Alcohol and 1,2-Diol Dehydrogenases: Synthetic Use as Oxidants

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Abstract: The redox equilibrium between alcohol – carbonyl groups is greatly used by enzymes and chemists to prepare useful compounds. Carbonyls are often intermediate groups whose reactivity can be used to synthesize complex structures; in contrast, alcohols are more easily found in the products of interest because their coordinative ability is used both in biology and in chemistry. Dehydrogenase activities are an interesting alternative to chemical redox reactants because they are often chemo-, regio-, and stereo-selective. These enzymes allow for two different uses: direct, as racemates resolving agents; inverse, as enantioselective reducing agents. We will focus on their use as oxidative agents, considering both the well-known alcohol dehydrogenases and the less known and less used 1,2-diol dehydrogenases. An account of both classes will be presented.

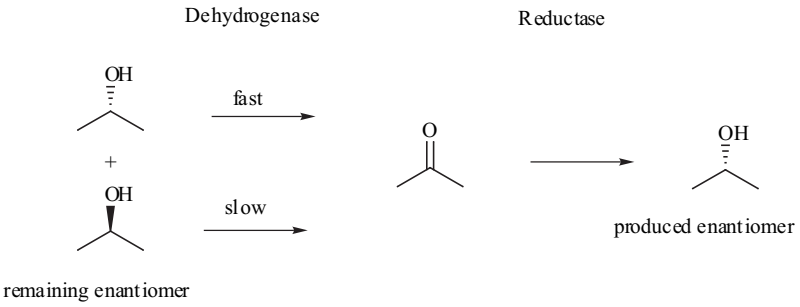
Keywords: Chiral alcohols, chiral 1,2-diols, enzymatic methods, alcohol oxidation, dehydrogenases, racemate resolution.

1. INTRODUCTION

Alcohols and carbonyls are interesting intermediates in organic synthesis. They contain reactive functions that can be often transformed in other functions in chemo- and stereo-selective mode. In the context of the everyday increasing demand for enantiopure compounds, the possibility of producing enantiopure chiral alcohols using enzymatic procedures has become an appealing alternative to classical chemical synthesis. There are two main approaches to prepare chiral alcohols: by reduction of the corresponding prochiral carbonyls; by resolution of racemic mixtures [1-4]. This last option can be pursued by the selective oxidation of one enantiomer, leaving the other enantiomer unchanged. In chemical reactivity, the alcohol oxidation and the carbonyl reduction are two separate reactions that need different catalysts and different reaction conditions. In contrast, in enzymes, the catalyzed reaction is often reversible and the catalyst can work both ways. This property presents also another interesting aspect: when the reaction is stereoselective, it recognizes the same geometry, thus producing and consuming the same stereoisomer. It is consequently possible to access both enantiomers using the same catalyst following the two alternative reaction directions (Scheme (**1**)).

Also in this research area, most of the enzymes derive from microbes, because their enzymes are easily located and produced [5]. In the field of alcohol oxidation, the microbial dehydrogenases are nearly ubiquitous and they present flexible substrate recognition and high efficiency.

Comparing the two methods of producing chiral alcohols using biocatalysts, i.e the optical resolution of racemic alcohols or their derivatives and the direct synthesis of chiral alcohols from prochiral compounds, we can point to different advantages. The advantage of the racemate resolution is that the biocatalysts might be easy to use, since the starting material, racemic alcohol, is the same as that in conventional chemical resolution method. The advantage of the latter is that its theoretical yield of chiral alcohols is 100%, whilst the maximum yield in resolution is 50% of the starting material. However, the two methods have been enhanced to the point to overcome their respective disadvantages; racemate resolution has been taken to 100% yield through the use of dynamic deracemization [1], whilst reductive biocatalysts have been improved and optimized through the use of molecular biology. Production of chiral



Scheme 1. Kinetic resolution of alcohol racemates by ADHs.

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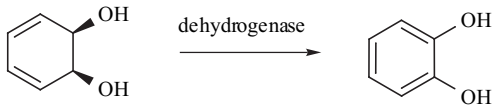
alcohols through racemate resolution using biocatalysts has been thoroughly studied [6-14].

Practically, no work has been done using 1,2-diol dehydrogenases. These biocatalysts have not been thoroughly studied because they catalyze the transformation of 1,2-diols into their respective aromatic 1,2-dihydroxy derivatives (Scheme (**2**)).



Scheme 2. Product of 1,2-diol dehydrogenases.

However, if their use could be made practical, they can represent a new and original source of different chemoselectivity with respect to well-known alcohol dehydrogenases.

2. THE ENZYMES

To give a complete outlook of the potential and applicability of these enzymatic activities, it is worth to introduce the enzymes involved in the oxidation of alcohols.

a. Alcohol Dehydrogenases

The first enzyme class is constituted by the alcohol dehydrogenases (ADH). These enzymes are known and used since several years; it is sufficient to mention the production of acetic acid by the oxidative fermentation of ethanol. Nevertheless, the enzyme structures and functions have been studied only at the end of the last century, using the new tools of molecular biology [15, 16].

These enzymes are present in all organisms, where they are involved in one of the most common activities in living beings: the transformation of alcoholic groups into their corresponding carbonyl and/or carboxyl derivatives. Of course, the most commonly used ADHs in biocatalysis derive from microorganisms that are an available, low cost source of enzymatic activities. The ADH specificities are highly varied depending on the original microorganism, but their basic function is very similar (Scheme (**3**)): the enzyme in its oxidized state oxidizes the substrate being accordingly reduced, then it returns to its original state at the expense of a co-factor that is reduced.

The reaction is an equilibrium that is shifted in one or the other direction by the amount ratio of its participants. This fact is very important, because it in principle allows for the tuning of the reaction toward the desired direction. In this sense, the role played by the co-factor is fundamental; it is thus important to introduce the ADHs dividing them by the involved co-factor. Many of the most well-known ADHs are $NAD(P)^+$ dependent enzymes; however, recently, many PQQ (pyrroloquinoline quinone) ADHs have been reported. Both classes perform the same transformation; but both specificities and mechanisms are different.

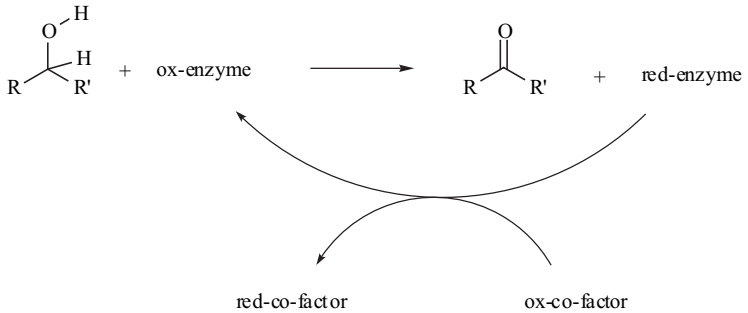
$NAD(P)^+$ Dependent ADHs

The great majority of the commercially available ADHs are $NAD(P)^+$ dependent enzymes. $NAD(P)^+$ are mostly soluble components of the enzyme, in contrast to FAD and FMN that are co-enzymes bound to the oxidoreductase [17]. In addition, much work has been done to convert $NADP^+$ dependent enzymes into NAD^+ dependent enzymes, because NAD^+ is cheaper and more easily available [18]. Depending on the original source, the enzymes show different specificities. Horse-liver ADH readily oxidizes a broad variety of cyclic alcohols and 2- or 3- hydroxy esters. *T. brockii* ADH prefers open chain methyl and ethyl alcohols. In order to oxidize bulky alcohols, it is possible to use an NAD-dependent ADH from *Rhodococcus erythropolis*, or an NADP dependent ADH from *Lactobacillus*; i.e. *L. kefir* ADH accepts a broad variety of alcohols, such as 1-phenyl ethanol (and similar ring substituted compounds), aliphatic open-chain alcohols, 2- and 3-hydroxy esters, cyclic alcohols.

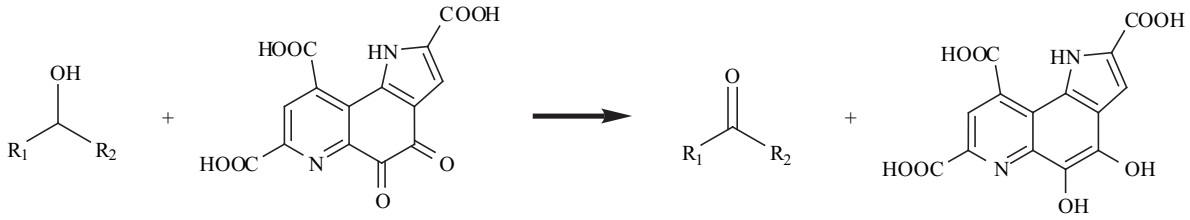
PQQ Dependent ADHs

This recently discovered class of ADHs [19] uses as co-factor PQQ (see Scheme (**4**)), or related compounds. PQQ has a quite high midpoint redox potential (+90 mV) compared to pyridine nucleotide (-320 mV) and flavin (-45 mV). They are mostly periplasmic proteins and require less energy, or even no energy, to effect the oxidation. They can be further subdivided by: the type of the quinone co-factor; the presence of an additional prosthetic group; and their localization. In Table **1**, some of the known PQQ-dependent ADHs are reported.

Substrate specificity is quite broad. A short list is reported in Table **2**; here, it is possible to observe the high number of compounds that can be oxidized by these enzymes. They can be subdivided into several types, depending on their structure and their prosthetic group. The



Scheme 3. Common mechanism of the ADH alcohol oxidation.



Scheme 4. PQQ (pyrroloquinoline quinone) co-factor of ADHs.

Table 1. Classes of PQQ-Dependent ADHs

Enzyme class	*Form*	*Enzyme*	*Prosthetic group*	**Source**
Quinoproteins	Soluble			
		Glucose dehydrogenase	PQQ	*Acinetobacter calcoaceticus*
		Methanol dehydrogenase	PQQ	Methylotrophs
		Alcohol dehydrogenase Type I	PQQ	*Pseudomonas* species
		Sorbose dehydrogenase	PQQ	*Gluconobacter* species
		Methyl amine dehydrogenase	Tryptophan tryptophyl quinone (TTQ)	Methylotrops
		Aromatic amine dehydrogenase	TTQ	*Alcaligenes* species
		Amine oxidase	Topaquinone (TPQ)	Various species
		Lysyl oxidase	Lysine tyrosyl quinone (LTQ)	Mammals
	Membrane-bound			
		Glucose dehydrogenase	PQQ	Various species
		Glycerol dehydrogenase	PQQ	*Gluconobacter* species
		Quinate dehydrogenase	PQQ	*A. calcoaceticus* *Gluconobacter* species
Quinohemoproteins	Soluble			
		Alcohol dehydrogenase Type II	PQQ/heme c	Various species
		Lupanine hydroxylase	PQQ/heme c	*Pseudomonas* species
		Amine dehydrogenase	TPQ/heme c	*P. putida* *Paracoccus denitrificans*
	Membrane-bound			
		Alcohol dehydrogenase Type III	PQQ/heme c	*Acetobacter* species *Gluconobacter* species
		Sorbitol dehydrogenase	PQQ/heme c	*Gluconobacter* species

Table 2. Substrates of PQQ-Dependent ADHs

Alcohols	*Sugar and sugar alcohols*
methanol	L-sorbose
ethanol	glycerol
1-butanol	D-glucose
1,2-propanediol	maltose
polyethylenglycol	D-fructose
tetrahydrofurfuryl alcohol	D-mannitol
polyvinyl alcohol	meso-erythritol
1-propanol	lactose

types that show the broadest specificity are ADH I and II, especially this latter can oxidize several different primary and secondary alcohols and aldehydes. There are also many PQQ-dependent quinoproteins that can oxidize sugars and sugar alcohols. The structures of some of these proteins have been recently reported based on X-ray analyses [20-22]. It is interesting to note that single mutations can greatly change their substrate recognition capability [21].

It is important to compare the function of quinoproteins with pyridine nucleotide- and flavin-dependent enzymes. Since both protein classes have similar functions, the important difference is the diverse redox potential that the two co-factors show; this characteristic can be important to allow for fast oxidation reactions by quinoproteins, modulating the response of different bacteria to various environments.

b. 1,2-Diol Dehydrogenases

A second class of alcohol dehydrogenases is specific for diol recognition; in particular, these enzymes perform the transformation of dihydrodiols into the corresponding aromatic dihydroxy derivatives (Scheme (**2**)); consequently, the scope of these activities is very different. Nevertheless, the reaction should follow a similar mechanism and its discussion can be of interest in the same presentation. DDHs are NAD(P)-dependent enzymes that operate the second step in aromatic compounds degradation by microorganisms. Because the first step is an NADH-dependent dioxygenation, it is clear that the second step has advantage from the restoration of the consumed NAD. Dehydrogenases that catalyze the oxidation of cis-diols from different aromatic compounds (benzoic acid [23], naphthalene [24], toluene [25], chloridazon [26], phenanthrene [27] and benzene [28]) have been purified from *Pseudomonas* species. A comparison of these enzymes shows that in many cases, they are specific towards cis-dihydrodiols and NAD, with pH optima between 7.9 and 9.8. In addition, these dehydrogenases are usually homotetramers, and are members of the type II short-chain alcohol dehydrogenase family [29].

3. ENZYMATIC DERACEMIZATION

a. Alcohol Oxidation

To obtain chiral molecules by biocatalytic reactions, we can imagine two methods, i.e. the desymmetrization of *meso*- and prochiral compounds [30, 31] and the kinetic resolution of racemates, [4]; the latter is much more frequent, probably because the synthesis of racemates is easier than that of *meso*- and prochiral substrates. Notwithstanding, this kinetic resolution has several inherent disadvantages for practical applications. In fact, an ideal resolution process should provide a single enantiomeric product in 100% yield. This goal can be hindered by different drawbacks:

(i) The theoretical yield of each enantiomer can never exceed a limit of 50%.

(ii) Separation of the product from the remaining substrate may be difficult, in particular for those cases where simple extraction or distillation fails and chromatographic methods are required [32].

(iii) In the majority of processes, only one stereoisomer is desired. When also the second isomer may be used through an enantioconvergent synthetic path, this can be often done at the expense of additional labor and yield [33].

(iv) For kinetic reasons, the enantiomeric purity of substrate and/or product is low at 50% conversion and the resolution must be stopped at an earlier point, making more complicated the separation and purification of the desired compound [34].

In recent years, resolution techniques have been further developed with the objective of completely transforming a racemate into a single isomer. This operation is commonly called 'deracemization'; in Table **3** the difference between resolution, desymmetrization and deracemization is sketched [35]. The general principle of these methods is that the substrate enantiomers are transformed through two different stereochemical pathways. For example, whereas the stereochemistry of the R enantiomer remains the same during its transformation, the S enantiomer reacts with *inversion* of configuration; thus, both enantiomers give a product with a single absolute configuration in 100% theoretical yield.

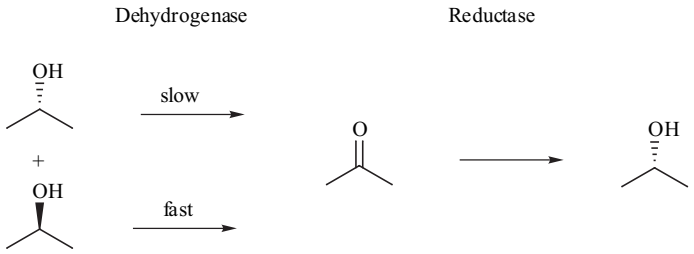
In the case of alcohol dehydrogenases, we usually have a significant difference in the recognition of the alcohol enantiomers; one of them is rapidly oxidized to the corresponding ketone, whilst the other reacts much more slowly. This in principle allows for the recovery of the slow reacting enantiomer in pure stereochemical form and in 50 % yield, achieving the resolution step.

To obtain the complete deracemization, the produced ketone must be recycled; this second step can be achieved following different strategies. The most obvious of them is the chemical reduction of the ketone to give a new racemate that, compared to the starting material, is composed by 25% of each enantiomer. The new racemate can be again submitted to the resolution step resulting, summed to the previous operation, in a 75% of the slow reacting enantiomer and a 25% of ketone. Successive repetitions of this sequence will rapidly arrive at very high enantiomeric purity (e.g. ~97% of the slow reacting enantiomer after five steps). This ideal situation is quite well approximated by experiments that, in a limited amount of steps, arrive at >95% enantiomeric purity in appreciable total yield (~80%). The greatest drawback of this approach is the need to perform separated bio- and chemical transformations; in fact, the reductive step is nearly always incompatible with the enzymatic reaction conditions.

An interesting alternative is the performance of both steps, oxidation and reduction, by enzymatic reactions. In this case, it is possible to conceive a more direct way to get complete deracemization. In fact, due to the enantioselective recognition of enzymes, both oxidative-reductive steps will be stereospecific: in the first, only one enantiomer is oxidized to the corresponding ketone, in the second, this last is enantioselectively reduced to only one isomer. Choosing the correct complementary enzymes, the only product will be the non-reacting isomer of the first enzyme (see Scheme (**5**)).

Table 3. Differences Between Different Methods to Prepare Enantiopure Alcohols

Method	*Substrate*	*R Product enantiomer*	*S Product enantiomer*	*Remaining substrate*	*Mechanism*
Kinetic resolution	Racemate	50 %	0 %	S	100 % retention or inversion
Desymmetrization	Prochiral or meso compound	100 %	0 %	none	Enantioselective transformation
Deracemization	Racemate	100 %	0 %	none	50 % retention 50 % inversion



Scheme 5. Use of different ADHs to completely deracemize an alcohol racemate.

It is clear that the same result will be also obtained using the second transformation on its own. Due to the possibility of using the ADHs to perform the inverse (reductive) reaction, there are few examples of their application in the oxidative direction. There are two main application areas: the first concerns the transformation of sugars, and the second, the oxidation of primary alcohols to either aldehydes or acids. The motivation behind these two uses is different; in fact, in the first case, the selection of the enzymatic preparation is favored by the availability of the compounds together with the enzyme selectivity, whilst in the second case, the mild reaction conditions are the main support of the bioconversion alternative.

PQQ-dependent ADHs show recognition for a good range of sugars, or polyhydroxy compounds. D-Arabitol dehydrogenase (ARDH) from *G. suboxydans* IFO 3257 [36] can oxidize various sugar alcohols to their corresponding oxidation products, such as glycerol to dihydroxyacetone, D-arabitol to D-xylulose, D-sorbitol to L-sorbose, and D-mannitol to D-fructose, following the Bertrand–Hudson's rule [37] in sugar alcohol oxidation (i.e., the most favorable configuration for oxidation has the erythro form and R-configuration of two secondary hydroxyl groups adjacent to the primary alcohol).

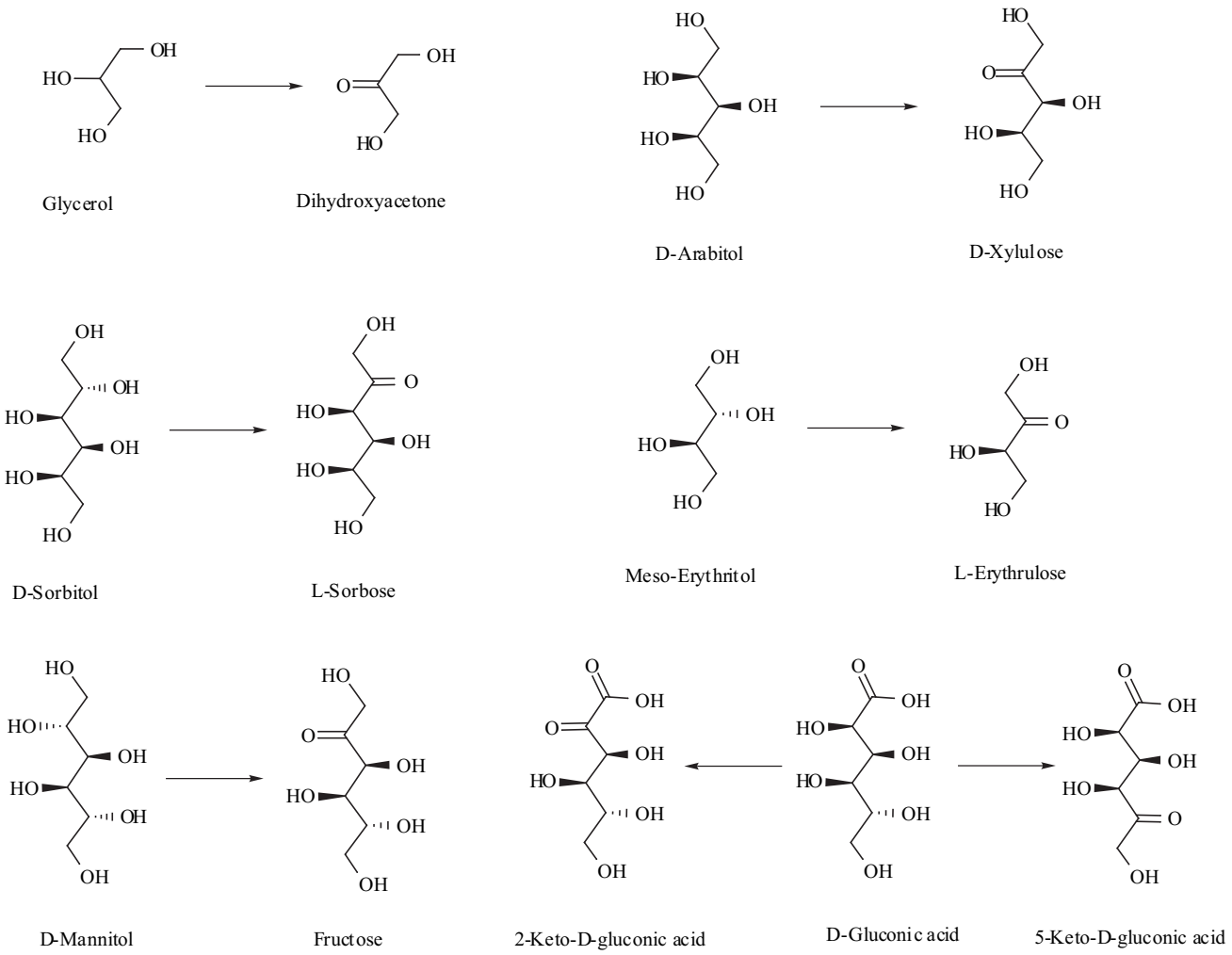
Membrane-bound glycerol dehydrogenase (GLDH) from *Gluconobacter industrius* IFO 3260 was reported to have the same wide substrate specificity as ARDH [38]. It is worth noting that the enzyme catalyzes D-gluconate oxidation to yield 5-keto-D-gluconate, whereas 2-keto-D-gluconate is produced by a flavoprotein D-gluconate dehydrogenase.

Because L-erythrulose is not readily available from commercial sources, it is important to investigate the fermentation profile of L-erythrulose production, to identify the enzyme responsible for meso-erythritol oxidation. *G. frateurii* CHM 43 was screened among thermotolerant *Gluconobacter* and mesophilic strains as a high L-erythrulose producer from meso-erythritol when grown at 37 ˚C [36].

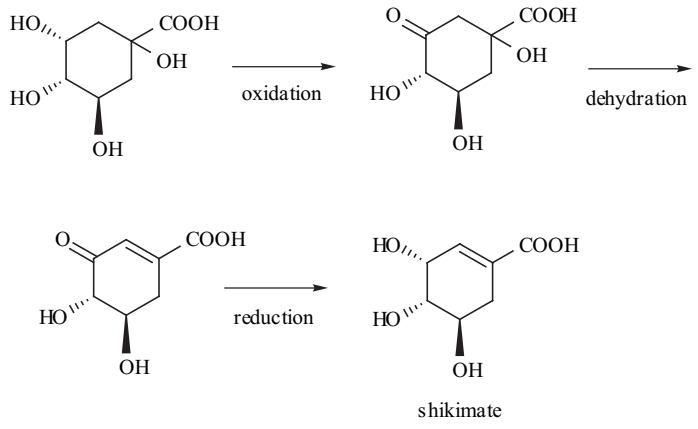
ARDH catalyzes L-sorbose production from D-sorbitol; a different FAD-containing D-sorbitol dehydrogenase (FAD-SLDH) [39], catalyzes the oxidation of D-sorbitol to D-fructose. D-Fructose production by oxidative fermentation is competitive with D-glucose isomerase, because this oxidation is irreversible, unlike D-glucose isomerase. Most strains of the genus *Gluconobacter* contain another D-sorbitol oxidizing enzyme yielding L-sorbose, which is very important in industrial production of vitamin C (Scheme (**6**)).

Several aerobic bacterial strains carrying quinate dehydrogenase (QDH) have been selected, i.e. *Gluconobacter melanogenus* IFO 3294, *G. oxydans* IFO 3292, *G. oxydans* IFO 3244, and *A. calcoaceticus*. A coupling reaction from quinate to shikimate is illustrated in Scheme (**7**). It shows the transformation of quinate to 3-dehydroquinate, 3-dehydroshikimate, and shikimate by oxidative fermentation, making accessible the new syntheses for many antibiotics, herbicides, and aromatic amino acids derived from the shikimate pathway.

The second area of application of oxidation by ADHs concerns the transformation of simple alcohols into the corresponding aldehydes or acids. As mentioned, this reaction is attracting interest because of its mildness. Besides the well-known oxidation of ethanol, other short-chain alcohols are transformed into the corresponding acids by ADHs. For example propionic acid, that can be produced from oxidation of sugars in anaerobic environment, can be prepared from propanol using dehydrogenases present in *Gluconobacter oxidans* in high yield [40]. The optimization of its preparation requires the accurate setting of the transformation conditions, including co-substrate (glycerol) addition, accurate pH choice, counter-ion neutralizing choice, and feeding methodology selection.

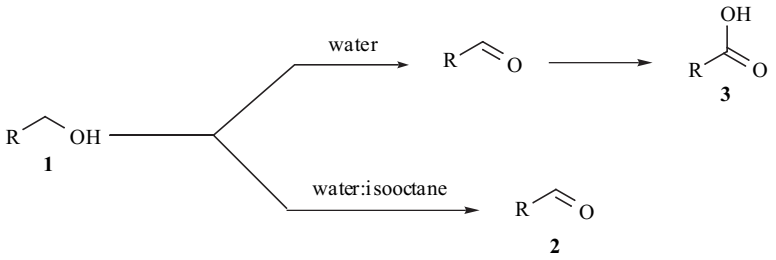


Scheme 6. Chemoselective oxidation of sugars by *Gluconobacter* strains.



Scheme 7. Preparation of shikimate from quinate by chemoenzymatic approach.

In a different approach, several primary alcohols have been transformed into either aldehydes or acids [41]. In this case, the correct choice of the reaction conditions allowed for the preparation of one of the products at will. Using a two-phase preparation, it is possible to stop the reaction at the aldehyde level, whilst in water, the main product is the acid. Reaction time also affects the final result, because better aldehyde yield is obtained by stopping the reaction after short time. The efficiency of this procedure is really surprising if we consider that the chemical oxidation of aldehydes to acids is faster than the alcohol – aldehyde step. Some more hints can be expected from the analysis of the solvent role; particularly noting that isooctane is a very poor solvent for alcohols. This scheme has been applied to some substrates using two different microorganisms (see Scheme (**8**) and Table **4**).



R = a) 1-pentanol,
b) 3-methyl-1-butanol,
c) 1-hexanol,
d) geraniol,
e) benzyl alcohol,
f) 2-phenylethanol,
g) 2-phenylthioethanol,
h) cinnamyl alcohol,
i) (R+S)-2-phenyl-1-propanol

Scheme 8. Transformation of primary alcohols into either aldehydes or acids.

Table 4. Water and Water:Isooctane Enzymatic Oxidation of Compounds of Scheme 8

	Acetobacter						*Gluconobacter*					
	Water			**Water:isooctane**			**Water**			**Water:isooctane**		
Substrate	**Product**	**Yield[a]**	**Time (h)**	**Product**	**Yield[b]**	**Time (h)**	**Product**	**Yield[a]**	**Time (h)**	**Product**	**Yield[b]**	**Time (h)**
1a	**3a**	>97	3	**2a**	74	1	**3a**	>97	4	**2a**	93	0.75
1b	**3b**	>97	3	**2b**	90	1	**3b**	>97	4	**2b**	90	1
1c	**3c**	>97	3	**2c**	87	1	**3c**	>97	3	**2c**	91	0.75
1d	**3d**	>97	24	**2d**	72	4	**3d**	16	24	**2d**	29	5
1e	**3e**	25	24	**2e**	<5	24	**3e**	<5	24	**2e**	<5	24
1f	**3f**	>97	3	**2f**	90	0.75	**3f**	>97	5	**2f**	85	2
1g	**3g**	>97	2	**2g**	93	0.75	**3g**	>97	5	**2g**	96	1
1h	**3h**	>97	8	**2h**	77	0.75	**3h**	20	24	**2h**	24	4
1i	**3i**	40	24	**2i**	<5	24	**3i**	33	24	**2i**	<5	24

[a]Yields (%) determined by standard GLC analysis; carboxylic acids were analysed after conversion to the corresponding methyl ester after treatment with CH_2N_2.
[b]The yields of the aldehydes are related to the sum of the products detected in the aqueous and organic phase.

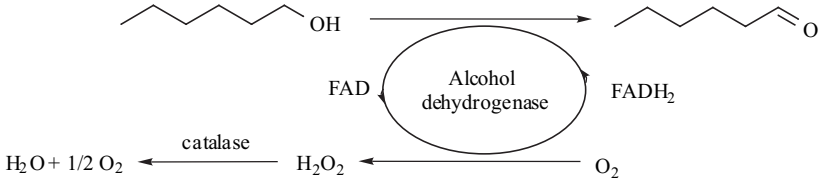
In addition to the production in very mild conditions of the desired product (aldehyde or acid), the possibility to perform a deracemization operation as evidenced with compound **1i** is noteworthy, that gives the (S)-alcohol in good enantiomeric excess (95%) and conversion yield (40%). This approach has been applied also in other cases [42-44].

A last interesting example of alcohol oxidation by enzymes is the transformation of n-hexanol into n-hexanal using isolated enzymes in biphasic and micellar systems [45]. The oxidation uses two enzymes: the first is responsible for the redox step (it is an ADH), and the second is used to eliminate the hydrogen peroxide formed in the first step (it is a catalase enzyme) in order to enhance the reaction yield (see Scheme (**9**)).

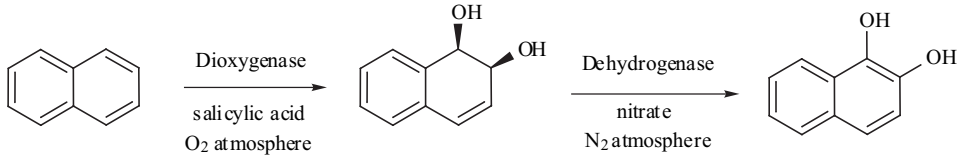
The first phase is always the phosphate buffer in water, where the enzymes are present; the second phase is either the substrate (n-hexanol), or the substrate mixed with a surfactant (Brij 35) that forms a micellar system. Both systems give the desired product in good yield; however, the use of the surfactant does not improve the yield and therefore, the direct biphasic system appears the best choice. On the other hand, the presence of the catalase enzyme is necessary to reach appreciable n-hexanal production (from 0.015 mmol/g to 0.2 mmol/g).

b. 1,2-Diol Oxidation

The dehydrogenation of 1,2-dihydro-1,2-dihydroxy compounds derived from the dioxygenation of aromatic compounds is a well-known step in the biodegradation of these recalcitrant compounds. In contrast, this activity has been scarcely used to produce the corresponding 1,2-dihydroxy derivatives. The principal reason behind the dislike of DDHs can be easily found in the difficulty of isolation and purification of the air-sensitive products. For what the argument of the present paper is concerned with, it must be clear that the applicability of these enzymes to



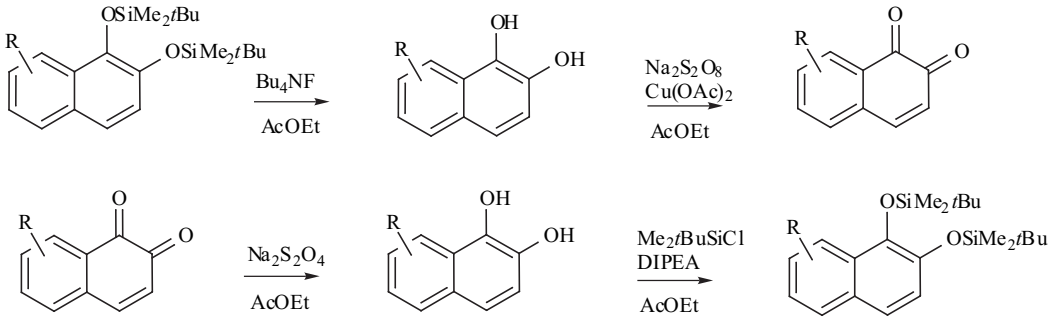
Scheme 9. Importance of hydrogen peroxide elimination to improve alcohol oxidation by FAD-dependent ADHs.



Scheme 10. Two steps oxidation of naphthalene to 1,2-dihydroxy naphthalene by recombinant strains.

produce chiral compounds by deracemization is not straightforward. In fact, the substrates are usually produced by bioconversions that are, in their own, enantioselective; this means that the available diol has only one configuration and this will presumably be destroyed by its DDH. Nevertheless, some very interesting applications can be reported.

1,2-Dihydroxy aromatic derivatives can be of interest because they are often precursors of pharmaceutical compounds. Consequently, their chemoenzymatic preparation has been reported. Many substituted 1,2-dihydroxynaphthalenes have been prepared using a finely tuned recombinant strain [46, 47] (Scheme (**10**)). In this work, the authors have isolated the products of the bioconversion by their transformation into stable compounds. These last can be either silyl derivatives, or quinones. All the different structures can be interconverted by usual chemical methods (Scheme (**11**), Tables **5**, **6**).



R = H, 7-CH_3, 7-CH_2CH_3, 8-Cl, 7-Br, 8-Br, 7-NO_2, 7-OCH_3, 7-COOMe

Scheme 11. Preparation of 1,2-quinones and of 1,2-dihydroxy derivatives starting from the complementary compounds.

Table 5. Silyl Derivatives of 1,2-Dihydroxynaphthalenes Prepared by Direct Reaction of 1,2-Dihydroxy Derivatives

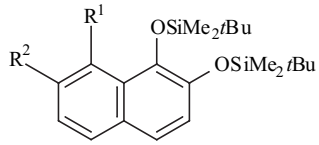
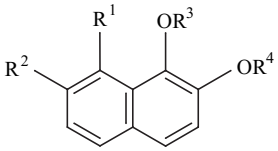
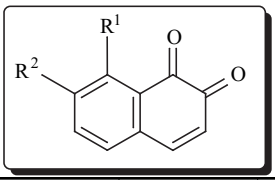
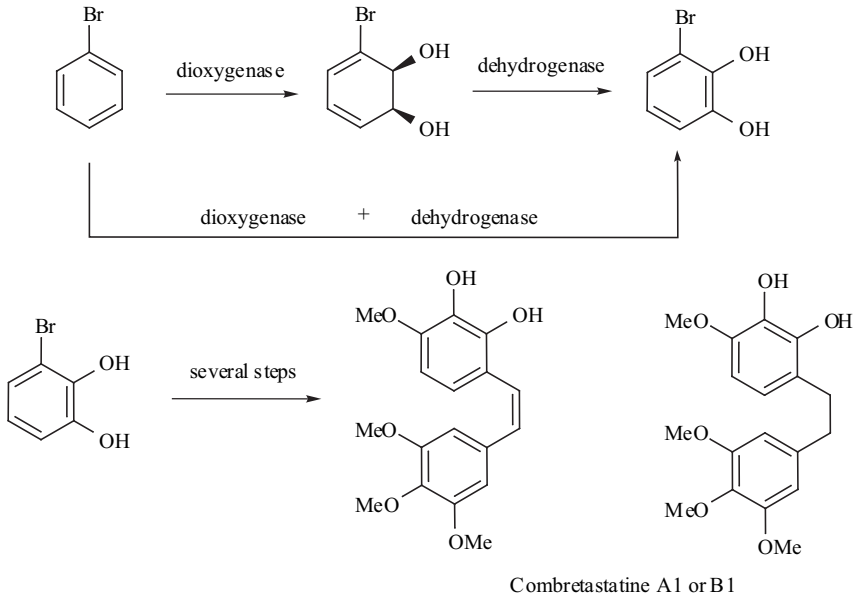
	**R^1**	H	H	Br	Cl	H	H
	R^2	H	Br	H	H	NO_2	$COOCH_3$
 R^3= *t*$BuMe_2Si$, R^4= H or R^3= H, R^4= *t*$BuMe_2Si$	**R^1**	H	H	H	CH_3CH_2	H	H
	R^2	H	CH_3	CH_3CH_2	H	Br	OCH_3

Table 6. 1,2-Naphthoquinones Prepared from 1,2-Dihydroxynaphthalenes

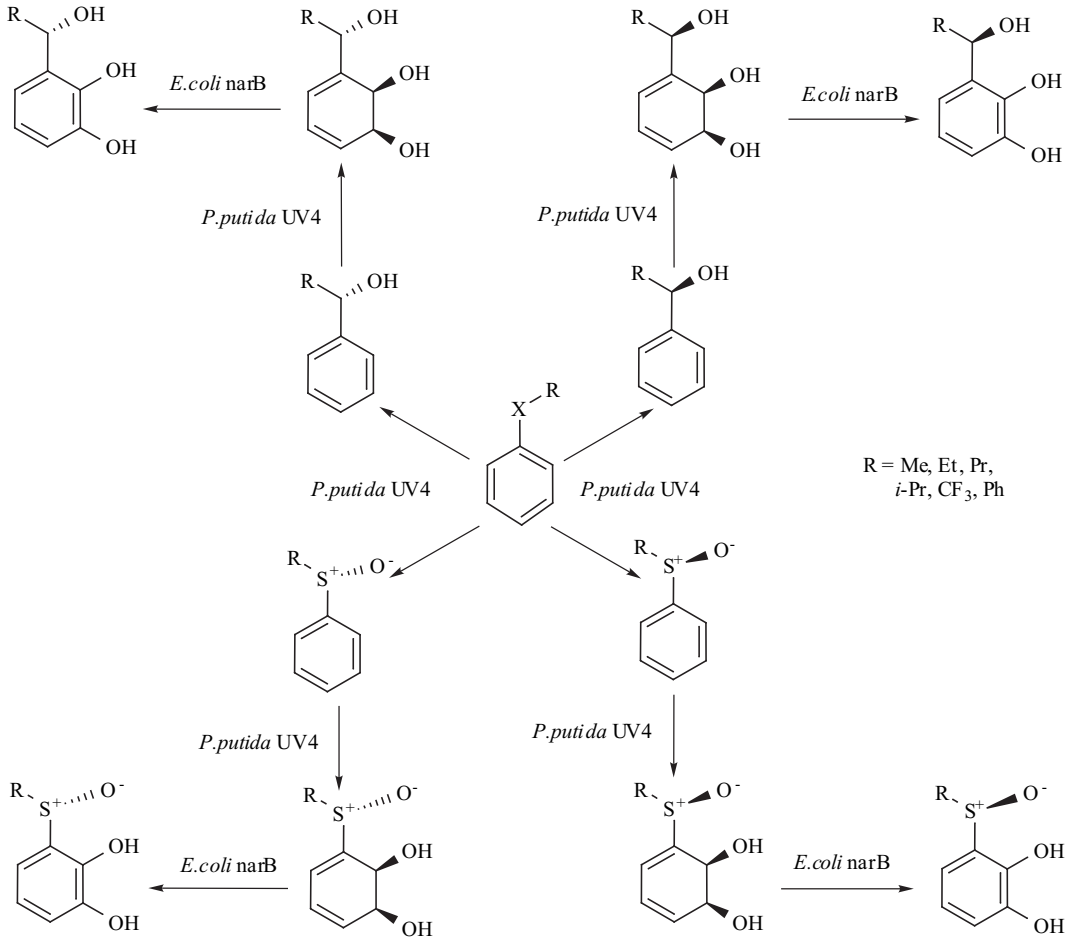


R^1	H	H	H	H	Br	Cl	H	H	H
R^2	H	CH_3	CH_3CH_2	Br	H	H	NO_2	OCH_3	$COOCH_3$



Scheme 12. Chemoenzymatic synthesis of Combretastatine A1 and B1 using two activities: toluene dioxygenase and dehydrogenase for the preparation of the starting synthon.

In a more focused synthesis, Bui *et al.* [48] prepared an intermediate of Combretastatine A1 and B1 using two successive biotransformations either in a two-strain sequence, or using only one strain carrying two activities (see Scheme (**12**)).



Scheme 13. Preparation of enantiopure benzyl alcohols and sulfoxides using sequential dioxygenation and dehydrogenation enzymatic reactions.

An example of application of DDHs to the deracemization of racemates is reported by Raschke *et al.* [49]. These authors use a cis-chlorobenzene dihydrodiol dehydrogenase to selectively oxidize the "natural" enantiomer of several diols. In particular, the reaction has been applied to: *cis*-(1*R*,2*S*)-1,2-indandiol, *cis*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene, *para*-substituted *cis*-toluene dihydrodiols (F, Cl, Br, I, CH_3), (+)-*cis*-2',3'-dihydroxy-2',3'-dihydro-2-chlorobiphenyl, (+)-*cis*-2',3'-dihydroxy-2',3'-dihydro-3-chlorobiphenyl, and (+)-*cis*-2',3'-dihydroxy-2',3'-dihydro-4-chlorobiphenyl. In all cases where both enantiomers are available, one of the enantiomer is preferably transformed; this is particularly interesting in the case of 1,2-indandiol, because the racemate can be easily prepared by chemical synthesis from the commercially available indene; only the (1R,2S)-enantiomer is converted, allowing the isolation of enantiopure (1S,2R)-enantiomer.

Finally, an interesting and convincing application of DDHs is reported by Boyd *et al.* [50]. Also in this case, the use of two enzymatic activities in whole cell bioconversion allows for the production of interesting compounds. But, the obtained molecules are now chiral. The synthetic scheme is straightforward and beyond doubt demonstrates the power of the enzymatic approach in the solution of not standard problems (Scheme (**13**)).

The synthetic project expects the use of a mutant strain (*P.putida* UV4) that contains a toluene dioxygenase activity; this first converts alkyl benzenes into the corresponding benzyl alcohols and then into the dihydrodiols; the same transformations are possible with thioalkyl benzenes. A second strain, this time a recombinant *E.coli*, contains a 1,2-diol dehydrogenase that transforms the obtained diols into the aromatic 1,2-dihydroxy derivatives. The final products are still chiral because they carry a carbon or a sulphur atom in the benzylic position that is asymmetric. Yields and enantiomeric excesses depend on the R nature and on the bioconversion conditions; usually, the first intermediate, either the alcohol or the sulfoxide, are not isolated, directly obtaining the tri-oxygenated products. The dehydrogenation step is carried by using a mild oxidative enzyme, allowing for the isolation of enantiopure derivatives.

4. FUTURE PERSPECTIVES

The field of chiral alcohol preparation is clearly the subject of intense activity. In addition, the biotechnological approach is also increasing its importance. We expect to see in the future more and more applications of the enzyme approach to this field. The present question is: is it possible to further the current level of this technology? The answer can be divided into two main chapters: the scientific and the technological developments. It is unquestionable that the research in the biotechnological application to the chiral alcohol preparation will continue. In particular, we can expect the selection of new ADHs with different properties: enhanced recognition towards more substrates and enhanced productivity, concerning both specific enzyme activity and reaction condition stability. At the same time, new, more efficient experimental procedures will be developed to overcome the current limitations in the fields of co-factor regeneration, reaction reversibility, new recombinant strains showing better stability and performance, enzyme genetic or protein manipulation. The technology is also going to change and improve in order to transform laboratory experiments into pilot and industrial scale experiments. Here, it will be fundamental to study and understand the system variables affecting the yield and performance; cell and enzyme immobilization, biphasic fermenter systems, high density cell conversions, continuous conversion procedures, more sophisticated substrate delivery and product recovery systems, will contribute to take the biotechnological approach to a more competitive level. Finally, 1,2-diol dehydrogenases and other dehydrogenases have still to be analyzed in their promise as new redox enzymes.

5. CONCLUSION

In this review, we analyzed the present state of the enzymatic racemate resolution of chiral alcohols. The use of ADHs as oxidative enzymes was shown as the opportunity of preparing alcohol enantiomers. The introduction of 1,2-diol dehydrogenases represent an incentive to search for new applications of enzymatic activities, also outside their "native" reactivity.

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REFERENCES

[1] Strauss, U.T.; Felfer, U.; Faber, K. *Tetrahedron: Asymmetry*, **1999**, *10*, 107.
[2] Schoffers, E.; Golebiowski, A.; Johnson, C. R. *Tetrahedron,* **1996**, *52*, 3769.
[3] Kagan, H. B.; Fiaud, J. C. *Top. Stereochem*., **1988**, *18*, 249.
[4] (a) Horeau, A. *Tetrahedron,* **1975**, *31*, 1307; (b) Sih, C. J.; Wu, S.-H. *Top. Stereochem*., **1989**, *19*, 63.
[5] Demain, A.L. *Biotechnol. Adv*., **2000**, *18*, 499.
[6] Csuk, R.; Glänzer, B.I. In *Stereoselective biocatalysis*; Patel R.N. Ed.; Dekker: New York, **2000**, p 527.
[7] D'Arrigo, P.; Pedrocchi-Fantoni, G.; Servi, S. In *Stereoselective biocatalysis*; Patel R.N. Ed.; Dekker: New York, **2000**, p. 365.
[8] Grunwald, J.; Wirts, B.; Scollar, M.P.; Klibanov, A.M. *J. Am. Chem. Soc*., **1985**, *108*, 6732.
[9] Hummel, W. *Trends Biotechnol*., **1999**, *17*, 487.
[10] Kula, M.R.; Kragl, U. In *Stereoselective biocatalysis*; Patel, R.N. Ed.; Dekker: New York, **2000**, p. 839.
[11] Mori, K. In *Stereoselective biocatalysis*; Patel R.N. Ed.; Dekker: New York, **2000**, p. 59.
[12] Patel, R.N.; McNamee, C.G.; Benerjee, A.; Howell, J.M.; Robinson, R.S.; Szarka, L.J. *Enzyme Microb. Technol*., **1992**, *14*, 731.
[13] Wong, C.H.; Drueckhammer, D.G.; Sweers, H.M. *J. Am. Chem. Soc*., **1985**, *107*, 4028.
[14] Kataoka, M.; Kotaka, A.; Hasegawa, A.; Wada, M.; Yoshizumi, A.; Nakamori, S.; Shimizu, S. *Biosci. Biotechnol. Biochem*., **2002**, *66*, 2649.
[15] Matsushita, K.; Toyama, H.; Adachi, O. In: *Advances in microbial physiology*, Rose, A.H.; Tempest, D.W. Eds., Academic Press: London, **1994**, *36*, p. 247.
[16] Matsushita, K.; Toyama, H.; Adachi, O. *Appl. Microbiol. Biotechnol*., **2002**, *58*, 13.
[17] Hummel, W. *TIBTECH*, **1999**, *17*, 487.

[18] Luyten, M. A.; Bur, D.; Wynn, H.; Parris, W.; Gold, M.; Friesen, J.D.; Jones, J. B. *J. Am. Chem. Soc.,* **1989***, 111*, 6800.
[19] Matsushita, K.; Adachi, O. In: *Principles and applications of quinoproteins*; Davidson, V. Ed.; Dekker: New York; **1993**, p. 47.
[20] Goodwin, P.M.; Anthony, C. *Adv. Microb. Physiol*., **1998**, *40*, 1.
[21] Keitel, T.; Diehl, A.; Knaute, T.; Stezowski, J.J.; Hohne, W.; Gorisch, H. *J. Mol. Biol*., **2000**, *297*, 961.
[22] Zhi-wei, C.; Baruch, P.; Mathews, F.S.; Matsushita, K.; Yamashita, T.;Toyama, H.; Adachi, O. *Acta Crystallogr. Sect. D*, **1999**, *55*, 1933.
[23] Reiner, A.M. *J. Biol. Chem*., **1972**, *247*, 4960.
[24] Patel, T.R.; Gibson, D.T. *J. Bacteriol*., **1974**, *119*, 879.
[25] Rogers, J.E.; Gibson, D.T. *J. Bacteriol*., **1977**, *130*, 1117.
[26] Eberspacher, J.; Lingens, F. *Hoppe-Seylers Z. Physiol. Chem*., **1978**, *10*, 1323.
[27] Nagao, K.; Takizawa, N.; Kiyohara, H. *Agric. Biol. Chem*., **1988**, *10*, 2621.
[28] Fong, K.P.Y.; Tan, H.-M. *FEBS Letter*, **1999**, *451*, 5.
[29] Persson, B.; Krook, M.; Jornvall, H. *Eur. J. Biochem*., **1991**, *200*, 537.
[30] Schoffers, E.; Golebiowski, A.; Johnson, C. R. *Tetrahedron,* **1996**, *52*, 3769.
[31] Kagan, H. B.; Fiaud, J. C. *Top. Stereochem*., **1988**, *18*, 249.
[32] Faber, K. *Ind. J. Chem., Sect. B,* **1992**, *31B*, 921.
[33] Cotterill, I. C.; Jaouhari, R.; Dorman, G.; Roberts, S. M.; Scheinmann, F.; Wakefield, B. J. *J. Chem. Soc., Perkin Trans. 1,* **1991**, 2505.
[34] Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc*., **1982**, *104*, 7294.
[35] Stecher, H.; Faber, K. *Synthesis,* **1997**, 1.
[36] Adachi, O.; Moonmangmee, D.; Shinagawa, E.; Toyama, H.; Yamada, M.; Matsushita, K. *Biochim. Biophys. Acta*, **2003**, *1647*, 10.
[37] Hann, M.R.; Tilden, B.E.; Hudson, C.S. *J. Am. Chem. Soc*., **1938**, *60*, 1201.
[38] Ameyama, M.; Shinagawa, E.; Matsushita, K.; Adachi, O. *Agric. Biol. Chem*., **1985**, *49*, 1001.
[39] Shinagawa, E.; Matsushita, K.; Adachi, O.; Ameyama, M. *Agric. Biol. Chem*., **1982**, *46*, 135.
[40] Švitel, J.; Šturdik, E. *Enzyme Microbiol. Technol*., **1995**, *17*, 546.
[41] Gandolfi, R.; Ferrara, N.; Molinari, F. *Tetrahedron Lett*., **2001**, *42*, 513.
[42] Geerlof, A.; Jongejan, J. A.; Van Dooren, T. J. M.; Petronella, C. *Enzyme Microb. Technol*., **1994**, *16*, 1059.
[43] Adlercreutz, P. *Appl. Microbiol. Biotechnol*., **1989**, *30*, 257.
[44] Molinari, F.; Villa, R.; Aragozzini, F.; Le`on, R.; Prazeres, D. M. F. *Tetrahedron*: *Asymmetry,* **1999**, *10*, 3003.
[45] Karra-Chaabouni, M.; Pulvin, A.; Meziani, A.; Thomas, D.; Touraud, D.; Kunz, W. *Biotech. Bioeng*., **2003**, *81*, 27.
[46] Di Gennaro, P.; Galli, E.; Orsini, F.; Pelizzoni, F.; Sello, G.; Bestetti, G. *Res. Microbiol*., **2000**, *151*, 383.
[47] Cavallotti, C.; Orsini, F.; Sello, G.; Di Gennaro, P.; Galli, E.; Bestetti, G. *Tetrahedron*, **1999**, *55*, 47.
[48] Bui, V.P.; Hudlicky, T.; Hansen, T.V.; Stenstrom, Y. *Tetrahedron Lett*., **2002**, *43*, 2839.
[49] Raschke, H.; Fleischmann, T.; Van der Meer, J.R.; Kohler, H.-P.E. *Appl. Environ. Microbiol*., **1999**, *65*, 5242.
[50] Boyd, D.R.; Sharma, N.D.; Ljubez, V.; Byrne, B.E.; Shepherd,S.D.; Allen, C.C.R.; Kulakov, L.A.; Larkin, M.J.; Dalton, H. *Chem. Comm*., **2002**, 1914.

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