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Asymmetric reduction of ketones via whole cell bioconversions and transfer hydrogenation: complementary approaches

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Abstract—Prochiral aryl and dialkyl ketones were enantioselectively reduced to the corresponding alcohols using whole cells of the white-rot fungus *Merulius tremellosus* ono991 as a biocatalytic reduction system and ruthenium(II)–amino alcohol and iridium(I)–amino sulfide complexes as metal catalysts in asymmetric transfer hydrogenation. Comparison of the results showed that the corresponding chiral alcohols could be obtained with moderate to high enantioselectivities (e.e.s of up to 98%). The biocatalytic and transfer hydrogenation approaches appear to be complementary. The biocatalytic approach is the most suitable for the enantioselective reduction of chloro-substituted (aryl) ketones, whereas in the reduction of α,β-unsaturated compounds excellent results were obtained using the catalytic hydrogenation protocol.

1. Introduction

Enantiomerically pure alcohols are useful chiral building blocks in organic synthesis that can be used as key intermediates in the synthesis of more complex enantiopure bioactive compounds. Accordingly, there is considerable interest in highly efficient routes to chiral alcohols. They can be synthesized in enantiomerically pure form from prochiral ketones either biologically, using a biocatalytic system, or chemically via stereoselective reduction, using either a catalytic system or a stoichiometric amount of reducing agent.

The enantioselective reduction of ketones using Me-CBS-oxazaborolidine, developed by Corey et al.,[1,2] is an excellent example of the latter method. Here we have used this methodology to establish the absolute configurations of some of the chiral alcohols obtained via the biocatalytic route.

Many examples of enantioselective microbial reductions have been described in the literature.[3–8] Bernardi et al. described the reduction of (*Z*)-3-fluoro-4-phenyl-1-(*p*-tolylsulphonyl)but-3-en-2-one using the yeast *Geotrichum candidum* to form (−)-(*S*)-3-fluoro-4-phenyl-1-(*p*-tolylsulphonyl)but-3-en-2-ol with 98% e.e. The opposite enantiomer (+)-(*R*)-3-fluoro-4-phenyl-1-(*p*-tolylsulphonyl)but-3-en-2-ol could be formed in 95% e.e. using the white-rot fungus *Phanerochaete chrysosporium*.[5] White-rot fungi are lignin degrading basidiomycetes and contain an extensive reductive enzyme system.

Recently, we described the biocatalytic properties of white-rot fungi in aryl acid reductions.[8] Ketone reduction during lignin degradation has been suggested[9] and the de novo preparation of both chloro-substituted arylketones and chiral chloro-substituted aryldiols by

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white-rot fungi has been reported.[10] In addition, the fungus *P. chrysosporium* possesses an interesting and unprecedented hydroquinone reductase activity.[11] From the scarce literature precedents known, it can be inferred that white-rot fungi may possess a versatile biocatalytic system for the enantioselective reduction of various kinds of functionalized ketones. So far, relatively little is known about the reductive part of the ligninolytic system, and in particular enantioselective ketone reduction by white-rot fungi has hardly been described.[5] Therefore, we embarked on a study of the enantioselective reduction of a variety of functionalized ketones. These ketones are valuable synthons for the synthesis of a variety of pharmaceuticals and other biologically active molecules.

Asymmetric transfer hydrogenation using an organic hydrogen source has also proven to be a valuable chemical method for enantioselective reduction. Recently, we developed two catalytic systems that proved to be very suitable for the transfer hydrogenation of aryl alkyl ketones.[12–14] For the ruthenium(II)-catalyzed asymmetric transfer hydrogenation, a series of new amino alcohol ligands was synthesized and optimized, which resulted in the most effective chiral amino alcohol ligand so far, for the reduction of acetophenone.[12] Additionally, a new class of N,S-chelates was developed for the iridium(I)-catalyzed reduction of unsymmetrical ketones.[13] The amino alcohol NH-benzyl (1*R*,2*S*)-norephedrine ligand, **A**, and the amino sulfide (1*R*,2*S*)-2-amino-1-phenyl-1-benzylthio-propane and (1*R*,2*S*)-2-amino-1,2-diphenyl-1-benzylthioethane ligands, **B**, are shown in Fig. 1.

Herein, a screening study for the enantioselective reduction of aryl alkyl ketones, dialkyl ketones, functionalized ketones and α,β-unsaturated ketones by white-rot fungi is presented. The results of the biocatalytic conversions are compared to ruthenium(II)–amino alcohol- and iridium(I)–amino sulfide-catalyzed asymmetric transfer hydrogenation for the reduction of prochiral (aryl and dialkyl) ketones. In addition, novel results regarding the asymmetric transfer hydrogenation of α,β-unsaturated ketones are presented, showing that the biocatalytic system and the metal based catalysts can be used in a complementary way for the synthesis of a variety of functionalized enantiomerically pure alcohols.

2. Results and discussion

A screening of ketone reductase activity of different basidiomycete strains was first performed. Since acetophenone **1a** is generally used as a model substrate, and both 1-(3′-chloro-4′-methoxyphenyl)-1-propanone **2a** and 1-(3′,5′-dichloro-4′-methoxyphenyl)-1-propanone **3a** are metabolites of white-rot fungi,[10] these ketones were used as potential substrates (Fig. 2). The ketones were added (as a concentrated solution in acetone) to whole cell cultures and the incubation was carried out for 72 hours at 28°C. The reactions were monitored by periodically taking samples and subsequent analysis by HPLC, GC and/or ^{1}H NMR. All reactions were performed on an analytical scale. Eight different basidiomycete strains, which are reported in Section 4, were screened for reductive activity (results not shown). The white-rot fungus *Merulius tremellosus* reduced the ketones with the highest reductive activity. Subsequently, six different *M. tremellosus* strains were screened on the same substrates for both reductive activity and enantioselectivity (Table 1). The ketones **1a**, **2a** and **3a** were enantioselectivily reduced by all strains but the total recovery and e.e. decreased after 48 hours of incubation. Presumably, the ketones and alcohols are being metabolized by the ligninolytic system of the fungus. All three ketones yielded the corresponding (*S*)-alcohols with an e.e. of ≥95%. For determining the absolute configuration of the products, the resulting alcohols were compared to independently synthesized enantiomerically enriched samples obtained via reduction of ketones **2a**, **3a** and **5** using Corey's oxazaborolidine approach (see Section 4).[1,2] Absolute configurations of the product alcohols were attributed by analogy with the reduction of compound **1a**.

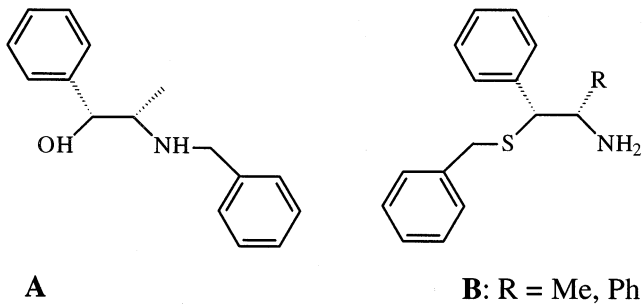


Figure 1.

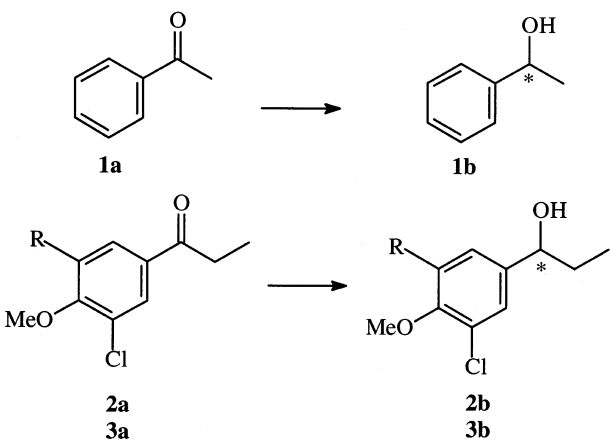


Figure 2.

Table 1. Screening *Merulius tremellosus*: reduction of arylketones to their corresponding chiral alcohols

Strain	Alcohol **1b** produced (mM)	Ketone **1a** recovered (mM)	Prod. yield (%)	Alcohol **2b** produced (mM)	Ketone **2a** recovered (mM)	Prod. yield (%)	Alcohol **3a** produced (mM)	Ketone **3b** recovered (mM)	Prod. yield (%)
M. tremellosus ono991	0.41	0.34	61	0.70	0.21	53	0.40	0.25	88
M. tremellosus K124i	0.29	0.50	58	0.47	0.23	61	0.66	0.24	86
M. tremellosus CBS250.36	0.13	0.68	40	0.32	0.41	54	0.29	0.62	76
M. tremellosus K152	0.14	0.74	54	0.27	0.47	57	0.19	0.56	43
M. tremellosus K152i	0.10	0.66	29	0.12	0.73	44	0.12	0.65	34
M. tremellosus ATTC4725	0.11	0.36	17	0.10	0.41	17	0.14	0.51	29

The microbial reduction of acetophenone **1a** has been described for the yeast *G. candidum*. Under standard conditions (*R*)-1-phenylethanol was obtained in 52% yield with 28% e.e. Remarkably, changing culture conditions and cultivation under argon yielded 98% of the enantiomeric (*S*)-1-phenylethanol with 95% e.e.[15] To the best of our knowledge, the microbial reduction of ketones **2a** and **3a** has not been reported. In addition, the enantioselective reduction of acetophenone and chlorinated aryl ketones by white-rot fungi is a novel observation.

M. tremellosus ono991, isolated in the Wageningen laboratory, was used in further experiments because it is the fastest growing strain and one of the best reducers of the different ketones. The performance of *M. tremellosus* ono991 was compared to the asymmetric transfer hydrogenation using a ruthenium(II) catalyst with an amino alcohol ligand and an iridium(I) catalyst with amino sulfide ligands, developed at the Amsterdam laboratories. Both the biocatalytic and the transfer hydrogenation systems were tested for the enantioselective reduction of various α-aryl, dialkyl, α,β-unsaturated and chloro-substituted functionalized ketones. Transfer hydrogenation experiments were carried out using propan-2-ol as a hydrogen donor, a solution of ketone (0.1 M in dry propan-2-ol), the catalyst precursor (i.e. $[RuCl_2(p\text{-cymene})]_2$ or $[IrCl(COD)]_2$, (0.5 mol%)), the chiral ligand (1 mol%), **A** for ruthenium and **B** for iridium, and *tert*-BuOK (2.5 mol%), which were stirred at room temperature under argon. The degree of conversion and the product e.e.s were monitored during the reaction by GC, HPLC and/or ^{1}H NMR.

2.1. Enantioselective reduction of aryl ketones

The results of the biocatalyzed and metal-catalyzed reductions of aryl ketones (Fig. 3) are shown in Table 2. The corresponding alcohols were obtained with e.e.s of up to 98%. The choice of substrate did not strongly affect the outcome of the reactions. In most cases, use of the ruthenium catalyst gave rise to a higher selectivity than the use of the iridium catalyst. Both the fungus and the metal based catalysts are suitable systems for aryl ketone reduction. The latter method can be used to produce complementary absolute product configuration to the former: using *M. tremellosus* as a catalyst, the (*S*)-enantiomeric alcohols were obtained, whereas the metal based catalysts containing ligands with (1*R*,2*S*)-configuration, used in this study, afforded the (*R*)-enantiomers. Using the opposite ligand configuration would result in product with (*S*)-absolute configuration.

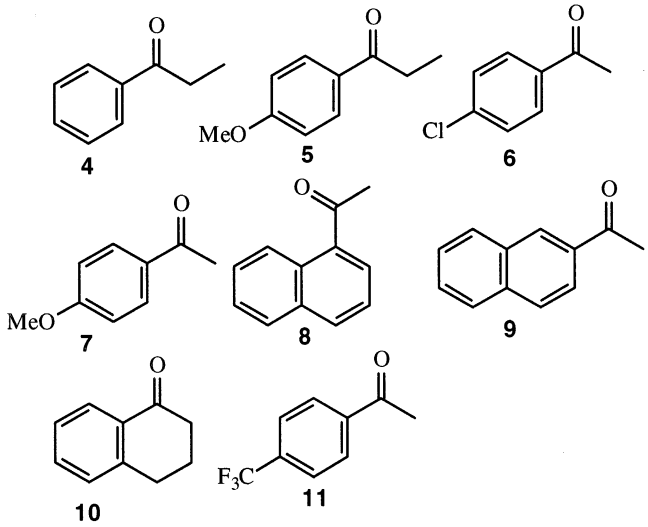


Figure 3.

2.2. Reduction of dialkyl ketones

Whole cell systems of the acetic acid bacteria *Gluconobacter oxydans*, *Acetobacter aceti*, *Acetobacter pasteurianus* and *Acetobacter peroxydans* were capable of reducing a series of dialkyl ketones.[16–18] The corresponding alcohols were produced with e.e.s varying from 11 to 99%. In general, the metal-catalyzed reduction of dialkyl ketones occurred with low enantioselectivity. A few exceptions have been described in the literature. Using an oxazolinylferrocenylphosphine[19] or

Table 2. Alcohol production from biocatalyzed and metal-catalyzed asymmetric reduction of aryl ketones

Ketone	*M. tremellosus*		Ruthenium[a]		Iridium[b]	
	Product yield (%)	% e.e.	Conv. (%) [2 h]	% e.e.	Conv. (%) [1 h]	% e.e.
1	61	95 (*S*)	91	95 (*R*)	82	80 (*R*)
2	53	96 (*S*)	N.d.	N.d.	N.d.	N.d.
3	88	97 (*S*)	N.d.	N.d.	N.d.	N.d.
4	N.d.	N.d.	98	90 (*R*)	95	92 (*R*)
5	49	98 (*S*)	N.d.	N.d.	N.d.	N.d.
6	N.d.	N.d.	96	86 (*R*)	99	77 (*R*)
7	56	98 (*S*)	68	93 (*R*)	52	88 (*R*)
8	51	90	96	96 (*R*)	99	97 (*R*)
9	63	Rac.	94	93 (*R*)	97	87 (*R*)
10	68	95	68	96 (*R*)	39	37 (*R*)
11	34	92	N.d.	N.d.	N.d.	N.d.

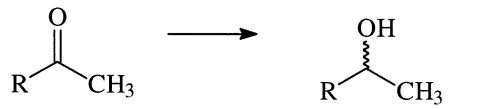
[a] Data obtained from Reference 10.
[b] Data obtained from Reference 11.

a combination of Ru(II) and a phosphinooxazoline,[20] cyclohexyl methyl ketone could be reduced giving products with e.e.s of up to 66%. Recently, e.e.s of up to 92% were obtained in the reduction of *tert*-butyl methyl ketone, using an in situ generated Rh-PennPhos catalyst.[21]

The results of the reduction of dialkyl ketones **12a**–**15a** (Fig. 4) are shown in Table 3. *M. tremellosus* converted, respectively, 50 and 35% of ketones **12a** and **14a** into the corresponding alcohols. For technical reasons, the e.e. of the products could not be measured. The fungus was not able to reduce cyclohexyl methyl ketone and only relatively low enantioselectivities were obtained in the ruthenium- and iridium-catalyzed transfer hydrogenation of dialkyl ketones **12a**–**15a**. Clearly, both the fungal system and the metal based catalysts are not suitable catalysts for the reduction of dialkyl ketones.

2.3. Enantioselective reduction of α,β-unsaturated olefinic and acetylenic ketones

Few examples in the literature describe the microbial reduction of α,β-unsaturated and acetylenic ketones such as substrates **16**–**18** (Fig. 5). (*E*)-4-Phenyl-3-buten-2-one could successfully be reduced to its corresponding alcohol **16a**, with e.e. of up to 94%, using a crude extract of the yeast *Saccharomyces cerevisiae*.[22] Acetylenic derivatives containing one aromatic substituent and non-terminal triple bonds are known for their antimicrobial and especially fungistatic properties.[23] Therefore, acetylenic compounds such as **18** may be poor substrates for fungal reduction using whole cell cultures. In contrast, an isolated alcohol dehydrogenase from the bacterium *Thermoanaerobium brockii* produced the (*S*)-enantiomer of 3-butyn-2-ol from its corresponding ketone.[24]



12a R= *n*-butyl **12b** R_1= *n*-butyl

13a R= *iso*-butyl **13b** R_1= *iso*-butyl

14a R= *sec*-butyl **14b** R_1= *sec*-butyl

15a R_1 = cyclohexyl **15b** R_1= cyclohexyl

Figure 4.

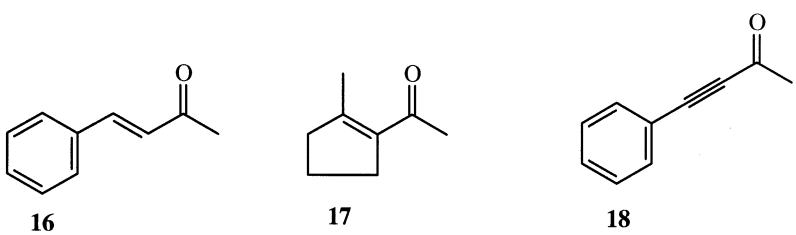
Due to their conformational flexibility and sensitivity to basic conditions, the chemical enantioselective reduction of simple α,β-unsaturated ketones has remained problematic. Using $RuCl_2$(xylbinap)(1,2-diamine) catalysts (1,2-diamine = 1,1-dianisyl-2-*iso*-propyl-1,2-ethylenediamine (DAIPEN), 1,2-diphenylethylene-diamine (DPEN) or *trans*-cyclohexane-1,2-diamine), Noyori et al. achieved e.e.s of over 90% in the asymmetric hydrogenation of α,β-unsaturated ketones.[25] However, reaction times of around 15 hours or more and a hydrogen pressure of 8–10 atmospheres is required to obtain high yields and e.e.s.

Chiral propargylic alcohols are useful building blocks for the synthesis of various structurally diverse organic compounds, with interesting biological activities.[25] A straightforward approach to synthesize these chiral compounds chemically would be asymmetric hydrogenation. However, so far none of the currently available catalyst systems could both chemo- and enantioselectively reduce α,β-acetylenic ketones into their corresponding alcohols. The first asymmetric transfer hydrogenation of acetylenic ketones was described by Noyori et al. By using chiral Ru(II) catalysts and propan-2-ol as the hydrogen donor, structurally diverse acetylenic ketones were reduced highly

Table 3. Alcohol production from biocatalyzed and metal-catalyzed asymmetric reduction of dialkyl ketones

Ketone	*M. tremellosus*		Ruthenium		Iridium	
	Conv. (%)	% e.e.	Conv. (%) [3 h]	% e.e.	Conv. (%) [3 h]	% e.e.
12	50	N.d.[a]	65	25	41	14
13	N.d.	–	25	33	12	9
14	35	N.d.[a]	N.d.	N.d.	N.d.	N.d.
15	–	–	58	23	58	33

[a] Due to technical problems, the enantioselectivities of these reductions could not be determined.



16 **17** **18**

Figure 5.

selectively to their corresponding propargylic alcohols with e.e.s of over 95%. Using this method, the C≡C bond was left intact.[26] This class of compounds has also been prepared by reductive cleavage of chiral acetylenic acetals, the metal hydride reduction of acetylenic ketones, enantioselective alkynylation of aldehydes and enzymatic transformations and the hydroboration of α,β-ynones.[26]

The white-rot fungus *M. tremellosus* ono991 was not able to convert compound **16** to the desired corresponding unsaturated alcohol **16b**, but, unexpectedly, a mixture of the saturated ketone **16c** (55 mol%) and the hydroxy-substituted ketone **16d** (45 mol%) was formed, as revealed by $^{1}H/^{13}C$ NMR analysis of the crude reaction mixture (Fig. 6). The remarkable formation of the Michael adduct **16d** was shown to be the result of a bio-mediated conversion, since this product could not be detected in abiotic control experiments, where no (or autoclaved) fungal cells were present. In contrast to reduction with the yeast *S. cerevisiae*, fungal reduction did not result in formation of alcohol **16b**.

Reduction of the acetylenic ketone **18** did not result in the formation of the desired alcohol. In all of the products the triple bond was destroyed and a complex mixture of compounds was found containing different ketones and alcohols, including the completely saturated alcohol **16e**. Evidently, the fungal system cannot be used for the synthesis of enantiomerically pure unsaturated alcohols. However, the remarkable bio-mediated Michael-type addition of H_2O to the α,β-unsaturated system merits further investigation.

Both the ruthenium(II) and iridium(I) catalysts successfully reduced the α,β-unsaturated ketones **16** and **17**, which are relatively insensitive to basic conditions, into the corresponding unsaturated alcohols (Table 4). The reductions also proved to be fully chemoselective towards the C=O bond. The reduction of **16** yielded product e.e.s of up to 35%. Conversion of the more rigid substrate **17** gave rise to e.e.s of up to 71%.

Using the metal catalysts, the acetylenic ketone **18** could successfully be reduced to form the desired acetylenic alcohol. The highest enantioselectivity was obtained using the ruthenium(II)–amino alcohol catalyst, resulting in an e.e. of 98%. The reaction using the iridium(I) catalyst reduced the acetylenic ketone giving the acetylenic alcohol with an e.e. of 90%. In contrast to the fungal system, both metal-catalyzed reductions proved to be fully chemoselective towards the reduction of the C=O bond, making the metal based approach the method of choice for the synthesis of this class of unsaturated chiral alcohol.

2.4. Enantioselective reduction of functionalized ketones

Asymmetric reduction of substrates such as 2-chloroacetophenone **19** and 3-chloropropiophenone **20** gives products that may be converted to valuable synthetic intermediates such as chiral epoxides. Biocatalytic enantioselective reductions of these compounds have been described previously. Substrate **19** was enantioselectively reduced to the corresponding (*S*)-alcohol (99% e.e.) using acetone powder of the yeast *G. candidum* as catalyst and NAD^+ as co-substrate.[27] Enantioselective reduction of compound **20** using different yeasts resulted in the corresponding (*S*)-alcohol and the corresponding (*R*)-alcohol with, respectively, 99.9 and 68% e.e.[28]

(*R*)-2-Chloro-1-phenylethanol could be synthesized chemically with a high e.e. of 95% using a polymer supported version of Noyori's TsDPEN with formic acid/triethylamine as a hydrogen donor (TsDPEN = *N*-(*p*-tolylsulfonyl)-1,2-diphenyl-ethylenediamine).[29] A drawback of this system is that generally long reaction times of 15 hours or more are required in order to obtain high yields.

M. tremellosus was able to stereoselectively reduce both chloro-substituted ketones **19** and **20** to their corresponding alcohols **19b** and **20b** (Table 5). The chloro-substituted alcohol **19b** was obtained with an e.e. of

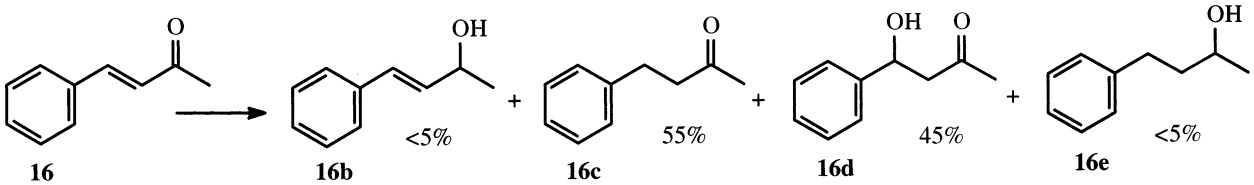


Figure 6.

Table 4. Alcohol production from biocatalyzed and metal-catalyzed asymmetric reduction of α,β-unsaturated and acetylenic ketones

Ketone	*M. tremellosus*		Ruthenium		Iridium	
	Product yield (%)	% e.e.	Conv. (%)	% e.e	Conv. (%)	% e.e.
16	<5	N.d.	79 (5 h)	35 (*R*)	40 (5 h)	25 (*R*)
17	N.d.	N.d.	78 (16 h)	42 (*R*)	98 (16 h)	71 (*R*)
18	–	–	74 (16 h)	98 (*S*)	63 (16 h)	90 (*S*)

Table 5. Alcohol production from biocatalyzed and metal-catalyzed asymmetric reduction of functionalized ketones

Ketone	*M. tremellosus*		Ruthenium		Iridium	
	Conv. (%)	% e.e.	Conv. (%)	% e.e.	Conv. (%)	% e.e.
19	95	88	–	–	–	–
20	30	82	–	–	–	–

88%. Dechlorination of ketone **19** resulted in the formation of acetophenone **19c** as a side-product (5%). Reduction of compound **20** yielded the dechlorinated alcohol **20c** as the main product (60%). 30% of the ketone was converted to the corresponding chloro-substituted alcohol **20b**, obtained with an e.e. of 82%. Also, the dechlorinated ketone **20d** was produced as a side-product in 5% yield (Fig. 7). Dehalogenation by white-rot fungi has been described previously. The white-rot fungus *P. chrysosporium* is capable of reductive dechlorination of chlorinated hydroquinones.[30] Also, dehalogenation by whole cells of bacteria has been reported.[31]

Neither ruthenium(II)- nor iridium(I)-catalyzed reduction of substrates **19** and **20** resulted in formation of the corresponding alcohol, which might be a result of catalyst deactivation due to oxidative addition of the alkyl chlorides. The promising results obtained with the fungal systems merits further investigation towards the optimization of the whole cell bioconversion approach for the synthesis of this type of substituted chiral alcohol.

3. Conclusion

From the results obtained in this study it can be concluded that both the biocatalytic and the metal-catalyzed systems are able to selectively reduce a broad spectrum of prochiral ketones resulting in complementary product configurations. The α-aryl ketones are reduced by both systems, yielding the (*S*)-isomer on using the biocatalyst and the (*R*)-isomer on using the metal catalysts. The biocatalyst appeared to be the most suitable for enantioselective reduction of the chloro-substituted ketones. The α,β-unsaturated ketones could be reduced, although the desired unsaturated alcohols could not be obtained. Dialkyl ketones are also part of the substrate spectrum. The selectivity of these conversions is unknown so far.

In contrast, metal-catalyzed transfer hydrogenation could not be used for the reduction of chloro-substituted ketones, whereas the reduction of dialkyl ketones proceeded with low stereoselectivity. However, both the iridium and ruthenium catalysts reduced α,β-unsaturated systems with moderate to high enantioselectivity, in particular the acetylenic alcohol **18a** could be obtained with an e.e. of 98%. Also, the reactions proceeded with full chemoselectivity towards the C=O bond.

Of the biocatalysts examined, ketone reductase from *M. tremellosus* ono991 has the broadest substrate specificity. For commercial application of the white-rot fungus as a biocatalyst in ketone reductions, yields must be optimized. At the moment, experiments for enhancement of the reductive activity and characterization and purification of the ketone reductase from *M. tremellosus* are in progress. Also, the remarkable biomediated Michael addition of water to the double bond of the α,β-unsaturated system merits further investigation.

The developed metal catalysts also exhibit a wide scope since they were able to produce a variety of functionalized alcohols with moderate to high enantioselectivities. However, to realize the demand of the chemical indus-

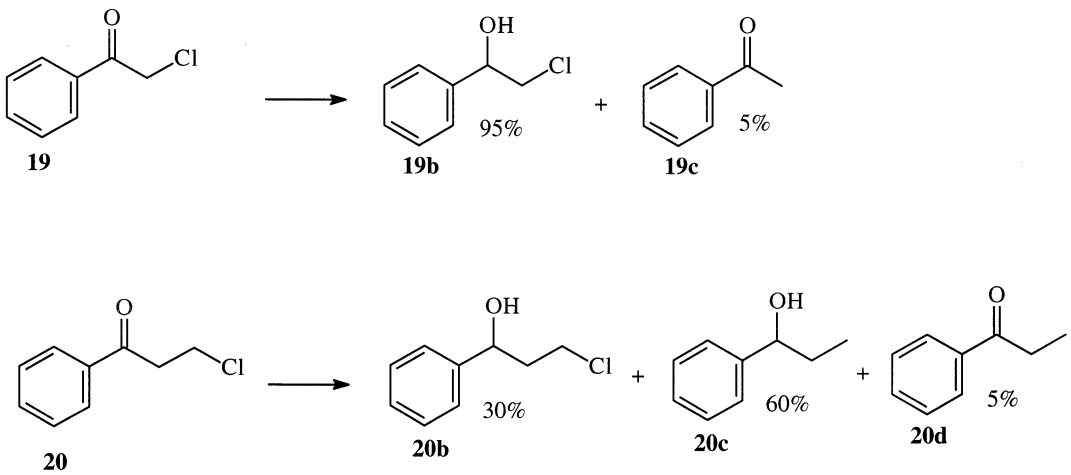


Figure 7.

tries, in most cases, improvement in the enantioselectivity of reductions with these systems is desirable.

4. Experimental

4.1. General

HPLC was performed on a Hewlett Packard HPLC Chemstation (Pascal Series) (Waldbronn, Germany) equipped with a HP 1100 pumping station, series diode array, a HP 1100 detector and a HP 1100 data processor. The column (200 mm×3 mm) used for analysis of ketones and alcohols was filled with ChromSpher C18-PAH (5 μm particles) and was obtained from Chrompack (Middelburg, The Netherlands). Ketones and alcohols were analyzed with the following gradient: 90:10, 0:100 and 90:10 water:acetonitrile at 0, 15 and 25 minutes, respectively. The flow rate was 0.4 mL min^{-1} and the column temperature was 30°C. The UV absorbance was monitored at wavelengths of 215, 265 and 280 nm. The column (460 mm×150 mm) used for analysis of aryl acids was filled with Intersil ODS-3 (5 μm particles) and was obtained from Phase Sep (Deeside, UK). Product yields are defined as the amount of produced alcohol per amount of converted ketone.

Enantiomer analysis of chiral alcohols was performed with a Chiralcel OB-H column (460 mm×150 mm), filled with a cellulose derivate coated on 5 μm silica gel from Mallinckrodt Baker BV (Deventer, NL). All chiral alcohols were analyzed under isocratic conditions with 90% hexane and 10% propan-2-ol (0.5 mL min^{-1}, 30°C).

Compound identifications were based on matching retention times and UV spectra with those of standards. $^{1}H/^{13}C$ NMR spectra were recorded on a Bruker avance 600 MHz NMR spectrometer.

Metal-catalyzed reactions were completed under an atmosphere of argon in flame dried Schlenk flasks, using anhydrous solvents. Propan-2-ol was freshly distilled from CaH_2 prior to use. ^{1}H NMR spectra were recorded on a Bruker AHX 300 spectrometer. Chemical shifts are in ppm relative to TMS as internal standard. Gas chromatography was performed using a Carlo Erba GC 6000 Vega 2 instrument, 25 m column: Cyclosil-B and Chiraldex-GTA (chiral, dialkylalcohols) and a Carlo Erba HRGC Mega 2 instrument, 25 m column: BPX35 SGE (achiral). HPLC analysis was performed using a Gilson HPLC apparatus and a Chiralcel OD column.

4.2. Organisms and culture conditions

Strains were obtained from different culture collections: Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; American Type Culture Collection, Rockville, Maryland, USA; Culture Collection of Industrial Microbiology Wageningen, Wageningen University and Research Centre, The Netherlands; *Merulius tremellosus* strain K124i, K152 and K152i were kindly supplied by Professor A. Hatakka, University of Helsinki, Finland. Fungal strains were maintained at 4°C on agar slants. The agar medium contained (L^{-1}) 20 g glucose, 5 g mycological peptone (Oxoid Ltd., Basingstoke, Hampshire, UK), 2 g yeast extract (Gibco BRL, Life Technol. Ltd., Paisley, Scotland, UK), 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 15 g agar. Fungal strains were grown in a high nitrogen peptone medium containing (L^{-1}) 20 g glucose, 5 g mycological peptone, 2 g yeast extract, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot H_2O$, with the addition of 0.058 g NaCl.[32]

Serum bottles (100 mL), containing medium (10 mL), were inoculated with a cylindrical agar plug (diameter 4 mm), which was taken from the outer periphery of an agar medium plate covered with the mycelium of the fungal strain. Fungal cultures were incubated statically in the dark with loosely capped bottles. For experiments with functionalized ketones the batches were nitrogen flushed directly after incubation, since parallel experiments (results not shown) showed an increased yield for this reduction when performed under anaerobic conditions. Control cultures (no addition of ketones) were incubated and treated like ketone-containing cultures to monitor for de novo production of metabolites. Sterile abiotic controls in the presence of ketones were also monitored to check for chemical reduction of ketones. Unless indicated otherwise, the experiments were carried out in triplicate.

4.3. Screening for ketone reduction

The white-rot fungi *M. tremellosus*, *Bjerkandera* BOS55, *Trametes hirsuta*, *P. chrysosporium*, *Dichomitus squalenes*, *Trametes versicolor*, *Phlebia brevispora* and *Stereum hirsutum* were used for the first screening. The second screening was performed by using several strains of the species *M. tremellosus* (reported in Table 1). Culture bottles (100 mL) containing high nitrogen peptone medium (10 mL) were inoculated with a cylindrical agar plug (diameter 4 mm). Cultures were incubated in the dark at 25°C. To 4-day-old cultures, 1 mM ketone was added as a concentrated solution in acetone, to a concentration of 1 mM to the culture. The final concentration of acetone was 0.5% v/v, which was not toxic when added to mycelium.[33] Samples (150 μL) of extracellular fluid were taken periodically and were centrifuged and analyzed by HPLC and ^{1}H NMR. E.e. ratios were determined by HPLC using a chiral column.

4.4. Starting materials

Commercially available ketones **1a**, **4**–**11**, **12a**–**14a**, **16**–**20** (Aldrich Chemie) and **15a** (Avocado Research Chemicals Ltd) were purchased. Their corresponding alcohols (Aldrich Chemie), 1-(3′-chloro-4′-methoxyphenyl)-1-propanone **2a** and 1-(3′,5′-dichloro-4′-methoxyphenyl)-1-propanone **3a** were prepared as previously described.[10]

The enantiopure corresponding product alcohols of ketones **2a**, **3a** and **5**, used as reference compounds, were obtained by enantioselective borane reduction. From results described by Corey et al.[1,2] it can be concluded that the asymmetric reduction using BH_3·THF and (*R*)-2-Me-CBS-oxazaborolidine afforded the corresponding (*S*)-alcohol with high yields and selectivity. By reducing the ketones according to this method, using BH_3·THF and (*R*)-2-Me-CBS-oxazaborolidine, we obtained product alcohols with high e.e. of >94%, which were, considering the above, most likely the (*S*)-enantiomers. Data of chiral HPLC analysis (R_ts on Chiralcel OB-H, hexane:propan-2-ol = 9:1) are; compound **2b**: (*R*)-isomer 10.4 min, (*S*)-isomer 11.9 min; compound **3b**: (*R*)-isomer 10.7 min, (*S*)-isomer 12.1 min; and (*R*)-isomer 14.3 min, (*S*)-isomer 16.1 min for the corresponding product alcohol of compound **5**.

4.5. 4-Phenyl-4-hydroxy butanone 16c

Characteristic NMR data (recorded on a Bruker avance 600 MHz NMR spectrometer): ^{1}H NMR (δ $CDCl_3$) in ppm: CH, 5.09; CH_3, 2.13; CH_2, 2.80. ^{13}C NMR (δ $CDCl_3$) in ppm: CH, 70.4; CH_2, 52.5; C=O, 207; CH_3, 31.

4.6. General procedure for ruthenium(II)-catalyzed enantioselective reduction of ketones

A solution of (*p*-cymene)-ruthenium(II) chloride dimer (0.0125 mmol) and amino alcohol ligand **1** (0.030 mmol) in dry propan-2-ol (5 mL) was heated at 80°C for 1 h under argon. After cooling the mixture to room temperature, propan-2-ol (44 mL), the ketone (5 mmol) and *tert*-BuOK (0.1 M in propan-2-ol, 0.75 mL, 0.075 mmol) were added. The reaction was run at room temperature under argon for the time indicated and monitored by GC, NMR and/or HPLC.

4.7. Iridium(I)-catalyzed enantioselective reduction of ketones using propan-2-ol as a hydrogen donor

A solution of $[IrCl(COD)]_2$ (0.01 mmol, 6.7 mg) and the amino sulfide ligand (**A** or **B**) (0.05 mmol) in dry propan-2-ol (5 mL) was heated at 80°C for 30 min under argon. After cooling the mixture to room temperature, it was diluted with propan-2-ol (33.75 mL) and a ketone (4 mmol) and *tert*-BuOK (0.1 M in propan-2-ol, 1.25 mL, 0.125 mmol) were added. The reaction was run at room temperature under argon for the time indicated and monitored by GC, NMR and/or HPLC.

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