Pergamon

Tetrahedron: *Asymmetry* 10 (1999) 4667–4675

TETRAHEDRON: *ASYMMETRY*

Asymmetric acyloin condensation catalyzed by phenylpyruvate decarboxylase

Zhiwei Guo, Animesh Goswami, K. David Mirfakhrae and Ramesh N. Patel *

Enzyme Technology, Process Research, Process Research & Development, Bristol-Myers Squibb Pharmaceutical Research Institute, New Brunswick, NJ 08903, USA

Received 5 October 1999; accepted 12 November 1999

Abstract

Cells obtained from growth of *Achromobacter eurydice*, *Pseudomonas aromatica* and *Pseudomonas putida* on L-phenylalanine containing medium catalyzed the enzymatic acyloin condensation of phenylpyruvic acid **1** and acetaldehyde **2** by phenylpyruvate decarboxylase to produce 3-hydroxy-1-phenyl-2-butanone **3**. The acyloin condensation by *Achromobacter eurydice* and *P. aromatica* was stereoselective, providing the 3*R* enantiomer **3a** with enantiomeric excess (ee) of 95% and 84%, respectively. A partially purified enzyme was prepared from the cell free extract of *Achromobacter eurydice.* The acyloin product **3a** was obtained in 45% yield with an ee of 91% by using this partially purified preparation of phenylpyruvate decarboxylase.

1. Introduction

Chiral unsymmetrically substituted acyloins (α-hydroxyketones) are important classes of intermediates in organic synthesis due to their bifunctional aspect, especially having one chiral center amenable to further modification. Enzyme mediated acyloin formation could provide an advantageous, environmentally friendly method to prepare chiral acyloins.[1,2] Acyloin formations mediated by yeast pyruvate decarboxylase (EC 4.1.1.1)[3–5] and bacterial benzoylformate decarboxylase (EC 4.1.1.7)[6–9] have been reported. Though phenylpyruvate decarboxylase (EC 4.1.1.43)[10,11] for decarboxylation of phenylpyruvic acid has been known for a long time, there is no report in the literature on the acyloin condensation catalyzed by phenylpyruvate decarboxylase. The present work describes the first report of asymmetric acyloin condensation catalyzed by phenylpyruvate decarboxylase.

* Corresponding author. E-mail: patelr@bms.com

0957-4166/99/$ - see front matter
PII: S0957-4166(99)00548-0

2. Results and discussion

Several *Pseudomonas* species and *Achromobacter eurydice* were grown in medium containing L-phenylalanine to induce the phenylpyruvate decarboxylase enzyme. The cell mass production was higher when the cells were grown in medium containing yeast extract. The cells were used to catalyze the acyloin condensation between phenylpyruvic acid **1** and acetaldehyde **2** to provide the acyloin product 3-hydroxy-1-phenyl-2-butanone **3** (Fig. 1).

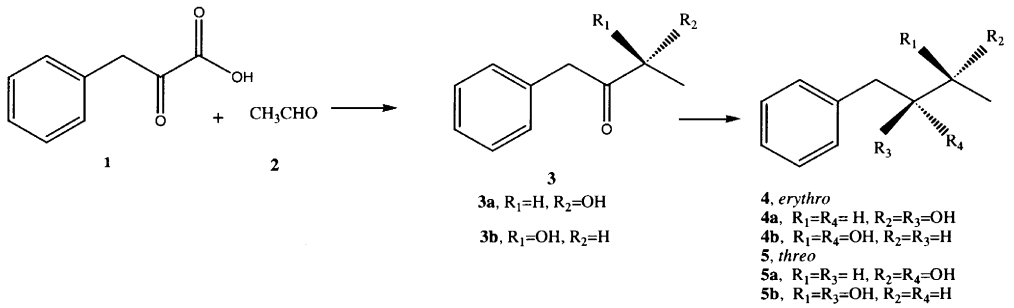

Fig. 1. Acyloin condensation with phenylpyruvate decarboxylase

Authentic racemic product **3** was prepared using known[12] chemical synthetic schemes. Analytical methods were developed for identification of product **3**. Analytical HPLC methods were also developed to separate the two enantiomers of the acyloin product **3**.

Acyloin condensation was carried out with the cell suspensions of various microorganisms from the genus *Pseudomonas* and *Achromobacter*. The results are shown in Table 1. There was no acyloin product formed in the absence of cells. In the presence of cells, acyloin products were identified. The yield of the acyloin product was calculated by comparing the HPLC area with that of a standard curve made by using the chemically synthesized **3**. Product **3** was obtained in 1–7% yield during the initial screening studies. There are two probable reasons for the low yield. Phenylpyruvic acid **1** decomposes under the reaction conditions and the amounts of actual phenylpyruvate decarboxylase enzyme in cells used during the screening experiments were low. Sodium salt of phenylpyruvic acid was found to have better stability under the reaction conditions. The yield of the acyloin product was improved to 45% using higher concentration of partially purified enzyme and the sodium salt of phenylpyruvic acid as described later.

The acyloin product from *Achromobacter eurydice* SC 16386 was isolated. The structure of product **3** was established by comparison of its NMR and mass spectral data with that of the authentic **3** synthesized by the chemical route. The ee of this sample was determined to be 95% by HPLC. The retention times of the major (97.5%) and minor (2.5%) enantiomers were 42.5 min and 27.7 min (HPLC method 4), respectively. The racemic authentic sample of **3** prepared by chemical synthesis showed two peaks at 27.7 and 42.5 min of 1:1 ratio when analyzed by the same HPLC method. The enantiomeric analysis of product **3** from *Pseudomonas aromatica* SC 16387 showed the same major enantiomer as that obtained with *Achromobacter eurydice* with an ee of 84%. Thus, the acyloin products were formed with high enantioselectivity in both cases. The amounts of acyloin products in other cases were too small to make definitive comments about their enantiomeric purity at this point.

The absolute configuration of the acyloin product was established by isolating the product from the enzymatic reaction and subsequent chemical reduction to a diol of known stereochemistry. It was reported that α-hydroxyketones could be reduced by $Zn(BH_4)_2$ to *erythro* diols as the major and *threo* diols as the minor products.[13] The chemically synthesized (±)-**3** was reduced by $Zn(BH_4)_2$ to the *erythro*-(±)-**4**

Table 1
Initial screening of microorganisms for acyloin condensation by phenylpyruvate decarboxylase

Microorganism	SC #	Growth Medium	Yield of 3a	EE of 3a
Achromobacter eurydice	16386	Phenylalanine-Yeast	7.0%	95%
Pseudomonas aromatica	16387	Phenylalanine-Yeast	3.6%	84%
Pseudomonas putida	13940	Phenylalanine-Yeast	1.2%	ND
Pseudomonas putida	13914	Phenylalanine-Yeast	1.0%	ND
Pseudomonas putida	13914	Phenylalanine	0.5%	ND
Pseudomonas putida	16157	Phenylalanine-Yeast	Trace	ND
Pseudomonas putida	16157	Phenylalanine	1.1%	ND
Pseudomonas putida	16154	Phenylalanine-Yeast	Trace	ND
Pseudomonas putida	16154	Phenylalanine	1.0%	ND
Pseudomonas putida	16012	Phenylalanine	1.6%	ND
Pseudomonas putida	16376	Phenylalanine-Yeast	Trace	ND
Pseudomonas putida	16013	Phenylalanine	Trace	ND

ND Not Determined

as the major (70%) and *threo*-(±)-**5** as the minor (30%) products. All compounds were characterized by NMR. An HPLC method (method 5) was also developed for the analysis of **4a**, **4b**, **5a**, and **5b**.

Achromobacter eurydice SC16386 cells were grown in a large scale fermentor with yeast extract medium to provide increased cell mass. These cells and modified reaction conditions using phenylpyruvic acid sodium salt were employed for the enzymatic acyloin condensation, and a reaction product consisting of two compounds was isolated in 18% total yield. These were separated to provide the acyloin product **3a** (10.7% isolated yield, 88% ee by HPLC) and the diol **5a** (7.2% isolated yield, diastereomerically and enantiomerically pure by HPLC and ^{1}H NMR). The diol **5a** was probably formed by reduction of the initially formed acyloin product **3a** by the oxidoreductase(s) present in *Achromobacter eurydice* SC16386 cells. The enzymatic acyloin product **3a** with 88% ee was reduced by $Zn(BH_4)_2$ to the *erythro*-**4a** and *threo*-**5a** diols as the major (70%) and minor (30%) products, respectively. The absolute configurations of the *erythro*-**4a** and *threo*-**5a** diols were determined to be 2*S*,3*R* and 2*R*,3*R*, respectively, by comparing the specific rotation data to those of the literature values.[14] Thus, the absolute configuration of the acyloin product **3a** was determined as 3*R*.

Cell extracts of *Achromobacter eurydice* were prepared, and the phenylpyruvate decarboxylase was partially purified from the cell free extract as described in the Experimental. This partially purified phenylpyruvate decarboxylase was used for the acyloin condensation to provide the acyloin product **3a** and phenylacetic acid as by-product. The latter probably formed by oxidation of phenylacetaldehyde obtained by decarboxylation of phenylpyruvate. The acyloin product **3a** was obtained in 45% yield with an ee of 91%. Further purification of the enzyme is in progress.

The acyloin condensation catalyzed by phenylpyruvate decarboxylase enzyme probably proceeds the same way as pyruvate decarboxylase and benzoylformate decarboxylase catalyzed acyloin condensations[6] involving enzyme catalyzed decarboxylation of phenylpyruvic acid ($PhCH_2COCO_2H$) to the thiamine–enzyme bound complex $PhCH_2CH(OH)$–TPP–enzyme, followed by its reaction with the aldehyde (RCHO) to provide the acyloin product $PhCH_2COCH(OH)R$. The products of phenylpyruvate catalyzed acyloin condensation can be used for the synthesis of biologically active acyloins[16] and various substituted carbohydrates and carbohydrate mimetics.[17] The present chemical methods of syntheses of acyloins[12,18] generally give racemic products. The enzyme catalyzed acyloin condensation, such as the one described here, can provide stereoselective products and are of great value for asymmetric synthesis.

3. Experimental

3.1. Chemicals

Chemicals were purchased from VWR and/or Aldrich.

3.2. Microorganisms

Microorganisms were obtained from our culture collection. Some microorganisms were obtained from ATCC. The SC number denotes the number in our culture collection.

3.3. Analytical methods

NMR spectra were recorded in $CDCl_3$ using a Bruker AC-300 NMR spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C or a Varian 500 MHz NMR spectrometer operating at 500 MHz for ^1H and 125 MHz for ^{13}C. LC–MS analysis was performed by BMS analytical department.

3.4. HPLC

Method 1 was performed on a reversed phase phenylhexyl column (5 μm, 15 cm×0.46 cm, Luna) at 50°C using 0.1% H_3PO_4 and CH_3CN (75:25) as eluent at a flow rate of 1 mL/min and UV detection at 210 nm. Retention times for compounds **1** and **3** were 11.7 min and 10.9 min, respectively.

Method 2 was performed on a reversed phase C-18 column (5 μm, 15 cm×0.21 cm, Advantage 300) at 50°C with a flow rate of 0.5 mL/min using a gradient elution as follows: 98% solvent A (0.1% trifluoroacetic acid in water) and 2% solvent B (0.1% trifluoroacetic acid in CH_3CN) for the first 5 min, then increasing to 85% solvent A and 15% solvent B in 15 min. UV detection was at 210 nm. Retention times for compounds **1** and **3** were 10.7 min and 14 min, respectively.

Method 3 was performed on a reversed phase C-18 column (5 μm, 15 cm×0.46 cm, Kromasil) at 40°C using 0.1% H_3PO_4 and CH_3CN (75:25) as eluent at a flow rate of 1.0 mL/min and UV detection at 210 nm. Retention times for compounds **1**, phenyl acetic acid and **3** were 5.0 min, 6.7 min and 7.5 min, respectively.

Method 4 for separation of stereoisomers was performed on a Chiralpak AD column (25 cm×0.46 cm, Daicel) at ambient temperature with a mixture of hexane:isopropanol:ethanol:trifluoroacetic acid (98.5:0.8:0.6:0.1) as eluent at a flow rate of 1.5 mL/min and UV detection at 210 nm. Retention times for the two isomers of compound **3** were 27.7 min and 42.5 min.

Method 5 for separation of stereoisomers was the same as method 4 except the eluent was a mixture of hexane:isopropanol:ethanol (95.4:4.0:0.6) and the flow rate was 1.0 mL/min.

HPLC methods 1–3 were used for identification and quantitation of different components while methods 4 and 5 were used for enantiomeric excess (ee) analyses.

3.5. Chemical synthesis of 3-hydroxy-1-phenyl-2-butanone *3*

To a stirred solution of trimethylsilyl cyanide (20 mmol, 1984 mg, 2667 μL) in 80 mL CH_2Cl_2 at 0°C, acetaldehyde (20 mmol, 880 mg, 1117 μL) was added followed by TEA (2 mmole, 202 mg, 278 μL). The resulting mixture was stirred at 0°C for 2 h. The solvent was removed under reduced pressure. The residue was dissolved in 20 mL anhydrous ether and added to a stirred solution of benzylmagnesium

chloride (30 mL, 1.0 M in ether) at 24°C. The reaction mixture was refluxed for 2 h, cooled to room temperature. Water (20 mL) was added with caution followed by 20 mL of 4 M HCl under vigorous stirring. The progress of the reaction was followed by TLC. The hydrolysis was complete after 1 h. The product was extracted with ethyl acetate (2×40 mL). The extract was washed successively with water, 5% sodium bicarbonate, water, 1 M HCl, water, brine, dried over $MgSO_4$, and concentrated to dryness. Flash chromatography on silica and elution with CH_2Cl_2 gave 2.48 g of a colorless oily product which still contained some by-product benzyl alcohol as impurity. The pure **3** (390 mg) was obtained by a second flash chromatography on silica and elution with hexane:MTBE (4:1 to 2:1), followed by a third flash chromatography on silica and elution with CH_2Cl_2:MTBE (9:1). The pure **3** was homogeneous by TLC and HPLC, ^{1}H NMR: δ 1.44 (d, J=7.4 Hz, 3H, CH_3), 3.42 (1H, OH, D_2O exchangeable), 3.78 (d, J=15.5 Hz, 1H, CH_2-B), 3.84 (d, J=15.5 Hz, 1H, CH_2-A), 4.38 (m before D_2O exchange, q after D_2O exchange, J=7.4 Hz, 1H, CH), 7.28–7.4 (m, 5H); ^{13}C NMR δ 19.66 (CH_3), 44.45 (CH_2), 72.15 (CHOH), 127.2, 128.7 (2C), 129.3 (2C), 133.0, 210.0 (CO). MS m/z 165 (M+H).

3.6. Chemical reduction of (±)-3 by $Zn(BH_4)_2$

The ether solution of $Zn(BH_4)_2$ (160 mL) was prepared from $ZnCl_2$ (30 mmol) and $NaBH_4$ (2.7 g) as described in the literature.[15] To a stirred solution of (±)-**3** (377 mg in 2.3 mL ether) at 0°C, 5 mL of the above $Zn(BH_4)_2$ solution was added. The resulting mixture was stirred at 0°C and the progress of reaction was monitored by TLC. After 2 h, additional 3 mL of $Zn(BH_4)_2$ was added and the reaction was continued for one more hour to near completion. Water (1 mL) was added dropwise followed by 30 mL of ethyl acetate. The organic phase was washed successively with 1 M HCl (2×5 mL), water (5 mL), brine (5 mL), dried over $MgSO_4$, and concentrated to dryness. Flash chromatography on silica and elution with hexane:ethyl acetate (1:1) gave 265 mg of 1-phenyl-2,3-butanediol. The ^{1}H NMR spectrum of the product showed that it was a mixture of the *erythro*-**4** (70%) and *threo*-**5** (30%) by comparison with literature data.[14] TLC and HPLC (method 2, with retention time 12.0 min for **4** and 13.6 min for **5**, respectively) showed the same 7:3 ratio of the two diastereoisomers. This mixture was subjected to flash chromatography and eluted with CH_2Cl_2:MTBE (4:1). The fast moving portion (fraction 29–34, 8 mL per fraction) gave **5** as a colorless oil, 60 mg, HPLC method 2: 95% **5** and 5% **4**, ^{1}H NMR ($CDCl_3$–2% D_2O): δ 1.28 (d, 3H), 2.78 (dd, 1H), 2.90 (dd, 1H), 3.60 (m, 1H), 3.70 (m, 1H), 7.2–7.4 (m, 5H), which were in agreement with the literature data for *threo* isomers. This oily sample (±)-**5** showed two major peaks at 19.2 and 22.1 min of 1:1 ratio when analyzed by the HPLC method 5. The slow moving portion (fraction 39–46) gave a white solid, 120 mg, HPLC method 2: >95% **4**, ^{1}H NMR ($CDCl_3$–2% D_2O): δ 1.26 (d, 3H), 2.72 (dd, 1H), 2.85 (dd, 1H), 3.8–3.95 (m, 2H), 7.2–7.4 (m, 5H), which were in agreement with the literature data for *erythro* isomers. This solid sample (±)-**4** showed two major peaks at 21.3 and 26.0 min of 1:1 ratio when analyzed by the HPLC method 5.

3.7. Growth of microorganisms on L-phenylalanine agar plate

The composition of medium for agar plate was as follows: L-phenylalanine 3.3 g, $(NH_4)_2SO_4$ 1 g, agar noble 3 g, 5 mL H33A solution (made by dissolving 100 g each of K_2HPO_4 and KH_2PO_4 in water to a volume of 1 L), 5 mL H33B solution (made by dissolving 40 g $MgSO_4 \cdot 7H_2O$, 2 g NaCl, 2 g $FeSO_4 \cdot 7H_2O$, 2 g $MnSO_4 \cdot 4H_2O$ in water to a volume of 1 L), dissolved in water by heating and brought to a volume of 1 L. The pH was adjusted to 7.0. The media was autoclaved at 121°C for 15 min. Twenty cultures including nine bacteria and 11 yeasts were inoculated on agar plates. Six cultures, one *Pseudomonas* sp. and five *Pseudomonas putida*, grew well on the plate after six days.

3.8. Growth of microorganisms in medium containing L-phenylalanine as the sole carbon source

The first liquid growth stage was in 20 mM L-phenylalanine medium made by dissolving 3.3 g L-phenylalanine, 2 g KH_2PO_4, 1 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4$ in water to a volume of 1 L. The pH was adjusted to 7.0 and the medium was autoclaved at 121°C for 15 min. Microorganisms grown on the L-phenylalanine agar plate above were inoculated in 100 mL of L-phenylalanine medium and incubated at 28°C and 200 rpm for four days.

This first stage grown culture (5 mL) was transferred to 100 mL of sterile 60 mM L-phenylalanine medium made in the same way as above except containing 9.9 g of L-phenylalanine and grown at 28°C and 200 rpm for four days. The cells and broth were separated by centrifugation at 15,000 g for 25 min and stored at −70°C before use.

3.9. Growth of microorganisms in medium containing L-phenylalanine and yeast extract

The L-phenylalanine yeast extract medium was made by dissolving 3 g L-phenylalanine, 3 g yeast extract, 0.9 g L-glutamic acid, 4 g KH_2PO_4, 2 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4$ in water to a volume of 1 L. The pH was adjusted to 7.0 and the medium was autoclaved at 121°C for 15 min. Microbial cultures from thawed frozen vials were inoculated into 100 mL of the sterile medium and grown at 28°C, 200 rpm for two or three days. For the first transfer, the grown culture (5 mL) was transferred to 100 mL of the sterile medium and grown in the same way. Each successive transfer was done in the same way. After three transfers, the cells and broth were separated by centrifugation at 15,000 g for 25 min and stored at −70°C before use.

3.10. Acyloin condensation mediated by microbes grown in medium containing L-phenylalanine as the sole carbon source

In a 50 mL Erlenmeyer flask, cells (0.4 g wet weight) were suspended in 10 mL 0.1 M sodium phosphate buffer pH 7.0. $MgCl_2 \cdot 6H_2O$ (6 mg, final concentration 3 mM), thiamine pyrophosphate chloride (TPP, 1 mg, final concentration 0.2 mM) and phenylpyruvic acid (33 mg, final concentration 20 mM) were added. The mixture was incubated for 10 min at 200 rpm at 28°C. Acetaldehyde (56 μL, final concentration 100 mM) was added and the biotransformation was carried out at 200 rpm and 28°C. Samples (2 mL each) were taken at 2, 4, 6, and 24 h. To each sample 1 mL 5% citric acid (pH adjusted to 3.5) was added and extracted with 3 mL ethyl acetate. The organic extract was washed with 2 mL brine and concentrated by evaporation under a stream of nitrogen.

The reaction product was dissolved in 1 mL CH_3CN, filtered and analyzed by HPLC (method 1) along with an authentic sample of **3** as a standard (retention time 10.9 min). No acyloin product was formed in control samples containing no cells.

3.11. Acyloin condensation mediated by microbes grown in medium containing L-phenylalanine and yeast extract

Cells (0.5–1.0 g) were suspended in 5 mL of 0.1 M sodium phosphate buffer pH 7.0 and placed in a 50 mL Erlenmeyer flask. To the mixture, $MgCl_2 \cdot 6H_2O$ (3 mg, final concentration 3 mM), cocarboxylase (TPP, 2.3 mg, final concentration 1 mM) was added followed by a solution of phenylpyruvic acid (8.2 mg in 50 μL ethanol, final concentration 10 mM) under vigorous stirring. One control reaction was set up without cells. Acetaldehyde was added in three portions: 10 μL after the mixture was incubated for

10 min, 45 μL at 4 h and 45 μL at 20 h. The biotransformation was carried out at 200 rpm 28°C for 24 h. The mixture was acidified with 1 mL 1 M HCl and extracted with ethyl acetate (2×4 mL). The ethyl acetate extract was washed with 5% sodium bicarbonate and water. Removal of ethyl acetate gave the reaction product. The reaction product was dissolved in CH_3CN and analyzed by HPLC methods 2 and 4.

3.12. *Reaction product from* Achromobacter eurydice *SC 16386 biotransformation*

The product obtained from *Achromobacter eurydice* SC 16386 biotransformation was analyzed by HPLC, mass spectrometry and one and two dimensional ^1H NMR. MS m/z 165 (M+H). ^1H NMR: δ 1.44 (d, J=7.4 Hz, 3H, CH_3), 3.42 (bs, 1H, OH), 3.78 (d, J=15.5 Hz, 1H, CH_2-B), 3.84 (d, J=15.5 Hz, 1H, CH_2-A), 4.38 (m, 1H, CH), 7.2–7.38 (m, 5H).

3.13. *Growth of* Achromobacter eurydice *SC16386 in 100 L medium in fermentor*

The medium (per L) contained 3 g L-phenylalanine, 20 g yeast extract, 5 g L-glutamic acid, 4 g KH_2PO_4, 2 g $(NH_4)_2SO_4$, and 0.2 g $MgSO_4$. The microbial culture of *Achromobacter eurydice* SC16386 from a thawed frozen vial was inoculated into 100 mL of the sterile medium and grown at 28°C and 200 rpm for two days. This grown culture was transferred (5% inoculum) into fresh sterile medium and grown in the same way for three successive transfers. The scale of each successive transfer was increased to make 5 L inoculum for final transfer to 100 L sterilized medium in a large fermentor. The fermentor was also maintained at 28°C for 48 h. The cells were separated from broth by centrifugation at 15,000 g for 25 min. The wet cells (1076 g) were stored at −70°C before use.

3.14. *Isolation of acyloin product* ***3a*** *and diol* ***5a*** *from the acyloin condensation mediated by* Achromobacter eurydice *SC16386 cells grown in fermentor*

Achromobacter eurydice SC16386 cells (300 g) were suspended in 200 mL water and 500 mL sodium phosphate buffer (0.2 M, pH 7.0) in a 2 L glass reactor maintained at 28°C and equipped with a mechanical stirrer and a condenser (4°C). $MgCl_2 \cdot 6H_2O$ (600 mg) and TPP (460 mg) were added to the reactor. Sodium phenylpyruvate monohydrate was added in two batches (2.04 g each at 0 and 24 h). Acetaldehyde was added in six batches (2.8 mL each at 0, 4, 8, 24, 28, and 32 h). After 48 h, the reaction mixture was acidified with 50 mL of 4 M HCl and extracted with ethyl acetate (900 mL). The organic layer was washed with 5% sodium bicarbonate (2×100 mL), water (100 mL), 1 M HCl (100 mL) and brine (100 mL), dried over $MgSO_4$ (15 g) and charcoal (2 g), filtered and concentrated to dryness under reduced pressure. Flash chromatography of the residue on silica with CH_2Cl_2:MTBE (4:0 to 4:1) eluent gave 510 mg crude product **3a**, and a more polar by-product *threo*-diol **5a** (240 mg, 7.2% yield).

The crude product **3a** was purified on silica eluted with hexane:MTBE (4:1) to afford pure **3a** (350 mg, 10.7%), $[\alpha]_D^{24}$ –90.5 (c 1.54, $CHCl_3$) and –23.6 (c 1.56, MeOH). The ee of this product was determined as 88% by HPLC (method 5). The retention time of the major (94%) and minor (6%) enantiomers were 18.4 and 14.8 min, respectively. The racemic authentic sample of **3** showed two peaks at 14.8 and 18.4 min of 1:1 ratio when analyzed by the same HPLC method.

The *threo*-diol **5a**: $[\alpha]_D^{24}$ +33.7 (c 1.02, $CHCl_3$) (lit.[14] data $[\alpha]_D^{24}$ +35.53 (c 1.162, $CHCl_3$)), ^1H NMR ($CDCl_3$–2% D_2O): δ 1.28 (d, 3H), 2.78 (dd, 1H), 2.90 (dd, 1H), 3.62 (m, 1H), 3.70 (m, 1H), 7.2–7.4 (m, 5H). The *threo*-diol **5a** showed one single peak only on HPLC (method 5) at 19.2 min.

3.15. Reduction of acyloin condensation product **3a** to diols **4a** and **5a** by $Zn(BH_4)_2$

The acyloin product **3a** (164 mg, ee 88%) was reduced with 5 mL of the same $Zn(BH_4)_2$ solution as described previously at 0°C for 30 min. The crude product mixture of **4**:**5** was obtained in about 7:3 ratio. The mixture was separated by flash chromatography on silica, and elution with CH_2Cl_2:MTBE (5:1) gave the fast moving diol **5a** (42 mg, colorless liquid) and the slow moving diol **4a** (50 mg, white solid).

The fast moving diol **5a**: one major peak at 19.2 min by HPLC method 5, $[\alpha]_D^{24}$ +20.1 (c 1.50, $CHCl_3$) (lit.[14] data for **5a** 2*R*,3*R* $[\alpha]_D^{24}$ +35.53 (c 1.162, $CHCl_3$)).

The slow moving diol **4a**: one major peak at 26.0 min by HPLC method 5, $[\alpha]_D^{24}$ –33.6 (c 1.74, $CHCl_3$) (lit.[14] data for **4a** 2*S*,3*R* $[\alpha]_D^{25}$ –41.94 (c 1.130, $CHCl_3$)).

3.16. Partial purification of phenylpyruvate decarboxylase enzyme from Achromobacter eurydice SC16386 cells

Achromobacter eurydice SC16386 cells (100 g) were suspended in 500 mL buffer A (50 mM potassium phosphate, 2 mM $MgSO_4$, 1 mM DTT, 0.1 mM TPP, pH 6.8) and homogenized by three passages through a microfluidizer (Model 110F, Microfluidics Co., Newton, Massachusetts). The resulting suspension was centrifuged at 15,000 g for 30 min. The supernatant was applied on a DE52 cellulose column (14×5.0 cm) previously equilibrated with buffer A. The column was washed with 450 mL buffer A followed by a gradient elution from 100% buffer A (300 mL) to 100% buffer B (0.8 M NaCl in buffer A, 300 mL). The protein concentration and enzyme activity of different fractions were assayed. The major activity was found in 100 mL of the eluate.

3.17. Acyloin condensation by the partially purified phenylpyruvate decarboxylase enzyme from Achromobacter eurydice SC16386

A portion of the active fraction obtained from the DE-52 cellulose column chromatography was used for this experiment. Acetaldehyde (11 μL) was added to a mixture of the active fraction (500 μL), water (289 μL), potassium phosphate buffer (100 μL, 500 mM, pH 6.8), $MgCl_2$ (30 μL, 100mM), TPP (20 μL, 50 mM), and sodium phenylpyruvate (50 μL, 100 mM) in a screw-capped 4 mL glass vial. After stirring at 24°C for 20 h, the reaction was terminated by addition of aqueous HCl (50 μL, 1 M) and MeOH (950 μL). The resulting mixture was filtrated and subjected to HPLC method 3 to determine the amounts of various components. In order to remove the phenylacetic acid, the aqueuous reaction mixture was extracted with ethyl acetate (2×3 mL). The ethyl acetate extract was washed with 5% $NaHCO_3$ (2×2 mL) and brine. After removal of solvent, the residue was analyzed by HPLC. HPLC method 3 showed only one major peak of acyloin **3** at 7.5 min, and the ee value of 91% was determined for the major enantiomer **3a** by HPLC method 5.

Acknowledgements

We are indebted to Mr. Steve Schwarz and Mr. David Brzozowski for their help in growing the microorganisms, to Dr. Venkata Nanduri for his help in the partial purification of the enzyme and to the BMS analytical department for providing the NMR and mass spectral analyses.

References

1. Csuk, R.; Glanzer, B. I. *Chem. Rev*. **1991**, *91*, 49–97.
2. Servi, S. *Synthesis* **1990**, 1–25.
3. Juni, E. *J. Biol. Chem.* **1961**, *236*, 2302–2308.
4. Crout, D. H. G.; Dalton, H.; Hutchinson, D. W.; Miyagoshi, M. *J. Chem. Soc. Perkin Trans*. **1991**, 1329–1334.
5. Rogers, P. L.; Shin, H. S.; Wang, B. *Adv. Biochem. Eng. Biotechnol.* **1997**, *56*, 33–59.
6. Prosen, E.; Ward, O. P.; Collins, S.; Dewdney, N. J.; Hong, Y.; Wilcocks, R. *Biocatalysis* **1993**, *8*, 21–29.
7. Hegeman, G. D. *J. Bacteriol*. **1966**, *91*, 1140–1160.
8. Weiss, P. M.; Garcia, G. A.; Kenyon, G. L.; Cleland, W. W.; Cook, P. F. *Biochemistry* **1988**, *27*, 2197–2205.
9. Tsou, A. Y.; Ransom, S. C.; Gerlt, J. A.; Buechter, D. D.; Babbitt, P. C.; Kenyon, G. L. *Biochemistry* **1990**, *29*, 9856–9862.
10. Asakawa, T.; Wada, H.; Yamano, T. *Biochim. Biophys. Acta* **1968**, *170*, 375–391.
11. Heider, J.; Boll, M.; Breese, K.; Breinig, S.; Ebenau-Jehle, C.; Feil, U.; Gadon, N.; Laempe, D; Leuthner, B.; Mohamed, M. E.; Schneider, S.; Burchhardt, G.; Fuchs, G. *Arch. Microbiol*. **1998**, *170*, 120–131.
12. Gill, M.; Kiefel, M. J.; Lally, D. A. *Tetrahedron Lett*. **1986**, *27*, 1933–1934.
13. Nakata, T.; Tanaka, T.; Oishi, T. *Tetrahedron Lett*. **1983**, *24*, 2653–2656.
14. Awano, K.; Yanai, T.; Watanabe, I.; Takagi, Y.; Kitahara, T.; Mori, K. *Biosci., Biotechnol., Biochem*. **1995**, *59*, 1251–1254.
15. Gensler, W. J.; Johnson, F.; Sloan, A. D. B. *J. Am. Chem. Soc.* **1960**, *82*, 6074–6081.
16. Uchida, R.; Shiomi, K.; Sunazuka, T.; Inokoshi, J.; Nishizawa, A.; Hirose, T.; Tanaka, H.; Iwai, Y.; Omura, S. *J. Antibiotics* **1996**, *49*, 886–889.
17. Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. *Chem. Rev.* **1996**, *96*, 443–473.
18. Vankar, Y. D.; Chaudhuri, N. C.; Singh, S. P. *Synth. Commun.* **1986**, *16*, 1621–1626.