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Fungal deracemization of benzoin

Ayhan S. Demir,[a,b,*] Haluk Hamamci,[b,c] Ozge Sesenoglu,[a] Rahsan Neslihanoglu,[b] Beril Asikoglu[b] and Doga Capanoglu[b]

[a]*Middle East Technical University, Department of Chemistry, 06531 Ankara, Turkey*
[b]*Middle East Technical University, Department of Biotechnology, 06531 Ankara, Turkey*
[c]*Middle East Technical University, Department of Food Engineering, 06531 Ankara, Turkey*

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Abstract—An enzyme system of *Rhizopus oryzae* (ATCC 9363) catalyzes the inversion of the chirality of benzoin via a deracemization reaction and, depending on the pH of the medium, both enantiomers of benzoin are obtained in good yield and high ee starting from *rac*-benzoin. © 2002 Elsevier Science Ltd. All rights reserved.

The importance of enantiopure α-hydroxy ketones as precursors of versatile building blocks in asymmetric synthesis is well established. They are important structural units in many biologically active natural products.[1]

The methods of preparation of optically active compounds are classified into two broad categories: optical resolution of racemic compounds and asymmetrization of prochiral compounds. Biocatalysts are widely utilized in both cases.[2] When the starting material is a racemic mixture, the most popular enzymatic approach to obtain the optically active compound is kinetic resolution. However, simple kinetic enzymatic resolutions are restricted to a maximum yield of 50% per enantiomer. More useful is the coupling of racemization with resolution, known as dynamic resolution.[3] By dynamic resolution, it is essential that the starting material racemizes under the reaction conditions, while the product does not. The most straightforward is the synthesis of the target molecule in racemic form and its conversion to the optically active form.[3]

In this work, we report the preparation of both enantiomers of benzoins via a novel enzymatic deracemization reaction using the fungus *Rhizopus oryzae*. This deracemization reaction inverts the chirality of one enantiomer of a racemate to the other antipode, resulting in an optically active compound starting from a racemic mixture and it is entirely different from the above-mentioned dynamic resolution. The most common enzymatic deracemization processes already reported are the oxidation of *sec*-alcohols followed by enantioselective reduction to the antipode.[4] Here we present an attractive alternative option for the fungus mediated synthesis of both enantiomers of benzoin via deracemization of racemic benzoin.

Several methods have been developed for the preparation of optically active benzoins. The stereoselective oxidation of optically active enolates, oxidation of prochiral enolates using optically active oxaziridines, selective oxidation of chiral titanium enolates, and asymmetric oxidation of silyl enol ethers can be cited as some such methods.[5–7] As an alternative to chemical methods, optically active benzoins are prepared enzymatically by reduction of α-diketones and by kinetic resolution of racemic α-hydroxy and α-acetoxy ketones.[8,9] As we reported earlier, selective hydrolysis of the acetoxy ketones by the fungus *R. oryzae* yields hydroxy ketones in high enantiomeric excess.[10] We also presented the first general synthesis of enantiomerically pure benzoin and substituted benzoins from aromatic aldehydes via benzoylformate decarboxylase (BFD) and benzaldehyde lyase (BAL) catalyzed C–C bond formation.[11]

Based on the preliminary information available to us from our previous work with fungus-mediated conversion reactions, a series of fungi were screened for the enantioselective hydrolysis of acetoxy ketones. During this work, we found a deracemization activity in the fungus *R. oryzae* (NRRL 395) on *rac*-benzoin. The enzyme system of *R. oryzae* catalyzes the inversion of

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* Corresponding author. Tel.: +90 312 2103242; fax: +90 312 2101280; e-mail: asdemir@metu.edu.tr

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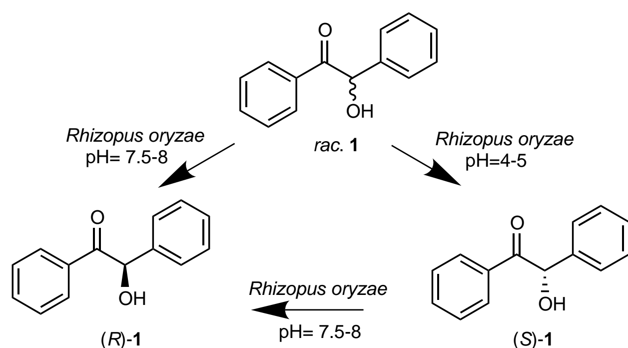


Scheme 1.

the chirality of benzoin. To the best of our knowledge, this is the first example of inverting the chirality of either enantiomer of benzoin.

R. oryzae (ATCC 9363) was inoculated in a medium containing 0.2% ammonium sulphate, 0.065% monobasic potassium phosphate, 0.025% magnesium sulphate, 0.005% zinc sulphate, 0.5% glucose and 1.5% agar. The streaked plates were incubated at 30°C for 3–4 days for spore production and then stored at 4°C until use. The surface of petri plates containing spores were rubbed with a sterile inoculation loop and then transferred to a 1 L sterile beaker containing 400 mL growing medium (for 400 mL medium: 2 g yeast extract, 8 g glucose, 2 g sodium chloride, 4 g tryptone, diluted to 400 mL with distilled water and sterilized in an autoclave for 15 min, 1 atm, 121°C) and the organism was grown in a rotary shaker at 35°C for 2 days. After 2 days, *rac*-benzoin (2 mmol) dissolved in 5 mL DMSO was added (optimum pH of the reaction medium was found to be 7.5–8.5). Conversions were monitored by TLC and LC MS (equipped with a chiral column using authentic (*R*)- and (*S*)-benzoin as reference).[12] Disappearance of the (*S*)-enantiomer and increased formation of the (*R*)-enantiomer was observed during the reaction and shaking was resumed until no more change was observed (consumption of all (*S*)-enantiomer, 21 days). (*R*)-Benzoin **1** was isolated in 73–76% yields and in 95–97% ee. As a control experiment, (*S*)-benzoin was used as a substrate and after the reaction only (*R*)-benzoin was isolated in 74% yield and 97% ee. Under similar conditions no change was observed by using (*R*)-benzoin as substrate, and (*R*)-benzoin was recovered in 77% yield (Scheme 1).

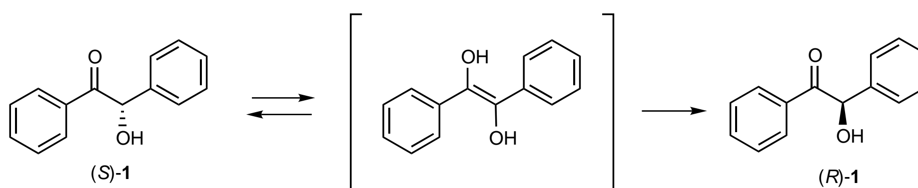
This reaction was carried out at different pH values, and interestingly, we found an opposite selectivity if the reaction was carried out at pH 4–5. Under these conditions *rac*-benzoin was converted into its (*S*)-enantiomer (15 days). The best yield was found to be 71% and the highest ee for the product was 85%. In all cases, some of the products were lost during the work-up procedure and a small amount of diol was detected by LC MS.

Although the mechanism for the deracemization is not clear at present, there are two possible paths for the asymmetrization of the substrates. One is deracemization of the substrate via formation of an ene-diol (Scheme 2) and the other is the enantioselective degradation of one enantiomer. The latter is supposed to be a minor path, if at all, based on the yield and the ee mentioned above.

However, the deracemization reaction of *rac*-benzoin proceeded cleanly, and the enantiomeric excess of the product reached up to 97% after a 21 day incubation. Moreover, upon changing the pH, the absolute configuration of the product was changed as well. This means that the spatial arrangement of the ligands around the asymmetric centre depends on the acidity of the medium. We suggest that the pH dependency is due to some change in the enzyme, or it is more likely that the fungal strain contains many different enzymes, which are active at different pH values.

The mode of interaction between the enzyme and the substrate is not clear at present. However, interestingly, changing only the pH of the medium brings about a dramatic change in the enantioselectivity. In all cases no reaction occurred in the absence of the biocatalyst.

In conclusion, we have found an enzyme system that gives both enantiomers of benzoin depending on the pH of the medium via a novel deracemization reaction. This process can be performed under mild reaction conditions using an extremely simple procedure and gives good to excellent yields and enantiomeric excesses. Further investigation on the scope and limitation of these reactions, as well as a mechanistic study is underway.



Scheme 2.

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12. pH values of the reactions were adjusted using 1N NaOH solution and lactic acid solution. After the reaction was complete, the fungal biomass was filtered off and the mixture was extracted two times with 200 ml of ether. The combined organic layers were washed with brine, dried over $MgSO_4$ and the crude product was purified by flash column chromatography (1:8 EtOAc:hexane). All physical and chemical properties of benzoins were identical with commercially available materials. Ee values were determined by HPLC using a chiral column; Chiralpak AD column, UV detection at 254 nm, eluent: hexane/2-propanol = 9:1, flow 0.80 mL min^{-1} 20°C. R_t (*R*)-benzoin: 25.75 min, R_t (*S*)-benzoin: 32.27 min.