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Kinetic Resolution of Chiral α-Hydroperoxy Esters by Horseradish Peroxidase-Catalyzed Enantioselective Reduction to α-Hydroxy Esters

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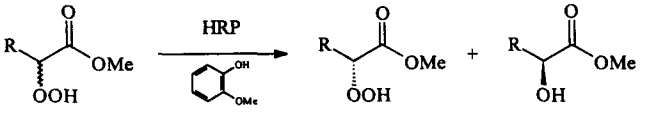
Abstract: The kinetic resolution of the methyl α-hydroperoxy esters **4** has been investigated by horseradish peroxidase (HRP)-catalyzed reduction to the corresponding optically active α-hydroxy esters **5** in the presence of guaiacol. The method allows for the first time the preparation of enantiomerically pure (*ee* > 97%) methyl (*R*)-2-hydroperoxybutyrate (**4a**). The HRP enzyme is sensitive to the steric demand of the ester alkyl side chain, as manifested by ethyl (*ee* > 97%) and isopropyl (*ee* 79%), while the *tert*-butyl derivative is not accepted by the enzyme (no reduction).

The optically active α-hydroxy ester structural unit is widespread in natural products and has during the last years been frequently used as a convenient building block in organic synthesis.[1] Consequently, efficient methods for the construction of enantiomerically pure α-hydroxy esters are in demand.[2] Although the related α-hydroperoxy ester functionality is also found in naturally occuring compounds [3,4], no synthesis of optically active α-hydroperoxy esters is known to date. To bridge this gap, we report herewith an effective enzymatic kinetic resolution of racemic α-hydroperoxy esters (Eq. 1).

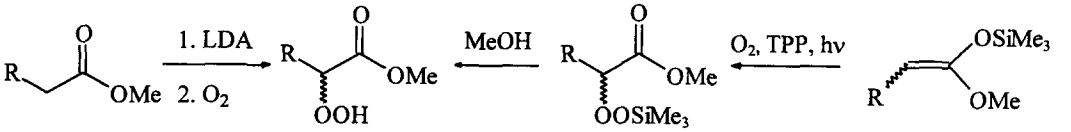


(Eq. 1)

The development of enzyme-assisted asymmetric reactions for the preparation of optically active compounds is currently under intensive investigation in organic chemistry.[5] Thus, horseradish peroxidase (HRP) and chloroperoxidase (CPO) have been successfully employed for oxidation purposes.[6] For example, in the CPO-catalyzed oxidation of prochiral sulfides with racemic 1-arylethyl hydroperoxides, the (*R*) sulfoxides (*ee* 13-99%) as well as the (*S*) hydroperoxides (*ee* 24-91%) and the corresponding (*R*) alcohols (*ee* 17-80%) were isolated in optically active form.[7]

Recently we have reported that HRP/guaiacol catalyzes the kinetic resolution of chiral secondary hydroperoxides.[8] The reduced alcohol as well as the remaining hydroperoxide were obtained in high optical purity (*ee* > 95%). This novel horseradish peroxidase-catalyzed reduction methodology was presently employed for the kinetic resolution of α-hydroperoxy esters.

The α-hydroperoxy esters **4a-c** were prepared either by the photooxygenation of silyl ketene acetals and subsequent desilylation [9] or by the autoxidation of lithium ester enolates [10] (Scheme 1). The unknown derivatives **4a,b** were fully characterized. [11]

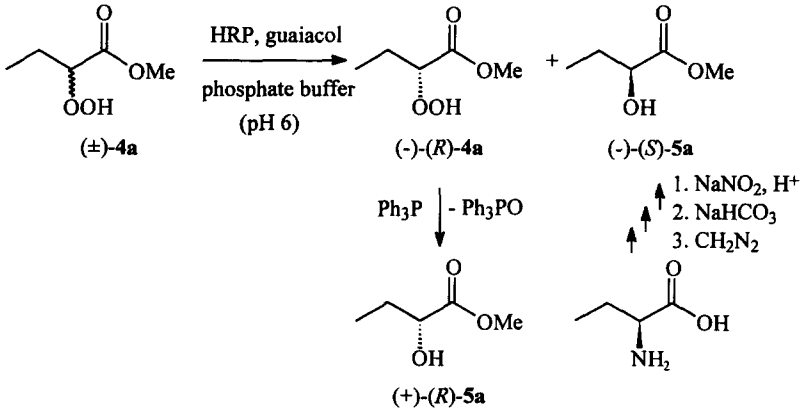


1 **4** **3** **2**

1 - 4	a	b	c
R	Et	*i*Pr	*t*Bu

Scheme 1: Synthesis of the α-Hydroperoxy Esters **4a-c**

The kinetic resolution of the racemic α-hydroperoxy ester **4a** by enantioselective reduction to α-hydroxy ester **5a**, catalyzed with HRP (Sigma, Type II, RZ 1.5-2.0) in the presence of guaiacol (Scheme 2), gave both products in high (*ee* > 97%) optical purity (Table 1). However, the *ee* values of the products



(±)-**4a** (-)-(*R*)-**4a** (-)-(*S*)-**5a**

(+)-(*R*)-**5a**

Scheme 2: HRP-Catalyzed Kinetic Resolution of Chiral α-Hydroperoxy Ester **4a**

Table 1: HRP-Catalyzed Resolution of Chiral α-Hydroperoxy Esters **4a-c** in the Presence of Guaiacol. [a]

	α-Hydroperoxy Ester	Conversion [b]	*ee* (%)	
Entry	(±)-**4**	(%)	(-)-(*R*)-**4**	(-)-(*S*)-**5**
1	**a**: R = Et	50	97	97
2	**b**: R = *i*Pr	50	79	64
3	**c**: R = *t*Bu	0 [c]	--	--

[a] Guaiacol was used in equimolar amounts with respect to α-hydroperoxy ester **4**, the molar ratio of (±)-**4** : HRP was 12000:1, reaction scale was between 0.1 and 3.0 mmol. [b] Photometrically determined according to literature.[8a,12] [c] Even after 24 h no reaction.

derived from the kinetic resolution of the α-hydroperoxy ester **4b** were significantly lower, while the α-hydroperoxy ester **4c** did not react at all with HRP/guaiacol.

To obtain optimal substrate concentrations for the enzymatic transformations of the racemic α-hydroperoxy esters **4a,b**, the kinetic parameters were determined (Table 2). The data reveal that at constant

Table 2: Kinetic Parameters [a] for the Horseradish Peroxidase-Catalyzed Reduction of α-Hydroperoxy Esters (±)-**4a,b** in the Presence of Guaiacol

Substrate	K_M [mmol]	V_{max} [b] [μmol min^{-1}]	k_{cat} [min^{-1}]	k_{cat}/K_M [mM^{-1} min^{-1}]
(±)-**4a**	18.6 ± 1.4	35.0 ± 1.0	1400	75.3
(±)-**4b**	60.0 ± 10	43.3 ± 3.6	1732	28.9

[a] The kinetic parameters for the α-hydroperoxy esters (±)-**4** were determined at a guaiacol concentration of 500 μM. [b] The initial rates were monitored by following the appearance of the guaiacol oxidation product ($\varepsilon = 2.66 \times 10^4$ M^{-1} cm^{-1}) at 470 nm in 0.1 M phosphate buffer (pH 6).[13] V_{max} und K_M were calculated by using the Duggleby program.

guaiacol concentration, the enzyme parameters K_M and V_{max} depend on the R substituent. Moreover, fortunately the α-hydroperoxy esters **4a-c** do not react at room temperature with guaiacol in the absence of HRP and the enzyme-catalyzed kinetic resolution could be conducted between 15 and 20 °C. The stoichiometric ratio of 1 : 1 for the α-hydroperoxy esters **4** and guaiacol was chosen in order to limit the α-hydroperoxy ester consumption to 50% and thereby avoid overreduction.

The absolute configuration of the α-hydroperoxy ester (-)-(*R*)-**4a** and the corresponding α-hydroxy ester (-)-(*S*)-**5a** was determined by synthesis of an authentic sample of (-)-(*S*)-**5a** from (+)-(*S*)-α-aminobutyric acid as starting material according to the literature (Scheme 2).[14] The absolute configuration of the α-hydroperoxy esters (-)-(*R*)-**4a,b** were assigned after triphenylphosphine reduction (retention of the configuration [15]) to the α-hydroxy ester (+)-(*R*)-**5** (Scheme 2). The enantiomeric excesses of the α-hydroperoxy esters (-)-(*R*)-**4a,b** and the corresponding α-hydroxy esters (-)-(*S*)-**5a,b** were determined from the ratio of the areas corresponding to the two enantiomers in the HPLC chromatogram (Chiralcel OD column, 250 x 4 mm; eluent: *n*-hexane/isopropyl alcohol (90:10); flow rate: 0.6 ml/min). The measured *ee* values and the established configurations manifest that the HRP enzyme recognizes preferentially the (+)-(*S*)-α-hydroperoxy ester **4a,b**. Consequently, the kinetic resolution affords the optically active (-)-(*R*)-α-hydroperoxy esters **4a,b** and (-)-(*S*)-α-hydroxy esters **5a,b**. This enantioselectivity of the HRP for the chiral oxidant **4** is in contrast to that observed for 1-phenylalkyl hydroperoxides [8], for which HRP recognizes the (*R*) enantiomer. Presumably such chiral recognition is not only due to the steric demand of the substrate, but also electronic factors through weak bonding between the substrate ester functionality and the amino acid functionalities at the active site of the

enzyme appear to play a role. This is a feature which deserves further probing by judicious design of appropriate chiral hydroperoxides with polar functional groups and substituents with different steric demand.

The reactions were performed also on a semipreparative scale (3 mmol). After conventional workup and purification by column chromatography, the labile optically active α-hydroperoxy esters **4a,b** were isolated in yields up to 58% and the corresponding α-hydroxy esters **5a,b** up to 69%.

In summary, horseradish peroxidase recognizes highly selectively sterically unencumbered (*S*)-α-hydroperoxy esters **4**, which allows kinetic resolution of racemic mixtures by means of enantioselective reduction to furnish the corresponding α-hydroxy esters **5**. The enzyme-catalyzed reduction provides for the first time optically active α-hydroperoxy esters on the semipreparative scale.

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11. **4a**: IR (neat) : ν = 3600-3300 cm^{-1} (OOH), 2975, 2885, 1740 (C=O), 1635, 1440, 1220, 1150, 1000, 870.- ^1H NMR (250 MHz, $CDCl_3$): δ = 0.96 (t, *J* = 7.5 Hz, 3 H), 1.75 (m, 2 H), 3.76 (s, 3 H, OMe), 4.45 (dd, *J* = 7.5 Hz, 5.2 Hz, 1 H), 9.54 (s, 1 H, OOH).- ^{13}C NMR (63 MHz, $CDCl_3$): δ = 9.9 (q), 23.7 (t), 52.4 (q, OMe), 84.8 (d), 173 (s).- $C_5H_{10}O_4$ (134.1) calc. C 44.77 H 7.51 found C 44.57 H 7.68.
    4b: IR (Film): ν = 3600-3200 cm^{-1} (OOH), 2970, 2880, 1740 (C=O), 1440, 1370, 1275, 1215, 1150, 1020.- ^1H NMR (250 MHz, $CDCl_3$): δ = 0.96 (d, *J* = 7.0 Hz, 3 H), 1.01 (d, *J* = 7.0 Hz, 3 H), 2.13 (d sept, *J* = 7.0 Hz, 5.1 Hz, 1 H,), 3.80 (s, 3 H, OMe), 4.38 (d, *J* = 5.1 Hz, 1 H), 9.21 (s, 1 H, OOH).- ^{13}C NMR (63 MHz, $CDCl_3$): δ = 18.0 (q), 18.9 (q), 30.3 (d), 52.2 (q, OMe), 88.6 (d), 172.5 (s).- $C_6H_{12}O_4$ (148.2) calc. C 48.64 H 8.16 found C 48.37 H 8.31.
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