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Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 18 (2007) 2537–2540

Enantiocomplementary preparation of (*S*)- and (*R*)-mandelic acid derivatives via α-hydroxylation of 2-arylacetic acid derivatives and reduction of α-ketoester using microbial whole cells

Yongzheng Chen,[a,b] Jinggang Xu,[a] Xiaoying Xu,[a] Yu Xia,[a] Hui Lin,[a] Shiwen Xia[a,*] and Lixin Wang[a]

[a]*Key Laboratory for Asymmetric Synthesis and Chirotechnology of Sichuan Province, Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences, Chengdu 610041, China*
[b]*Graduate School of Chinese Academy of Sciences, Beijing 100039, China*

Received 13 August 2007; accepted 20 September 2007

Abstract—Forty one microorganisms belonging to different taxonomical groups were used to carry out the enantioselective reduction of methyl benzoylformate to afford the corresponding (*R*)-methyl mandelate, with moderate to high ee. In contrast, the monooxygenase enzyme in *Helminthosporium* sp. CIOC3.3316 catalyzed the hydroxylation of methyl 2-phenylacetate to (*S*)-methyl mandelate. This combination of oxidation and reduction biotransformations thus provides a method for preparing the enantiomers of chiral α-hydroxy acid derivatives.

1. Introduction

The growing interest in asymmetric synthesis has promoted a great development in biotransformations in organic synthesis and has been widely used for the synthesis of chiral compounds.[1] Enantiomerically pure α-hydroxy acids and their derivatives are important building blocks for the synthesis of a wide variety of bioactive molecules and other fine chemicals.[2] Moreover, Mandelic acid and its derivatives have been found to act as versatile intermediates for pharmaceuticals and resolving agents in chiral resolution processes.[3] Due to the great interest in these compounds, several methods have been developed to obtain the enantiomerically pure form. These include the kinetic resolution of racemic α-hydroxy acid ester,[4] hydrolysis of cyabohydrin,[5] and reduction of prochiral α-ketoesters.[6] However, methyl mandelate and its derivatives can hardly be gained by C–H oxidation of their corresponding prochiral aromatic carboxylic acid derivatives.[7]

The regio- and stereoselective hydroxylation of 2-phenylacetic acid derivatives is the simplest route for preparing optically active mandelic acid derivatives. However, regio- and stereoselective hydroxylation on activated C–H remains a challenge in synthetic chemistry.[8] On the other hand, hydroxylation reaction can be a useful tool for this type of transformation.

Adam et al. reported the α-oxidation of long-chain carboxylic acids to a useful optically active 2-hydroxy acids by α oxidase of peas (*Pisum sativum*).[9] Recently, Arnold et al. reported the first enantioselective oxidation of **1** to (*S*)-**2** with engineered cytochrome P450 BM-3;[7b] however, engineered cytochrome P450 BM-3 shows low to moderate yields and moderate to high ee (82–90%) on 2-phenylacetic esters. Holland's group reported the hydroxylation of long-chain alkanenitriles (C_6–C_{10}) to give the corresponding (*R*)-2-alkanols in low yields (8–19%) and high enantiomeric purity (ee >95%) by *Helminthosporium* sp. NRRL4671.[10]

Herein, we report a microbial strain (*Helminthosporium* sp. CIOC3.3316), which can hydroxylate methyl 2-phenylacetate to (*S*)-methyl mandelate with high enantioselectivity (92% ee). Moreover, several microorganisms, which can reduce methyl benzoylformate to (*R*)-methyl mandel-

* Corresponding author. Fax: +86 28 85211877; e-mail: swxia2008@hotmail.com

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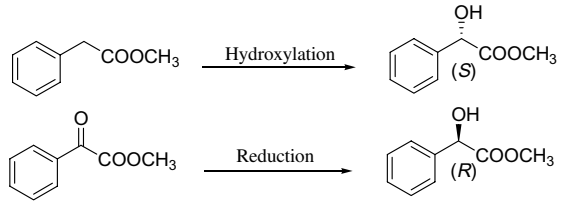
doi:10.1016/j.tetasy.2007.09.029

ate, have also been studied in this paper. This combination of oxidation and reduction biotranformations thus provides a method for preparing the enantiomers of chiral α-hydroxy acid derivatives (Scheme 1).



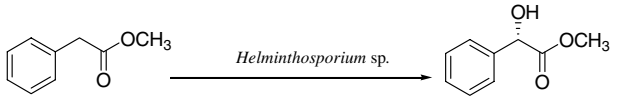
Scheme 1. Enantioselective hydroxylation and reduction.

2. Results and discussion

2.1. Oxidation

From the results shown in Table 1, the *Helminthosporium* sp. CIOC3.3316 performed an (*S*)-preference with high enantioselectivity. Strains (entries 1, 3–5) showed little to no activity. However, using *Helminthosporium* sp. CIOC3.3316, 62% yield and 92% ee of methyl mandelate were obtained (entry 2).

Table 1. Oxidation of methyl 2-phenylacetate by *Helminthosporium* sp.



1 **2** (*S*)-methyl mandelate

Entry	Microorganism	Yield	ee[c]
1	*Helminthosporium* sp. CIOC3.1033	0	—
2	*Helminthosporium* sp. CIOC3.3316	82[a](63)[b]	92(*S*)
3	*Helminthosporium* sp. CIOC3.3319	<5%	nd
4	*Helminthosporium* sp. CIOC3.3114	0	—
5	*Helminthosporium* sp. CIOC3.1669	<5%	nd

[a] Determined by HPLC analysis.
[b] Isolated yield.
[c] Determined by HPLC.

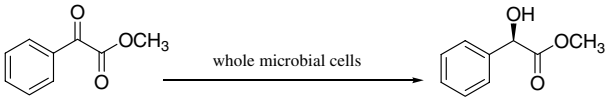
Recently, Arnold et al. reported that the first enantioselective oxidation of **1** to (*S*)-**2** (90% ee) with engineered cytochrome P450 BM-3 (BM-3; isolated from *Bacillus megaterium*) is capable of enantioselective hydroxylation at the α-position of certain carboxylic and peptide groups.[7b] Compared with what Arnold et al. reported, we used a wild type strain (*Helminthosporium* sp. CIOC3.3316) as a biocatalyst, and (*S*)-**2** was prepared by using resting whole cells with 92% ee. In addition, whole-cell fermentations offer a different approach to overcoming the cofactor obstacle: living organisms provide natural recycling systems for all factors required.[11] The tedious process of protein purification can be avoided, and applications are not limited by possible enzyme instability. In many cases, cells can be easily cultivated and stored, hence representing a renewable source of the catalytic entity.

With the whole cells of a microbial strain (*Helminthosporium* sp. CIOC3.3316) expressing high hydroxylase activity as discovered in our group as the biocatalyst, a novel microbial hydroxylation system will be constructed. Reaction conditions, the interrelations of electronic and stereo effects with regio- and enantioselectivity of the hydroxylation of aromatic and aliphatic carboxylic acid esters are also currently under investigation.

2.2. Reduction

The results of the microbial screening for the reduction are summarized in Table 2. In terms of reduction yields, among the 41 microorganism strains evaluated, 10 of them catalyzed the reduction of ketoester **3** to the corresponding hydroxy acid ester **4** with high yields (>90%, entries 3–12); six of which (*Geotrichum candidum* CIOC2.616, *Geotrichum candidum* CIOC2.1062, *Geotrichum candidum* CIOC SY1, *Saccharomyces cerevisiae* CIOC2.1396, *Yarrowia lipolytica* CIOC2.1506) reduced ketoester **1** to the corresponding (*R*)-mandelic acid ester with good enantioselectivities (90–95% ee, entries 3–5, 7, 9, 11).

Table 2. Reduction of benzoylformate by whole microbial cells



3 **4** (*R*)-methyl mandelate

Entry	Microorganism	Yield[a] (%)	ee[a] (%)
1	*Trichosporon cutaneum* CIOC2.25	84	92(*R*)
2	*Geotrichum candidum* CIOC2.1080	69	91(*R*)
3	*Geotrichum candidum* CIOC2.616	92	95(*R*)
4	*Geotrichum candidum* CIOC2.1062	97	94(*R*)
5	*Geotrichum candidum* CIOC SY1	92	94(*R*)
6	*Saccharomyces cerevisiae* CIOC2.399	97	85(*R*)
7	*Saccharomyces cerevisiae* CIOC2.1396	93	90(*R*)
8	*Saccharomyces cerevisiae* CIOC2.1090	93	85(*R*)
9	*Saccharomyces cerevisiae* CIOC SY12	96	90(*R*)
10	*Saccharomyces cerevisiae* CIOC SY13	93	74(*R*)
11	*Yarrowia lipolytica* CIOC2.1506	93	95(*R*)
12	*Pichia farinosa* CIOC2.1463	93	70(*R*)

[a] Determined by HPLC.

In terms of stereoselectivity, eight strains (*Trichosporon cutaneum* CIOC2.25, *Geotrichum candidum* CIOC2.1080, *Geotrichum candidum* CIOC2.616, *Geotrichum candidum* CIOC 2.1062, *Geotrichum candidum* CIOC SY01, *Saccharomyces cerevisiae* CIOC2.1396, *Yarrowia lipolytica* CIOC2.1506) follow 'Prelog's rule' leading to the (*R*)-mandelate while no one strain shows an anti-Prelog specificity leading to the (*S*)-mandelate. These results are consistent with 'Prelog's rule'. This simple model states that the majority of dehydrogenases deliver the hydride ion to the *re*-face of a prochiral ketoester. *Geotrichum candidum* CIOC2.616 is selected as the best strain to further preparative synthesis of the (*R*)-methyl mandelate.

3. Conclusion

In conclusion, the enantiomers of methyl mandelate were obtained by reduction of methyl benzoylformate and oxidation of methyl 2-phenylacetate. Several strains catalyzed the enantioselective reduction of methyl benzoylformate with high conversion and enantiomeric purity to give the (*R*)-methyl mandelate. Moreover, it has been shown that *Helminthosporium* sp. CIOC3.3316 is capable of hydroxylating methyl 2-phenylacetate to (*S*)-methyl mandelate. Enantioselective hydroxylation in the α-position of carboxylic acid ester provided a novel reaction type with microbial whole cells, and it opens a new route to (*S*)-mandelic acid derivates. More importantly, there have been significant developments in the direct synthesis of (*R*) or (*S*)-methyl mandelate using microbial cells under solvent free conditions. As a result, it will contribute to the development of green and sustainable synthetic processes.[12]

4. Experimental

4.1. General

Unless otherwise noted, reagents were purchased from commercial suppliers and used without further purification. Compound **1**, compound **3**, and *rac*-and (*R*)-methyl mandelate were purchased from Aldrich Chemical Co. TLC was performed on glass-backed silica plates. Column chromatography was performed by using silica gel (200–300 mesh) with ethyl acetate/petroleum ether as eluent. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker-300 (300/75 MHz) spectrometer using $CDCl_3$ as a solvent and TMS as an internal standard.

4.2. 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); fungi were grown in a medium of potato with 2% (w/v) glucose. Strains were maintained on nutrient agar slants at 4 °C. Erlenmeyer flasks (250 mL), each containing 100 mL of the appropriate sterilized cultivation medium were inoculated with the tested microorganism and incubated in an orbital shaker (160 rpm) at 28 °C. After 48 h of growth (yeast, bacteria) and 72 h (fungi), respectively, the cells were harvested by centrifugation and washed twice with cool physiological saline (0.85%).

4.3. Typical procedure for the microbial screening

To a suspension of microorganism cells (yeasts, bacteria and fungi) in 10 mL of 50 mM potassium phosphate buffer (pH 7.0), was added benzoylformate **1** (0.1 mmol), or methyl 2-phenylacetate **3** (0.1 mmol), respectively. The reaction mixtures were incubated in an orbital shaker (160 rpm) at 30 °C for 24 h. After centrifugation at 8000 g for 8 min, the supernatant was saturated with NaCl and then extracted three times with ethyl acetate. Chemical yields and ee of the product were determined by GC or HPLC analysis.

4.4. Isolation and characterization of the products

The biotransformation of methyl 2-phenylacetate and methyl benzoylformate was carried out for 24 h on a 300 mg (2.0 mmol) and 328 mg (2.0 mmol) scale, respectively. The product was extracted with ethyl acetate, dried and concentrated. The crude of the reaction mixture was purified by flash chromatography (25% EtoAc/petroleum ether). This gave isolated yields of 209 mg (1.42 mmol) and 268 mg (1.61 mmol), respectively (62–81% yield). This concentrated sample was used for HPLC analysis to determine its enantiomeric purity. (*S*)-methyl mandelate: $[\alpha]_D^{20} = +130.2$ (*c* 0.8, methanol), (*R*)-methyl mandelate: $[\alpha]_D^{20} = -135.6$ (*c* 1.0, methanol). The ^{1}H NMR and ^{13}C NMR of methyl mandelate are as follows: ^{1}H NMR ($CDCl_3$, 300 Hz), δ 3.45 (s, 1H), 3.76 (s, 3H), 5.18 (s, 1H), and 7.37–7.40 (m, 5H). ^{13}C NMR ($CDCl_3$, 75Hz): δ 174.0, 138.2, 128.5, 128.4, 126.6, 72.8, 53.0.

4.5. Determination of ee of the product formed

The absolute configurations were assigned by chiral-phase HPLC analysis using authentic (*R*)-**2** or (*S*)-**4** as a standard. The product from the biotransformation of methyl 2-phenylacetate and methyl benzoylformate was analyzed on HPLC using a chiral column, chiralcel OD-H (φ 0.46 cm × 25 cm) from Daicel, Japan. The mobile phase used was hexane/isopropanol (90:10), 1 mL/min monitored at 254 nm. The retention times for racemic methyl mandelate were 11.53 min and 13.11 min.

Acknowledgment

We are grateful for financial support from the National Natural Science Foundation of China (No. 20672110).

References

1. (a) Faber, K. *Biotransformation in Organic Chemistry*; Springer: Berlin, 1997; (b) Giacomo, C.; Sergio, R. *Angew. Chem., Int. Ed.* **2000**, *39*, 2226; (c) Kaoru, N.; Rio, Y.; Tomoko, M.; Tadao, H. *Tetrahedron: Asymmetry* **2003**, *14*, 2659.
2. Copolla, G. M.; Schuster, H. F. *α-Hydroxy Acids in Enantioselective Synthesis*; VCH: Weinheim, 1997.
3. (a) Inghardt, T.; Johansson, A. PCT Int. Appl. WO2003044145, 2002; (b) Inghardt, T.; Nystom, J. E. PCT Int. Appl. WO2000042059, 2000.
4. (a) Huerta, F. F.; Laxmi, Y. R. S.; Backvall, J.-E. *Org. Lett.* **2000**, *2*, 1037; (b) Robert, F. C.; Kevin, F.; Tord, I.; Olle, K.; Kristina, N.; John, E. R.; Larry, Y. *Tetrahedron Lett.* **2003**, *44*, 5477; (c) Tang, L.; Deng, L. *J. Am. Chem. Soc.* **2002**, *124*, 2870; (d) Huang, H.-R.; Xu, J.-H.; Xu, Y.; Pan, J.; Liu, X. *Tetrahedron: Asymmetry* **2005**, *16*, 2113.
5. (a) Wang, M.-X.; Lu, G.; Ji, J.-C.; Huang, Z.-T.; Meth-Cohn, O.; Colby, J. *Tetrahedron: Asymmetry* **2000**, *11*, 1123; (b)

Zhu, D.; Ankati, H.; Mukherjee, C.; Yang, Y.; Biehl, E. R.; Hua, L. *Org. Lett.* **2007**, *9*, 2561; (c) Kaul, P.; Banerjee, A.; Banerjee, U. C. *Biomacromolecules* **2006**, *7*, 1536; (d) DeSantis, G.; Zhu, Z.; Greenberg, W. A.; Wong, K.; Chaplin, J.; Hanson, S. R.; Farwell, B.; Nicholson, L. W.; Rand, C. L.; Weiner, D. P.; Robertson, D. E.; Burk, M. J. *J. Am. Chem. Soc.* **2002**, *124*, 9024.

6. (a) Yang, W.; Xu, J.-H.; Xie, Y.; Xu, Y.; Zhao, G.; Lin, G.-Q. *Tetrahedron: Asymmetry* **2006**, *17*, 1769; (b) Zhu, D.; Stearns, J. E.; Ramirez, M.; Hua, L. *Tetrahedron* **2006**, *62*, 4535; (c) Xiao, M.-T.; Huang, Y.-Y.; Shi, X.-A.; Guo, Y.-H. *Enzyme Microb. Technol.* **2005**, *37*, 589; (d) Zhu, D.; Yang, Y.; Buynak, J. D.; Hua, L. *Org. Biomol. Chem.* **2006**, *4*, 2690; (e) Glueck, S. M.; Pirker, M.; Nestl, B. M.; Ueberbacher, B. T.; Larissegger-Schnell, B.; Csar, K.; Hauer, B.; Stuermer, R.; Kroutil, W.; Faber, K. *J. Org. Chem.* **2005**, *70*, 4028; (f) Vasse, J. L.; Levacher, V.; Bourguignon, J.; Dupas, G. *Chem. Commun.* **2002**, 2256.
7. (a) Adam, W.; Boland, W.; Hartmann-Schreier, J.; Humpf, H.-U.; Lazarus, M.; Saffert, A.; Saha-Moller, C. R.; Schreier, P. *J. Am. Chem. Soc.* **1998**, *120*, 11044; (b) Landwehr, M.; Hochrein, L.; Otey, C. R.; Kasrayan, A.; Backvall, J.-E.; Arnold, F. H. *J. Am. Chem. Soc.* **2006**, *128*, 6058.
8. Burton, S. G. *Trends Biotechnol.* **2003**, *12*, 543.
9. Adam, W.; Saha-Moller, C. R.; Schreier, P. *Tetrahedron: Asymmetry* **1997**, *7*, 2287.
10. Holland, H. L.; Kohl, A.; Larsen, B. G.; Andreana, P.; Gu, J.-X. *J. Mol. Catal. B: Enzym.* **1997**, *2*, 253.
11. Mihovilovic, M. D.; Müller, B.; Stanetty, P. *Eur. J. Org. Chem.* **2002**, *22*, 3711.
12. (a) Riva, S. *Trends Biotechnol.* **2006**, *24*, 219; (b) Anastas, P. T.; Kirchhoff, M. M. *Acc. Chem. Res.* **2002**, *35*, 686.