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Biocatalytic deracemisation of α-hydroxy esters: high yield preparation of (*S*)-ethyl 2-hydroxy-4-phenylbutanoate from the racemate

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Abstract—Biocatalytic deracemisaton of the racemic ethyl ester of 2-hydroxy-4-phenylbutanoic acid gives the (*S*)-enantiomer exclusively in >99% e.e. and 85–90% yield. Ethyl and methyl esters of mandelic acid and the methyl ester of 2-hydroxy-4-phenylbutanoic acid also gave the (*S*)-enantiomer exclusively. Whole cells of *Candida parapsilosis* (ATCC 7330) were used to effect this biotansformation. © 2002 Published by Elsevier Science Ltd.

1. Introduction

Optically active α-hydroxy acids and their esters are important building blocks for the asymmetric synthesis of a wide variety of bioactive molecules.[1] Several methods, chemical[2–4] and enzymatic,[5] have been reported for the synthesis of these compounds. Enantiomerically pure 2-hydroxy-4-phenylbutanoic acid/ester is an important pharmaceutical intermediate used in the synthesis of anti-hypertensive drugs and ACE inhibitors[6] and can be prepared by the asymmetric reduction of the corresponding α-oxo acid by both chemical and biocatalytic methods.[6,7] We have reported the reduction of the 2-*oxo*-4-phenylbutanoic acid ethyl ester to the 2-hydroxy compound (e.e. >99%) using cells of *D. carota*.[8] Enzymatic resolution of the racemate is also known,[9,10] but, as in every resolution the maximum possible yield of each enantiomer is only 50%. In order to increase the yield of one enantiomer beyond 50%, there have been attempts to prepare enantiomerically pure compounds based on the principle of dynamic kinetic resolution.[11,12] Enzymatic resolution in combination with ruthenium-catalysed racemisation of some α-hydroxy acid esters has been reported.[13] The deracemisation of racemic mandelic acid has been effected using a two enzyme system.[14] Deracemisation by a single biocatalyst has also been reported. (*R*)-3-Pentyn-2-ol was obtained enantioselectively from the corresponding racemic alcohol by the use of *Nocardia fusca*,[15] while arylethanols have been deracemised using *Geotrichum candidum*[16,17] and *Sphingomonas pausimobilis*.[18] Plant cell cultures have also been used for the deracemisation of various alcohols.[19] 1,2-Diols have been deracemised using *Corynosporium cassicola* DSM 62475[20] and (*S*)-1,2-pentanediol has been produced from the racemic 1,2-diol using *Candida parapsilosis*.[21] In our attempts to prepare enantiomerically pure 2-hydroxy-4-phenylbutanoic acid/esters we explored the possibility of deracemising the racemic acid/ester using *C. parapsilosis*. The only α-hydroxy acid/ester studied as a substrate for biocatalytic deracemisation so far is mandelic acid and its derivatives and this was done using a two enzyme system.[14] We report herein the first example of α-hydroxy acid esters deracemised by *C. parapsilosis*. The racemic ethyl ester of 2-hydroxy-4-phenylbutanoic acid afforded the (*S*)-enantiomer (> 99% e.e., 85–90% isolated yield) upon treatment with *C. parapsilosis* (ATCC 7330). The ethyl and methyl esters of mandelic acid and the methyl ester of 2-hydroxy-4-phenylbutanoic acid also gave the (*S*)-enantiomer exclusively.

2. Results and discussion

Racemic 2-hydroxy-4-phenylbutanoic acid ethyl ester was converted into the (*S*)-enantiomer ($[\alpha]_D^{25} = +7.5$ (*c* 1, EtOH), >99% e.e. as compared to the literature value[10]) as the sole product on incubation for 1 h with resting cells of *C. parapsilosis* (ATCC 7330). The time of the reaction was optimised based on the enantiomeric

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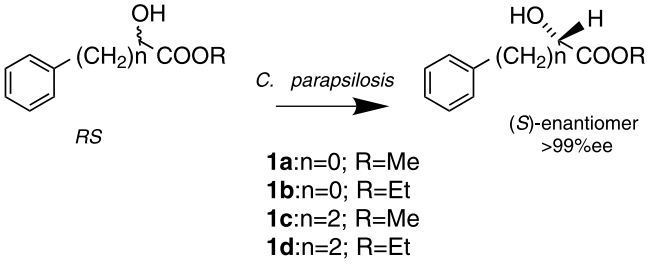
excess (e.e.) of the product (Table 1), which shows the data for the formation of (*S*)-2-hydroxy-4-phenylbutanoic acid ethyl ester from the racemate. The other α-hydroxy acid esters studied were the methyl and ethyl esters of mandelic acid which also showed complete conversion after 1 h to the (*S*)-enantiomer ($[\alpha]_D^{25}=+143$ (*c* 1, MeOH); $[\alpha]_D^{25}=+134$ (*c* 3, $CHCl_3$)), respectively, which is >99% e.e. as compared to the reported values.[22,23] The methyl ester of 2-hydroxy-4-phenyl butanoic acid was also converted into the (*S*)-enantiomer as evidenced by HPLC (Scheme 1). The products were analysed by HPLC using a chiral (Chiracel OD) column and solvent composition reported by us.[10] In order to rule out the decomposition of the 'other' enantiomer, i.e. the (*R*)-enantiomer, separate experiments were done on a larger scale with 2-hydroxy-4-phenylbutanoic acid ethyl ester and the isolated yield was determined (85–90%) as described in Section 4. For the other three esters the blank runs did not show any degradation products from the substrate.

Table 1. Time course of the deracemisation reaction of (*RS*)-2-hydroxy-4-phenylbutanoic acid ethyl ester

Entry	Reaction time (min)	% e.e. of (*S*)-2-hydroxy-4-phenylbutanoic acid ethyl ester
1	10	30
2	20	58
3	40	86
4	60	>99



Scheme 1. Deracemisation of racemic α-hydroxy acid esters using whole cells of *C. parapsilosis* (ATCC 7330).

The racemic esters were dissolved in the minimum amount of water-miscible organic solvent (10% of the final reaction volume), which was added to the aqueous cell suspension. The use of water immiscible solvents led to prolonged reaction times, e.g. in hexane it takes 5 h to reach 97% e.e. With ethanol, >99% e.e. is achieved in 1 h. The work up is simple, involving centrifugation of the cells and straightforward extraction of the product using an organic solvent. The free acids, 2-hydroxy-4-phenylbutanoic acid and mandelic acid, did not show any deracemisation reaction. It is interesting to note that in an earlier study[21,24] *C. parapsilosis* (IFO 0708) produced (*S*)-1,2-pentanediol from the racemic 1,2-diol. The exact mechanism of this reaction is not known as yet but, given the fact that oxidoreductases are abundantly present in microorganisms including the *Candida* sp.,[25] it is likely that the mechanism involves an enantioconvergent stereoinversion[26,27] as proposed for (*RS*)-1,2-diols, i.e. the (*R*)-hydroxy enantiomer in the racemate is oxidised to the corresponding oxo-compound by a (*R*)-specific NAD^+-linked dehydrogenase and then further reduced to the (*S*)-α-hydroxy ester by an (*S*)-specific NADPH-dependent reductase. Thus, the only enantiomer formed by this biotransformation is the (*S*)-enantiomer. When 2-oxo-4-phenylbutanoic acid ethyl ester was incubated with the resting cells of *C. parapsilosis*, the product formed was the (*S*)-hydroxy ester in >99% e.e. In fact in a related study we are trying to establish the generality of this asymmetric reduction. In the case of *G. candidum*,[26] however, stereoinversion (aerobic conditions) in racemic arylethanols results in the formation of (*R*)-alcohols, suggesting the involvement of an alcohol oxidase rather than an alcohol dehydrogenase. We are currently in the process of elucidating the mechanism for the *C. parapsilosis*-mediated deracemisation reaction of α-hydroxy acid esters.

3. Conclusion

In conclusion, we have shown that racemic α-hydroxy acid esters, i.e. methyl and ethyl esters of 2-hydroxy-4-phenyl butanoic acid and mandelic acid in the presence of *C. parapsilosis* produce valuable chiral synthons—the (*S*)-enantiomer in enantiomerically pure form (>99% e.e.). The isolated yield in the case of ethyl 2-hydroxy-4-phenylbutanoate (85–90% isolated yield) proved that this one-step reaction is in fact a deracemisation process, whereby the 'other' enantiomer is converted to its mirror image and not destroyed. It is important to note that this reaction was not seen in free acids, i.e. 2-hydroxy-4-phenylbutanoic acid and mandelic acid even though the hydroxy group in these compounds is a secondary alcohol group. This suggests that other functional groups on the α-carbon influence the enzymatic deracemisation.

4. Experimental

4.1. General

The strain of *C. parapsilosis* (ATCC 7330) used was bought from ATCC, USA. All chemicals used for media preparation were bought locally. The racemic and enantiomerically pure samples of mandelic acid esters were bought from Aldrich Chemical Co., USA and used as such. Racemic 2-hydroxy-4-phenylbutanoic acid ethyl and methyl esters were prepared from benzaldehyde and pyruvic acid by standard procedures. The (*R*)-enantiomer of this acid ester was bought from Aldrich Chemical Co., USA. ^1H NMR spectra were measured on a 400 MHz Jeol instrument.

4.2. Growth conditions of *C. parapsilosis*

Cells of *C. parapsilosis* were grown in optimised YMB medium in shake flasks and were grown for 24 h in a

shaker at 200 rpm, 25°C. Resting cells used for the biotransformation were harvested after 24 h, centrifuged and used as a suspension in sterile distilled water.

4.3. A typical biotransformation experiment

Racemic 2-hydroxy-4-phenylbutanoic acid ethyl ester (3 mg; 0.014 mmol)) in organic solvent (0.1 ml) was added to an aqueous (sterile distilled water) suspension (0.2g/ml wet weight) of resting cells of *C. parapsilosis* (ATCC 7330). The final reaction volume was 1.0 ml. The reaction mixture was incubated at 25°C in an orbital shaker (200 rpm) for 1 h after which time the cells were separated and the product extracted into ether, concentrated and analysed for enantiomeric purity and chemical yield. Appropriate control experiments were carried out using (a) cells in the reaction medium without the substrate and (b) substrate in the reaction medium without cells. The HPLC profile of the first control experiment revealed that nothing from the cells on extraction co-eluted with the product. The second control experiment indicated that the substrate did not give any products in water. The other three esters were also used as substrates in a similar manner.

4.4. Time course of the reaction

The reaction mixture as given above in a typical biotransformation experiment was incubated at 25°C in an orbital shaker (200 rpm) for different intervals of time. After incubation the samples were withdrawn, the cells separated by centrifugation and the supernatant extracted and analysed for enantiomeric purity. The time of the reaction was optimised based on the enantiomeric purity of the product (Table 1).

4.5. Isolation and characterisation of the products

The biotransformation of 2-hydroxy-4-phenylbutanoic acid ethyl ester was carried out for 1 h (>99% e.e.) on 300 mg (1.44 mmol) and 500 mg (2.4 mmol) scale. The product was extracted into diethyl ether, dried and concentrated. This gave isolated yields of 255 mg (1.22 mmol) and 450 mg (2.16 mmol), respectively (85–90% yield). This concentrated sample was used for HPLC analysis to determine its enantiomeric purity. The ^{1}H NMR of ethyl ester of 2-hydroxy-4-phenyl butanoic acids is as follows: (TMS in $CDCl_3$): δ 1.25 (t, 3H), 1.85–2.22 (m, 2H), 2.75 (t, 2H), 4.25 (dd, 1H), 4.10–4.30 (q, 2H) and 7.10–7.30 (m, 5H). The other three substrates were compared with the standards by HPLC and their yields are reported in Table 2. NMR of the standard methyl ester of 2-hydroxy-4-phenylbutanoic acid is as follows: (TMS in $CDCl_3$): δ 1.75–2.15 (m, 2H), 2.7 (t, 2H), 3.8 (s, 3H), 4.12 (dd, 1H) and 7.1–7.4 (m, 5H). The ^{1}H NMR data for the standard methyl and ethyl esters of mandelic acid were as reported.[28,29]

4.6. Determination of e.e. of the product formed

The product from the biotransformation of 2-hydroxy-4-phenylbutanoic acid ethyl ester and the other three esters, was analysed on HPLC using a chiral column, Chiracel OD from Daicel, Japan. The mobile phase used was hexane:isopropanol:trifluoro acetic acid (99:1:0.1) @ 2 ml/min monitored at 254 nm for all the four acid esters. The retention times for the four racemic acid esters were: 2-hydroxy-4-phenylbutanoic acid ethyl ester (9.05 and 13.99 min); 2-hydroxy-4-phenylbutanoic acid methyl ester (10.05 and 14.65 min); ethyl mandelate (10.27 and 19.14 min); and methyl mandelate (10.27 and 19.23 min). For comparison, an enantiomerically pure standard was used and it was found that the (*S*)-enantiomer was the early eluting enantiomer in all the four acid esters.

Table 2. Deracemisation of α-hydroxy acid esters with *C. parapsilosis* (ATCC 7330)

Entry	Compound	Yield (%)	Time (h)	Configuration
1	$n=0$; R = Me*	70	1	*S*
2	$n=0$; R = Et*	74	1	*S*
3	$n=2$; R = Me*	73	1	*S*
4	$n=2$; R = Et	85–90	1	*S*

* 100 mg of the substrate was used.

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