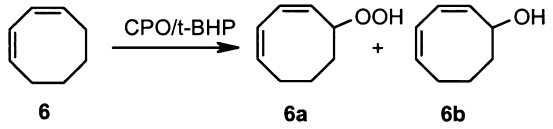
For cycloheptadiene **5** a 60% conversion of substrate was observed after 72 h. Cycloheptene oxide was not detected in the reaction mixture and only diols (−)-**5a**, **5b** and (+)-**5c** in a ratio of 2:1:1 were observed (Scheme 2).

As in the case of cyclohexadiene, it is possible to presume the formation of a parent epoxide, which undergoes rapid chemical hydrolysis with partial rearrangement to give the 1,2- and 1,4-diols. The same 79% ee value measured for (−)-**5a** and (+)-**5c** indicates that a regiospecific hydrolytic oxirane ring opening occurs.



Scheme 2. Oxidation of 1,3-cycloheptadiene catalyzed by CPO.

The course of the CPO-catalyzed oxidation of *cis*,*cis*-1,3-cyclooctadiene **6** is quite surprising, since after 6 days a 53% conversion of the substrate was observed with formation of a product different from the expected epoxide or diols in 43% yield (Scheme 3), together with small amounts (5%) of the racemic alcohol **6b**. The main product was identified as **6a** by comparison with a synthetic standard, while the absence of any optical rotatory power highlighted its racemic nature.[10,11]



Scheme 3. Oxidation of *cis*,*cis*-1,3-cyclooctadiene in the presence of CPO.

The unexpected course of the reaction prompted us to initiate a deeper investigation. When the reaction was performed in the absence of CPO, only a 7% yield of **6a** was obtained after 6 days, thus confirming the contribution of CPO to the product formation. In a subsequent experiment we treated **6b** with *t*-BHP in the presence of CPO and no trace of **6a** could be detected. Finally, two separate experiments were performed in the presence of *t*-BuOH or TROLOX® as radical scavengers.[12] Both reactions, as reported in Table 2, gave amounts of **6a** comparable with the value observed under the standard conditions. The above considerations suggest that a direct, but non-selective, transfer of -OOH from CPO to the substrate outside the active site occurs, similar to that observed in CPO-catalyzed oxidative halogenation reactions.[12]

Table 2. Oxidation[a] of **6** with CPO/*t*-BHP in the presence of radical scavengers

Radical scavenger	Time (h)	% **6a**[b]
None	72	31
20% *t*-BuOH	72	27
TROLOX® 7.5 mM	72	34

[a] 0.2 mmol of **6**; CPO 800 U, citrate buffer 0.1 M pH 5; *t*-BHP, 0.2 mmol, was added to the reaction mixture in two aliquots at 24 h intervals.

[b] The reaction conversions were determined from the ^{1}H NMR spectra of aliquots of the reaction mixture extracted directly with $CDCl_3$.

3. Conclusions

The results obtained reveal that the activity of *Caldariomyces fumago* peroxidase in the enantioselective oxidation of *meso*-1,3-cyclodienes is strongly influenced by the nature of the substrate. Selective epoxidation of one double bond of the diene is the main activity of the enzyme, with good enantioselectivity for C6 and C7 1,3-cyclic dienes **1** and **5**. The presence of substituents on the ring affects the stereoselectivity of the reaction, allowing enantioselectivity in the case of dihydroxy derivative **2**, whereas no selective desymmetrization was evidenced for the acetoxy- and methoxy derivatives **3** and **4**.

With the larger ring system *cis*,*cis*-1,3-cyclooctadiene, **6**, the biocatalyzed reaction with *C. fumago* chloroperoxidase followed an unexpected course, permitting peroxidation at the allylic position. This aspect of the behavior of CPO is presently under investigation in our laboratory.

4. Experimental

4.1. Material and methods

CPO from *C. fumago* (suspension in 0.1 M sodium phosphate buffer, pH 4) was purchased from Sigma. Substrates **2**, **5** and **6** were products of Aldrich. Ester **3** was obtained by conventional acetylation of *cis*-1,3-dihydroxycyclohexadiene **2** with pyridine and acetic anidride; methoxy derivative **4** was prepared by methylation of **2** with methyl iodide in the presence of a catalytic amount of cesium carbonate. *t*-Butyl hydroperoxide was an Aldrich solution 70% v/v in water.

Chiral GC and GC–MS analyses were performed on Megadex DMP β-dimethylpenthyl βCDX OV1701 capillary column and ZEBRON™ 5%-phenyl–95%-dimethylpolisiloxane, respectively. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD at 250.13 and 62.9 MHz, respectively, on a Bruker AMX-250 instrument using TMS as internal reference. Chiral NMR shift reagent was europium(III) tris-[3-(heptafluoropropyl)hydroxymethylene]-(+)-camphorate, Eu(hfc)$_3$. Optical rotations were measured on a DIP 135 JASCO instrument.

4.2. Enzymatic oxidation of cyclic dienes

In a typical oxidation reaction diene (0.1 mmol) was added to citrate buffer solution (0.1 M, pH 5.0, 2 ml) under magnetic stirring at room temperature. After a few minutes CPO (400 U) was added and then *t*-BHP (0.1 mmol in two aliquots at 24 h intervals). The conversion of the substrates was monitored by GC analysis of aliquots extracted by diethyl ether and using *n*-decane as internal standard. Reactions were stirred vigorously at room temperature (25°C) until profiles of the reactions were unchanged according to GC analysis.

Preparative scale experiments (2 mmol) were performed without the use of internal standards. The reactions were stopped at a defined time, extracted with diethyl ether or ethyl acetate, the organic solutions dried over Na_2SO_4, evaporated to dryness, and the residue subjected to silica gel chromatography eluting with ethyl acetate/hexane in different ratios.

4.2.1. (1*R*,2*S*,3*S*,4*S*)-Tetrahydroxycyclohex-5-ene, (+)-2b. Obtained in 25% yield and 78% ee by a 96 h reaction. $[\alpha]_D = +55.7$ (*c* 0.2 in MeOH). ^{1}H, ^{13}C NMR

and optical rotation data were in agreement with the literature values for (+)-Conduritol F.[13]

4.2.2. (1α,2α,3β,4α)-1,2-Diacetoxy-3,4-dihydroxycyclohex-5-ene, 3b. Compound **3b** was prepared by a 48 h reaction and was isolated in 65% yield, racemic (by GC chiral analysis). ^{1}H NMR: δ 2.03 (s, 3H), 2.08 (s, 3H) 3.97 (dd, $J=11.0$, 7.5 Hz, 1H), 4.21 (dt, $J=7.5$, 2.0 Hz, 1H), 4.90 (dd, $J=11.0$, 4.5 Hz, 1H), 5.58 (dd, $J=5.0$, 4.5 Hz, 1H), 5.77 (ddd, $J=10.0$, 5.0, 2.0 Hz, 1H), 5.94 (dd, $J=10.0$, 2.0 Hz, 1H). Anal. calcd for $C_{10}H_{14}O_6$: C, 52.17; H, 6.13. Found: C, 52.08; H, 5.90%.

4.3. (1*R*,2*R*)-Cyclohept-3-ene-1,2-diol, (−)-5a, (1*R*,4*S*)-cyclohept-2-ene-1,4-diol 5b, (1*R*,4*R*)-cyclohept-2-ene-1,4-diol, (+)-5c

Compounds (−)-**5a**, **5b** and (+)-**5c** were prepared by a 72 h reaction.

(−)-5a: 30% yield, ee 79% (by chiral GC analysis of diacetyl derivative); $[\alpha]_D=-32.3$ (c 0.4 in $CHCl_3$); ^{1}H NMR: δ 1.39 (m, 1H), 1.66 (m, 2H), 1.92 (m, 1H), 2.18 (m, 2H), 2.54 (bs, 1H), 2.73 (bs, 1H), 3.37 (dt, $J=9.5$, 3.5 Hz, 1H), 4.16 (m, 1H), 5.59 (dt, $J=11.5$, 2.5 Hz, 1H) 5.85 (m, 1H); ^{13}C NMR: δ 24.1, 27.7, 36.7, 72.8, 75.4, 131.1, 133.0. Anal. calcd for $C_7H_{12}O_2$: C, 65.60; H, 9.44. Found: C, 65.51; H, 9.30%. Absolute (*R*,*R*) configuration of (−)-**5a** was assigned after hydrogenation with C/Pd and comparing the optical rotation of the obtained product ($[\alpha]_D=-30.1$, c 0.3 in $CHCl_3$) with literature data for (1*S*,2*S*)-1,2-dihydroxycycloheptane.[14]

5b: 14% yield. The physical data were in agreement with those reported.[15]

(+)-5c: 15% yield, ee 79% (by chiral GC analysis of diacetyl derivative); $[\alpha]_D=+54.8$ (c 0.3 in $CHCl_3$). ^{1}H NMR: δ 1.60–1.81 (m, 6H), 4.42 (dd, $J=8.0$, 1.2 Hz, 2H), 5.76 (d, $J=1.2$ Hz, 2H). Anal. calcd for $C_7H_{12}O_2$: C, 65.60; H, 9.44. Found: C, 65.50; H, 9.32%. In the light of the well known hydrolysis mechanism reported for the cyclodiene oxides, the absolute configuration of (+)-**5c** was assigned as *R*,*R*.[16]

4.4. Cycloocta-2,4-dien-1-yl hydroperoxide, 6a, cycloocta-2,4-dien-1-ol, 6b

Compound (±)-**6a** was prepared by a 144 h reaction.

(±)-**6a**, 43% yield. The physical data were in agreement with those reported.[10] The racemic nature of **6a** was determined by chiral GC analysis of the corresponding alcohol obtained by reduction with triphenylphosphine in diethyl ether.[17]

Acknowledgements

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