Asymmetric Synthesis with the Enzyme *Coprinus* Peroxidase: Kinetic Resolution of Chiral Hydroperoxides and Enantioselective Sulfoxidation

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Received February 2, 1999

The enzyme *Coprinus* peroxidase (CiP) was employed for the kinetic resolution of racemic hydroperoxides **1** and the asymmetric sulfoxidation of prochiral sulfides **4**. Eleven hydroperoxides **1a**−**k** were reduced by CiP and guaiacol as reductant under conditions of kinetic resolution with enantioselectivities of up to >98% for the (*S*)-hydroperoxide **1** and 90% for the (*R*)-alcohol **2**. In the absence of a reductant, the hydroperoxide **1a** afforded with CiP enantiomerically enriched hydroperoxide **1a** (ee up to 54%) and alcohol **2a** (ee up to 40%), as well as ketone **3a** (which is also formed simultaneously in all other reactions) and molecular oxygen. Catalase activity was established for CiP with hydrogen peroxide. When aryl alkyl sulfides **4** were used as oxygen acceptors, three products, sulfoxides **5**, alcohols **2,** and hydroperoxides **1,** were obtained, all in enantiomerically enriched form. The highest ee value (89%) was achieved for the sulfoxide derived from naphthyl methyl sulfide (**4f**). Thus, CiP may be utilized for the asymmetric synthesis of optically active hydroperoxides **1**, alcohols **2**, and sulfoxides **5**.

Introduction

In recent years, enzymes have increasingly been used for the synthesis of optically active compounds.[1] In particular, peroxidases have proven to be versatile biocatalysts for a number of redox transformations.[2] Among the best-known peroxidases is chloroperoxidase from *Caldariomyces fumago* (CPO), which, for example, catalyzes enantioselective epoxidations,[3] sulfoxidations,[4] and benzylic hydroxylations.[5] Also, horseradish peroxidase (HRP) has been intensively studied for the kinetic resolution of hydroperoxides.[6] In many of these reactions, good to excellent enantioselectivities have been obtained; unfortunately, this selectivity advantage comes at the dear price of limited scope of substrate acceptance by the enzyme. Moreover, many enzymes accept only one of the substrate enantiomers, such that the other antipode is usually inaccessible. However, recently we have discovered that the microorganism *Bacillus subtilis* possesses peroxidase activity in which the opposite enantiomer of the hydroperoxide is recognized as by the horseradish peroxidase.[7] This favorable finding encouraged us to screen other peroxidases for their selectivity and substrate acceptance to enable the biocatalytic synthesis of both optically active enantiomers for a wide variety of functionalized substrates.

The *Coprinus* peroxidase (CiP), which has been isolated from the basidiomycete *Coprinus cinereus*,[8] is known from its X-ray crystal structure[9] to have a rather large substrate opening and should, therefore, be a favorable candidate to accept a broad range of substrates, also sterically encumbered ones. We have applied the enzyme *Coprinus* peroxidase for the kinetic resolution of racemic hydroperoxides and the enantioselective oxidation of prochiral sulfides and report herein our results.

Results and Discussion

Kinetic Resolution of Hydroperoxides. In our efforts to provide optically enriched hydroperoxides, which may be used as oxidants in asymmetric synthesis,[10] we have shown that the kinetic resolution of racemic hydroperoxides with horseradish peroxidase (HRP) constitutes a useful way to achieve this goal.[6] Unfortunately,

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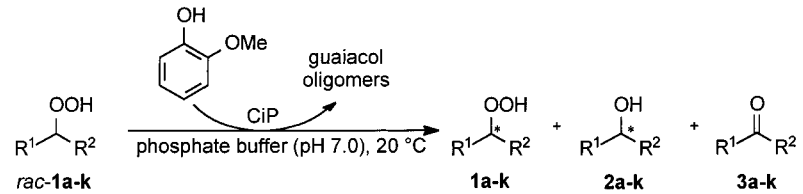
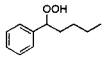
10.1021/jo990201p CCC: $18.00
Published on Web 06/06/1999

Table 1. CiP-Catalyzed Kinetic Resolution of Hydroperoxides 1 in the Presence of Guaiacol[a]



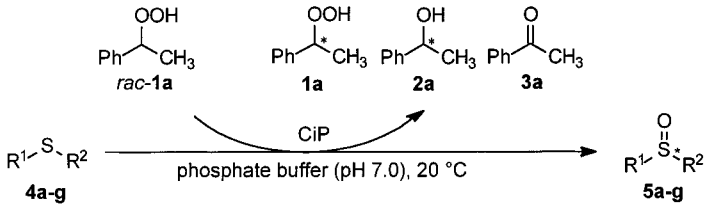
entry	hydroperoxide		ROOH:CiP (mol ratio)	t (h)	product distribution (%)[b] ROOH	ROH	R=O	enantiomeric excess (%)[c] ROOH [(*S*)-(-)]	ROH [(*R*)-(+)]
1		**(1a)**	1900:1	4	47	50	3	>98	90
2		**(1b)**	1750:1	2	50	47	3	84	92
3		**(1c)**	1650:1	20	34	41	25	56	67
4		**(1d)**	500:1	72	36	31	33	31	54
5		**(1e)**	200:1	408	37	20	43	<5	<5
6		**(1f)**	600:1	96	31	22	47	<5	15
7		**(1g)**	400:1	68	30	50	20	90 (*R*)[d]	64 (*S*)[d]
8		**(1h)**	1200:1	20	52	33	15	68 (*R*)[e]	65 (*S*)[e]
9		**(1i)**	200:1	336	-	-	>95	-	-
10		**(1j)**	500:1	144	-	-	>95	-	-
11		**(1k)**	450:1	48	83	4	13[f]	-	-

[a]The hydroperoxide was dissolved in a 1 M phosphate buffer (pH 7.0) and an equimolar amount of guaiacol was added, followed by CiP as an aqueous solution. [b]Determined from the ^{1}H-NMR spectrum of the crude product mixture. [c]Determined by HPLC anylsis on a Chiralcel OD-H or OB-H column. [d]The selectivity is opposite to all other cases. [e]The sign of the optical rotation for (*R*)-**1g** is negative and for (*S*)-**2g** positive. [f]Acetophenone.

HRP does not catalyze the kinetic resolution of sterically hindered and, particularly, tertiary hydroperoxides.[6a] For this purpose, we have employed CiP, since, as already mentioned, its X-ray crystal structure exposed a rather large opening for substrate entry.[9a] To establish the catalytic efficiency and the substrate selectivity of CiP, we examined a series of sterically and electronically varied racemic hydroperoxides **1**, with emphasis on those substrates that are not satisfactorily converted by HRP.

The results (Table 1) show that the hydroperoxide structure dramatically influences the enantioselectivity and the enantiomeric preference of the CiP enzyme. An increase of the chain length of the alkyl group in the case of the alkyl aryl hydroperoxide leads to a drastic reduction of the reactivity and selectivity. Whereas for the hydroperoxides **1a**−**c** with a methyl group good to excellent enantioselectivities were obtained at low enzyme concentration in relatively short reaction times (entries 1−3), for the substrates with sterically more demanding alkyl groups, e.g., **1d**−**f**, low or even no selectivity was observed (entries 4−6). By comparison, the variation of the aryl group does bring about significant changes in the efficiency of enantiomeric control, as exemplified by the hydroperoxides **1a**−**c** (entries 1−3), but not as pronounced as does the alkyl group in the derivatives **1d**−**f** (entries 4−6). Interestingly, the enzyme CiP shows better reactivity and selectivity toward the cyclic hydroperoxide **1g** than toward the corresponding acyclic hydroperoxide **1d** (entries 7 and 4, respectively). Apparently, the conformationally more rigid cyclic structure fits well into the enzyme pocket. Sterically more demanding hydroperoxides such as **1i**,**j** show almost no conversion to the corresponding alcohols even at large enzyme concentrations and long reaction times (entries 9 and 10). Instead, the transformation of the hydroperoxide to the corresponding ketone was observed. Considerable amounts of ketone were also obtained for substrates **1d**−**f** with alkyl groups larger than methyl (entries 4−6). Unfortunate for our purposes, the tertiary hydroperoxide **1k** (entry 11) was only ineffectively converted to the corresponding alcohol **2k**.

Table 2. CiP-Catalyzed Sulfoxidation with Racemic (1-Phenyl)ethyl Hydroperoxide[a]



entry	sulfide	sulfide:CiP (mol ratio)	convn (%)[b] sulfide	product distribution (%)[b] ROOH	ROH	R=O	enantiomeric excess (%)[c] SO [(*S*)-(-)]	ROOH [(*S*)-(-)]	ROH [(*R*)-(+)]
1		2000:1[d]	>95	8	78	14	<5	57	10
2	(**4a**)	-[e]	95	17	83	<2	-	-	-
3		150:1	51	5	59	36	39	82	29
4	(**4b**)	190:1	68	28	57	15	79	>98	68
5	(**4c**)	200:1	>95	38	52	10	79	95	65
6	(**4d**)	200:1	<2	47	33	20	-	55	65
7	(**4e**)	100:1	23	6	54	40	17	75	32
8	(**4f**)	180:1	53	35	46	19	89	81	76
9	(**4g**)	200:1	24	8	54	38	<10	46	36

[a] The (1-phenyl)ethyl hydroperoxide (**1a**) was dissolved in 20 mL of a 1 M phosphate buffer (pH 7.0), 0.45 equiv. of sulfide was added, followed by CiP as an aqueous solution; the reaction mixture was stirred for 4 h. [b]Determined from the ^{1}H-NMR spectrum of the crude product mixture. [c]Determined by HPLC anylsis on a Chiralcel OD-H or OB-H column. [d]The (1-phenyl)ethyl hydroperoxide (**1a**) was dissolved in 2 mL of a 1 M phosphate buffer (pH 7.0), 0.45 equiv. of sulfide was added, followed by CiP as an aqueous solution; the reaction mixture was stirred for 24 h. [e]Same conditions as in footnote d, but without enzyme.

Interestingly, a β-hydroxy group in the hydroperoxide **1h** does not decrease the reactivity and selectivity as much as prolongation of the alkyl chain in the ethyl derivative **1d** (entries 8 and 4, respectively). Thus, although steric effects play an important role, electronic factors are as well significant for enantiodifferentiation by the enzyme.

In all the hydroperoxides in Table 1, CiP preferentially accepts the *R* enantiomer as substrate with concurrent formation of the (*R*)-alcohol, while the (*S*)-hydroperoxide is left behind (entries 1−6, 8). An exception is the cyclic hydroperoxide **1g**, for which the *S* enantiomer is converted to the alcohol and the (*R*)-hydroperoxide remains (entry 7).[11] Evidently, the structural arrangement of the CiP enzyme pocket, which preferentially accommodates the short alkyl chain in the hydroperoxides **1a**−**d**, also accepts well the larger but flat phenyl group.

These results clearly establish the limitations of CiP-catalyzed kinetic resolution of hydroperoxides. Like HRP, CiP also shows very good enantioselectivity for aryl alkyl hydroperoxides with short alkyl chains. Sterically more encumbered and particularly tertiary hydroperoxides, however, are not or only unselectively reduced. The sense of the enantioselectivity is also the same for CiP as for HRP, except for the indanyl hydroperoxide **1g**. The larger substrate opening of the CiP enzyme, which had prompted us to examine sterically more demanding hydroperoxides, has not held up to our expectations.

Enantioselective Sulfoxidation. Peroxidases also possess the ability to catalyze oxygen-transfer reactions. Whereas CPO also catalyzes epoxidations,[3] most other peroxidases only oxidize sulfides.[4,12] We have investigated herein the oxygen-transfer propensity of CiP by using the enantioselective sulfoxidation as the model reaction. For this purpose, seven electronically and sterically varied prochiral sulfides **4** were oxidized by CiP (Table 2).

As oxidant, racemic (1-phenyl)ethyl hydroperoxide (**1a**) was used, which had been shown to be best in the CiP-

(11) It should be noted that for the hydroxy-functionalized hydroperoxide **1h** a change in the sequence priority applies, and thus, the stereoselection is the same.

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catalyzed kinetic resolution experiments. If successful, three optically active compounds would be prepared simultaneously, namely, the sulfoxide **5**, the alcohol **2,** and the hydroperoxide **1**. That this is feasible was nicely demonstrated by Wong et al. for the CPO enzyme.[4b]

In a preliminary experiment, the same reaction conditions were used for the enantioselective sulfoxidation of methyl phenyl sulfide (**4a**) as for the kinetic resolution of hydroperoxides (Table 2, entry 1). At complete sulfide conversion, only racemic sulfoxide **5a** was obtained. A control experiment without enzyme showed that under these conditions the sulfide was almost completely oxidized by the hydroperoxide (entry 2). This undesirable direct chemical sulfoxidation was minimized by employing low sulfide concentrations and a higher amount of enzyme (entry 3). Under these conditions, the CiP-catalyzed enantioselective sulfoxidation of methyl phenyl sulfide (**4a**) was achieved in moderate (39%) enantioselectivities for the sulfoxide **5a**. In the case of the alkyl aryl sulfides **4b** and **4c**, in which the phenyl rings are substituted with para electron-donating substituents, among the best (79%) enantioselectivities were obtained for the (*S*)-(−)-sulfoxides **5b** and **5c** (entries 4 and 5). Most pleasingly, high ee values were also found for the (*S*)-(−)-hydroperoxide **1a** (>95%) and for the (*R*)-(+)-alcohol **2a** (ca. 65%). In contrast, methyl *p*-nitrophenyl sulfide (**4d**) was not converted at all to the corresponding sulfoxide (entry 6), which may be ascribed to the deactivation of the sulfide toward oxidation due to the electron-withdrawing para substituent.[4a,c]

As was the case for the kinetic resolution of the hydroperoxides **1**, prolongation of the alkyl chain in the ethyl tosyl sulfide (**4e**) strongly reduced the reactivity and selectivity (entry 7). Benzyl methyl sulfide (**4g**) also proved to be a poor substrate for the CiP-catalyzed sulfoxidation (entry 9). Only low conversion and almost no enantioselectivity were observed. The best substrate was methyl naphthyl sulfide (**4f**), which was oxidized with the highest ee value of 89% (entry 8). The large steric difference between the naphthyl and the methyl groups in this prochiral sulfide is clearly advantageous for effective stereodifferentiation.

In all cases, the (*S*)-sulfoxide was formed, in contrast to the reactions catalyzed by CPO, which displays enantioselectivities up to >98% for a number of aryl sulfides[4c] in favor of the (*R*)-sulfoxide.[4] This makes the enzyme CiP a valuable native biocatalyst for the asymmetric synthesis of sulfoxides since the "opposite" enantiomer is made available. However, compared to CPO, the turnover number of CiP in the asymmetric sulfoxidation is lower and the substrate acceptance is more limited. The major advantage of the CiP-catalyzed sulfoxidation is that the hydroperoxide, sulfide, and enzyme are all mixed at once, while with CPO the cumbersome slow continuous addition of the oxygen donor is required.[4] Thus, the present CiP-catalyzed sulfoxidation contributes a biocatalytic process for the synthesis of selected (*S*) sulfoxides, which should serve as valuable synthons for the preparation of natural products and as powerful stereodirecting groups in asymmetric synthesis.[13]

Catalase Activity and Ketone Formation from the Alkyl Hydroperoxides. Unfortunately, up to 40% of the hydroperoxide **1a** used in the sulfoxidation reactions is converted to the corresponding ketone **3a**, whereby the oxidation of the sulfide to sulfoxide is substantially reduced. When, however, 1 equiv of hydrogen peroxide was used as oxidant instead of hydroperoxide **1a** in the CiP-catalyzed sulfoxidation, no conversion of the sulfide was observed although the H_2O_2 was completely consumed.[14] This prompted us to test whether the peroxidase CiP also possesses catalase activity, i.e., catalyzes the disproportionation of hydrogen peroxide to water and molecular oxygen. Indeed, the UV absorption of hydrogen peroxide at 240 nm decreased upon addition of CiP to a solution of hydrogen peroxide in phosphate buffer (pH 7.0), as displayed in Figure 1a.

Clearly, with hydrogen peroxide as oxygen source, the resulting *compound I* of the enzyme prefers to react with another molecule of hydrogen peroxide to produce O_2 through paths A and D rather than oxidize the sulfide to its sulfoxide along paths A and C in Scheme 1.

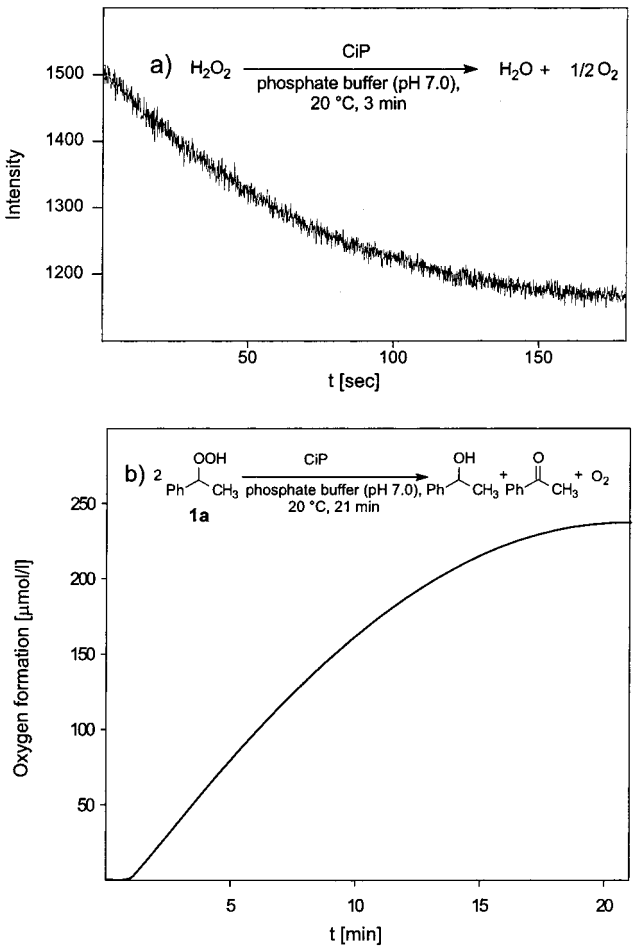

Figure 1. (a) CiP-catalyzed disproportionation of hydrogen peroxide [UV absorption at 240 nm, 200 μmol of H_2O_2, 60 μmol of CiP in 3 mL of 1 M phosphate buffer (pH 7.0)]. (b) Oxygen-gas evolution during the CiP-catalyzed disproportionation of (1-phenyl)ethyl hydroperoxide (**1a**) [18 μmol **1a**, 300 nmol CiP in 2 mL of 1 M phosphate buffer (pH 7.0)].

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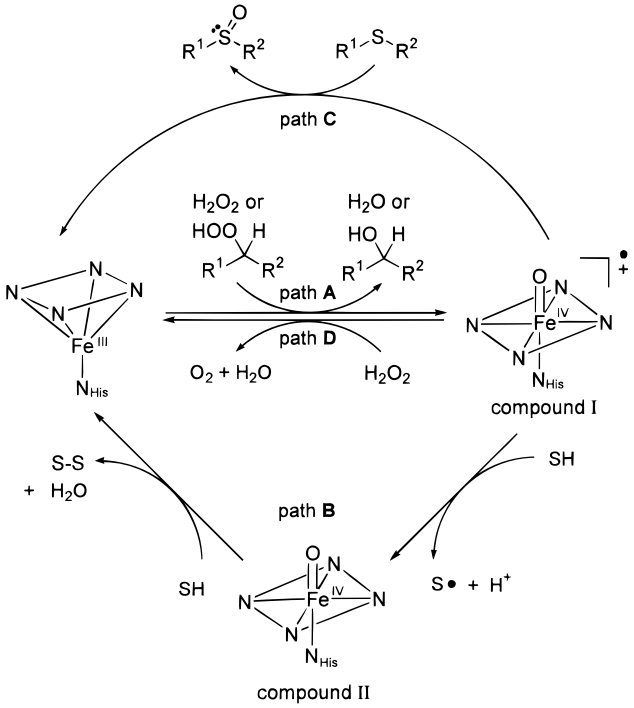
(14) While this manuscript was in preparation, the following paper appeared: Tuynman, A.; Vink, M. K. S.; Dekker, H. L.; Schoemaker, H. E.; Wever, R. *Eur. J. Biochem.* **1998**, *258*, 906−913. The authors describe the CiP-catalyzed formation of (*S*)-methyl phenyl sulfoxide with a yield of 84% and an ee of 73%, when an excess of H_2O_2 is added slowly, as catalase activity accounts for 85% of the H_2O_2 consumption.

Scheme 1. Catalytic Cycles of *Coprinus* Peroxidase (CiP): Peroxidase (A + B), Catalase (A + D), and Monooxygenase (A + C) Activity



In contrast, with the hydroperoxide **1a** as oxygen source, some asymmetric sulfoxidation takes place (Table 2); however, when CiP was allowed to react with hydroperoxide **1a** in the absence of a reductant, oxygen-gas evolution, which was monitored by means of an oxygen electrode, was observed (Figure 1b). To understand the origin of the oxygen evolution, a careful product study of the reaction between hydroperoxide **1a** and CiP was conducted (Table 3, entry 1).

Mechanistically significant, in addition to alcohol **2a** substantial amounts of ketone **3a** were also observed, even when the isolated pure enzyme was employed (entry 2), accompanied by oygen-gas release. The ratio of alcohol to ketone was 1:1, independent of the extent of conversion (entry 3). Also the sterically more hindered hydroperoxide **1f** gave the corresponding ketone **3f** in addition to the alcohol **2f**, but in an alcohol-to-ketone ratio of about 1:4 (entry 4). The appreciable ee values for the hydroperoxide **1a** and alcohol **2a** (entries 1−3) show clearly that an enzymatic stereodifferentiating process is involved.

In this context, Hager et al. were the first who reported the formation of molecular oxygen in the reaction between CPO and ethyl hydroperoxide.[15] They attributed the liberation of oxygen to the catalase activity of CPO toward alkyl hydroperoxides, as has been documented for hydrogen peroxide.[16] However, this seems questionable because early work claims that catalase itself does not catalyze the disproportionation of alkyl hydroperoxides into alcohol and O_2.[17] Also acetaldehyde was observed, presumed to be formed by CPO-catalyzed two-electron oxidation of the ethanol, which in turn derives from the ethyl hydroperoxide through reduction.[15] This, indeed, is the known mechanism for the reaction of alkyl hydroperoxides with catalases[18] and has also been reported to work enantioselectively with soybean lipoxygenase (SBLO).[19]

More recently, the mechanism of oxygen production was clarified. EPR-spectral evidence demonstrated the generation of peroxyl radicals in the reaction of peroxidases with hydroperoxides.[20] Moreover, it was shown that about 12% of the released oxygen is electronically excited singlet oxygen.[21] It was, thus, suggested that the peroxyl radicals, generated by enzymatic one-electron oxidation of the hydroperoxide, disproportionate according to the Russell mechanism[22] into ketone, alcohol, and O_2 in a ratio of 1:1:1 (Scheme 2).

This reaction, however, only constitutes a minor path for the formation of acetaldehyde; the major one involves the oxidation of ethanol, as suggested by Hager.[21]

In our experiments with CiP, however, the possibility that the ketone **3a** is formed by the oxidation of the alcohol **2a**[15,21,23] was discounted by the fact that the alcohol resisted CiP-catalyzed treatment with hydrogen peroxide or alkyl hydroperoxides **1b** and **1e** as oxygen sources. Thus, a plausible pathway for the formation of O_2 and ketone (Scheme 2) is through the intervention of peroxyl radicals, enzymatically generated by the CiP, which disproportionate into alcohol **2**, ketone **3,** and O_2 in a 1:1:1 ratio according to the Russell mechanism.[22] However, if the overall reaction in Scheme 2 were the only pathway for the ketone formation, an alcohol/ketone ratio of 2:1 would be expected, i.e., 1 mol of alcohol results from the reduction of the hydroperoxide for the formation of compound I and another mol from the enzymatically produced peroxyl radicals through the Russell cleavage, together with 1 mol of ketone. Instead, an alcohol/ketone ratio of 1:1 was obtained for hydroperoxide **1a** and even 1:4 for **1f**. This implicates that, besides the Russell cleavage, some of the ketone must be formed from the hydroperoxide by an additional pathway. That this pathway is also an enzyme-catalyzed reaction and not merely base-catalyzed dehydration of the hydroperoxide (Kornblum−DeLaMare reaction[24]), has been confirmed by means of a control experiment, in which each hydroperoxide **1a** and **1f** was stirred under the usual reaction conditions [phosphate buffer (pH 7.0)] without the CiP enzyme; after 4 h (**1a**) and even 3 days (**1f**), the hydroperoxides were recovered unchanged.

With the experimental data on hand, it is difficult to suggest which additional enzymatic pathway is responsible for the ketone formation. What may be stated definitely (see control experiment) is that the ketone is

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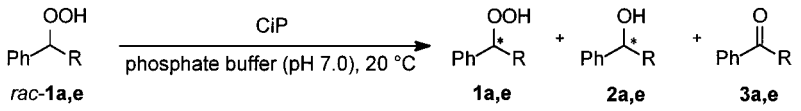
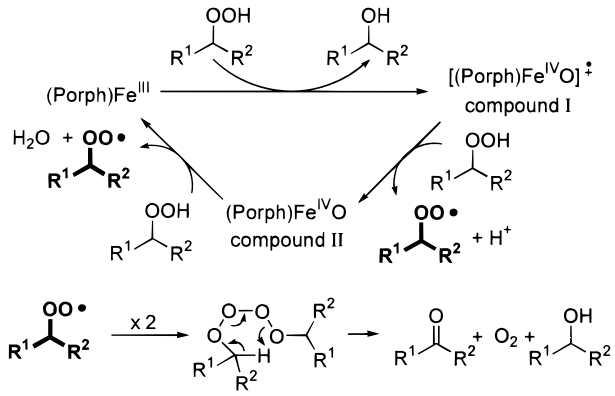
Table 3. CiP-Catalyzed Decomposition of the Hydroperoxides 1 to Alcohols 2 and Ketones 3 in the Absence of Guaiacol[a]



		ROOH:CiP		product distribution (%)[b]			enantiomeric excess (%)[c]	
entry	hydroperoxide	(mol ratio)	t(h)	ROOH	ROH	R=O	ROOH	ROH
							[(*S*)-(-)]	[(*R*)-(+)]
1		350:1	4	28	38	34	54	18
2	 (**1a**)	800:1[d]	96	35	33	32	24	54
3		164:1	120	0	51	49	-	40
4	 (**1f**)	150:1	48	71	6	23	-	-

[a]The hydroperoxide was dissolved in a 1 M phosphate buffer (pH 7.0), CiP was added as an aqueous solution, and the reaction mixture stirred for 4 h. [b]Determined from the ^{1}H-NMR spectrum of the crude product mixture. [c]Determined by HPLC anaylsis on a Chiralcel OD-H or OB-H column. [d]Isolated pure CiP with an activity of 2640 kPODU/g and a protein content of 639 mg/g was used.

Scheme 2. Formation of Peroxyl Radicals by CiP-Catalyzed Oxidation of Hydroperoxides and Their Disproportionation to Ketone, Alcohol, and Molecular Oxygen (Russell Mechanism)



not formed by enzymatic oxidation of the alcohol, which is known for the CPO-catalyzed transformation of primary alcohols to aldehydes.[15,23] Be this as it may, the enantioselective degradation of a chiral secondary hydroperoxide by a peroxidase in the absence of a reductant is unprecedented for such enzymes.

In summary, we have demonstrated that, besides the usual peroxidase activity, CiP also acts as catalase toward hydrogen peroxide. In its reaction cycle (Scheme 2), CiP uses alkyl hydroperoxides, on one hand, as substrates for the formation of compound I and, on the other hand, for the generation of peroxyl radicals by the reduction of compound I back to the native enzyme through compound II. The peroxidase activity of CiP may be used for the synthesis of optically active products by the kinetic resolution of hydroperoxides and enantioselective sulfoxidation, which provides (*S*)-(−)-hydroperoxides, (*R*)-(+)-alcohols, and (*S*)-(−)-sulfoxides in enantiomeric excess up to >90%. Clearly, the CiP peroxidase constitutes a promising enzyme for large-scale synthetic applications.

Acknowledgment. We thank NovoNordisk for the generous gift of CiP samples and the Deutsche Forschungsgemeinschaft (SFB-347 "Selektive Reaktionen Metall-aktivierter Moleküle") and the Fonds der Chemischen Industrie for financial support. We also thank Prof. P. Schreier, University of Würzburg, for help with the oxygen measurements.

Supporting Information Available: Experimental procedures with the tabulated HPLC conditions for the determination of the ee values of the hydroperoxides **1**, alcohols **2,** and sulfoxides **5** on chiral columns. This material is available free of charge via the Internet at http://pubs.acs.org.

JO990201P