Horseradish peroxidase (HRP)-catalysed enantioselective reduction of racemic hydroperoxy homoallylic alcohols: a novel enzymatic method for the preparation of optically active, unsaturated diols and hydroperoxy alcohols

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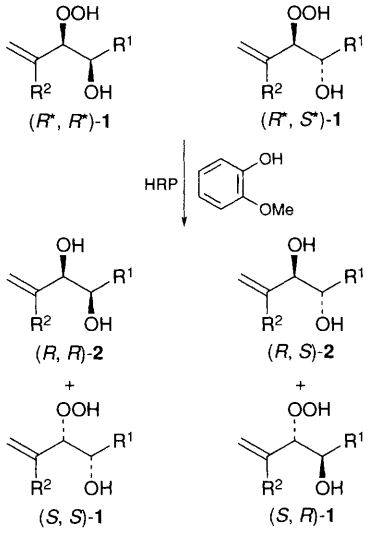
The kinetic resolution of chiral diastereoisomeric hydroperoxy homoallylic alcohols by horseradish peroxidase (HRP)-catalysed asymmetric reduction affords the optically active (*R,R*) or (*R,S*) allylic diols and (*S,S*) or (*S,R*) hydroperoxy homoallylic alcohols in high enantiomeric excess (up to 99%).

Peroxidases (E.C. 1.11.1.7) are heme-containing enzymes which catalyse the reduction of hydrogen peroxide and organic hydroperoxides in the presence of structurally diverse electron-donating substrates. Chloroperoxidase (CPO) and horseradish peroxidase (HRP) have been preferentially used as biocatalysts in asymmetric synthesis, for example, CPO catalyses the enantioselective epoxidation of unfunctionalized alkenes,[1] the stereoselective sulfoxidation of prochiral thioethers by racemic 1-arylethyl hydroperoxides[2] and regio- and stereo-selective halogenation.[3] Reports on enantioselective reactions catalysed by HRP are rather scarce; only the asymmetric sulfoxidation of prochiral sulfides[4] is known. Recently we reported the kinetic resolution of chiral alkyl aryl hydroperoxides[5] by HRP-catalysed enantioselective reduction to the corresponding alcohols. To date, the kinetic resolution of functionalized diastereoisomeric hydroperoxides with two chiral centres is still unknown. To assess the potential of the commercially available biocatalyst HRP in asymmetric synthesis, we have investigated the enzyme-catalysed reduction of chiral diastereoisomeric hydroperoxy homoallylic alcohols **1**, which are versatile intermediates for the Ti^{IV}-mediated hydroxyepoxidation.[6] The racemic hydroperoxides **1** were prepared diastereoselectively by the ene reaction of the corresponding allylic alcohols with singlet oxygen.[7] Here we report the enzymatic preparation of optically active hydroperoxy homoallylic alcohols **1** (to date no method is available for the asymmetric synthesis of such allylic hydroperoxides) and allylic diols **2** by enantioselective reduction of the former with HRP in the presence of guaiacol (Scheme 1).

Conversion rates, absolute configurations, enantiomeric excess (ee) and enantiomeric ratio (*E*) values for the HRP-catalysed kinetic resolution of hydroperoxides **1** are given in Table 1. Fortunately, hydroperoxides **1** did not react at room temperature with guaiacol in the absence of HRP; therefore, the enzymatic reactions could be conducted at room temperature (*ca.* 20 °C). The HRP enzyme exhibited excellent stereoselectivity for the reduction of the *threo* and *erythro* diastereoisomers of the racemic hydroperoxides **1**, with a high preference for the (*R*)-configuration of the hydroperoxy group. Accordingly, the kinetic resolution afforded the (*R,R*) or (*R,S*) allylic diols **2** with ee values between 84 and 99%, while the (*S,S*) and (*S,R*) hydroperoxy homoallylic alcohols were left behind nearly enantiomerically pure (ee >99%), *cf.* Table 1, entries 2–4 and 7–10.

Exceptional ee values were obtained for the hydroperoxides **1a** and **1e**. In contrast to *erythro*-**1a**, its *threo* diastereoisomer (entries 1 and 7) displayed a significantly lower enantioselectivity (ee >99% *vs.* 63%), which is also reflected in the values (30 *vs.* 10) of the enantiomeric ratios (*E*). Apparently, the *erythro* configuration of the functional groups (HOO and HO) facilitated selective binding of the (*R,S*) enantiomer at the active site of the enzyme. Moreover, steric effects on the HRP selectivity are more pronounced for the *threo* than the *erythro* diastereoisomers, as shown with derivative **1e**. Thus, methyl-branching at the α position of the double bond resulted in a significantly lower optical purity for the *threo* (ee 14%, *E* 2, entry 6) compared to the *erythro* analogue (ee 62%, *E* 14, entry 11). The unfavourable influence of branched substituents on the HRP stereoselectivity was already observed for alkyl aryl hydroperoxides;[5] however, alkyl substituents adjacent to the hydroxy group do not affect the enantioselectivity of HRP (entries 2–5 and 8–10).

To demonstrate the applicability of the enzymatic method for preparative purposes, *threo*-4-hydroperoxyhex-5-en-3-ol **1b** was reduced on semi-preparative scale. The results obtained with a 50-fold amount (400 mg, 3.03 mmol) of the hydroperoxide **1b** by using equimolar amounts of guaiacol (376 mg, 3.03 mmol) demonstrate convincingly that the HRP-catalysed kinetic resolution serves well for the preparation of the enantiomerically pure title compounds (entry 3). After conventional workup and purification by silica gel chromatography, the allylic hydroperoxides and the corresponding diols were isolated nearly optically pure in high yields (*ca.* 85% relative to 50% conversion). This implies that guaiacol oxidation products do not affect the reactivity and selectivity of this enzymatic



1, 2	a	b	c	d	e
R^1	Me	Et	Pr^i	Bu^t	Me
R^2	H	H	H	H	Me

Scheme 1

reaction even on the preparative scale. The enantioselectivity of HRP-catalysed reduction of allylic hydroperoxides was determined by employing multidimensional gas chromatography (MDGC) on chiral phases. The analytical protocol consisted of four steps: (i) extraction of the aqueous buffer solution with diethyl ether, (ii) separation of the unreacted hydroperoxides **1** from the corresponding diols **2** by normal phase HPLC, (iii) reduction of the temperature-labile allylic hydroperoxides **1** with triphenylphosphine and (iv) MDGC analysis on chiral columns. The absolute configurations of the allylic hydroperoxides **1** were assigned after triphenylphosphine reduction to the corresponding alcohols by employing the CD spectroscopic 'exciton chirality' method developed by Nakanishi *et al.*[8] The details of the configurational assignment of hydroxyfunctionalized allylic hydroperoxides **1** will be reported elsewhere.[9]

Table 1 Enantioselectivities of the HRP-catalysed kinetic resolution of hydroxy-functionalized hydroperoxides **1** in the presence of guaiacol[a]

Entry	Peroxide	ROOH : HRP (Mol ratio)	*t*/h[b]	Ee (%)[c,d]	
	threo-**1**			(*S*,*S*)-**1**[e]	(*R*,*R*)-**2**
1	**1a**	3400 : 1	3	63	67 (10)
2	**1b**	3040 : 1	3	>99	>99 (>200)
3	**1b**[f]		48	>99	93 (94)
4	**1c**	1866 : 1	3	>99	>99 (>200)
5	**1d**[g]	2500 : 1	20	91	84 (30)
6	**1e**	400 : 1	24	14	18 (2)
	erytho-**1**			(*S*,*R*)-**1**	(*R*,*S*)-**2**
7	**1a**	3200 : 1	3	>99	84 (30)
8	**1b**	3040 : 1	3	>99	89 (52)
9	**1c**	1866 : 1	3	>99	92 (79)
10	**1d**	2500 : 1	20	>99	89 (52)
11	**1e**	760 : 1	24	62	73 (14)

[a] All reactions were, unless indicated, conducted on a 0.06 mmol scale; conversion of the peroxides was 50% as determined photometrically; for the HRP reaction diastereoisomerically pure *threo*-**1** was used, while for *erythro*-**1** a 1 : 1 *threo–erythro* mixture were used. [b] Not optimized. [c] The enantiomeric excess was established by MDGC [d] Values given in parentheses are the enantiomeric ratios (*E* values, ref. 10). [e] The absolute configurations were assigned by CD spectroscopy after benzoylation of the hydroxy groups after reduction (ref. 9). [f] The reaction was conducted on a 3.03 mmol scale. [g] For HRP-catalysed reduction a 1 : 1 *threo–erythro* mixture was employed.

In summary, HRP exhibited a high enantioselectivity for (*R*,*R*) and (*R*,*S*) hydroperoxy homoallylic alcohols **1**. The enantioselectivity depends mainly on the steric demand of the substituent adjacent to the stereogenic hydroperoxy group and not on the hydroxy functionality or on the presence of additional chirality centres as established for the first time by our results. Important for synthetic applications is the fact that the enzyme-catalysed resolution can be performed on a preparative scale to provide optically active α,β-unsaturated (*S*,*S*) and (*S*,*R*) hydroxy-functionalized hydroperoxides **1**, as well as (*R*,*R*) and (*R*,*S*) diols **2**, for further asymmetric transformations, *e.g.* the $Ti(OPr^i)_4$-catalysed oxidation to optically active epoxy diols.[6] No other methods are presently available for the synthesis of optically active α,β-unsaturated hydroxy-functionalized hydroperoxides.

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