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Kinetic resolution of *vic*-diols by *Bacillus stearothermophilus* diacetyl reductase

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Abstract

The kinetic resolution of several racemic *syn*- and *anti*-1,2-diols by enzymatic oxidation with *Bacillus stearothermophilus* diacetyl reductase is described. The enantiomerically pure (R,R)- and (R,S)-diols are recovered in almost quantitative yield.

1. Introduction

Chiral vicinal diols are highly versatile building blocks in asymmetric synthesis.[1] The most important chemical route to these molecules, in terms of stereospecificity and enantioselectivity, is represented by the catalytic *cis*-dihydroxylation of olefins achieved by OsO_4 in the presence of cinchona alkaloids.[2] The major drawbacks of the chemical method, however, are the moderate enantiomeric excesses obtained in the synthesis of *anti*-1,2-diols from *cis*-olefins and the use of toxic metal catalysts,[3] which may be a source of pollution. A useful alternative for the synthesis of enantiopure vicinal diols is represented by enzymes, as recently demonstrated by the enantioselective reduction of prochiral α-diketones mediated by *Bacillus stearothermophilus* diacetyl reductase.[4] This enzyme is an efficient catalyst for the stereoselective reduction of these substrates to (S,S) vicinal diols and (S)-α-hydroxy ketones, in the presence of NADH as cofactor. The catalytic system was equally effective in single- or double-enzyme fashion, the latter supported by the glucose 6-phosphate/glucose 6-phosphate dehydrogenase recycling counterpart. As will be presented in this paper, however, the same enzyme can be successfully employed to obtain vicinal diols in the (R,R) or (R,S) configuration by kinetic resolution of the racemic mixture of diols via oxidation. There are few enzymes available that catalyze the oxidation of specific hydroxyl groups in polyols.[5,6] Glycerol dehydrogenase is specific for (R)-alcohols and oxidizes (R)-butan-1,2-diol

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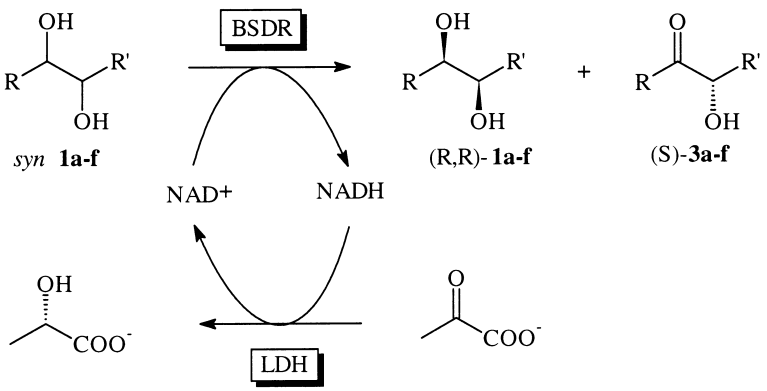
to 1-hydroxybutan-2-one. (S)-Butan-1,2-diol can be recovered unchanged, although in poor enantiomeric excess (ee 36%). Similarly, *cis*-cyclohexan-1,2 diol or *cis*-cyclopentan-1,2 diol are oxidized to (S)-α-hydroxy ketones in high enantiomeric excess but low chemical yield.[5]

2. Results and discussion

Bacillus stearothermophilus diacetyl reductase (BSDR) also proved to be a powerful catalyst in the oxidative sense, affording the corresponding α-hydroxy ketone and the unreacted alcohol with excellent enantiomeric excesses and good chemical yield. The overall reaction view is shown in Scheme 1.



Scheme 1.

Note that both the remaining alcohol and the product are chiral, thus avoiding the major drawback of the kinetic resolution concerning the recovery of optically active products with a maximum yield of 50%.[7] BSDR was confirmed as a very flexible enzyme, accepting a large variety of substrates bearing aliphatic, cyclic or aromatic substituents. Furthermore, all substituents being equal, the study has been carried out on the two distinct sets of substrates presented below: the *syn* and *anti* pairs.[8]

R	R'	*syn* (R,R) / (S,S)	*anti* (R,S) / (S,R)
CH_3	$CH_3(CH_2)_2$	**1a**	**2a**
CH_3	$CH_3(CH_2)_4$	**1b**	**2b**
CH_3	C_6H_5	**1c**	**2c**
CH_3CH_2	CH_3CH_2	**1d**	**2d**
	n	*anti* (R,R) / (S,S)	*meso*
	3	**1e**	**2e**
	4	**1f**	**2f**

Table 1
Kinetic resolution of the racemic *syn*-alcohols **1a–f** by oxidation with *Bacillus stearothermophilus* diacetyl reductase

substrate	time (h)	recovered alcohol (%)[a]	ee (abs. conf.)[b]	α-hydroxy ketone (%)[c]	ee (abs. conf.)[b]
1a	18	**1a** (50)	≥ 99 (R,R)	**3a** (10)	≥ 99 (S)
1b	18	**1b** (48)	≥ 99 (R,R)	**3b** (14)	≥ 99 (S)
1c	72	**1c** (49)	≥ 99 (R,R)	**3c** (3)	
1d	18	**1d** (49)	≥ 99 (R,R)	**3d** (13)	≥ 99 (S)
1e	27	**1e** (50)	≥ 99 (R,R)	**3e** (16)	≥ 99 (S)
1f	27	**1f** (48)	≥ 99 (R,R)	**3f** (18)	≥ 99 (S)

a. isolated yield; b. by GC analysis on chiral column, only one enantiomer is detected in all cases; c. GC yields

Table 2
Kinetic resolution of the racemic *anti*-alcohols **2a–f** by oxidation with *Bacillus stearothermophilus* diacetyl reductase

substrate	time (h)	recovered alcohol (%)[a]	ee (abs. conf.)[b]	α-hydroxy ketone (%)	ee (abs. conf.)[b]
2a	27	**2a** (49)	76 (R,S)	**3a** (23)[c]	≥ 99 (R)
2b	18	**2b** (49)	62 (R,S)	**3b** (13)[c]	≥ 99 (R)
2f	72	**2f** (10)	d	**3f** (88)[a]	≥ 99 (R)

a. isolated yield; b. by GC analysis on chiral column ; c. GC yields; d. *meso* alcohol.

In the catalytic cycle of the *syn* pair,[8] Scheme 1, the (S,S)-diol is stereospecifically oxidized on the hydroxy group proximal to the smaller substituent,[9] affording the unreacted (R,R)-diol in excellent chemical yield and enantiomeric excess, and the corresponding (S)-α-hydroxy ketone, as shown in Table 1. The NADH produced during the oxidation process is conveniently transformed to NAD^+ in the presence of the relatively inexpensive pyruvate and lactic dehydrogenase (LDH). Moreover, the recycling system has the additional advantage of being highly soluble in water, also owing to the pH, thus making the recovery of the products easy and practical.[10]

The specificity of BSDR for the (S) stereochemistry is further confirmed by the data obtained with the *anti* pair, where the exclusive elaboration of the (S)-hydroxy function adjacent to the smaller substituent is observed.[9] As a consequence, the (R,S)-diol is kinetically resolved from the racemate by virtue of the oxidation of the (S,R)-enantiomer to the (R)-α-hydroxy ketone, as shown in Table 2.

When this biocatalyzed oxidation is applied to the *meso* diol **2f**, desymmetrization of the substrate is achieved,[11] with the advantage of transforming, in principle, all the starting material into the desired homochiral product.

As expected, shorter reaction times increase the chemical yield of the α-hydroxy ketones to the detriment of the enantiomeric excess of the diols. As an example, the kinetic resolution of **1d** after 10 hours affords **3d** in 34% yield (ee 99%), but the corresponding (R,R)-**1d** in 75% enantiomeric excess. Despite the shorter reaction times, however, side reactions frustrate attempts to obtain most of the α-hydroxy ketones on a multigram scale. It is well known that enzymatic oxidations work best at elevated pH (8–9), where cofactors and products may be unstable.[12] To avoid this instability to some

extent, the pH of the medium has been shifted from the optimum value of 9.2 to pH 8.2, where the specific activity of BSDR is only partially reduced.[13] Also, under these experimental conditions, a progressive disappearance of the α-hydroxy ketones is observed, as confirmed by control experiments carried out on (R)-3-hydroxy-4-hexanone **3d**. A possible explanation for this side reaction might be the formation of an enediol, followed by further biotransformation.[14] A notable exception is the (R)-1-hydroxy-2-cyclohexanone **3f** that is recovered from the reaction mixture in almost quantitative yield.

In conclusion, several enantiomerically pure (R,R)- and (R,S)-*vic*-diols may be obtained by kinetic resolution via oxidation of the corresponding racemates, in the presence of *Bacillus stearothermophilus* diacetyl reductase.

3. Experimental

3.1. Materials and methods

Bacillus stearothermophilus is available from American Type Culture Collection (ATCC 2027). For synthetic reactions, the wet cells (20 g) obtained from four portions of 250 mL cultures are separated by centrifugation and washed with 200 mL of 0.15 M NaCl. The cells are suspended in 100 mL of TEA–HCl buffer, treated with 40 mg of lysozyme for 60 min at 22°C and recentrifuged. The supernatant (enzyme solution) was used without further treatment. Alternatively the supernatant was freeze-dried to a powder by lyophilization,[15] stored in a refrigerator, and used directly for chemical synthesis. Typically 1 mL of enzyme solution contains ca 0.2 units of enzyme (1 unit=1μmol of *endo*-bicyclo[3.2.0]hept-2-en-6-ol oxidized per minute).[4] The TEA–HCl buffer is composed of 50 mM triethanolamine, containing 0.1 mM EDTA, 1 mM β-mercaptoethanol and HCl to adjust the pH to 7.5. Further purification of the crude extract via DEAE (diethylaminoethyl) sepharose CL 6B and Cibachron-blue 3GA agarose column chromatography provided a pure protein.[13] Note that almost identical results were obtained using either the purified enzyme or the crude extract. Lysozyme, NAD^+ and pyruvate were from Sigma, lactic dehydrogenase (LDH) was from Boehringer Mannheim. Cyclohexanediol and cyclopentanediol were purchased from Aldrich. All other diols, except 2,3-octanediol, have been obtained from the commercially available corresponding diketones (Aldrich) by reduction with $NaBH_4$, followed by separation of the *syn* and *anti* pairs by column chromatography. The same reduction and separation methodology has been used to obtain the 2,3-octanediol from the 3-hydroxy-2-octanone.[4] Enantiomeric separations and excesses were determined by GC on a chiral column[4] and ee≥99% means that the corresponding enantiomer was not detected. The absolute configurations are determined by comparison of the signs of the specific rotations with the literature values, when available. In the other cases, the absolute configurations are assigned on the basis of the GC retention time in a homologous series, upon analysis of the racemates. NMR spectra were obtained on a 300 MHz instrument.

3.2. General procedure of oxidation

A portion of the enzyme solution (2 mL) was added to a flask containing 1 mmol of diol, 20 mg NAD^+, 1 mmol of pyruvate, 200 μg of lactic dehydrogenase in 100 mL of TEA–HCl buffer, pH 8.2. After the proper time, the reaction mixture was saturated with NaCl and extracted with ethyl acetate. The products were separated by chromatography on SiO_2 with ethyl acetate:cyclohexane (1:1) as eluent. The yields, absolute configurations and enantiomeric excesses are listed in the tables. Specfic rotations: (R,R)-**1a** $[\alpha]_D$=+16 (*c* 1.2, $CHCl_3$) confirmed by comparison with literature data on the (S,S)-enantiomer;[16] (R,R)-

1b $[\alpha]_D$=+18.5 (*c* 1.1, $CHCl_3$) confirmed by comparison with literature data;[17] (R,R)-**1c** $[\alpha]_D$=−55.9 (*c* 1.9, $CHCl_3$) confirmed by comparison with literature data;[18] (R,R)-**1d** $[\alpha]_D$=+12 (*c* 2, $CHCl_3$) confirmed by comparison with literature data on the (S,S) enantiomer;[16] (R,R)-**1e** $[\alpha]_D$=−33.3 (neat) confirmed by comparison with literature data;[19] (R,R)-**1f** $[\alpha]_D$=−38 (*c* 1.5, H_2O) confirmed by comparison with literature data;[20] (R,S)-**2a** $[\alpha]_D$=−10.4 (*c* 1.5, $CHCl_3$), the stereochemistry was assigned based on the GC retention time; (R,S)-**2b** $[\alpha]_D$=−22 (*c* 1.0, $CHCl_3$) confirmed by comparison with literature data on the (S,S)-enantiomer;[21] (R)-**3f** $[\alpha]_D$=+13 (neat) confirmed by comparison with literature data on the (S)-enantiomer.[5] ^1H and ^{13}C NMR are consistent with previously published data.

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