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Stereoselective Prostereogenic 3-Oxo Ester Reduction Mediated by a Novel Yeast Alcohol Dehydrogenase Derived from *Kluyveromyces marxianus* CBS 6556

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Abstract: A yeast alcohol dehydrogenase (ADH) has been purified up to a purification factor value of 21,731-fold from *Kluyveromyces marxianus* CBS 6556. The purification procedure consisted of two chromatographic steps (DEAE-anion exchange and affinity chromatography). The optimal pH was 7, its optimal temperature was 40 °C and its co-factor was NADPH. This novel ADH efficiently mediated the reduction of 3-oxo esters with a high degree of stereoselectivity, providing chiral alcohols having the (*S*) absolute configuration at the newly formed stereogenic centre by delivering the hydride from the *re*-face of the prochiral carbonyl compounds.

Keywords: alcohol dehydrogenase; asymmetric bioreduction; fermentation; *Kluyveromyces marxianus*; 3-oxo esters

Introduction

Nowadays the use of biological catalysts in the synthesis of pharmaceuticals, fine chemicals and chiral intermediates for drug development is very common.[1] Most of the work, published in the last thirty years in this field, has been focused on finding biocatalytic systems, microorganisms or isolated enzymes, with the ability of promoting the formation of new stereogenic centres with high stereoselectivity in achiral molecules.

One of the most investigated processes is the asymmetric bioreduction of prostereogenic ketones.[2–8] Many research groups have focused their attention on searching for microorganisms able to accomplish such a biotransformation, which may represent a valuable alternative to the deeply investigated *Saccharomyces cerevisiae*.[9]

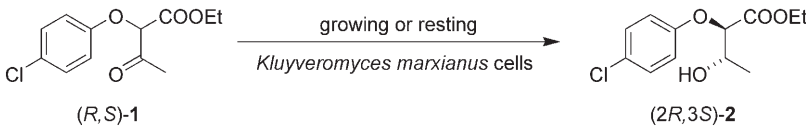
Recently, we reported that the yeast *Kluyveromyces marxianus* CBS 6556 reduced ethyl 2-(4-chlorophenoxy)-3-oxobutanoate (**1**) affording nearly enantiomerically pure ethyl (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxybutanoate (**2**, Scheme 1).[10] *Kluyveromyces marxianus* is not a widely used biocatalyst in organic synthesis as yet. This yeast is known for its capacity of growing on lactose as a sole carbon and energy source, which presents the possibility of utilizing cheese whey as a cheap source of nutrients for its cultivation.[11] It has been investigated for the preparation of useful intermediates in the synthesis of pharmaceuticals[12] but very few works have been published describing its usefulness in the asymmetric reduction of prostereogenic ketones and kinetic resolution.[10,13–21]

Herein, we report the partial purification of an alcohol dehydrogenase (ADH) from *Kluyveromyces marxianus* CBS 6556, responsible for such a highly enantioselective reduction of **1** (Scheme 1) and its comparison with an ADH enzyme isolated from a different strain of the same yeast, showing a reverse stereochemical preference in the bioreduction of ethyl acetoacetate[14] and ethyl 2-(phthalimido)methyl-3-oxobutanoate (**3**, Scheme 2).[15–16]

Results and Discussion

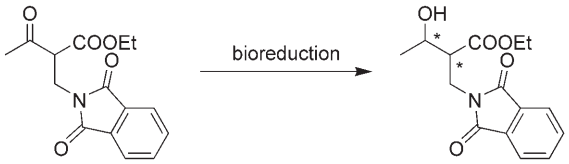
The reduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate (**1**) was performed using growing and resting cells of *Kluyveromyces marxianus* CBS 6556 (Scheme 1). The results are summarised in Table 1.[10]

Such results prompted us to develop a method for the isolation and purification of the alcohol dehydrogenase (ADH) involved in the above-mentioned bio-



(*R*,*S*)-**1** (2*R*,3*S*)-**2**

Scheme 1. Bioreduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate (**1**).



3 **4**

Scheme 2. Bioreduction of ethyl 2-(phthalimido)methyl-3-oxobutanoate (**3**) by *Kluyveromyces marxianus* KCTC 7155.

Table 1. Results of growing and resting cells of *Kluyveromyces marxianus* CBS 6556-mediated reduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate (**1**).[10]

Reaction conditions	Reaction time [h]	Yield[a] [%]	*de*[b] [%]	$ee_{(2R,3S)}$[c] [%]
Growing cells	8	73	>99	86
Resting cells	1.5	84	>99	97

[a] Yield refers to the product isolated by chromatography.
[b] Diastereoisomeric excesses (*syn*>*anti* pair) determined by ^{1}H NMR.
[c] Enantiomeric excesses were determined by HPLC.

reduction. Before starting the purification procedure, attempts were performed on the crude extract, obtained by mechanical disruption of *Kluyveromyces marxianus* CBS 6556 cells, to assess the best pH, temperature and conditions for the bioreduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate (**1**). As shown in Figure 1, **2** was quantitatively obtained when 0.1 M KH_2PO_4 buffer solution (pH 7.0) was employed. In this case, the reaction was complete within 45 min. Reaction times exceeded this time at all other pH values.

Once the best pH value had been found, the bioreduction was performed at different temperatures (Figure 2). As it can be seen, at 40 °C the reaction was complete within 30 min. At the temperatures above 40 °C the reaction rate was lower.

The isolation/purification procedure consisted of two steps, performed by chromatography using first an anion exchange and then an affinity resin (Table 2).

In the first step, the crude extract was partially purified over a DEAE-Sepharose anion exchange resin. The specific activity increased from 0.22 U/mg (in the crude extract) to 79.6 U/mg protein, with a purification factor value of 362. The specific activity was measured by monitoring the reduction of 2-(4-chlorophenoxy)-3-oxobutanoate (**1**) with GC (Scheme 1). The active fractions were then further purified by an affinity chromatography, using a 2′5′-ADP Sepharose 4B affinity resin, which shows high affinity towards NADPH-dependent enzymes. Specific activity increased to 4,781 U/mg, leading to a 21,731-fold total purification. However, in spite of the very high purification factor value, the desired enzyme was found to be only partially purified, since three spots were pres-

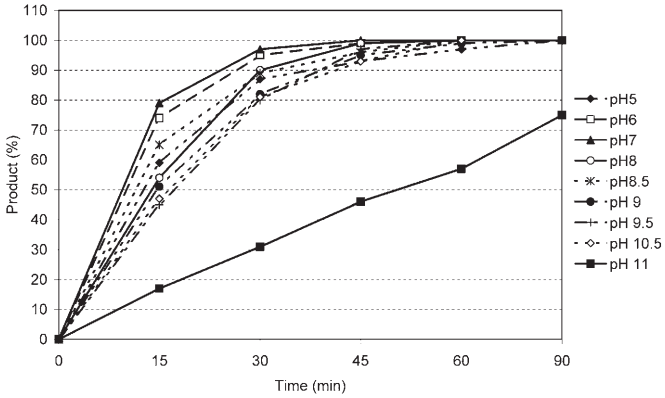


Figure 1. pH optimization: crude extract (500 μL), 0.1 M KH_2PO_4 (at the defined pH value) (500 μL), 3 μM NADPH (34.2 μL) and **1** (11 μL) in absolute ethanol (c = 0.1 mM) were incubated at 30 °C. Reaction progress was monitored by GC analysis.

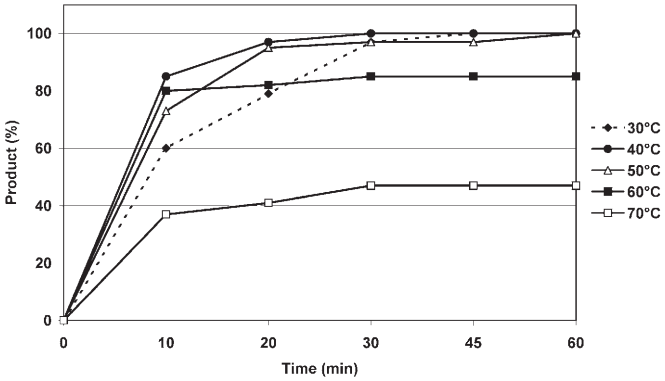


Figure 2. Temperature optimization: crude extract (500 μL), 0.1 M KH_2PO_4 (500 μL) (pH 7.0), 3 μM NADPH (34.2 μL) and **1** (11 μL) in absolute ethanol (c = 0.1 mM) were incubated at the defined temperature. Reaction progress was monitored by GC analysis.

Table 2. Purification steps of the novel ADH from *Kluyveromyces marxianus* CBS 6556, detected in fermentative reduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate (**1**).

Step	Protein[a] [mg]	Total units[b] [Unit]	Specific activity[c] [U/mg]	Purification[d] (fold)
Crude extract	42	9.24	0.22	–
DEAE Sepharose	5	398	79.6	362
2′5′-ADP Sepharose 4B	0.016	76.5	4,781	21,731

[a] Protein content was determined using the Bradford method (see Experimental Section).
[b] Total units were determined by multiplying specific activity by protein content.
[c] Specific activity at each step was determined by a spectrophotometric assay.
[d] Purification factor value is the ratio between specific activity at a given step and specific activity of the crude extract.

ent in the active fraction, as evidenced by SDS-PAGE analysis.

The obtained enzyme solution was used to perform the bioreduction of **1**. As expected, only (2*R*,3*S*)-**2** formed (Figure 3).[3] These results suggest that the identified enzyme is accountable for the highly stereoselective reduction of **1** observed in the presence of *Kluyveromyces marxianus* CBS 6556 whole cells.[10]

The absolute configuration of **2** formed in the bioreduction performed in the presence of *K. marxianus* was established to be (2*R*,3*S*) by single crystal X-ray analysis of the corresponding acid (quantitatively obtained by treating **2** with KOH) (Figure 4).[10] All the other absolute configurations were assessed by HPLC and single crystal X-ray analysis of authentic samples prepared from threonines as previously described.[22]

The observed stereoselectivity is different from the findings of Koh, who patented a novel ADH isolated from *Kluyveromyces marxianus* KCTC 7155, which produced a mixture of ethyl (2*R*,3*R*)- and (2*S*,3*R*)-2-(phthalimido)methyl-3-hydroxybutanoate (**4**) when catalysing the reduction of its precursor ethyl 2-(phthalimido)methyl-3-oxobutanoate (**3**) (Scheme 2).[15–16]

We then investigated the stereochemical behaviour of the ADH isolated, by using our purification protocol from *Kluyveromyces marxianus* CBS 6556, in the bioreduction of **3**. This reaction was performed under different conditions. The substrate **3** was first incubated in the presence of growing cells of *Kluyveromyces marxianus* CBS 6556 which gave **4** with 20% diastereomeric excess (*de*) and enantiomeric excess $ee_{(2R,3S)/(2S,3R)} = 22\%$ and $ee_{(2R,3R)/(2S,3S)} = 82\%$ (Figure 5 B).

Absolute configurations were assessed by comparison with HPLC data reported by Koh and co-workers.[15] As reported in the mentioned work the main formed stereoisomers had absolute configurations (2*R*,3*R*) and (2*R*,3*S*), even though a different strain of the microorganism was used.

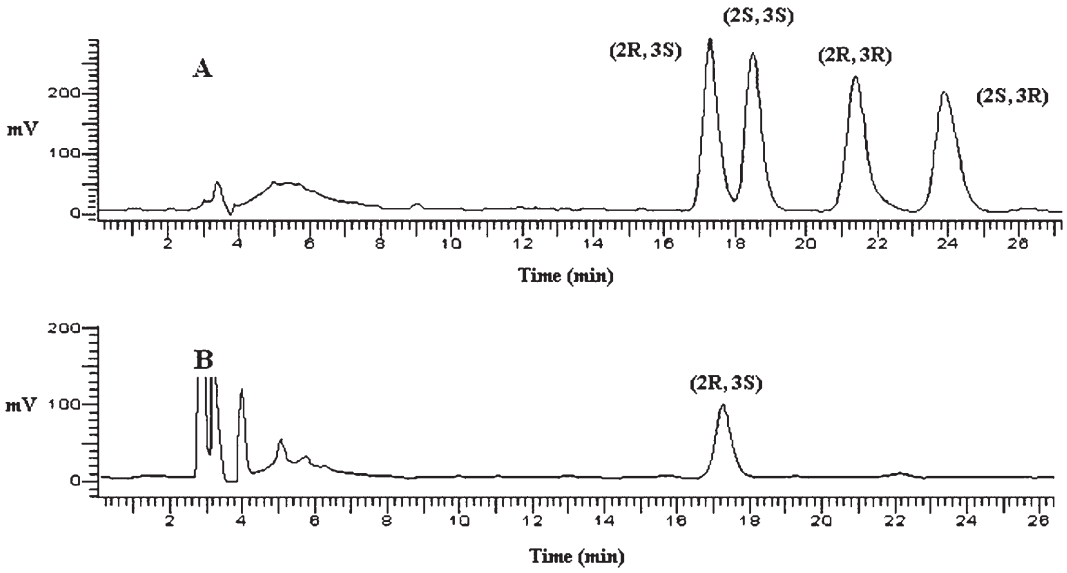


Figure 3. (**A**) HPLC chromatogram of diastereoisomeric mixture of ethyl 2-(4-chlorophenoxy)-3-hydroxybutanoate (**2**) obtained by $NaBH_4$ reduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate (**1**), and (**B**) HPLC chromatogram of ethyl (2*R*,3*S*) 2-(4-chlorophenoxy)-3-hydroxybutanoate (**2**) from reduction of racemic ethyl 2-(4-chlorophenoxy)-3-oxobutanoate (**1**) carried out in the presence of the enzyme sample obtained after two step anion-exchange/affinity chromatography within 1 hour (Table 2).

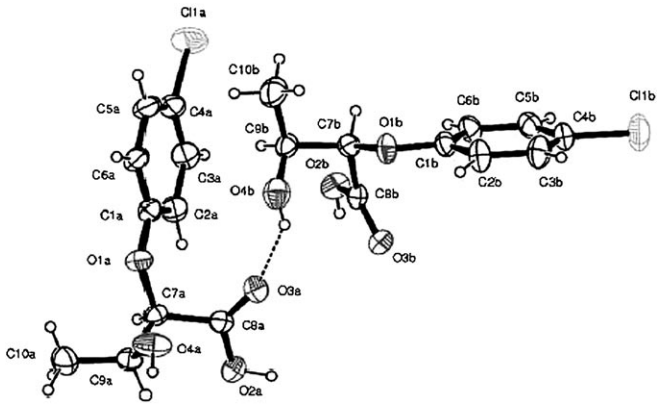


Figure 4. ORTEP view of the asymmetric unit with the atomic numbering scheme of (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxybutanoic acid. Thermal ellipsoids probability level at 30%.

Ethyl 2-(phthalimido)methyl-3-oxobutanoate (**3**), was also reduced in the presence of baker's yeast, which is known to generally produce (*S*)-3-hydroxy compounds by reducing 3-oxoesters.[3,23] In fact, under these conditions the expected products were indeed formed [$de = 29\%$, $ee_{(2R,3S)/(2S,3R)} > 99\%$ and $ee_{(2R,3R)/(2S,3S)} > 99\%$] (Figure 6).

Interestingly, the reduction of **3** carried out with the enzyme isolated from *Kluyveromyces marxianus* CBS 6556 under the above described conditions yielded the product with opposite stereochemistry, (2*R*,3*S*) and (2*S*,3*S*) being the main stereoisomers formed ($de = 35\%$, $ee_{(2R,3S)/(2S,3R)} = 88\%$ and $ee_{(2R,3R)/(2S,3S)} > 99\%$) (Figure 7).

In this study, *Kluyveromyces marxianus* CBS 6556 was selected for its outstanding reducing properties towards keto esters of our interest when compared

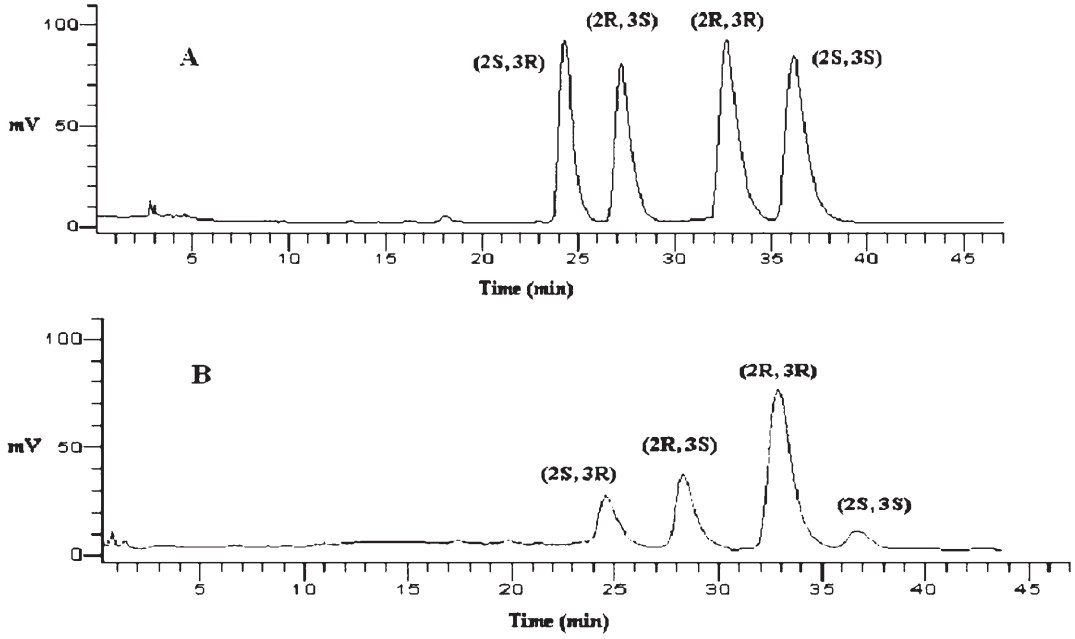


Figure 5. (**A**) HPLC chromatogram of diastereoisomeric mixture of ethyl 2-(phthalimido)methyl-3-hydroxybutanoate (**4**) obtained by $NaBH_4$ reduction of ethyl 2-(phthalimido)methyl-3-oxobutanoate (**3**), and (**B**) HPLC chromatogram of ethyl 2-(phthalimido)methyl-3-hydroxybutanoate (**4**) obtained carrying out the bioreduction of **3** in the presence of growing *Kluyveromyces marxianus* CBS 6556 cells.

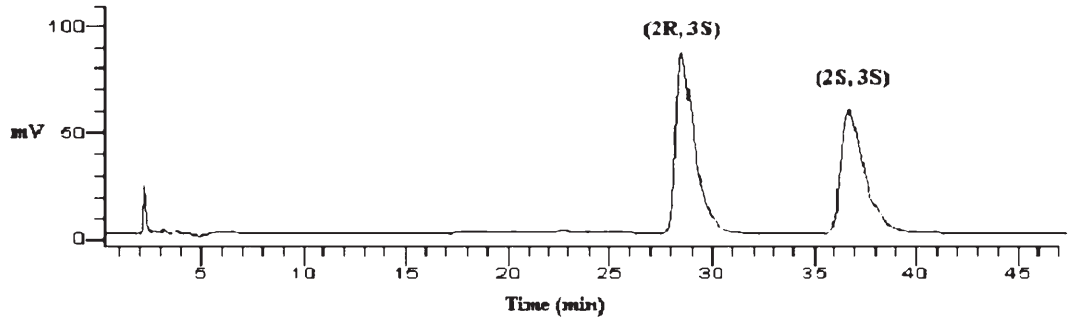


Figure 6. HPLC chromatogram of ethyl 2-(phthalimido)methyl-3-hydroxybutanoate (**4**) obtained from bioreduction of **3** in the presence of baker's yeast.

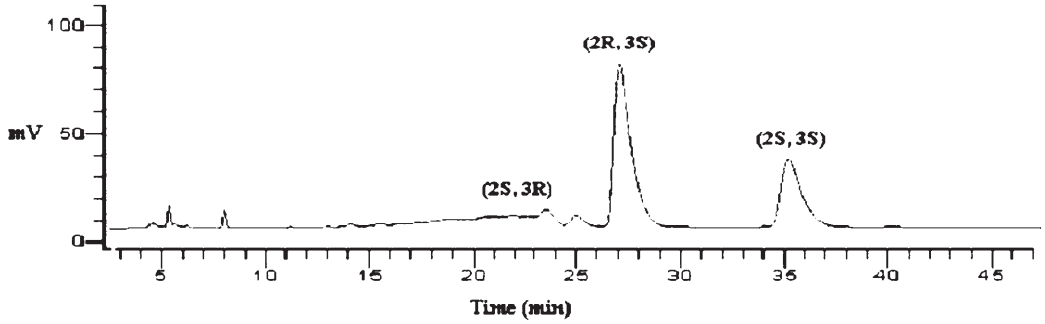


Figure 7. HPLC chromatogram of ethyl 2-(phthalimido)methyl-3-hydroxybutanoate (**4**) obtained from bioreduction of **3** performed in the presence of the ADH isolated from *Kluyveromyces marxianus* CBS 6556 following the procedure summarized in Table 2, and detailed in the Experimental Section.

with other yeast strains, including baker's yeast. The isolated enzyme shows an improvement in the rate of the reduction of **1** and allows the quantitative conversion of the keto ester avoiding the formation of by-products as observed when performing the reaction with different yeast strains: the use of whole cell catalyst could have the disadvantage of the presence of an enzymatic pool competing with the dehydrogenase affording undesidered products.[23,24]

Obtaining enantiopure compounds is a challenging task in organic chemistry, due to elevated cost of the reactant and/or starting materials. On the other hand, the preparation of optically active compounds is highly important in various synthetic preparative organic chemistry areas. In this sense, the possibility of a readily available reducing agent capable of reducing substrates like **1** in quantitative yield, high enantiomeric excess and in short times proves essential.

Conclusions

An NADPH-dependent alcohol dehydrogenase (ADH) was partially purified from *Kluyveromyces marxianus* CBS 6556.

It is able to perform the bioreduction of **1** yielding the nearly enantiopure 3-hydroxy ester **2** [$de > 99\,\%$, $ee_{(2R,3S)} = 97\,\%$]. The best identified reaction conditions were 40 °C in 0.1 M KH_2PO_4 buffer solution (pH 7.0). Under these conditions, the reaction was complete within 30 min.

On the other hand, the reduction of 3-oxo ester **3** performed by using our ADH (Table 2 and Figure 1), produced ethyl (2*R*,3*S*)- and (2*S*,3*S*)-2-(phthalimido)-methyl-3-hydroxybutanoate (**4**) as the main stereoisomers [$de = 35\,\%$, $ee_{(2R,3S)/(2S,3R)} = 88\,\%$ and $ee_{(2R,3R)/(2S,3S)} > 99\,\%$]. These results are different from those obtained by Koh and co-workers,[15] who used an ADH from *Kluyveromyces marxianus* KCTC 7155 which produces a mixture of (2*R*,3*R*)-**4** and (2*S*,3*R*)-**4** with a diastereoisomeric ratio (2*R*,3*R*)/(2*S*,3*R*) = 80:20 ($de = 60\,\%$).[16] These data led us to the conclusion that the enzyme we have identified and partially purified from *Kluyveromyces marxianus* CBS 6556 is a different ADH endowed with opposite stereochemical preference with respect to the one already reported, and that the yeast *Kluyveromyces marxianus* CBS 6556 constitutes a useful tool for the preparation of enantiomerically pure ethyl (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxybutanoate (**2**).

Wider investigations are currently in progress aimed at exploiting the substrate specificity and synthetic applications of *Kluyveromyces marxianus* CBS 6556 and the novel ADH, also compared to other well-known biocatalysts able to mediate the bioreduction reaction. Further work is also ongoing to definitively purify the novel identified ADH from *Kluyveromyces marxianus* CBS 6556.

Experimental Section

A Labfors Fermenter, equipped with all features necessary for a long-term sterile operations, with 3.6-L total volume and 2.5-L working volume was used. All the major variables related with the fermentation processes were monitored and stored by computer.

Ethyl 2-(4-chlorophenoxy)-3-oxobutanoate (**1**)[25] and ethyl 2-(phthalimido)methyl-3-oxobutanoate (**3**)[15] were prepared as previously reported. Spectroscopic and analytical data of ethyl 2-(4-chlorophenoxy)-3-hydroxybutanoate (**2**)[25] and ethyl 2-(phthalimido)methyl-3-hydroxybutanoate (**4**) are consistent with those already reported.[15] Reaction progress was monitored by TLC and GC analysis. Thin-layer chromatography (TLC) was performed on silica gel sheets with a fluorescent indicator (Stratochrom SIF, 60 F_{254} Merck); TLC spots were observed under ultraviolet light or visualised with I_2 vapour. GC analyses were performed by using an HP5 column (methyl silicone gum; 30 m × 0.25 mm × 250 μm film thickness) on a Hewlett Packard 5890 model, Series II. GC-MS analyses were performed on a Hewlett Packard 6890–5793 MSD. Column chromatography was performed using silica gel Merck 60 (0.063–0.200 μm).

^{1}H NMR spectra were recorded in $CDCl_3$ on a Varian Mercury 300 MHz spectrometer.

The *ee*s and absolute configurations of the products were determined by HPLC analysis performed on a Perkin–Elmer 200 series with a UV/Vis detector 785 A and a commercially available Chiralcel OD (Daicel) column under isocratic conditions employing *n*-hexane/2-propanol = 98:2 as mobile phase, flow rate 1 mL/min, $\lambda = 230$ nm for **2** and *n*-hexane/2-propanol = 95:5, flow rate 1 mL/min, $\lambda = 230$ nm for **4**. The retention time (t_R) of each stereoisomer was determined by using as $t_R°$ the retention time of the mobile phase prepared replacing *i*-PrOH with *i*-PrOH-d_8 revealed by the refractive index detector.

Optical density was measured at 620 nm by a Perkin–Elmer UV/Vis Lambda Bio 20 spectrophotometer.

DEAE Sepharose and 2′5′-ADP Sepharose 4B were purchased from Amersham Biosciences.

Microorganism Sources

Baker's yeast used to reduce the 3-oxoesters **1** and **3** was supplied by Lievitalia. *Kluyveromyces marxianus* CBS 6556 was obtained from a public type culture collection (CBS).

Culture Medium

The microorganisms were cultivated under aerobic conditions in a medium containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose. 2% Agar-Agar was added to the same medium for cells preservation on agar slants.

Procedures for the Bioreduction of Ethyl 2-(Phthalimido)methyl-3-oxobutanoate (3)

Baker's yeast-mediated reduction: Baker's yeast (17 g) was dispersed to give a smooth paste in tap water (32 mL). Ethyl 2-(phthalimido)methyl-3-oxobutanoate (**3**) (200 mg) was then added to the suspension which was then stirred at 250 rpm and kept at 37 °C. The reaction progress was monitored by GC analysis: 0.5 mL samples were extracted by adding ethyl acetate (1 mL) and then centrifuged at 10,000 *g* for 5 min. The supernatant (organic phase) was separated, dried over anhydrous Na_2SO_4 and then analysed by GC and TLC. The reaction was stopped after 24 h. The reaction mixture was extracted several times with EtOAc. The extracts were combined and dried over anhydrous Na_2SO_4. A yellow oil was obtained after removal of the solvent under reduced pressure.

Kluyveromyces marxianus *CBS 6556 whole growing cells-mediated reduction:* Cells preserved on agar slants at 4 °C were used to inoculate 250-mL flasks containing the cultivation medium (100 mL). The flasks were incubated aerobically at 30 °C on an orbital shaker and stirred at 250 rpm. 250-mL flasks containing the cultivation medium (100 mL) were then inoculated with 5 mL of the 24-h-old suspension and incubated in the same conditions for 24 h. A 1-L flask containing the cultivation medium (400 mL) was then inoculated with 5 mL of the latter suspension and incubated for 24 h and **3** (200 mg) dissolved in ethanol (1 mL) was added when the culture had the optical density of 7.2. The reaction was monitored by GC analysis: 0.5 mL samples were extracted by adding ethyl acetate (1 mL) and then centrifuged at 10,000 *g* for 5 min. The supernatant (organic phase) was separated, dried over anhydrous Na_2SO_4 and then analysed by GC and TLC. The reaction was stopped after 24 h. The content of the flask was centrifuged and the supernatant extracted three times with ethyl acetate.

Purified ADH-mediated reduction: NADPH (6 mg, 0.007 mmol) in 10 mM phosphate buffer pH 6.8 (25 mL) and **3** (0.2 mmol) were added to the enzyme solution obtained by affinity chromatography (5 mL, Table 2). The suspension was shaken at 30 °C for 11 days, monitoring the progress of the reaction by GC and TLC. The crude product was extracted with ethyl acetate after saturation of the suspension with NaCl. The organic phase was dried over anhydrous Na_2SO_4 and the solvent evaporated under reduced pressure.

Determination of Protein Content

The protein content was determined by using the method of Bradford,[26] calibrated by bovine serum albumin (BSA) as a standard (Bio-Rad Protein Assay kit).

Evaluation of Enzyme Activity

The enzyme activity was evaluated using both spectrophotometric (method A) and GC (method B) method.

Method A: spectrophotometric assay: The enzyme activity was determined by monitoring the decrease of absorbance at 340 nm during the reduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate (**1**). The standard assay mixture contained, in a total volume of 1.0 mL, the enzyme solution (0.89 mL), **1** (0.01 mL of 100 mM ethanol solution of **1**: 50 mg **1** in 2 mL EtOH) and 1 mM NADPH (0.1 mL, solution prepared in KH_2PO_4 buffer at pH 6.5).

Method B: GC assay: The enzyme activity was determined by measuring the percentage of product **2** formation after 1 h shaking suspension at 40 °C. The standard assay mixture contained the enzyme solution (1 mL), **1** (11 μL, 100 mM ethanol solution of **1**: 50 mg of **1** in 2 mL EtOH) and NADPH (35 μL) equimolar with **1**.

SDS-PAGE Analysis

Purification steps were analysed using SDS-PAGE on 10% polyacrylamide gels, and the protein bands were stained with either Coomassie Brilliant Blue R-250 or silver stain (Sigma ProteoSilver™ Silver Stain Kit).

Purification

Purifications were performed at 4 °C.

Buffers: Buffer A. 0.01 M KH_2PO_4 (pH 6.5). Buffer B. 0.01 M KH_2PO_4 (pH 6.5), 0.4 M NaCl. Buffer C. 0.01 M KH_2PO_4 (pH 6.5), 2 M NaCl.

***Step 1. Extraction*:** *Kluyveromyces marxianus* (24.5 g, wet mass weight) obtained from fermentation was washed twice with Buffer A (60 mL) and then centrifuged at 7,500 *g* for 5 min. The wet mass was suspended in Buffer A to a final concentration of 60% w/v and the cells were disrupted at 0 °C with 0.8 mm glass beads for 10 min. Glass beads were removed and the suspension was centrifuged at 12,500 *g* for 20 min at 0 °C.

***Step 2. DEAE-Sepharose chromatography*:** The crude extract (42.5 mL) was passed through a DEAE-Sepharose column, equilibrated with Buffer A. The adsorbed enzyme was eluted for the first 60 min only with Buffer A and then with a mixture of Buffer A and Buffer B (0–33 % Buffer B in 30 min; 33 % Buffer B for 20 min; 33–100 % Buffer B in 30 min). The active fractions were collected and diluted with Buffer A to 50 % v/v (final volume 100 mL).

Step 3. 2′5′-ADP Sepharose 4B: The enzyme solution (100 mL) was passed through a 2′5′-ADP Sepharose 4B column and equilibrated with Buffer A. The adsorbed enzyme was eluted for the first 60 min with Buffer A and then with a mixture of Buffer A and Buffer C (0–33 % Buffer C in 20 min; 33 % Buffer C for 30 min; 33–100 % Buffer C in 30 min). The active fractions were collected.

X-Ray Analysis

To establish the absolute configuration at C-7 and C-9 in an unambiguous manner, suitable crystals were grown and subjected to single crystal X-ray analysis, using a Nonius Kappa CCD area detector diffractometer equipped with a fine focus sealed graphite-monochromated Mo-K_α radiation (λ = 0.71073 Å). Data for (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxybutanoic acid were collected at 293(2) K. Data reduction and cell refinement were carried out with the programs DENZO[27] and COLLECT.[28] The structure was solved by the direct methods procedure of SIR97,[29] while the refinement processes were carried on full matrix least squares technique using SHELXL-97.[30] Detailed crystal data and geometrical parameters have been deposited (cif file).[31] The asymmetric unit of (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxybutanoic acid with the atomic numbering scheme is depicted in Figure 4.

Pertinent crystallographic data for (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxybutanoic acid: $C_{10}H_{11}ClO_4$, M_r = 230.64 g·cm^{-3}, orthorhombic, space group: $P2_12_12$, a = 13.2109(4), b = 24.8989(9), c = 6.8380(2) Å, cell volume = 2244.75(12) Å^3, Z = 8, T = 293(2) K, ρ_c = 1.365 g·cm^{-3}, μ = 0.331 mm^{-1}, θ range = 2.25°-27.52°, *hkl* indices $-17 \le h \le 17, -31 \le k \le 32, -8 \le l \le 8$, reflections (measured) = 12,009, reflections (unique) = 5031, reflections (unique $[F_o > 2\sigma\{|F_o|\}]$): 2563, R_{int} = 0.052, 344 parameters, R_1/wR_2 (all data): 0.1433/0.1059, R_1/wR_2 ($I > 2\sigma(I)$): 0.0529/0.0824, Flack parameter = −0.02(8), largest diff. peak/hole: 0.162/−0.245 e·Å^3.

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