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# Deracemization of (±)-mandelic acid using a lipase–mandelate racemase two-enzyme system

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**Abstract**

Deracemization of (±)-mandelic acid was achieved by using a novel two-enzyme process consisting of: (i) *Pseudomonas* sp. lipase catalyzed *O*-acylation of (±)-mandelic acid in diisopropyl ether; followed by (ii) mandelate racemase catalyzed racemization of the remaining unreacted (*R*)-mandelic acid in aqueous buffer. When this one-pot sequence was repeated four times, (*S*)-*O*-acetylmandelic acid was obtained in 80% isolated yield and >98% ee as the sole product. 

Deracemization processes which provide a single enantiomer in 100% enantiomeric excess and chemical yield from a racemate[1] have recently gained increasing attention due to their improved economic balance as opposed to kinetic resolution, which provides two enantiomers each in 50% maximum yield. In order to avoid the occurrence of an undesired isomer, it has been common practice to racemize the unwanted enantiomer and to subject it again to kinetic resolution in subsequent cycles until virtually all of the racemic material has been converted into a single stereoisomer.[2] For obvious reasons, this laborious procedure is not justified on a laboratory scale, but it is a viable option for industrial-scale processes, where the re-racemized material is simply added to the subsequent batch-resolution. One of the major disadvantages of this procedure is the fact that the unreacted stereoisomer has to be separated after kinetic resolution from the formed product,[3] because the most commonly employed (chemical) reaction conditions for racemization[4] would also destroy the enantio-enriched product. Aiming at the simplification of deracemization via kinetic resolution and re-racemization, we envisaged the use of biocatalytic racemization by employing a racemase,[5] which would allow in situ racemization of the non-reacted starting material in the presence of the formed product due to the unparalleled chemoselectivity of the enzyme and the mild (biocatalytic) reaction conditions. Consequently, the separation step can be omitted, thus simplifying this process significantly.

As depicted in Scheme 1, the following process was designed based on a stepwise action of lipase and racemase on (±)-mandelate: in a first step, kinetic resolution of (±)-mandelic acid was accomplished with excellent enantioselectivity ($E$>200) via *Pseudomonas* sp. lipase catalyzed acyl transfer[6] in diisopropyl

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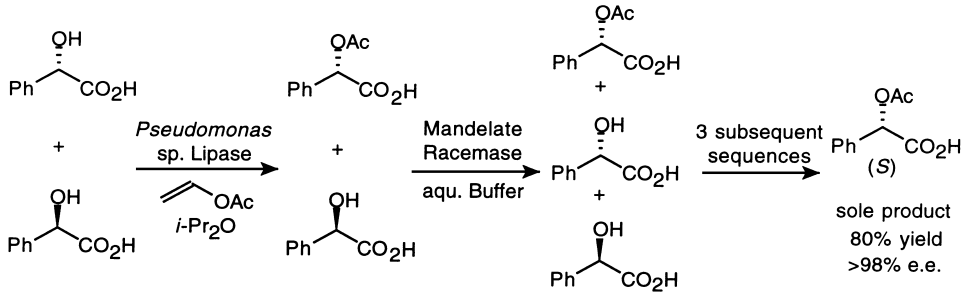
ether producing a 50:50 mixture of (*S*)-*O*-acetyl mandelic acid and (*R*)-mandelate. Then, the lipase was recovered by filtration, the organic solvent was replaced by aqueous buffer, and the remaining non-reacted (*R*)-mandelic acid was racemized using immobilized mandelate racemase[7,8] without affecting the formed (*S*)-*O*-acetyl mandelate.[9] When this process was repeated four times in total, (*S*)-*O*-acetyl mandelic acid was obtained in 80% isolated yield and >98% ee as the sole product.[10] Since, after four steps, 94% of a single isomer can be obtained in theory from a racemate, the overall efficiency of this process is 87%.




Scheme 1. Deracemization of (±)-mandelic acid via a lipase–racemase two-enzyme system

Both biocatalysts can be easily recovered by filtration which enables their reuse since enzyme deactivation is marginal. The scope and limitations of this deracemization process are currently being studied on several α-hydroxycarboxylic acids of industrial importance.

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## References

1. Strauss, U. T.; Felfer, U.; Faber, K. *Tetrahedron: Asymmetry* **1999**, *10*, 107–110.
2. For typical examples, see: Xie, Y.-C.; Liu, H.-Z.; Chen, J.-Y. *Biotechnol. Lett*. **1998**, *20*, 455–458; Kamphuis, J.; Boesten, W. H. J.; Kaptein, B.; Hermes, H. F. M.; Sonke, T.; Broxterman, Q. B.; van den Tweel, W. J. J.; Schoemaker, H. E. In *Chirality in Industry*; Collins, A. N.; Sheldrake, G. N.; Crosby, J., Eds.; Wiley: New York, 1992; pp. 187–208.
3. It should be kept in mind that the materials to be separated for racemization will be combined at the end of the process.
4. Ebbers, E. J.; Ariaans, G. J. A.; Houbiers, J. P. M.; Bruggink, A.; Zwanenburg, B. *Tetrahedron* **1997**, *53*, 9417–9476.
5. Racemases belong to the class of isomerases [EC 5.X.X.X].
6. (±)-Mandelic acid (1 g) was dissolved in diisopropyl ether (30 mL) containing vinyl acetate (13.2 mL), lipase Amano P (1 g) was added and the mixture was shaken at 150 rpm at rt for 8–12 h. When the reaction came to a standstill at 50% conversion, the enzyme was recovered by filtration and the solvent was evaporated. See: Jeromin, G. J.; Albertz, M. *J. Prakt. Chem*. **1992**, *334*, 526–528.
7. Mandelate racemase [EC 5.1.2.2] is a remarkably stable, cofactor-independent enzyme. For its purification and characterization, see: Hegeman, G. D.; Rosenberg, E. Y.; Kenyon, G. L. *Biochemistry* **1970**, *9*, 4029–4035; for a review, see: Kenyon, G. L.; Gerlt, J. A.; Petsko, G. A.; Kozarich, J. W. *Acc. Chem. Res*. **1995**, *28*, 178–186.
8. Mandelate racemase has recently been made available in large quantities ($23\times10^6$ U from a 10 L fermentation volume) through enzyme induction. Stecher, H.; Felfer, U.; Faber, K. *J. Biotechnol*. **1997**, *56*, 33–40. The enzyme accepts a wide variety of α-hydroxy-α-(hetero)aromatic and α-hydroxy-β,γ-unsaturated carboxylic acids. Felfer, U.; Goriup, M.; Strauss, R. V. A.; Orru, U. T.; Faber, K., unpublished results.

9. The mixture of (*S*)-*O*-acetyl mandelate and (*R*)-mandelate from the resolution step was taken up in Hepes buffer (50 mmol, pH 7.5, 100 mL) containing $MgCl_2$ (10 mmol), semipurified mandelate racemase (100 mg) immobilized onto DEAE-cellulose (1 g) was added and the mixture was shaken at 150 rpm at rt for 8–12 h. After filtration of the enzyme, the mixture was lyophilized, acidified (3 M HCl, 1 mL) and extracted into diisopropyl ether. The latter solution was subjected to the subsequent resolution step.
10. The remainder to 100% accounts for losses during final recovery. The simultaneous action of lipase and mandelate racemase, which would lead to a dynamic kinetic resolution, is not feasible due to the complete inactivity of mandelate racemase in organic solvents. Strauss, U. T.; Felfer, U., unpublished results.