Pergamon

*Tetrahedron: Asymmetry* Vol. 6, No. 2, pp. 603-608, 1995
Elsevier Science Ltd
Printed in Great Britain
0957-4166/95 $9.50+0.00

0957-4166(95)00044-5

# Enzyme-Catalyzed Kinetic Resolution of Racemic Secondary Hydroperoxides

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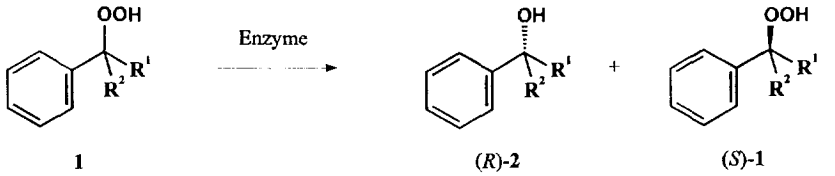
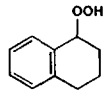
***Abstract:*** The kinetic resolution of 1,2,3,4-tetrahydro[1]naphthyl and other secondary and tertiary hydroperoxides has been investigated by acylation with isopropenyl acetate in the presence of various lipases, by chloroperoxidase-catalyzed asymmetric oxidation of sulfides, and by horseradish peroxidase (HRP)-catalyzed reduction in the presence of guaiacol. For preparative purposes, the latter method is the best one and allows for the first time the isolation and characterization of the enantiomerically pure (*S*)-1,2,3,4-tetrahydro[1]naphthyl hydroperoxide. Tertiary hydroperoxides are not accepted as substrates by HRP.

The preparation of optically active, chiral hydroperoxides as potential stereoselective oxidizing reagents is of interest for asymmetric synthesis. The direct preparation by reaction of optically active alcohols or their derivatives with hydrogen peroxide proceeds with inversion of configuration, unfortunately accompanied by a high degree of racemization.[1] Thus, the reaction of optically active 1,2,3,4-tetrahydro[1]naphthol or its hydrogen phthalate ester gave only a racemic hydroperoxide.[2] In the 1950's, the latter was partly resolved by asymmetric adsorption on right-handed quartz,[3] but the result was difficult to reproduce.[4] The kinetic resolution of tertiary hydroperoxides by the Sharpless epoxidation of allylic alcohols [5] gave only low e.e. values. Besides the analytical separation of the enantiomers on a chiral column,[6] we succeeded in resolving 1,2,3,4-tetrahydro[1]naphthyl hydroperoxide (**THPO**, **1a**) [7] on the preparative scale.

Recently, the use of enzymes for the kinetic resolution of hydroperoxides in organic solvents has been demonstrated. Baba *et al.* [8] resolved 1-phenylethyl hydroperoxide by acylation with isopropenyl acetate in the presence of a lipase. The chloroperoxidase-catalyzed asymmetric oxidation of sulfides by 1-phenylethyl hydroperoxide afforded under kinetic resolution conditions the hydroperoxide in high enantiomeric excess.[9] Subsequently, we have reported [10] on the kinetic resolution of secondary hydroperoxides by horseradish peroxidase in the presence of guaiacol. Here we present our results on the kinetic resolution of 1,2,3,4-tetrahydro[1]naphthyl hydroperoxide and other aralkyl hydroperoxides **1b-h** (Scheme 1) by three different enzymatic methods.




**1** (*R*)-**2** (*S*)-**1**

a: 

b: $R^1 = Me$ ; $R^2 = H$ c: $R^1 = Et$ ; $R^2 = H$

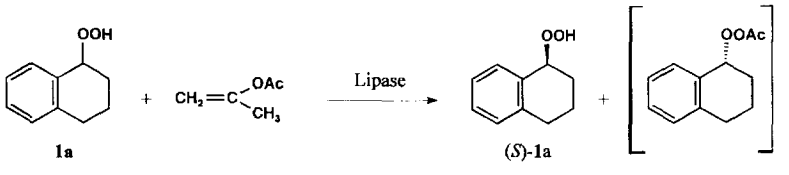
d: $R^1 = Pr$ ; $R^2 = H$ e: $R^1 = iPr$ ; $R^2 = H$ f: $R^1 = Et$ ; $R^2 = Me$

g: $R^1$ = Cyclohexyl ; $R^2 = Me$ h: $R^1 = CH_2OH$ ; $R^2 = Me$

**Scheme 1**

*Kinetic Resolution of* ***THPO (1a)*** *by Lipase-Catalyzed Acylation*

The use of lipase from *Pseudomonas fluorescens* (LPL Amano P) for the enzymatic resolution of racemic aralkyl hydroperoxides has been described and the optical activity of the remaining hydroperoxide was demonstrated by HPLC analysis after reduction with $LiAlH_4$.[8] We determined the ratio of the enantiomers directly after the reaction of hydroperoxide **1a** with isopropenyl acetate in toluene at room temperature in the presence of lipases (Scheme 2) by an earlier HPLC method.[6] The results are summarized in Table 1. The




**1a** (*S*)-**1a**

**Scheme 2**

lipases examined (entries 1-3) show different activity, but even at high conversion (entry 1) only a low e.e. value was obtained for the remaining hydroperoxide. More favorable results were obtained with pancreatin. After consumption of nearly 50% of the starting **THPO** (entry 4), the e.e. amounted only to 42% . However, the desired high e.e. (> 90%) was obtained at a high conversion, which permitted the isolation of the pure (*S*) enantiomer of **1a** (entry 5) but in very low yield. The configuration of the enantiomer was determined by reduction of the hydroperoxide with $Na_2SO_3$ to the corresponding alcohol and comparison with an authentic sample.

**Table 1.** Lipase-Catalyzed Resolution of **THPO (1a)** by Acylation with Isopropenyl Acetat in Toluene at Room Temperature.

| Entry | Lipase | Time (h) | Conversion (%)[a] | e.e. (%) |
|---|---|---|---|---|
| 1 | Lipase PS[b] | 96 | 83 | 37 |
| 2 | Lipase AY[b] | 138 | 55 | 22 |
| 3 | Lipase M[b] | 120 | 29 | 5 |
| 4 | Pancreatin[c] | 72 | 55 | 42 |
| 5[d] | Pancreatin | 120 | 85 | 96 |

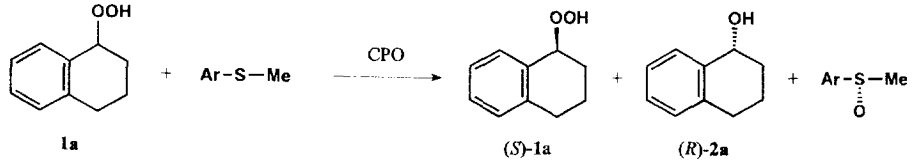
[a] Conversion of hydroperoxide 1a. [b] From Amano Enzyme Europe Ltd. [c] From Biochemie Bernd Belger, Kleinmachnow, Germany. [d] (*S*)-**1a** isolated: e.e. > 99%, m.p. 48-50°C, $[\alpha]_D^{20}$-19.2 (c 1, MeOH).

Change of the reaction conditons by variation of the acylating agent (vinyl acetate, acetic anhydride) did not increase the e.e. at comparable conversion. By using THF, MTBE or t-BuOH as solvents, only in the case of MTBE a similar degree of conversion was observed, but the e.e. value was lower compared to toluene. With the tertiary 1-methyl-1-phenylpropyl hydroperoxide (**1f**) or 1-cyclohexyl-1-phenylethyl hydroperoxide (**1g**) no reaction was observed, which confirms the previous results.[8]

*Chloroperoxidase-Catalyzed Oxidation of Aryl Methyl Sulfides with Racemic Hydroperoxides*

Wong *et al.*[9] described the use of chloroperoxidase (CPO) from *Caldariomyces fumago* for the oxidation of sulfides, in which the chiral hydroperoxides were resolved into the corresponding (*R*) alcohol and the unchanged (*S*) hydroperoxide. In the case of **1a**, they did not observe enantioselective sulfoxidation or hydroperoxide resolution.

We investigated this reaction with **THPO** and other hydroperoxides to assess the scope of this enzymatic transformation for the preparation of optically active hydroperoxides (Scheme 3). The reaction conditions




**Scheme 3**

reported by Wong *et al.*[9] were used in the case of 1-phenylethyl hydroperoxide (**1b**). The reaction of the other racemic hydroperoxides was carried out at room temperature because at 4 °C the reaction rate was too low.

**Table 2.** CPO-Catalyzed Oxidation of Aryl Methyl Sulfides by Racemic Hydroperoxides

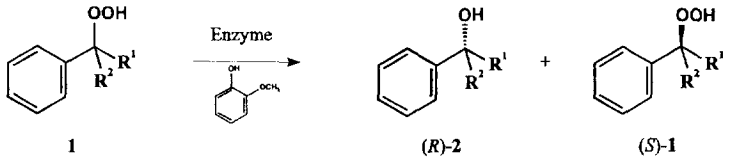
| Entry | Hydroperoxide | Sulfide | e.e. (%) (*R*)-Sulfoxide[a] | e.e. (%) (*S*)-**1** |
|---|---|---|---|---|
| 1 | **1a** | Phenyl Methyl | 64 | 4 |
| 2 | **1a** | *p*-Tolyl Methyl | 38 | 4 |
| 3 | **1b** | Phenyl Methyl | 92 | > 99 |
| 4 | **1b** | *p*-Tolyl Methyl | 90 | > 99 |
| 5 | **1f** | Phenyl Methyl | 38 | 4 |
| 6 | **1f** | *p*-Tolyl Methyl | 18 | 0 |

[a] At 50% conversion

The results in Table 2 show that the oxidation to the sulfide and the simultaneous resolution of the hydroperoxide proceeded in high enantioselectivity exclusively with 1-phenylethyl hydroperoxide (**1b**), as shown in Table 2 (entries 3 and 4). For hydroperoxide **1a** (entries 1 and 2), the stereoselectivity of the sulfoxidation was moderate (in contrast to Wong *et al.*[9] we observed with **THPO** considerable selectivity); however, no kinetic resolution of the hydroperoxides was observed. In the case of the tertiary hydroperoxide **1f** (entries 5 and 6) low e.e. values were obtained. Unfortunately, the CPO-catalyzed preparation of optically active (*S*)-1-phenylethyl hydroperoxide (**1b**) cannot be used on a larger scale, since low concentrations of the substrates must be used.

*Horseradish Peroxidase-Catalyzed Reduction of Racemic Hydroperoxides*

Recently we have reported[10] on the horseradish peroxidase-catalyzed kinetic resolution of racemic hydroperoxides by enantioselective reduction in the presence of guaiacol. In order to assess the substrate selectivity of HRP, we have studied the HRP-catalyzed reduction of hydroperoxide **1a** and other aralkyl hydroperoxides (Scheme 4). The reactions were carried out as previously reported.[10]




**1** (*R*)-**2** (*S*)-**1**

**Scheme 4**

In Table 3 are given reaction times, chemical yields, absolute configurations, and the e.e. values. It is evident that the steric demand of the substituents decisively influences both yield and enantioselectivity. In contrast to the 1-phenylethyl (**1b,** entry 3) and 1-phenylpropyl (**1c**, entry 4) hydroperoxides, 1-phenylbutyl and 2-methyl-1-phenylpropyl derivatives **1d** and **1e** (entries 5,6) could not be resolved. On the other hand, hydroperoxide **1a,** which is bicyclic, was enantioselectively reduced to the corresponding alcohol (*R*)-**2a,** so that the hydroperoxide (*S*)-**1a** was obtained in optically pure form (entry 1). Thus, HRP-catalyzed kinetic resolution of **THPO** is a convenient method for the preparation of optically pure **THPO**, not only in analytical but also in preparative amounts (entry 2).

**Table 3.** HRP-Catalyzed Reduction of Alkaryl Hydroperoxides in the Presence of Guaiacol.[a]

| Entry | Hydroperoxide[b] | | 1 : HRP | Time | Conversion[c] | e.e. | (%) |
|---|---|---|---|---|---|---|---|
| | (mmol) | | (mmol) | (h) | (%) | (*S*)-**1** | (*R*)-**2** |
| 1 | **1a** | (0.06) | 2400 : 1 | 3 | 50 | > 95 | 97 |
| 2 | **1a**[d] | (6.00) | 6000 : 1 | 2 | 68 | 95 | 82 |
| 3 | **1b**[e] | (0.03) | 12000 : 1 | 0.05 | 50 | > 99 | > 99 |
| 4 | **1c** | (0.06) | 6000 : 1 | 2.5 | 50 | 93 | 95 |
| 5 | **1d** | (0.06) | 2400 : 1 | 1.5 | 50 | 0 | f |
| 6 | **1e** | (0.06) | 480 : 1 | 3 | 50 | 15 | 14 |
| 7 | **1g** | (0.04) | 160 : 1 | 24 | 7 | < 5 | < 5 |
| 8 | **1g** | (0.04) | 80 : 1 | 24 | 8 | < 5 | < 5 |
| 9 | **1h** | (0.06) | 360 : 1 | 36 | 8 | 5 | > 90 |
| 10 | **1h** | (0.06) | 80 : 1 | 30 | 11 | 9 | > 90 |

[a] Guaiacol was used in equimolar amounts with respect to **1**. [b] The hydroperoxides were prepared according to literature procedure.[1] [c] Photometrically determined according to literature procedure (Ref. 11). [d] For the procedure *cf.* Experimental. [e] Reported in Ref. 10. [f] Not determined.

After this successful kinetic resolution of secondary hydroperoxides, we attempted the HRP-catalyzed reduction of tertiary hydroperoxides in order to assess whether such bulkier substrates can also be resolved by this method. HRP-catalyzed reaction of 1-cyclohexyl-1-phenylethyl hydroperoxide (**1g**) and the hydroxy-functionalized 2-(1-hydroxy-2-phenyl)propyl hydroperoxide (**1h**) in the presence of guaiacol were studied. The conversion rate of both derivatives was low even after long reaction times and addition of more enzyme (entries 8 and 10). The corresponding alcohol of the hydroxy-functionalized hydroperoxide **1h** was obtained in enantiomerically enriched form (entry 10), whereas the sterically more hindered hydroperoxide **1g** yielded racemic alcohol. Thus, the tertiary hydroperoxide **1h**, was reduced enantioselectively; however, due to the very low conversion, this hydroperoxide was not accessible in significant optical activity by such an enzymatic reduction.

In conclusion, we have obtained for the first time the pure (*S*) enantiomer of **THPO** (**1a**) by HRP-catalyzed kinetic resolution, which is not accessible in preparative amounts by lipase- and CPO-catalyzed methods. Unfortunately, attempts to resolve tertiary hydroperoxides by the HRP-catalyzed reduction have failed because the peroxidase does not accept such sterically encumbered substrates.

## Experimental

**Materials and Methods:** Horseradish peroxidase was purchased from Sigma (RZ 2.0) and used as received. The hydroperoxides were prepared by a modified literature procedure.[1] Enantiomeric excess for the hydroperoxides and alcohols were determined with HPLC by using a Chiralcel OD column (0.46 x 25 cm) and a photodiodearray detector; eluent: n-hexane/isopropyl alcohol (90:10), flow rate 0.6 ml/min.

**General Procedure for the Lipase-Catalyzed Acylation of THPO:** To a solution of THPO (328 mg, 2 mmol) and isopropenyl acetate (150 mg, 1.5 mmol) in an organic solvent (10 ml) was added lipase (0.5-1g) and the mixture was stirred at room temperature for 72-138 h. After filtration of the lipase, the conversion of the THPO was determined by iodometric titration, the filtrate was concentrated and the enantiomeric excess of the remaining THPO was determined by HPLC method as described above.

**General Procedure for the CPO-Catalyzed Oxidation of Aryl Methyl Sulfides with Racemic Hydroperoxides:** A sample of 100 µl CPO (600 U, Sigma) was dissolved in 40 ml of 0.05 M citrate buffer (pH 5) and the mixture was stirred at room temperature for 5 min. Aryl methyl sulfide (0.02 mmol) was added and the mixture was stirred for another 10 min. To this solution was added the racemic hydroperoxide (0.02 mmol) and the reaction progress was monitored by measuring the disappearance of the sulfide at $\lambda$ = 250 nm on a Shimatzu UV-2102 PC spectrophotometer. The reaction was stopped at ca. 50% conversion and the mixture was extracted with $CH_2Cl_2$ (3 x 30 ml).The organic layer was dried over $Na_2SO_4$ and the solvent was removed under reduced pressure. Enantiomeric excess for the hydroperoxides and the sulfoxides were determined by HPLC.

**General Procedure for the Analytical Scale Resolution of Hydroperoxides with HRP:** Hydroperoxide (0.03 - 0.06 mmol) and guaiacol (0.03 - 0.06 mmol) were dissolved in 2 ml 0.1 M phosphate buffer (pH 6) and subsequently HRP (0.25 - 1.25 x $10^{-4}$ mmol) was added. The reaction mixture was allowed to stand at 20 °C for 0.05-36 h and then extracted with $CH_2Cl_2$ (3 x 2 ml). The combined organic phases were dried over $Na_2SO_4$ and the solvent was removed (ca. 20 °C/17 Torr). The enantiomeric excess of the hydroperoxides and alcohols was determined with the HPLC method as described above.

**General Procedure for the Preparative Scale Resolution of Hydroperoxide 1a with HRP:** A suspension of THPO (986 mg, 6.00 mmol), guaiacol (745 mg, 6.00 mmol), HRP (40 mg) and 40 ml of phosphate buffer (pH 6) was stirred at room temperature for 2 h. The mixture was extracted with $CH_2Cl_2$ (3 x 10 ml) and the combined organic phases were dried over $Na_2SO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, 0.063-0.200 mm, 1:1 hexane-ethyl acetate). The THPO-containing fractions were further purified by flash chromatography (silica gel, 0.032-0.062 mm, 8:2 hexane-ethyl acetate) to yield 75.0 mg (15%) of (*S*)-**1a** as colorless crystals after recrystallization from hexane; m.p. 48-50 °C, $[\alpha]_D^{20}$ -21.4 (c 1, MeOH), e.e. >99% ( determined by the HPLC method as described above).

**Acknowledgement.** This work was supported by the *Deutsche Forschungsgemeinschaft* (Schwerpunktprogramm "Peroxidchemie: Mechanistische und präparative Aspekte des Sauerstofftransfers" and Sonderforschungsbereich 347 " Selektive Reaktionen metallaktivierter Moleküle) and the *Fonds der Chemischen Industrie*. E. H. thanks the *Amano Enzyme Europe Ltd.* for generous gifts of enzyme samples.

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(*Received in UK* 19 *December* 1994)