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Preparation the Key Intermediate of Angiotensin-Converting Enzyme (ACE) Inhibitors: High Enantioselective Production of Ethyl (*R*)-2-Hydroxy-4-Phenylbutyrate with *Candida boidinii* CIOC21

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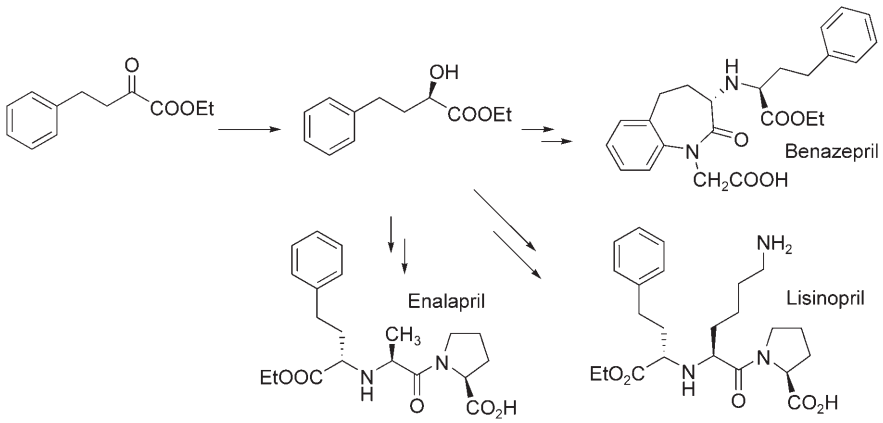
Abstract: Forty microorganisms belonging to different taxonomical groups were used to catalyze the enantioselective reduction of ethyl 2-oxophenylbutyrate to afford the corresponding ethyl 2-hydroxy-4-phenylbutyrate. Several microorganisms led to over 99% *ee* of ethyl (*S*)-2-hydroxy-4-phenylbutyrate. Especially, we firstly found that the *Candida boidinii* CIOC21 could be effectively used for the enantioselective preparation the ethyl (*R*)-2-hydroxy-4-phenylbutyrate in pure aqueous medium with 99% *ee*, a key intermediate in the production of angiotensin-converting enzyme (ACE) inhibitors.

Keywords: ACE inhibitors; bioreduction; *Candida boidinii*; ethyl (*R*)-2-hydroxy-4-phenylbutyrate; *Saccharomyces cerevisiae*

Introduction

The growing interest in asymmetric synthesis has promoted a great development in biotransformations in organic synthesis and they have been used for the synthesis of chiral compounds.[1] The optically pure α-hydroxy acid ester structural unit is widespread in natural products and has been frequently used as a convenient building block in organic synthesis in the last years.[2] Moreover, ethyl (*R*)-2-hydroxy-4-phenylbutyrate has been found to act as a versatile intermediate for the synthesis of angiotensin-converting enzyme (ACE) inhibitors (Scheme 1), such as benazepril, enalapril, lisinopril, ramipril and quinapril, etc., which are widely used to treat hypertension and congestive heart failure.[3].

A number of routes to ethyl 2-hydroxy-4-phenylbutyrate has been developed, including reduction of 2-



Scheme 1. Process for the production of ACE inhibitors.

oxo-4-phenylbutanoic acid and its derivates,[4] asymmetric hydrogenation,[5] multistep asymmetric synthesis,[6] chemical and enzyme resolution.[7] Especially ethyl (*S*)-2-hydroxy-4-phenylbutyrate could be easily prepared by the reduction of many microorganisms with excellent yield and enantiomeric excesses.[8] However, only few microorganisms were described as efficient biocatalysts in the reduction of ethyl 2-oxophenylbutyrate to ethyl (*R*)-2-hydroxy-4-phenylbutyrate,[7] Lacerda and co-workers reported the reduction of ethyl 2-oxo-4-phenylbutyrate with *K. marxianus, P. anomala, P. angusta* to give the ethyl (*R*)-2-hydroxy-4-phenylbutyrate in low to moderate enantioselectivity (35% *ee*, 35% *ee*, and 81% *ee*, respectively).[9] Besides, Chadha et al. reported that the enantioselective reduction of ethyl 2-oxophenylbutyrate to ethyl (*R*)-2-hydroxy-4-phenylbutyrate by using *Daucus carota* (wild carota) with excellent chemical yield (90%) and *ee* (99%).[10] However, in this process, a large amount of cells was needed, while this procedure was time-consuming (10 days). Herein, we first report the enantioselective preparation of ethyl (*R*)-2-hydroxy-4-phenylbutyrate through bioreduction of ethyl 2-oxophenylbutyrate in pure aqueous medium with high activity and enantioselectivity (yield 92%, 99% *ee*) by *Candida boidinii* CIOC21 in a comparatively short time (12 h).

Results and Discussion

During the reduction of ethyl 2-oxo-4-phenylbutyrate, *Candida boidinii* CIOC21 was found to be a promising redox biocatalyst with great synthetic potential due to its excellent chemical yield and enatiomeric excess. The results are summarized in Table 1. Some microorganisms (entries 22–29) showed an (*S*)-preference with moderate to high yields and excellent enantioselectivity (93–99%). However, other microorganisms (entries 30–38) showed (*R*)-preference. Especially, using *Candida boidinii* CIOC21, 92% yield and 99% *ee* of ethyl (*R*)-2-hydroxy-4-phenylbutyrate were obtained.

At the same time, (*R*)-**2** and (*S*)-**2** with high yields and optical purities were obtained respectively, using *Candida boidinii* CIOC21 (92% yield, 99% *ee*) and *Saccharomyces cerevisiae* CIOC SY2 (94% yield, 99% *ee*) (Scheme 2).

The best strain selected for further preparative synthesis of the (*R*)-**2** was *Candida boidinii* CIOC21 (92% yield, 99% *ee*). The effects of reaction time, reaction temperature, pH value, and the substrate concentration were also investigated to optimize the reaction conditions. Under the optimal conditions (12 h, pH 7.0, 30 °C, substrate concentration 20 mM, wet cells:substrate = 20:1), (*R*)-**2** with 92% yield and 99% *ee* was obtained by the reduction of **1** in aqueous medium using *Candida boidinii* CIOC21.

The effects of the cosolvents were subsequently investigated. Usually, a small amount of an organic solvent is often used to ensure sufficient solubility of keto ester substrates.[11] From the results shown in Table 2, addition of 10% ethanol or DMP increased the activity but sharply decreased the enantioselectivity (entry 2 and entry 7). By contrast with ethanol, DMP, MTBE and diisopropyl ether (entries 2, 3, 6, 7), correspondingly better reactivity and enantioselectivity were obtained in DMSO and DMF (entries 4, 5). However, (*R*)-**2** with 92% yield and 99% *ee* was obtained in aqueous medium using *Candida boidinii* CIOC21. In conclusion, in terms of the reactivity and enantioselectivity, the aqueous medium was the optimal medium. Most importantly, there have been significant developments in the direct synthesis of ethyl (*R*)-2-hydroxy-4-phenylbutyrate using microbial cells under solvent-free conditions. Hence, it will contribute to the development of green and sustainable synthetic processes.[12]

Conclusions

In conclusion, a practical biocatalytic synthesis of optically active ethyl (*R*)-2-hydroxy-4-phenylbutyrate has been developed. The synthesis is based on a reduction of the corresponding ethyl 2-oxo-4-phenylbutyrate. The reaction proceeds smoothly in a pure aqueous solvent media and gives the desired product (*R*)-**2** with high conversion (92%) and an excellent enantioselectivity (up to 99% *ee*). It is a simple and environmentally-friendly method to prepare the optically active ethyl (*R*)-2-hydroxy-4-phenylbutyrate, a key intermediate in the production of angiotensin-converting enzyme (ACE) inhibitors. This example is one more proof that the biocatalytic approach is a valuable method for the synthesis of optically active α-hydroxy acid esters. At the same time, (*R*)-**2** and (*S*)-**2** with high yields and optical purities were obtained using different microbial strains (Scheme 2). Future work in this area should be devoted to investigating the properties and characteristics of the reductase in *Candida boidinii* CIOC21. A better understanding of the underlying biochemical mechanism would also be of interest.

Experimental Section

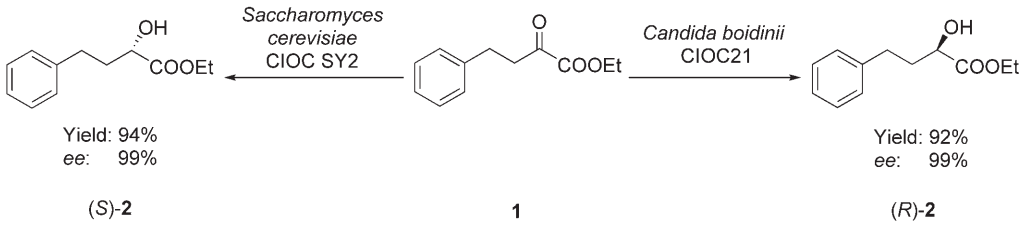
General Remarks

^{1}H and ^{13}C NMR spectra were recorded on a Bruker-300 (300/75 MHz) spectrometer using $CDCl_3$ as a solvent and TMS as internal standard. TLC was performed on glass-

Table 1. Microbial reduction of 2-oxo-4-phenylbutyrate.

Entry	Microorganism	Time [h]	Yield [%][a]	*ee* [%][a] [Absolute configuration]
1	*Geotrichum candidum* CIOC1035	12	35	4 (*S*)
2	*Candida boidinii* CIOC616	12	59	10 (*S*)
3	*Variovoras paradoxus* CIOC1842	24	26	10 (*S*)
4	*Geotrichum candidum* CIOC1175	12	86	14 (*S*)
5	*Saccharomyces cerevisiae* CIOC346	12	70	15 (*S*)
6	*Trichosporon cutaneum* CIOC25	12	62	17 (*S*)
7	*Achrombaccter xylosoxidans* subsp. *denitrificans* CIOC768	24	66	17 (*S*)
8	*Alcaligenes faecalis* CIOC924	24	36	20 (*S*)
9	*Candida boidinii* CIOC1644	12	61	27 (*S*)
10	*Alcaligenes faecalis* CIOC1799	24	83	28 (*S*)
11	*Stenotrophomonas maltophilia* CIOC1788	24	80	32 (*S*)
12	*Candida boidinii* CIOC26	12	67	34 (*S*)
13	*Candida magnoliae* CIOC1919	12	82	36 (*S*)
14	*Geotrichum candidum* CIOC1062	12	63	38 (*S*)
15	*Candida boidinii* CIOC1647	12	59	38 (*S*)
16	*Pichia farinosa* CIOC1463	12	86	41 (*S*)
17	*Candida boidinii* CIOC1646	12	63	46 (*S*)
18	*Geotrichum candidum* CIOC616	12	56	48 (*S*)
19	*Saccharomyces cerevisiae* CIOC396	12	82	61 (*S*)
20	*Geotrichum candidum* CIOC108	12	46	77 (*S*)
21	*Alcaligenes faecalis* CIOC767	24	68	87 (*S*)
22	*Saccharomyces cerevisiae* CIOC1090	24	90	88 (*S*)
23	*Saccharomyces cerevisiae* CIOC S1	12	41	95 (*S*)
24	*Microbacterium terregens* CIOC1901	24	26	98 (*S*)
25	*Yarrowia lipolytica* CIOC1502	12	90	99 (*S*)
26	*Geotrichum candidum* CIOC CIOC11	12	57	99 (*S*)
27	*Saccharomyces cerevisiae* CIOC1396	12	50	99 (*S*)
28	*Saccharomyces cerevisiae* CIOC399	12	49	99 (*S*)
29	*Saccharomyces cerevisiae* CIOC SY2	12	94	99 (*S*)
30	*Alcaligenes faecalis* CIOC1786	24	95	10 (*R*)
31	*Alcaligenes faecalis* CIOC1837	12	22	13 (*R*)
32	*Achrombaccter xylosoxidans* subsp. *denitrificans* CIOC2679	24	88	16 (*R*)
33	*Candida boidinii* CIOC22	12	44	16 (*R*)
34	*Trichosporon cutaneum* CIOC570	12	13	39 (*R*)
35	*Arthrobacter* sp. CIOC1008	24	91	48 (*R*)
36	*Alcaligenes faecalis* CIOC2006	24	37	55 (*R*)
37	*Achromobater xylosoxidans* subsp. *xylosoxidans* CIOC2007	24	89	56 (*R*)
38	*Candida boidinii* CIOC21	12	92	99 (*R*)
39	*Trichosporon cutaneum* CIOC1795	12	0	-
40	*Yarrowia lipolytica* CIOC1506	12	0	-

[a] Determined by GC analysis.



Scheme 2. Stereoselective reduction of keto ester **1**.

backed silica plates. Column chromatography was performed by using silica gel (200–300 mesh) with ethyl acetate/petroleum ether as eluent. The enantiomeric excess was determined by GC analysis on a Chirasil-Dex CB column. All other reagents were purchased from commercial sources and were used without further purification. Compound **1**

Table 2. Reduction by *Candida boidinii* CIOC21: effect of cosolvents on the synthesis of the ethyl (*R*)-2-hydroxy-4-phenylbutyrate.

Entry	Solvent [10% v/v]	Yield [%][a]	*ee* [%][a] Configuration
1	None	92	99 (*R*)
2	Ethanol	98	56 (*R*)
3	Methyl *tert*-butyl ether (MTBE)	29	21 (*R*)
4	DMF	86	99 (*R*)
5	DMSO	90	88 (*R*)
6	Diisopropyl ether	91	31 (*R*)
7	Dimethyl phthalate (DMP)	99	69 (*R*)

[a] Determined by GC analysis.

and ethyl (*R*)-**2**-hydroxy-4-phenylbutyrate were purchased from Aldrich Chemical Co, and the *rac*-2-hydroxy-4-phenylbutyrate was prepared by reduction with $NaBH_4$ in a methanol medium.

Cultivation of Microorganisms

Microorganisms screened were preserved in our laboratory. Yeasts were grown in a medium containing 0.2% (w/v) glucose, 2% (w/v) peptone, and 1% (w/v) yeast extract solution (pH 6.8–7.0); bacteria were grown in a medium containing 0.1% (w/v) sodium chloride, 0.5% (w/v) beef extract, 2% (w/v) peptone solution (pH 6.8). Strains were maintained on nutrient agar slants at 4 °C. Erlenmeyer flasks (250-mL) containing 100 mL of the appropriate sterilized cultivation medium were inoculated with the tested microorganism and incubated in an orbital shaker (180 rpm) at 27 °C. After 48 h of growth (yeast, bacteria), the cells were harvested by centrifugation and washed twice with cool physiological saline (0.85%).

Typical Procedure for the Microbial Screening

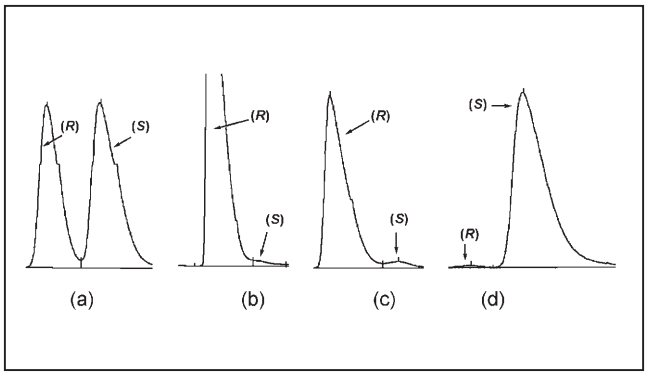
To a suspension of microorganisms cells (yeasts, bacteria) in 10 mL of 50 mM potassium phosphate buffer (pH 7.0), was added keto ester **1** (0.1 mmol) and glucose 0.5 g. The reaction mixtures were incubated in an orbital shaker (160 rpm) at 30 °C for 12 h. After centrifugation at $8000 \times g$ for 8 min, the supernatant was saturated with NaCl and then extracted three times with ethyl acetate. Chemical yield and *ee* of the product were determined by GC analysis.

Isolation and Characterization of the Products

The biotransformation of ethyl 2-oxophenylbutyrate was carried out for 12 h on a 41.2-mg (0.2 mmol) scale. The product was extracted with ethyl ether, dried and concentrated. The crude product of the reaction mixture was purified by flash chromatography (15% EtOAc/petroleum ether), this gave isolated yields of 35 mg (0.168 mmol). This concentrated sample was used for GC analysis to determine its enantiomeric purity. The ^{1}H NMR and ^{13}C NMR spectra of ethyl 2-hydroxyphenylbutyrate are as follows: ^{1}H NMR ($CDCl_3$, 300 Hz): $\delta = 1.29$ (3H, t, $J = 7.2$ Hz, OCH_2CH_3), 1.94–2.00 (1H, m, $ArCH_2CHH$), 2.10–2.15 (1H, m, $ArCH_2CHH$), 2.74–2.81(2H, m, OCH_2CH_3), 2.81(1H, s, O*H*), 4.17–4.25 (3H, m, $ArCH_2$, $CHOH$), 7.17–7.32(5H, m, Ar-*H*); ^{13}C NMR ($CDCl_3$, 75 Hz): $\delta = 175$, 141, 129, 128, 125, 69, 61, 35, 30, 14; MS: $m/z = 208$ (M^+).

Determination of *ee* of the Product Formed

The absolute configurations were assigned by chiral phase GC analysis using authentic (*R*)-**2** as a standard under conditions that gave almost near baseline resolution of the enantiomers (Scheme 3). The absolute configurations and optical purities of ethyl 2-hydroxyphenylbutyrate produced by microbial cell reductions were determined by GC (25 m × 0.25 mm, CP Chirasil-Dex CB column, Varian, USA. $T_c = 160$ °C).



Scheme 3. Chiral-phase GC analysis of α-keto ester reduction products. Samples were analyzed on a 25 m Chirasil-Dex CB column. (a) Racemic **2** prepared by $NaBH_4$ reduction of **1**. (b) (*R*)-**2** prepared by *Candida boidinii* CIOC21 reduction of **1** in aqueous medium. (c) (*R*)-**2** prepared by *Candida boidinii* CIOC21 reduction of **1** in aqueous medium with 10% (v/v) DMSO as a cosolvent. (d) (*S*)-**2** prepared by *Yarrowia lipolytica* CIOC1502 reduction of **1**.

Acknowledgements

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