Alcohol and 1,2-Diol Dehydrogenases: Synthetic Use in the Preparation of Chiral Alcohols by Carbonyl Reduction

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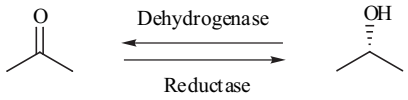
Abstract: Chiral alcohols are useful intermediates and auxiliaries in organic synthesis. Their preparation in enantiopure form is consequently of very high interest. Among the different enzymatic procedures, the use of dehydrogenase activities can be considered as the most attractive alternative. These enzymes can fruitfully work in inverse direction as enantioselective reducing agents. We will focus on the use of well-known alcohol dehydrogenases, describing their application in chiral alcohol preparation. A final discussion on the possibility of using these enzymes in either oxidative or reductive direction will be presented.

Keywords: Chiral alcohols, enantioselective preparation, carbonyl reduction, enzymatic methods, dehydrogenases.

1. INTRODUCTION

The current demand for enantiopure compounds stimulates continuous research for new methods of stereoselective synthesis; in this view, the production of enantiopure chiral alcohols by enzymatic procedures has gained increasing interest. The two principal ways to prepare chiral alcohols are: the selective reduction of the corresponding prochiral carbonyls; the resolution of racemic mixtures [1-4]. Redox enzymes can catalyse both the alcohol oxidation and the carbonyl reduction. This property can be fruitfully exploited to opportunely manage the catalysis in the desired direction (Scheme (**1**)).



Scheme 1. Alternative use of ADHs.

The synthesis of chiral alcohols from prochiral compounds has the important merit of suggesting a theoretical yield of 100%, but it is usually impossible to access both the enantiomers. However, reductive biocatalysts have been improved and optimised, mainly through the use of molecular biology, and nowadays, it is even possible to reverse the enzyme selectivity. Preparation of chiral alcohols through the selective reduction of prochiral carbonyl compounds using biocatalysts has been the object of many studies [5-13].

Most of the Alcohol DeHydrogenases (ADHs) are co-factor-dependent enzymes. Their mechanism of action can be schematised by the cycle reported in Scheme (**2**), where the role of the co-factor is to turn the enzyme back to its reduced form. The nature of the used co-factor is often used to classify enzymes. There are mainly two classes: $NAD(P)^+$ dependent enzymes, and PQQ (pyrroloquinoline quinone) dependent enzymes. These enzymes have been already introduced in a previous paper [14].

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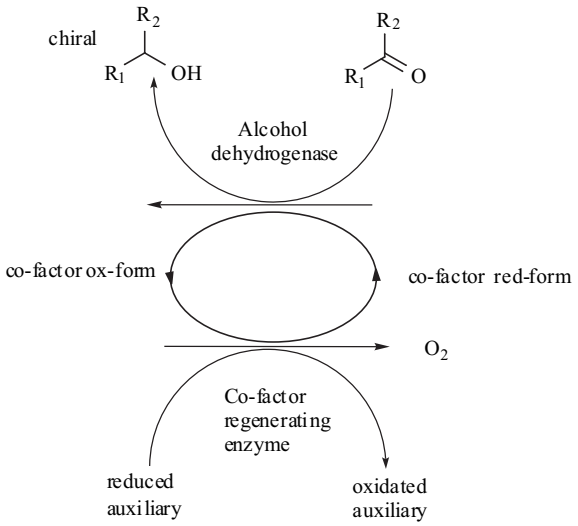

Scheme 2. Common mechanism of the ADH carbonyl reduction.

2. ENANTIOSELECTIVE ENZYMATIC ALCOHOL PREPARATION

Chiral alcohols are useful starting materials applicable to the synthesis of various pharmaceuticals that must be prepared in optically active form; therefore, the chiral alcohols used as synthons must be enantiopure compounds. Chemical preparation of chiral alcohols is possible through many different methods; however, production of chiral alcohols through the asymmetric reduction of prochiral carbonyl compounds using biocatalysts, such as microbial cells and commercially available oxidoreductases, represents a valid alternative and, as a consequence, has been carefully studied [5-12]. The reaction is classically based on two parallel actions: one is the carbonyl reduction operated by the oxidoreductase, and the other is the regeneration of the reducing power, i.e. of the enzyme co-factor (Scheme (**2**)).

This scheme can be considered common to both the whole cell and the isolated enzyme methods. Microorganisms already have both the activities and, in principle, it is possible to develop systems that produce the

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chiral alcohols without much effort. However, in the great majority of the cases, it is common practice to prepare recombinant strains that contain the oxidoreductase and these strains can show a limited activity because their regenerating machine is not sufficiently active. In these cases, it is important to also enhance the co-factor regenerating step, for example, introducing an external more efficient enzyme. In the isolated enzyme case, the need for the co-factor regeneration is essential.

Carbonyl Reduction

i. Whole Cells

The possibility of using whole cell biocatalysts is appealing, because it implicitly resolves the problem of co-factor regeneration. However, it is sometimes necessary to enhance the reductive power of the microorganism in order to obtain appreciable reaction yields and rates. In principle, for the construction of a bioreduction system using co-expressing *E. coli* transformant cells, it is required: (1) to select and characterise carbonyl reductases, (2) to clone their genes, (3) to co-express them with the co-factor regenerating gene, and (4) to optimise the conditions of the transformation in a reactor system [15].

One interesting example of the first point is the recently reported screening of a reductase that can reduce ketopantoyl lactone (KPL), ketopantoic acid (KPA), ethyl 20-ketopantothenate (KPaOEt), and 4-chloro-3-oxobutanoate ethyl ester (COBE) [15]. It is noteworthy that different microorganisms show different recognition power, different stereoselectivity, and different conversion rates in connection to each substrate. This result evidently shows the importance of selecting the best candidate among the available activities. In the same example, it is emphasised that it is possible to find reductases with different stereoselectivities (even of opposite sign) that lower the purity of the product; therefore, it is essential to isolate and clone the useful genes to get good results. A different approach is represented by the direct screening of the good microorganism without worrying about the nature of the enzyme involved. On this line, Matsuyama *et al.* [16] discuss the search for a microorganism that can effectively reduce 4-hydroxy-2-butanone that is the precursor of 1,3-butanediol (whose R-form is a precursor of azetidinone derivatives, important synthons in pharmaceutical chemistry). The screening concerned over 1000 microorganisms; at the end, the authors could select a candidate, *Candida parapsilosis* IFO 1396, to produce (*R*)-1,3-BDO with 97% *e.e.*

The third point, the co-expression of a co-factor regenerating enzyme, is of special importance when it is desired to scale the production of the chiral alcohols to practical levels. It has been verified in many cases that the limiting factor of carbonyl reduction is not the reductase activity, but the slow co-factor regeneration. In these cases, it is necessary to clone a second activity into the biocatalyst to overcome the rate limitation. An obvious choice is the selection of a competitive ADH that oxidises an inexpensive substrate generating the reduced form of the co-factor that is used by the reductase. Kataoka *et al.* [15] employ glucose dehydrogenase cloned in the same recombinant strain containing the active dehydrogenase. The authors observe that in the absence of GDH the reduction is completely depressed [17]. However, in many other cases, the presence of native regenerating systems is sufficient to guarantee the reaction success.

Finally, the alcohol production can be increased optimising the bioconversion system; this can be realised by carefully considering all factors that can influence the conversion. Among the most relevant there are: the substrate-product miscibility in water, their toxicity, the optimal environment for the cell and for the chemicals. These apparently simple factors however, require a careful consideration, because when working with whole cell, even small variations can greatly affect the reaction outcome. This is evident examining the great number of papers that concern the study of low-high temperature, low-high pH, multi-phase, continuous and fed batch conversions. Where the case demands, these aspects will be discussed in the following examples.

Reduction of COBE to (R)-CHBE ((R)-4-chloro-3-hydroxy butanoate ethyl ester) has been achieved using a recombinant strain containing a reductase from *Sporobolomyces salmonicolor* and GDH NADP regenerating genes [15]. The conversion system uses a biphasic solution (n-butyl acetate/water); the total yield is 300g/L of product with 92% *e.e.* in 16 h. The (S)-enantiomer is produced using a similar system containing an ADH from *Candida magnoliae*; the yield is 500g/L with 100% *e.e.* in 34 h.

The recombinant *E. coli* expressing CpSADH (*Candida parapsilosis* (S) alcohol dehydrogenase) produced ethyl (*R*)-CHBE from ECAA (ethyl 4-chloroacetoacetate), which was a derivative of diketene, with 2-propanol (IPA) [16]. Under suitable conditions, this recombinant *E. coli* reduces ECAA to ECHB in the (*R*)-configuration at 36.6 g/L and 95.2% yield with 99% *e.e.* Its maximum yield was reached after 14 h of incubation at 15°C. The same microorganism produced, in oxidative direction, (R)-1,3-butanediol from the racemate with 97% *e.e.*

Glycerol dehydrogenase from *Hansenula polymorpha* DL-1 was cloned in a recombinant *E.coli* and used in phosphate buffer (at pH 8.0) to both stereospecifically oxidise and reduce several compounds (Table **1**) [18].

A very interesting example concerns the use of a single microorganism to produce both enantiomers of secondary alcohols [19]. This result is achieved by tuning the conversion conditions. Thus, when *G.candidum* IFO 5767 cells are added to a mixture of a ketone (300 mg) and XAD-7 (18 g) in water (90 ml), and the mixture is shaken for 1 day at 30°C under a nitrogen atmosphere, the corresponding (*S*)-alcohol is produced. For example, the reduction of acetophenone (**4a**) gave (*S*)-phenylethanol ((*S*)) in >99% *e.e.*with a 98% chemical yield. In contrast, when the cells and a ketone are added into a Sakaguchi flask containing water (100 ml), and shaken for 1 day at 30°C, the corresponding (*R*)-alcohol is produced (e.g., (*R*) can be obtained with a 99% chemical yield in over 99% *e.e.*). These two procedures were applied to many other ketones with similar results (Table **2** and Scheme (**3**)).

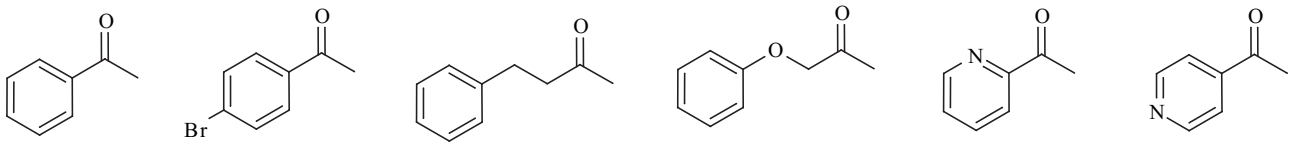
Table 1. Expression of the Gene of Glycerol Dehydrogenase in *E. Coli* HB101 and Reactivity of the Corresponding Enzyme

Substrate	Concentration [mM]	*E. coli* HB101 (pSE-PAD1) [U/mg]
Oxidation		
Glycerol	100	0.228
(*R*)-1,2-Propanediol	50	1.077
(*S*)-1,2-Propanediol	50	0.246
(*R*)-3-Chloro-1,2-propanediol	50	0.002
(*S*)-3-Chloro-1,2-propanediol	50	0.057
(*R*)- and(*S*)-1,2-Butanediol	100	0.200
(R)-1,3-Butanediol	50	0.259
(S)-1,3-Butanediol	50	0.042
(*2R, 3R*)-2,3-Butanediol	50	1.414
(*2S, 3S*)-2,3-Butanediol	50	0.059
meso-2,3-Butanediol	50	1.162
3-Hydroxy-2-butanone	50	0.020
(*R*)-2-Butanol	50	0.022
(*S*)-2-Butanol	50	0.024
(*R*)-1-Amino-2-propanol	50	0.051
(*S*)-1-Amino-2-propanol	50	0.018
(*R*)- and(*S*)-2-Amino-1-propanol	50	0.008
Reduction		
Dihydroxyacetone	20	0.979
Hydroxyacetone	20	0.364
2-Butanone	20	0.008
3-Hydroxy-2-butanone	20	0.696
2,3-Butanedione	20	1.906
4-Hydroxy-2-butanone	20	0.011

One unit of the enzyme activity is defined as the amount of enzyme that catalyses the formation or the decrease of 1 μmol of NADH per min at 30°C. Specific activity is expressed as units per mg of protein.

Recently, the use of plant cells has opened a new field of whole cell biocatalysis. The *Daucus carota* root (carrot) is an inexpensive material that showed interesting ADH activity. In particular, its use for the reduction of organochalcogeno acetophenones was applied to several compounds giving the (S)-configured alcohols in high *e.e.* and yield (Table **3**) [20]. The extremely easy reaction conditions make this approach a fundamental improvement in biocatalysis. The conditions are:

100 mg of the organochalcogeno ketone in ethanol (2.5 mL) in 1L Erlenmeyer flasks containing freshly cut carrot roots (50 g), and water (500 mL) at 32 °C on an orbital shaker (170 rpm).



4a **4b** **4c** **4d** **4e** **4f**

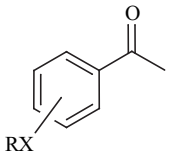
Scheme 3. Substrates reduced by *G.candidum* IFO 5767.

Table 2. Substrates Reduced by *G.candidum* IFO 5767 in Two Different Conditions: A in the Presence of XAD-7 Resin; B in the Absence of XAD-7 Resin[a]

Substrate	Method A				Method B			
	Yield(%)	Isolated(%)	*e.e.*(%)	Config	Yield(%)	Isolated(%)	*e.e.*(%)	Config
4a	98	74	>99	S	99	73	>99	R
4b	>99	92	92	S	>99	99	98	R
4c	96	90	>99	S	61	56	85	R
4d	>99	88	99	S	>99	82	95	R
4e	99	77	98	S	>99	89	99	R
4f	>99	79	99	S	98	60	>99	R

[a]The chemical yield, the *e.e.* and the absolute configurations were determined by GLC analysis.

Table 3. Reduction of Organochalcogeno Ketones 5a–i Using *D. Carota* Root into Chiral Organochalcogeno-α-Methylbenzyl Alcohols

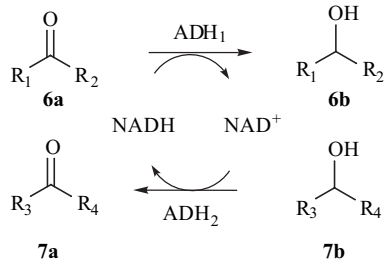
 Entry X = Se, S	R	Time(h)	Conversion(%)[a]	*e.e.*(%)[b]	Configuration
5a	ortho-MeSe	72	n.c.	—	—
5b	meta-MeSe	48	96	>99	(S)
5c	para-MeSe	48	83	>99	(S)
5d	ortho-PhSe	72	n.c.	—	—
5e	meta-PhSe	72	95	>99	(S)
5f	para-PhSe	72	72	>99	(S)
5g	ortho-MeS	72	8	>99	(S)
5h	meta-MeS	72	97	>99	(S)
5i	para-MeS	72	95	>99	(S)

[a]Conversion determined by GC; n.c.= no conversion. [b]Determined by chiral GC.

Soaked *Phaseolus aureus L* was also used to reduce aromatic ketones to the corresponding S-alcohols in very mild conditions [21]. (Washed *Phaseolus aureus L* 500 g. was allowed to soak in deionised water (4 L) for 24 h. Then, the ketone (~5 g) was added to the soaked *Phaseolus aureus L* in the above water, covered and allowed to shake for 24 h at room temperature (28 °C)). Yields and *e.e.s* are reported in the following Table **4**.

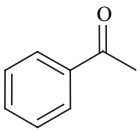
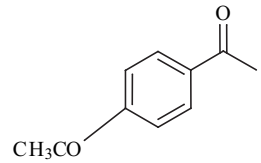
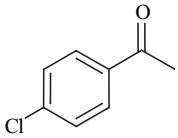
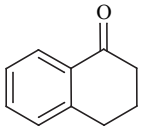
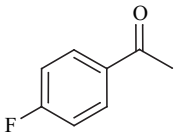
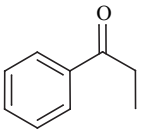
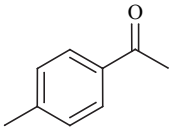
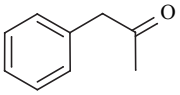
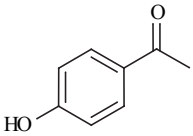
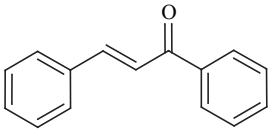
ii. Isolated Enzymes

ADHs are enzymes that can be isolated and purified; this fact together with the recent developments in co-factor regeneration resulted in a great number of experiments of ketone reduction. Also in this case, the selection of new ADHs is important, because the substrate specificity and the stereoselectivity can vary. In addition, the isolation and purification are operations that require attention and that can affect the enzyme efficiency and even its specific activity. The conversion techniques in this case are also more varied compared to whole cell conversions. In fact, even if the natural environment for enzymes is water, they can be also used in organic solvent, or even in ionic liquids; however, ADHs are still commonly used in water. In contrast, the co-factor regeneration systems have been thoroughly studied. In principle, there can be two approaches to solve the problem:



Scheme 4. Coupling of two redox reactions to solve the co-factor regeneration problem.

Table 4. Enantioselective Reduction of Various Aromatic Ketones with Soaked *Phaseolus aureus L*

Substrate	Time (h.)	Yield	*e.e.*	Substrate	Time (h.)	Yield	*e.e.*
	24	52	84		24	28	90
	24	50	90		46	55	98
	24	45	87		24	50	80
	24	51	95		24	48	98
	72	23	72		24	28	85

in the first, a second substrate, less expensive, can be used in a reverse reaction; in the second, a different ADH using a different substrate can be coupled with the wanted reaction (Scheme (**4**)).

Both the reactions are in equilibria and can be shifted to the desired direction adjusting the reaction conditions. In this case, if the desired product is **6b**, it is necessary to shift the first reaction to the right shifting the second reaction to the left. This result can be reached in many ways: the most trivial is to use a great amount of compound **7b**. The alternative is to choose a compound **7b** that generates a product **7a** that can be removed from the reaction environment. Examples of both approaches are available; in particular:

- use of GDH in the presence of great amount of glucose (4:1 with respect to the substrate)
- use of 2-propanol or cyclopentanol (15/20:1 with respect to the substrate)
- use of *Pyrococcus furiosus* hydrogenase in the presence of H_2 that produces H^+ as **7a**
- use of FDH (formate dehydrogenase) in the presence of formate that produces CO_2 as **7a**

Some recent examples are reported below.

In 1998, Shimizu *et al.* [22] reported the reduction of several carbonyl compounds using two ADHs from *S. salmonicolor* and *C. magnoliae* (Table **5**).

It is possible to note the compounds that are reduced are: β-ketoesters, α-dicarbonyls (also cyclic), aromatic aldehydes, and poli-hydroxy aldehydes. The relative activities are very different depending on the substrate and on the enzyme. The reaction is carried out in a biphasic system n-butyl acetate : water and the yield in the case of the substrate in the first row is ~80g/L with an *e.e.* of 86% at a 1.6 L scale.

A similar substrate range has been studied by Itoh *et al.* [23] that reports the reduction of diverse carbonyl compounds as sketched in Table **6**. They used a phenylacetaldehyde reductase from *Corynebacterium* ST-10 coupled with the oxidation of 2-propanol.

Similarly to the previous reported results by Shimizu *et al.*, the range of transformed carbonyls is wide, including both aromatic, aliphatic ketones, and β-ketoesters. The relative activities however span three order of magnitude (from 5 to 2250); compound enantiopurity is always very high (*e.e.* >98%) and the asymmetric carbon configuration is always the same. The reaction was also scaled to grams level in a reference experiment (compound **8**); here, it is interesting to note the study carried on the efficiency of the alcohol chosen for the "twin" NADH regeneration. Somehow surprisingly, 1-pentanol and 1-hexanol were much less effective than both shorter or longer chain alcohols (1-propanol or 2-heptanol). In addition, experiments showed that the endogenous NADH regeneration power of *E.coli* was insufficient to perform the reduction. Finally, the optimised conditions used for **8** were used together with the use of a

Table 5. Substrate Specificity of *S. salmonicolor* (AR) Reductase and *C. magnoliae* (CR) Reductase

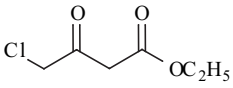
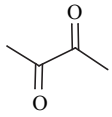
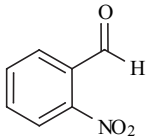
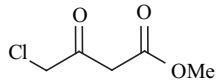
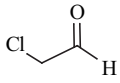
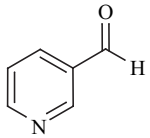
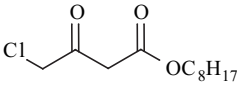
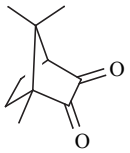
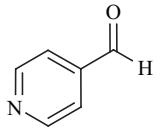
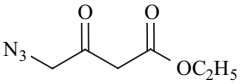
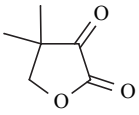
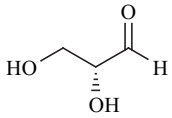
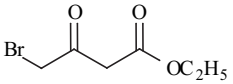
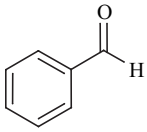
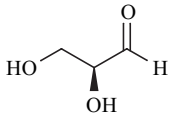
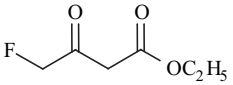
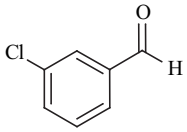
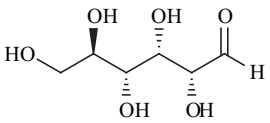
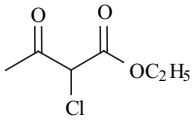
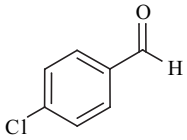
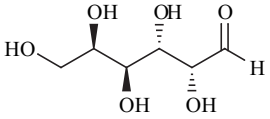
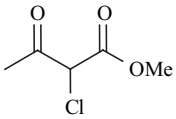
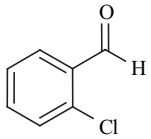
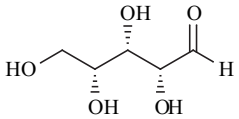
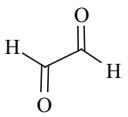
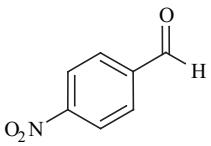
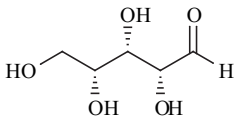
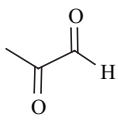
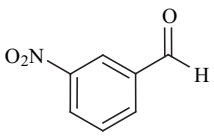
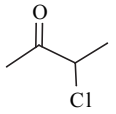
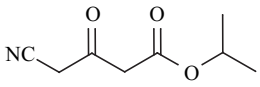
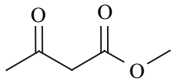
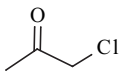
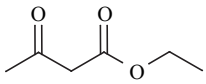
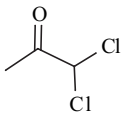
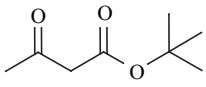
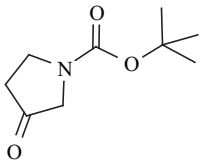
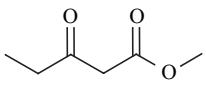
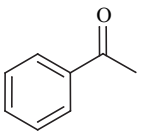
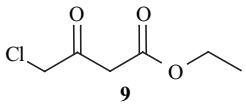
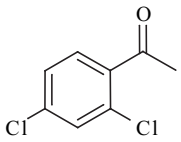
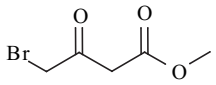
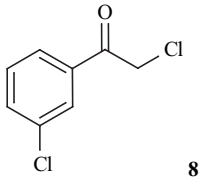
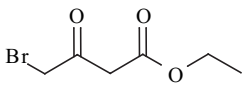
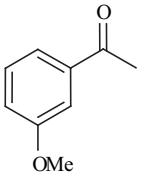
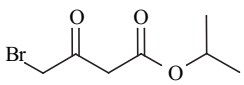
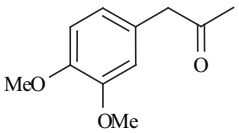
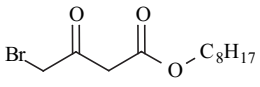
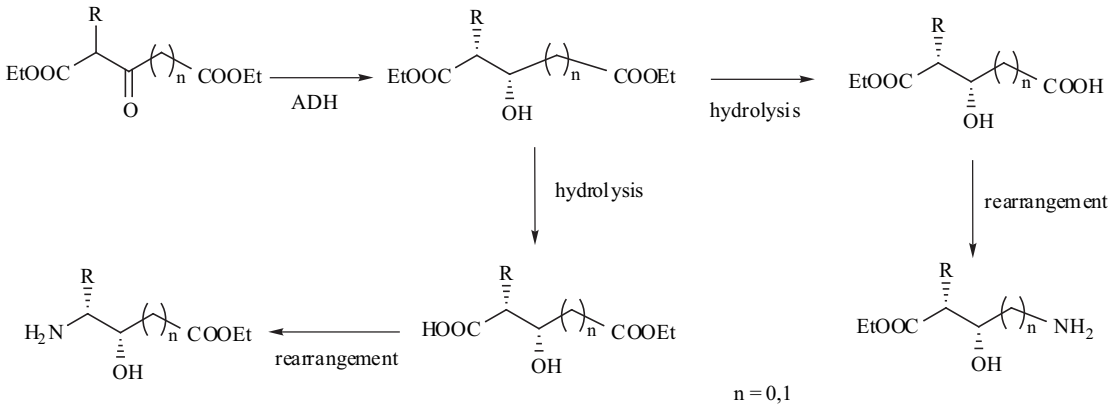
Substrate	Relative activity		Substrate	Relative activity		Substrate	Relative activity	
	AR	CR		AR	CR		AR	CR
	100	100		75	19		14	0
	25	11		17	0		228	0
	240	36		16	0		54	0
	65	n.d.		0	78		64	37
	75	n.d.		14	0		n.d.	65
	153	n.d.		56	0		81	0
	330	90		52	0		24	0
	74	11		58	0		472	0
	74	0		468	0		173	0
	219	0		63	0			

Table 6. Substrate Specificity of *Corynebacterium* ST-10 Phenylacetaldehyde Reductase

Substrate	Relative activity	*e.e.* %	Substrate	Relative activity	*e.e.* %
	122	n.d.		7	n.d.
	154	n.d.		188	n.d.
	868	99 (S)		449	n.d.
	2247	n.d.	 **10**	200	99 (S)
	5	n.d.		100	n.d.
 **9**	187	99 (R)		546	99 (S)
	18	n.d.	 **8**	258	99 (R)
	274	n.d.		744	99 (S)
	994	98.4 (R)		70	99 (S)
	545	n.d.			

two-phase environment and were applied to the reduction of compounds **9** and **10**, whose corresponding alcohols represent interesting industrial products.

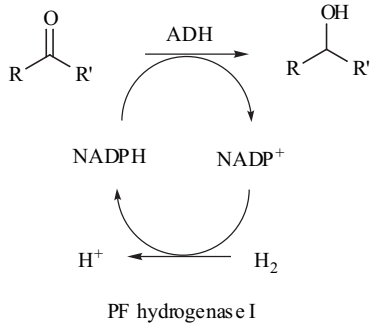
α-Hydroxy-β-amino and β-hydroxy-γ-amino acids are valuable intermediates in the synthesis of chiral compounds. The key reactions are: the preparation of the appropriate hydroxy-dicarboxyesters derivatives that are formed by enzymatic reduction of the corresponding keto derivatives; the partial hydrolysis of one ester group; and the Hofmann or Curtius rearrangement to the amino derivative [24] (Scheme (**5**)).



Scheme 5. Preparation of α-hydroxy-β-amino and β-hydroxy-γ-amino acids through enzymatic reduction and Hofmann or Curtius rearrangement.

As can be seen in the Scheme during the ketone reduction, two chiral centres are created giving rise to four potential diastereoisomers. However, depending on the structure, the results are from complete to good stereoselectivity. Ester chemical hydrolysis can be performed in very high regioselectivity originating the wanted monoester. The final rearrangement can be performed in both Hofmann and Curtius conditions producing the hydroxy-aminoacids. Depending on the hydrolysed ester group and on the distance between the ester and the keto groups, it is possible to prepare α, β or β-γ hydroxy amino acids.

Hummel [8] also reported a list of ADHs used in the preparation of various chiral alcohols. However, in this paper, the most interesting contribution to the present review concerns the engineering of the process; In particular, the preparation of L-tert-leucine by reductive amination of trimethyl pyruvate. The enzyme used is a leucine dehydrogenase from a *Bacillus* strain. The transformation is mainly limited by co-factor regeneration. In this example, the method employed uses membrane reactors. Ultrafiltration membranes with an exclusion limit of 5000–20000 Da retain the enzymes. The co-factor is either covalently coupled directly to the enzyme using an appropriate spacer, or to a water-soluble polymer such as polyethylene glycol (PEG). In two-phase membrane reactors, the co-factor can be separately regenerated by FDH after extraction of the product. The yield is in the order of hundreds grams per litre per day. Using a similar technique the author demonstrates the possibility of coupling oxidases and ADHs to get complete deracemisation



Scheme 6. Use of *P.furiousus* hydrogenase as co-factor regeneration system. The produced proton is easily removed by buffer.

of alcohol racemates (e.g. (R, S)-phenylethanol was converted into enantiopure (R)-phenylethanol).

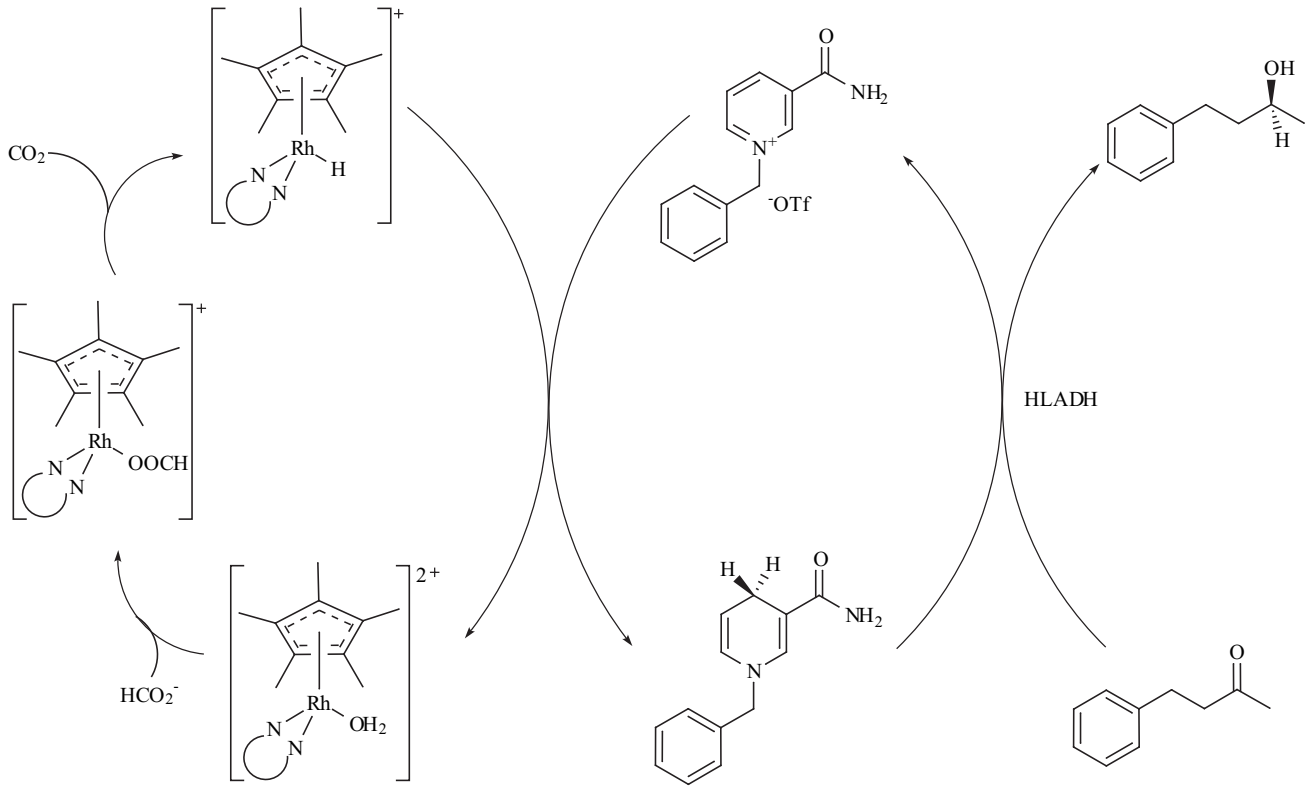
To conclude this chapter, some more specific examples will be reported.

The first example concerns a special enzyme for NADPH generation and regeneration. The enzyme is a hydrogenase from *Pyrococcus furiosus*, a marine hyperthermophilic strain [25]. The interesting difference is the special co-substrate needed by the enzyme to convert $NADP^+$ to NADPH. As already mentioned, this is an equilibrium reaction that must be taken to the right working on the reaction conditions. Here, the reaction is made irreversible by use of H_2 as co-substrate; the reaction generates H^+ that is easily subtracted from the mixture by pH control (See Scheme (**6**)).

The comparison of this regeneration system to a classical iso-propanol system indicates that the principal achievement consists in the complete shift of the reaction equilibrium to the right; in contrast, the reduction rate is not affected, as expected.

In the same area, a non-enzymatic regeneration system is described by Lo and Fish [26]. They used a classical Rhodium complex to regenerate the NADH consumed by an ADH. In particular, using a commercial Horse Liver Alcohol Dehydrogenase coupled with the Rh regeneration system, the authors could prepare some chiral alcohols in good yield and rate. The system is complex because it implicates the use of a substrate that is not NAD^+, but a biomimetic of it; as can be seen in Scheme (**7**), the Rh complex works on substrates that are similar to NAD^+ that can be recognised by the HLADH; this last being a requisite that can limit the use of this regeneration system.

The use of different enzymes to get inverted stereoselectivity has been already mentioned; however, two cases that describe the preparation of particular alcohols are worth of note. In the first, the two enantiomers of various propargilic alcohols are obtained using two enzymes from two diverse microorganisms (*Lactobacillus brevis* and *Candida parapsilosis*) [27]; in the second, two enzymes present in the same microorganism (*Geotrichum candidum*) produce different stereoisomers depending on the substrate nature, i.e. the (S)-enantiomer using methyl ketones and the (R)-enantiomer using trifluoromethyl ketones [28]. In this last case, the authors demonstrated that the shift from one to



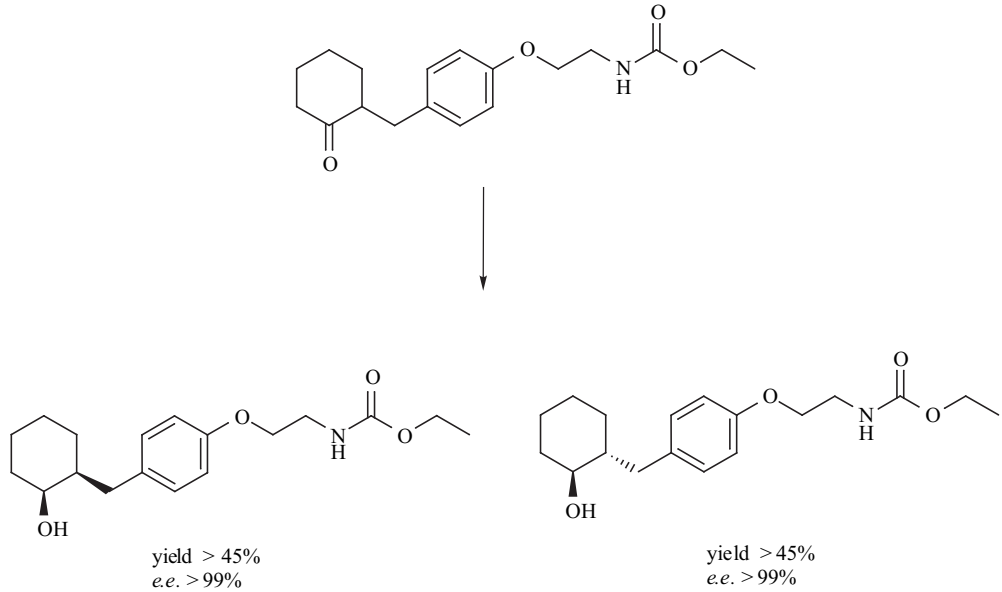
Scheme 7. Horse Liver Alcohol Dehydrogenase reaction coupled to Rhodium mediated co-factor regeneration.

the other selectivity is connected to the electronegativity of the fluorine atom; in fact, passing from 3 H atoms to 1F/2H, to 2F/1H, to 3F atoms, a corresponding gradual shift from S to R selectivity can be observed. In addition, the presence of chlorine atoms produces a similar effect as well as the presence of mixtures of F and Cl atoms.

Finally, an example of application of ADHs to the synthesis of more complex structures can be of interest. A Czech group uses both isolated enzymes and whole cells to the preparation of analogues of insect juvenile hormones [29-30]. As can be seen in the following Scheme (**8**), they prepared two stereoisomers of the same structures in good yield and high enantiopurity.

3. COMPARISON OF TECHNIQUES

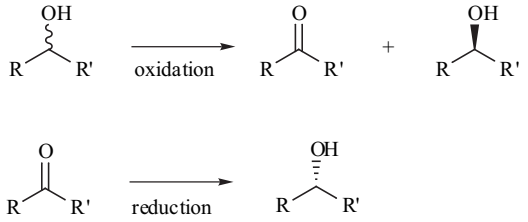
As it can be easily seen reading this paper and a preceding one [14], the preparation of chiral alcohols, particularly secondary alcohols, can be afforded through



Scheme 8. Preparation of analogues of insect juvenile hormones by stereoselective ketones reduction.

enzymatic methodologies: from compound deracemisation to asymmetric carbonyl reduction. Thus, it can be of interest an attempt to compare the advantages and disadvantages of the various techniques.

The first point concerns the alternative use of the enzymatic function in the direct, oxidative, or inverse, reductive, direction. The difference between the two is not merely a question of choice, because the obtained product is usually stereochemically different (Scheme (**9**)).



Scheme 9. Different products obtained using ADHs in oxidation or reduction sense.

It is thus possible to prepare either one or the other enantiomer. This fact is related to the recognition specificity of the enzyme that is usually the same in both directions. In addition, depending on the enzyme and on the substrate, the reaction reversibility can vary favouring one of the two reactions. Finally, the choice can be influenced by the efficiency of the stereoselection; the deracemization, in principle, can be adjusted at will; it is sufficient to optimise the cycles of the oxidation-reduction alternation; in contrast, the reduction cannot be changed without changing the enzyme. However, the reduction is usually more efficient producing the needed enantiomer without recycling operations.

The second issue concerns the whole cell – isolated enzyme dichotomy. Chemists have long preferred isolated enzymes because they can be used as common reactants. However, in part related to the enormous improvements of the biotechnology techniques, the use of whole cells is becoming more and more popular. In addition, the present methodology to prepare enzymes uses recombinant strains that can be also directly employed to carry the transformation on. It is nevertheless possible to compare the two methods in their practical differences.

Whole cell bioconversions have their advantages in the easy and cheap biocatalyst preparation, and in the mild and environmentally compatible conditions; in contrast, their disadvantages are in the absolute yields and in the managing of the living organisms that is uncommon for chemists. In recent years, many improvements have been applied to whole cell bioconversions; among them being, new immobilisation techniques, new fermenter exploitations, and higher control on the reaction conditions. In particular, great attention has been devoted to the increase of the absolute yield per unit volume. In fact, it is clear that in order to become competitive, the biotechnological approach must overcome the yield limits that, in many cases for whole cells, are related to substrate/product toxicity, to substrate bioavailability, and to product recovery.

In contrast, isolated enzymes have their advantages in the high absolute yield, in the easy handling and use, and in the possibility of addressing the reactivity request using "classical" chemical methods; their disadvantages are the difficulty and the cost of their preparation, their stability, and the need of introducing all the required components of the reaction in the reaction environment with particular emphasis on co-factor recycling. As in the case of whole cell, the disadvantages have been targeted to enhance the enzyme performance; thus, for example, the problem of co-factor regeneration has now many potential solutions.

4. CONCLUSION

In this review, we analysed the present state of the enzymatic preparation of enantiopure chiral alcohols. The use of ADHs as reductive enzymes was shown as the opportunity of preparing enantiopure alcohols. In addition, the possibility to use isolated enzymes or whole cells demonstrated the potential of the biological approach also from the viewpoint of the techniques. The number of examples are a significant evidence of the importance that the enzymatic preparation of enantiopure alcohols presently have. Finally, the comparison of the available alternatives was used to complete the outline of the current state of the research in this field.

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