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Microbial deracemisation of aromatic β-hydroxy acid esters

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Abstract

Aromatic β-hydroxy acid esters were found to undergo deracemisation using whole cells of *Candida parapsilosis*. The conditions for the deracemisation reaction were optimised where ∼75% isolated yield and >95% enantiomeric excess of the product was achieved. The effect of electron donating as well as electron withdrawing groups present in the standard substrate, ethyl 3-hydroxy 3-phenyl propionate was studied to establish the generality of the reaction. The enantiomeric excess of the product remains high (>95%) irrespective of the different substituents in the *para* position but substitution at the *ortho* position obstructs the process. Similarly, ethyl and methyl esters of the standard substrate undergo deracemisation reaction giving high ee of the product, but the benzyl ester of the standard substrate did not undergo deracemisation.

Keywords: *Candida parapsilosis*; Deracemisation; β-Hydroxy esters; Biotransformations; Biooxidation–reduction

1. Introduction

With the growing demand for the enantiomerically pure bioactive compounds, there is a need to develop an ecofriendly method for the preparation of such compounds. Optically pure β-hydroxy acid esters are very useful chiral synthons [1,2]. They are widely used in the synthesis of important pharmaceutical compounds like fluoxitine [3], L-carnitine [4], etc. These compounds can be synthesized by the reduction of their corresponding prochiral keto esters using chemical [5–7] as well as biocatalytic [8–10] methods. Enzymatic resolution [11] of the racemic β-hydroxy esters [12,13] is also known, however as in any resolution it has the limitation of low theoretical yield (<50%) of each enantiomer. These compounds have also been synthesized by chemo-enzymatic methods. Huerta and Backvall [14] has used Ruthenium catalyst along with the *Pseudomonas cepacia* lipase to reracemise the unwanted isomer. Since all the above methods have their own limitations, the development of better methods to synthesize these important chiral β-hydroxy esters is of growing interest. Deracemisation [15] is a methodology, which can in principle, provide a single enantiomer from the racemic intermediate in high yield and high optical purity. Deracemisation can be carried out chemically [16], chemo-enzymatically [17] and by using isolated enzymes [18]. Deracemisation of various racemic intermediates using biocatalysts (whole cells) [19,20] is more attractive to the modern scientists. Nakamura et al. [21] have reported the stereo-inversion of aliphatic β-hydroxy esters (methyl 3-hydroxy butanoate and methyl 3-hydroxy pentanoate) using *Geotrichum candidum* (IFO 5767). The deracemisation of α-hydroxy acid esters [22] has also been reported by our group.

Oxidoreductases are efficient biocatalysts for the selective oxidation and reduction of several organic substrates. The whole cells of *C. parapsilosis* are a good source of oxidoreductases and the purification and characterization of this class of enzymes has been reported [23]. Goswami et al. [24] have proved the deracemisation of 1,2-diols using the *C. parapsilosis* ATCC 52820. The whole cells of genetically modified [25] *C. parapsilosis* (IFO 1396) is known to reduce the β-keto esters to its optically pure β-hydroxy esters. Based on these reports we used *C. parapsilosis* as the biocatalyst for deracemisation. In this study, we report for the first time, the deracemisation of aromatic β-hydroxy acid esters using the whole cells of *C. parapsilosis* ATCC 7330 (Scheme 1).

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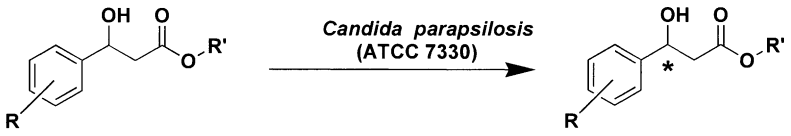


Entry	R	R'
1	H	Et
2	H	Me
3	*p*- MeO	Et
4	*p*-NO_2	Et
5	*p*-Me	Et
6	*o*-Me	Et

Scheme 1.

2. Experimental

2.1. Materials and methods

Ethyl 3-hydroxy 3-phenyl propionate (entry 1) was prepared by reducing the corresponding keto ester, which was bought from Fluka. Ethyl 3-oxo 3-(4-nitro phenyl) propionate and ethyl 3-oxo 3-(4-methoxy phenyl) propionate, were also bought from Fluka and were reduced using sodium borohydride and ethanol to get the corresponding racemic β-hydroxy acid esters. Methyl 3-hydroxy 3-phenyl propionate (2) was synthesized using our own procedure [26]. Ethyl 3-oxo 3-(4-methyl phenyl) propionate and ethyl 3-oxo 3-(2-methyl phenyl) propionate were synthesized using the method reported by Balaji and Chandra [27]. The keto esters were again reduced using sodium borohydride, ethanol and the corresponding racemic β-hydroxy esters were used for deracemisation. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker (400 MHz) spectrometer. Tetramethyl silane was used as an internal standard. IR spectra (neat) were recorded in a Shimadzu spectrometer. The optical purity was measured on a Jasco HPLC with PDA detector using Chiralcel OD-H and Chiralcel OJ-H chiral columns. The mobile phase was hexane:isopropanol, the proportion of solvents and the flow rate varies for different compounds. *C. parapsilosis* was procured from ATCC.

2.2. Growth conditions

Candida parapsilosis (ATCC 7330) was cultured in the YMB medium [22] and the strain was maintained on agar plates using the above described medium (2.1% agar). Sub-culturing was performed every 12 weeks and plates were stored at 4 °C.

2.3. A typical experimental procedure for biotransformation

The resting cells of *C. parapsilosis* (ATCC 7330) were suspended in distilled water (1 g/0.9 ml). Racemic β-hydroxy esters dissolved in organic solvents[2] (0.3/50 μl) were added to the above aqueous biomass. The final volume of the reaction mixture was 2.0 ml. The reaction mixture was incubated at 25 °C and 150 rpm (in a water bath orbital shaker) for 3 h. The reaction mixture was extracted using ethyl acetate, dried with anhydrous sodium sulphate, concentrated using rotovap and high vacuum. The product thus obtained was subjected to chiral analysis and the optical purity was determined. Appropriate control experiments with the reaction mixture containing all the components except (i) racemic β-hydroxy esters (ii) except the whole cells of *C. parapsilosis* established the optical purity of the product and the chemical yield.

2.4. Optimisation of the incubation time

The incubation period of the reaction was optimised by varying the reaction time from 1 to 12 h. The enantiomeric purity against each of the above time interval (1 h) was determined after necessary work up. Optical purity was found to be maximum at the end of 3 h and this was the standard reaction time for all the subsequent biotransformation reactions.

[2] Ethanol was the common solvent used for mixing the substrate except in case of methyl 3-hydroxy 3-phenyl propionate, in which case methanol was used for better yield.

3. Results and discussion

Biotransformation was carried out for 3 h at 25 °C with a number of aromatic β-hydroxy esters as mentioned in the experimental conditions under materials and methods. The isolated product was as analysed using chiral HPLC. High enantiomeric excess (>96%) of the product was obtained with most of the β-hydroxy acid esters. The isolated yield of the reaction is found to be in the range of 62–75% (Table 1). Improvement of the isolated yield is possible and is underway in our laboratory. Ethyl 3-hydroxy 3-phenyl propionate was chosen as a standard substrate for optimisation of the reaction. We have extended the *C. parapsilosis* mediated deracemisation to a variety of substituted aromatic β-hydroxy acid esters. The presence of electron withdrawing as well as electron donating groups in the *para* position of standard β-hydroxy ester does not seem to affect the optical purity of the product. We have also studied the effect of position of the substituent. The presence of a methyl group in the *para* position does not alter the optical yield of the product of deracemisation, but its presence in the *ortho* position slows down the reaction. The deracemisation of this compound (entry 6) did not yield high optical purity (∼10%) in the 3 h. Even when the incubation period was prolonged (12 h), the deracemisation reaction did not go to completion and resulted in poor enantiomeric excess (∼15%). The isolated yield of this product was comparable to the other products of deracemisation. The presence of the methyl group in the *ortho* position of the compound is closer to the chiral centre (the methylene group containing the –OH group). As per the proposed mechanism, the enzymes operate at the stereogenic centre and the presence of any bulky group in the *ortho* position of the compound probably creates a severe steric hindrance which could explain the failure of the reaction for this substrate. In addition to the ethyl ester of the standard compound the corresponding methyl ester also undergoes deracemisation resulting in very high enantiomeric excess.

Ethyl 3-hydroxy 3-phenyl propionate (1): ^{1}H NMR ($CDCl_3$) δ: 1.2 (t, 3H), 2.6–2.7 (d of dd, 2H, J=4.0, 9.0, 16.2 Hz), 3.49 (s, 1H), 4.12–4.18 (q, 2H), 5.09–5.13 (m, 1H), 7.2–7.3 (m_{ar}, 5H); ^{13}C NMR ($CDCl_3$) δ: 14.128, 43.425, 60.871, 70.315, 125.38–125.93, 127.38–127.93, 128.24–128.52, 142.621, 172.379. HPLC: Chiralcel OD-H; hexane:isopropanol (95:05); retention time of the racemic compound (in min): 19.973, 25.880.

Methyl 3-hydroxy 3-phenyl propionate (2): ^{1}H NMR ($CDCl_3$) δ: 2.65–2.78 (d of dd, 2H), 3.46 (s, 1H), 3.68 (s, 3H), 5.09 (m, 1H), 7.2–7.3 (m_{ar}, 5H); ^{13}C NMR ($CDCl_3$) δ: 43.28, 51.89, 70.31, 125.37–125.68, 127.82, 128.56, 142.64, 172.74. HPLC: Chiralcel OD-H; hexane:isopropanol (95:05), retention time of the racemic compound (in min): 26.320, 38.640.

Ethyl 3-hydroxy 3-(4-methoxy phenyl) propionate (3): ^{1}H NMR ($CDCl_3$) δ: 1.21–1.24 (t, 3H), 2.6–2.76 (d of dd, 2H), 3.49 (s, 1H), 3.76 (s, 3H), 4.12 (q, 2H), 5.05 (m, 1H), 6.83–6.85 (d, 2H), 7.25–7.27 (d, 2H). ^{13}C NMR ($CDCl_3$) δ: 14.08, 43.49, 55.18, 60.71, 69.92, 113.47–114.09, 126.65–127.23, 134.98, 159.10, 172.220. HPLC: Chiralcel OD-H; hexane:isopropanol (95:05), retention time of the racemic compound (in min): 25.053, 26.373.

Ethyl 3-hydroxy 3-(4-nitro phenyl) propionate (4): ^{1}H NMR ($CDCl_3$) δ: 1.24 (t, 3H), 2.68–2.74 (d of dd, 2H), 3.97 (s, 1H), 4.1 (q, 2H), 5.24 (m, 1H), 7.55–7.57 (d, 2H), 8.17–8.19 (d, 2H), ^{13}C NMR ($CDCl_3$) δ: 14.1, 43.07, 61.24, 69.42, 123.41–123.76, 126.30–126.58, 147.45, 149.94, 171.93. HPLC: Chiralcel OJ-H; hexane:isopropanol (95:05), retention time of the racemic compound (in min): 37.252, 40.227.

Ethyl 3-hydroxy 3-(4-methyl phenyl) propionate (5): ^{1}H NMR ($CDCl_3$) δ: 1.22–1.26 (t, 3H), 2.32 (s, 3H), 2.63–2.76 (d of dd, 2H), 3.36 (s, 1H), 4.13–4.18 (q, 2H), 5.06–5.08 (m, 1H), 7.13–7.15 (d, 2H), 7.23–7.25 (d, 2H) ^{13}C NMR ($CDCl_3$) δ: 14.08, 43.49, 55.18, 60.71, 69.92, 113.47–114.09, 125.63–126.21, 132.78, 157.20, 171.220. HPLC: Chiralcel OD-H; hexane:isopropanol (97:03), retention time of the racemic compound (in min): 164.326, 185.787.

Ethyl 3-hydroxy 3-(2-methyl phenyl) propionate (6): ^{1}H NMR ($CDCl_3$) δ: 1.24–1.28 (t, 3H), 2.33 (s, 3H), 2.63–2.67 (d of dd, 2H), 3.31 (s, 1H), 4.15–4.20 (q, 3H), 5.31–5.34 (m, 1H), 7.11–7.49 (m_{ar}, 4H). ^{13}C NMR ($CDCl_3$) δ: 14.16, 18.98, 42.19, 60.90, 67.00, 125.26, 126.38, 127.56, 130.45, 134.30, 140.57, 172. 58. HPLC: Chiralcel OJ-H; hexane:isopropanol (90:10), retention time of the racemic compound (in min): 15.587, 18.867.

Table 1
Deracemisation of β-hydroxy esters

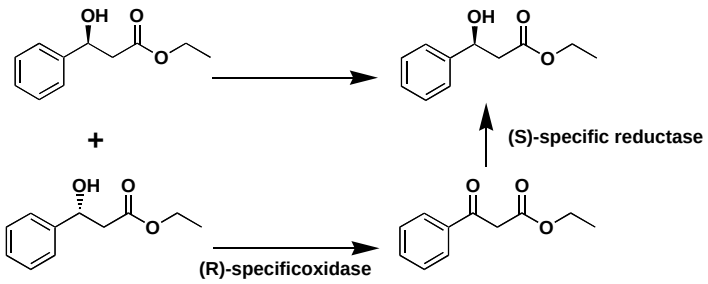
Entry	R	R′	Yield (%)	ee (%)	Elution of HPLC peaks	
					Early	Later
1	H	Et	62	96	Major (*S*)	
2	H	Me	70	99	Major (*S*)	
3	*p*-MeO	Et	65	98		Major[a] (*S*)
4	*p*-NO_2	Et	68	99		Major[a] (*S*)
5	*p*-Me	Et	75	99		Major[a] (*S*)
6	*o*-Me	Et	75	15		Major[a] (*S*)

ee: enantiomeric excess measured by HPLC.

[a] The absolute configurations were assigned based on the reported literature [28].

3.1. Proposed mechanism

After our initial extensive study on the effect of substituents with the standard substrate towards deracemisation, we have extended our study towards the mechanistic aspect of the reaction. The proposed mechanism for deracemisation of stereoinversion [29] can be applied to these substrates. The concept of deracemisation proposed currently is that of a two enzyme system which operate in the whole cell of *C. parapsilosis*. One of the enzyme catalyses the selective oxidation of one stereoisomer while the other enzyme



Scheme 2.

catalyses the selective reduction of this oxidised intermediate. In the case of the standard substrate (Scheme 2), between the two stereo isomers the (*R*)-isomer is oxidized selectively by a (*R*)-specific reductase to its corresponding keto ester which on further reduction by a (*S*)-specific reductase gives the (*S*)-enantiomer exclusively. Towards the support of the proposed mechanism, we have carried out the reduction of a ethyl 3-oxo-3-phenyl propionate [30] which resulted in the (*S*) enantiomer exclusively confirming the presence of (*S*)-specific reductase. When the (*S*)-enantiomer (>99% pure) of the standard substrate is used as substrate for deracemisation, the product recovered retained the configuration as well as the optical purity without any loss, clearly supporting the presence of (*S*)-specific reductase which does not oxidize the (*S*)-hydroxy ester to the corresponding keto ester.

4. Conclusion

A biocatalytic method was developed for the deracemisation of β-hydroxy acid esters using whole cells of *C. parapsilosis*. The conditions for the deracemisation reaction were optimized where ∼75% isolated yield and >95% enantiomeric excess of the product was achieved. The generality of the reaction was established by using different substituents in the standard substrate ethyl 3-hydroxy 3-phenyl propionate. The presence of electron donating and electron withdrawing groups in the *para* position does not affect the deracemisation reaction while substitution in the *ortho* position hinders the same. Ethyl and methyl esters of the standard substrate undergo deracemisation reaction to give high ee of the product, the benzyl ester of the same did not undergo deracemisation A mechanism has been proposed for the deracemisation of β-hydroxy esters which involves the oxidation of only one of the stereoisomer followed by its reduction to give the stereoisomer with the opposite configuration. The other antipode remains intact.

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[30] The reduction of aromatic β-keto esters was done successfully using the whole cells of *Candida parapsilosis*, unpublished result.