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# Chloroperoxidase-Catalyzed Asymmetric Synthesis of a Series of Aromatic Cyclic Sulfoxides

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**Abstract:** The chloroperoxidase (CPO) catalyzed oxidation of a series of rigid aromatic sulfides of well-defined geometry has been studied by the use of enantioselective gas chromatography for the determination of product composition and sulfoxide enantiomeric excess. The almost planar 1-thiaindane was found to be an excellent substrate, giving a quantitative yield of the (-)-(R)-1-oxide in 99% e.e. The sterically more demanding next higher homolog with a six-membered heterocyclic ring, 1-thiatetrahydronaphthalene (1-thiochroman), also gave a sulfoxide in high e.e. (≥96%) but in a much lower yield, indicating a preserved stereorecognition ability but a lower turnover rate. A carbonyl group in the 4-position (1-thiochroman-4-one) had no further effect on the CPO-mediated sulfoxidation. Enantioselectivity was lost for the symmetric disulfide, 1,3-benzodithiol, indicating an equal accessibility of the Fe=O complex towards both sulfur atoms and an active site centred above the heme-iron atom. In all cases with pronounced enantioselectivity the reaction gave the (R)-sulfoxide as observed previously with alkyl aryl sulfides. A change of oxygen source from hydrogen peroxide to t-butyl hydroperoxide and a longer reaction time gave a higher chemical but a lower optical yield, most likely due to an increased competition by the uncatalyzed oxidation reaction in this case. 

## INTRODUCTION

Chloroperoxidase (CPO; EC 1.11.1.10) is a hemoprotein oxidoreductase, isolated from the marine fungus *Caldariomyces fumago*, which exhibits both catalase and peroxidase as well as monooxygenase activities.[1] Of particular interest from the point of view of asymmetric synthesis is the enantioselective transfer of oxygen recently found in sulfoxidation[2] and epoxidation reactions.[3] Thus far, the sulfoxidation reaction has been carried out mainly with aryl alkyl sulfides and the enzyme has been demonstrated to show low tolerance towards variation in the structure.[2b] In all cases investigated, the dominating product enantiomer is the (R)-sulfoxide.[2b-d] Mechanistic studies involving $^{18}O$-labeling experiments have strongly indicated that sulfoxidation by CPO/$H_2O_2$ takes place via direct transfer of the ferryl oxygen to the substrate.[4]

In view of the high substrate selectivity previously reported, we have been interested in exploring the scope of the asymmetric sulfoxidation reaction by the use of a series of rigid and sterically well-defined aromatic bicyclic sulfides as substrates. The results from this investigation are presented in the following.

## RESULTS AND DISCUSSION

CPO was found to catalyze the oxidation of 2,3-dihydrobenzothiophene **1** to the corresponding sulfoxide **5** with high chemical yields and enantiomeric excesses, Table 1. High enantiomeric excess (e.e.) was maintained, but the chemical yield dropped significantly, when the five-membered ring was increased to a six-membered one as in 1-thiochroman **2**. Compared to **2**, no significant change in e.e. and chemical yield in the oxidation of 1-thiochroman-4-one **3** was observed, although differences of both steric and electronic

nature are present. This indicates the methylene group in **2** to be space-demanding enough to account for the low yield. The second sulfur in the five-membered ring of 1,3-benzodithiol (**4**) had a strong negative effect on CPO catalyzed asymmetric oxidation. This confirms the proposed model of an active site centred above the heme-iron atom.[5] Further, an equal accessibility of the two sulfur atoms from the Fe=O moiety would explain the lost enantioselectivity. The X-ray structure of CPO obtained recently[6] will probably spread some light upon these topological questions within a near future.

**Table 1.** Optical and chemical yields in CPO-catalyzed oxidation of a series of bicyclic sulfides

| Sulfide | No. | Sulfoxide | Yield[a]/% | E.e./% |
|---|---|---|---|---|
|  | **1** | (-)-(R)-**5** | 99.5 (1.5) | 99 |
|  | **2** | (-)-(R)-**6** | 10 (0.0) | 96 |
|  | **3** | (-)-(R)-**7** | 9 (0.4) | 95 |
|  | **4** | **8** | 24 (15) | 3 |

a. Values within parentheses correspond to yields obtained in the absence of CPO

No sulfone was detected as product and the R-sulfoxide was the dominating enantiomer in the oxidation of **1-3**. The resolution achieved in the GC analysis is illustrated by Figure 1. The absolute configuration of (-)-**7** was determined to be R- by comparison of its CD-spectrum with those of (-)-(R)-**5**[7] and (-)-(R)-**6**,[7b] Figure 2. Due to the low e.e. of **8**, the sign of the optical rotation of the favoured enantiomer could not be determined.

The high e.e. suggested **2** to be a good substrate to CPO, although the sulfoxide was obtained in low yield. The reaction conditions were systematically altered in an attempt to clarify if any external factors caused this low yield, Table 2. Compound **2** showed low solubility in aqueous buffer, but addition of co-solvents[2b,8] did not enhance the yield. Acetone instead showed a negative effect on the reaction.[2b] Neither did increased temperature or prolonged reaction time alter the outcome of the reaction. The high e.e. shown in Table 2 (entry no 8) after 22 h is most likely a result from the catalase activity which rapidly decreases the $H_2O_2$ concentration in the CPO-catalyzed reaction but not in the reference reaction without CPO. The competition from the uncatalyzed reaction path should therefore always be less than what is found from the reference. To suppress catalase activity, $H_2O_2$ was kept at a lower concentration through slower addition, but with no significant success (entry no 9). With higher concentration of $H_2O_2$ the uncatalyzed reaction became more pronounced, resulting in a decreased e.e. (entry no 10). A change of oxygen source to t-BuOOH gave lower yield and e.e. (entry no 11). A prolonged reaction time increased the yield, which probably is due to an increased competition by the uncatalyzed oxidation[2a-c] in this case where no catalase activity acts on the oxidant.

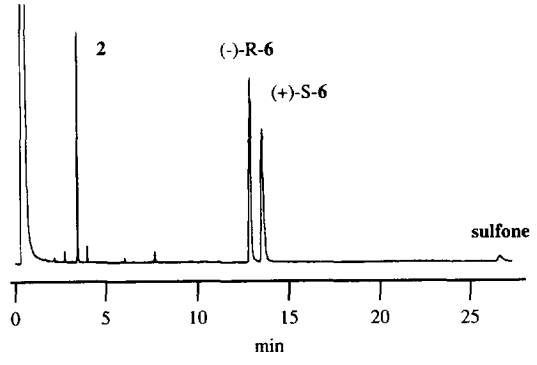


Fig. 1. Chromatogram[9d] of **2**, **6** and the corresponding sulfone used in evaluation of results from the oxidation reactions

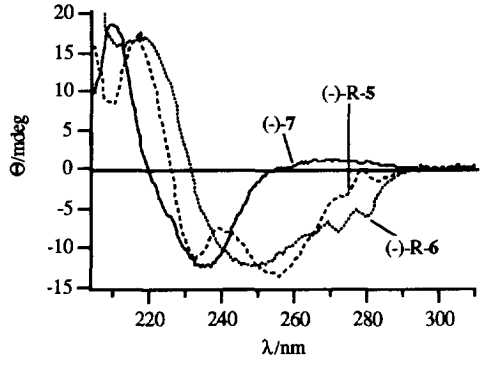


Fig. 2. CD-spectra of (-)-(R)-**5**, (-)-(R)-**6** and (-)-**7** in ethanol

In conclusion, this work shows that asymmetric oxidation of rigid bicyclic sulfides can be catalyzed by CPO to give (R)-sulfoxides in high enantiomeric excess, considerably higher than, for example, structurally related ortho-substituted thioanisoles.[2a-c] The results demonstrate an increased applicability of CPO, since previously only lower enantioselectivity has been reported for asymmetric syntheses of **5**,[7b,10] **6**[7b,10,11] and **7**.[12]

**Table 2.** Enantiomeric excesses and chemical yields in CPO catalyzed oxidation of **2** under specified conditions at pH 5.0

| Entry no. | Oxidant | Time | Temp./°C | co-solvent | Yield/% | E.e./% | Yield[a]/% |
|---|---|---|---|---|---|---|---|
| 1 | $H_2O_2$ | 65 min | 20 | | 10.0 | 96.0 | 0.0 |
| 2 | " | " | 25 | | 8.8 | 94.5 | 0.3 |
| 3 | " | " | 30 | | 11.0 | 93.5 | 0.6 |
| 4 | " | " | 20 | 10% acetone | 3.6 | 96.3 | 0.3 |
| 5 | " | " | " | 20% acetone | 1.6 | 78.0 | 0.5 |
| 6 | " | " | " | 10% t-BuOH | 11.6 | 97.6 | 0.4 |
| 7 | " | " | " | 20% t-BuOH | 10.9 | 95.3 | 0.3 |
| 8 | $H_2O_2$[b] | 22 h | " | | 12.7 | 96.7 | 15.8 |
| 9 | $H_2O_2$[c] | 8 h | " | | 12.2 | 92.1 | 2.6 |
| 10 | $H_2O_2$[d] | 5 h | " | | 12.9 | 61.8 | 7.4 |
| 11 | t-BuOOH | 65 min | " | | 2.5 | 73.6 | 0.7 |
| 12 | t-BuOOH | 6 days | " | | 21.5 | 60.7 | 7.0 |

a. Yield obtained in the absence of CPO, b. $H_2O_2$ added during the first 55 min, c. $H_2O_2$ added in portions every 20 min, d. 10 equivalents (250 mmol) of $H_2O_2$, added in portions every 5 min.

## EXPERIMENTAL

**Instrumentation.** Chiral gas chromatography was performed with the use of a Varian mod. 3400 gas chromatograph equipped with fused silica capillary columns[9a-d] (2 ml $H_2$/min) and a Varian mod. DS 654 computer. HPLC was performed with the use of a Scientific Systems Inc. Model 200 high-pressure pump, a Rheodyne injector (100 µl loop) and a Spectra Physics 8450 UV/vis detector coupled to a Hewlett Packard mod. 3395 integrator.[13]

NMR spectra were obtained with a 400 MHz Varian VXR-400 spectrometer. Optical rotations were measured by a Perkin-Elmer 241 polarimeter in a 1 dm quartz microcell. CD spectra were recorded using a JASCO mod. 720 spectropolarimeter and a quartz cell of 2 mm pathlength. The UV spectrophotometer used was a Perkin-Elmer Lambda 11. Mass spectra were obtained with use of a VG Zab Spec instrument (EI, 70 eV). For GC-MS a HP 5890 Series II gas chromatograph equipped with a 25 m SE-30 column was used.

**Materials.** Chloroperoxidase from *Caldariomyces fumago* was obtained from Sigma as a crude suspension and was used as such. The activity was determined to 30 u/µl (RZ 1.0).[14] t-Butyl alcohol (99.7%), t-butyl hydroperoxide (3M in isooctane), mercuric chloride (99%) and benzene-1,2-dithiol (97%) were from Fluka. Sodium metaperiodate (99.8%) was from Merck. Acetone (99.5%) and $H_2O_2$ (30% (w/w)) were obtained from Riedel-de Haën. 1-Benzothiophene (95%), thiochroman-4-one (97%) and diiodomethane (99%) were available from Aldrich. Ethanol was of spectroscopic grade, HPLC solvents were of HPLC quality (Fisons) and other solvents used were of p.a. quality.

**Syntheses.** Compound **1** was obtained by sodium reduction of benzothiophene.[15] The crude product was purified through recrystallization of the $HgCl_2$-complex of **1**, which removed excess of starting material. The product obtained (15%) consisted of 99.7% **1** (GC[9a]). NMR: δ($CDCl_3$)= 3.30 (2H, d), 3.36 (2H, 2d), 7.02 (1H, t), 7.12 (1H, t), 7.20 (1H, d), 7.23 (1H, d).

Oxidation of **1** to **5** was carried out with $NaIO_4$ in a water/ethanol mixture.[7b] The product (32%) contained 93% of **5** and 7% of the corresponding sulfone according to GC.[9a] The presence of sulfone was indicated by IR, which in addition to a strong band at 1025 $cm^{-1}$ (SO str.) also showed two bands at 1130 $cm^{-1}$ and 1300 $cm^{-1}$ (symm. and antisymm. $SO_2$ str.). MS: Calculated for $C_8H_8OS$: 152.030 Found: 152.034. NMR: δ($CDCl_3$)= 3.26-3.40 (3H, m), 3.86 (1H, m), 7.44 (1H, t), 7.49 (1H, t), 7.52 (1H, d), 7.85 (1H, d).

Compound **2** was obtained from **3** by Clemmensen reduction[15,16a] using amalgamated zinc (83% (w/w) in Zn)[16b] in toluene and 22% HCl. The product (91%) was 98.8% in **2** (GC[9a]). NMR: δ($CDCl_3$)= 2.11 (2H, p), 2.82 (2H, t), 3.04 (2H, m), 6.96-7.11 (4H, m). For comparison, a Wolff-Kishner reduction of **3** gave, after purification with flash chromatography, only 8% of a product containing 84% of **2**.[9b]

Oxidation of **2** to **6** with $NaIO_4$ was performed in presence of t-butanol as co-solvent, to increase the solubility. After work-up the product (72%) contained 99.5% **6** (GC[9b]). NMR: δ($CDCl_3$)= 2.85 (1H, ddd), 3.00 (1H, ddd), 3.07 (1H, dt), 3.17 (1H, ddd), 7.23 (1H, d), 7.35 (1H, t), 7.41 (1H, t), 7.72 (1H, d).

Sulfide **3** was oxidized to **7** with $NaIO_4$ in a 60% yield and the product contained 95% **7** and 5% of the corresponding sulfone (GC[9c]). NMR: δ($CDCl_3$)= 2.94 (1H, m), 3.50 (3H, m), 7.69 (1H, t), 7.80 (1H, t), 7.91 (1H, d), 8.20 (1H, d).

Compound **4** was synthesized from benzene-1,2-dithiol, diiodomethane and sodium ethoxide[16b] in dry ethanol under argon atmosphere.[17] The product obtained (65%) contained 99.2% **4** (GC[9b]) after Kugelrohr distillation under vaccum (ca. 110°C/1 mmHg). NMR: δ($CDCl_3$)= 4.48 (2H, s), 7.00 (2H, dd), 7.21 (2H,dd). GC-MS: Calculated for $C_7H_6S_2$: 153.991 Found: 152.969 (M-1, loss of a hydrogen yields a highly resonance stabilized carbocation). The characteristic isotope pattern from sulfur verified the identification.

Compound was oxidized with $NaIO_4$ and pure **8** (GC[9b]) was obtained (46% yield).[18a] NMR: δ($CDCl_3$)= 4.19 (1H, d), 4.34 (1H, d), 7.31 (1H, t), 7.49-7.53 (2H, m), 7.90 (1H, d). GC-MS: Calculated for $C_7H_6OS_2$: 169.986 Found: 169.975.

Higher oxidation products of **4** were prepared through oxidation with peracetic acid and used as references. The reactions were followed by GC[9c] and the products were identified by GC-MS. The enantiomers of *trans*-1,3-dioxide **9** and 1,1,3-trioxide **10** were both baseline separated in the chromato-grams and no *meso*-**9** was detected during synthesis.[18b] Extraction between water and diethyl ether was used as work-up procedure. The main part of *trans*-**9** and **10** was observed in the aqueous phase, explained by their high polarity. The disulfone **11**, on the other hand, was recovered from the ether phase. *Trans*-**9** was further purified (98.5%) by flash chromatography (silica gel, acetone). GC-MS: Calculated for $C_7H_6O_2S_2$: 185.981 Found: 185.963; Calculated for $C_7H_6O_3S_2$: 201.967 Found: 201.976; Calculated for $C_7H_6O_4S_2$: 217.971 Found: 217.952.

**Enzymatic oxidation.** The sulfide (25 μmol) and CPO (30 u) were magnetically stirred in 2.7 ml 0.1 M citrate buffer, pH 5.0. The mixture was kept at ambient temperature, 20°C, or thermostated to the specified temperature. $H_2O_2$ (50 μmol) was added in portions (each 25 μl of a 0.163 M solution) every 5 min for 55 min. The reaction was quenched after 65 min with a saturated $Na_2SO_3$ solution and extracted with dichloromethane (2x2 ml). The organic phase was dried ($MgSO_4$) and filtered. Oxidation under the same conditions was performed in the absence of CPO for comparison.

**Determination of product yield and enantiomeric excess.** The product composition from the enzymatic oxidation was determined by chiral GC. Racemic sulfoxides with traces of sulfones were synthesized as references. Both chemical yields and enantiomeric excesses were determined in a single chromatogram for the enzymatic oxidations of **1**,[9d] **2**,[9c-d] **3**[9d] and **4**,[9d] respectively. The organic phase was analyzed directly, as evaporation was shown to cause a change in the product composition.

**Determination of absolute configuration.** The sign of optical rotation was determined to be (-) at 589 nm for the enzymatic oxidation product of **1** (acetone),[7a] **2** (acetone),[7b] and **3** ($CH_2Cl_2$), but in case of **4** the e.e. was too low to permit any determination of sign. The absolute configuration of (-)-**7** was determined from a comparison with the CD-spectra of (-)-**5** and (-)-**6** in ethanol. Purification of the sulfoxides was performed by semi-preparative HPLC[13] and the purity ascertained by GC before CD-spectra were recorded.

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9. (a) 10 m DB-5 column (J&W Scientific); (b) 5 m oktakis-(3-O-butyryl-2,6-di-O-pentyl)-γ-cyclodextrin (Lipodex E) obtained from Prof. W. König, University of Hamburg; (c) 25 m Lipodex E[9b]; (d) 10 m heptakis (6-O-tert-butyldimethylsilyl-2,3-O-acetyl)-β-cyclodextrin obtained from Prof. König.

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