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Fungi mediated conversion of benzil to benzoin and hydrobenzoin

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Abstract—An enzyme system of four fungi catalyses the reduction of benzil to benzoin, as well as benzoin to hydrobenzoin. Depending on the pH of the medium, both enantiomers of benzoin can be obtained in good yield and high ee starting from benzil via a reduction, as well as from *rac*-benzoin via a novel deracemization reaction. Starting from benzil, (*R*)-, (*S*)- and *rac*-benzoin only (*R*,*R*)-hydrobenzoin was obtained in high ee and chemical yield.

1. Introduction

Biocatalysis is one of the most important stereoselective preparations of optically active compounds.[1] Among these methods, the reduction of benzil **1** using biocatalysts is highly desirable. Enantiomerically pure hydrobenzoins has been proven to be very useful chiral auxiliaries[2] and ligands[3] for stereoselective organic synthesis. These diols are accessible through resolution,[4] dihydroxylation of olefins,[5] reduction of benzils and through carbon–carbon bond formation.[6] Baker's yeast, which is a widely used biocatalyst, reduces various carbonyl compounds such as monoketones, diketones and ketoesters to the corresponding chiral alcohols.[7] However, in the reduction of benzil, Baker's yeast gave only racemic benzoin. Inoue et al.[8] and Konishi et al.[9] have recently reported the reduction of benzil to (*S*)-benzoin using the entire cell of *Bacillus cereus*, but benzoin was not further reduced to hydrobenzoin. In connection with our work in the area of benzoins,[10] we have reported in a short communication the preparation of both enantiomers of benzoins via a novel enzymatic deracemization reaction using the fungus *Rhizopus oryzae* (ATCC 9363).[11] During the deracemization reactions, we also observed a low yield formation of hydrobenzoin. In connection with this work, we report herein an attractive alternative option for the fungus-mediated synthesis of both enantiomers of benzoin **2** and (*R*,*R*)-hydrobenzoin **3**.

2. Results and discussion

To assess the ability of selected microorganisms to convert benzil into benzoin and hydrobenzoin, four different species of fungi were selected; *R. oryzae* (ATCC 9363), *R. oryzae* (72465), *Rhizomucor miehei* (72460) and *Rhizomucor pusillus* (72561). These four species were preferred due to their kinship and because of the preliminary work conducted concerning *R. oryzae* (ATCC 9363) mediated bioconversion.[10f,11] The ease and commercial availability of all the possible stereoisomeric products of benzoin and hydrobenzoin makes the analysis of the processes simple. Initially, all four microorganisms were screened and shown to produce benzil–benzoin conversion reactions. After the optimization of the parameters for microbial conversion, the best results were obtained using the following conditions.

Microorganisms were inoculated in a medium containing 0.2% ammonium sulfate, 0.065% monobasic potassium phosphate, 0.025% magnesium sulfate, 0.005% zinc sulfate, 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 utilized. The surface of the petri plate containing spores was rubbed

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with a sterile inoculation loop and then transferred to a 1 L sterile beaker containing a 400 mL growing medium, in which the organism was grown in a rotary shaker at 35 °C for 2 days. After 2 days, benzil (2 mmol) dissolved in 5 mL DMSO was added. Conversions were monitored by TLC and HPLC [equipped with chiral column using authentic (*R*)- and (*S*)-benzoin, as a reference]. After the appropriate time, benzoin was isolated as a colourless solid. All physical and chemical properties of benzoin were identical with commercially available materials. The ee values were determined by HPLC using a chiral column.

Under these conditions, the *Rhizopus* species furnished (*R*)-benzoin in 98 to >99% ee while the *Rhizomucor* species furnished (*S*)-benzoin in 71–73% ee (Table 1, entries 1, 3, 5 and 7). During the conversions it was found that each microorganism worked at a different pH. *R. oryzae* (ATCC 9363) and *R. oryzae* (72465) did not change the pH after the incubation, while both *Rhizomucor miehei* and *Rhizomucor pusillus* first decreased the pH of the medium to 4.5 for 3 days, in which time most of the benzil was converted into (*S*)-benzoin with 71–73% ee. The pH then increased to 8.0–8.5 after 5 days and remained constant at this pH for 15 days. At this high pH value, partial racemization of (*S*)-benzoin occurred (Scheme 1).

In addition to the above-described conditions the conversion reactions were also carried out at different pH levels (Table 1) and it was found that the pH of the medium strongly affected the stereochemical outcome of the products (pH values of the reactions were adjusted using a 1 M NaOH solution and a lactic acid solution). While *R. oryzae* (ATCC 9363) and *R. oryzae* (72465) afforded at a low pH (4.2–5.0) (*S*)-enantiomer of benzoin in 80–85% ee (Table 1, entries 2 and 4), both *Rhizomucor miehei* (72460) and *Rhizomucor pusillus* (72561) showed the same selectivity at a pH of 8.0–8.5 [(*S*)-benzoin formation]. However the ee value of benzoin decreased to 33–37% (Table 1 entries 6 and 8).

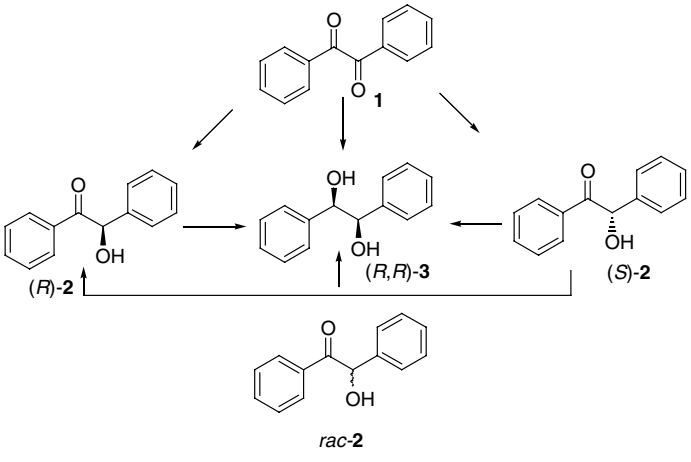
The benzil–benzoin bioconversion reactions, under optimized conditions with all four microorganisms, also furnished hydrobenzoin in a low yield. This reaction was repeated at different pH levels with prolonged times without breaking the reaction at the benzoin step, with the *Rhizopus* species at a pH of 6.5–8.5 and the *Rhizomucor* species at a pH of 8.0–8.5 to furnish hydrobenzoin as a major product. After work-up and purification, hydrobenzoin was isolated in 71–77% yields and in 96–99% ees with 71/29–99/1 *dl*/*meso* ratios (Table 2). The absolute configurations of the products were determined as (*R*,*R*)- by using HPLC with a chiral column. The best results were obtained with the *Rhizopus* species.

Table 1. Fungi mediated reduction of benzil

Entry	Fungus	Reaction time (days)	pH	(*R*)-Benzoin		(*S*)-Benzoin	
				Ee (%)[a]	Yield (%)	Ee (%)[a]	Yield (%)
1	*Rhizopus oryzae* (ATCC 9363)	6	6.5–8.5	>99	81		
2	*Rhizopus oryzae* (ATCC 9363)	5	4.2–5.0			80	75
3	*Rhizopus oryzae* (72465)	7	6.8–8.5	98	83		
4	*Rhizopus oryzae* (72465)[b]	5	4.1–5.0			85	78
5	*Rhizomucor miehei* (72460)	2	4.3–4.6			71	72
6	*Rhizomucor miehei* (72460)[b]	3	8.0–8.5			33	75
7	*Rhizomucor pusillus* (72561)	3	4.3–4.5			73	77
8	*Rhizomucor pusillus* (72561)[b]	3	8.0–8.5			37	73

[a] Determined by HPLC analysis using Chiralpak AD column, UV detection at 254 nm, eluent: hexane/2-propanol = 9:1, flow 0.80 mL min^{-1}. t_R (*R*)-benzoin: 25.70 min, t_R (*S*)-benzoin: 32.21 min.

[b] Obtained from TUBITAK Marmara Research Center, Turkey.



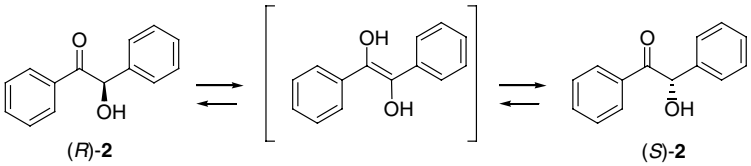
Scheme 1.

Table 2. Fungal conversion of benzil to hydrobenzoin

Entry	Fungus	Reaction time (days)	pH	Hydrobenzoin		
				dl/*meso*[a]	Ee (%)[b]	Yield (%)
1	*Rhizopus oryzae* (ATCC 9363)	21	pH 6.5–8.5	99/1	>99	77
2	*Rhizopus oryzae* (72465)	18	pH 6.5–8.5	99/1	>99	74
3	*Rhizomucor miehei* (72460)	13	pH 8.0–8.5	71/29	96	76
4	*Rhizomucor pusillus* (72561)	11	pH 8.0–8.5	79/21	97	71

[a] Determined by ^{1}H NMR.

[b] Determined by HPLC analysis using a Diacel Chiralcel OJ column, UV detection at 254 nm, eluent: hexane/2-propanol = 9:1, flow 0.80 mL min^{-1} 20 °C. t_R (*R*,*R*)-hydrobenzoin: 26.19 min, t_R (*S*,*S*)-hydrobenzoin: 32.94 min.



Scheme 2.

Using both of the *Rhizopus* species, *rac*-benzoin was also used as a substrate and converted to hydrobenzoin. During this work, we found deracemization activity in both of the fungi on *rac*-benzoin. The enzyme system of these microorganisms catalyzed the inversion of the configuration of benzoin.[11] The conversion of *rac*-benzoin was monitored by TLC and LC MS [equipped with a chiral column using authentic (*R*)- and (*S*)-benzoin as reference]. The disappearance of the (*S*)-enantiomer and the increased formation of the (*R*)-enantiomer were observed during the reaction while shaking was resumed until no more change was observed. (*R*,*R*)-Hydrobenzoin was isolated in a 73% yield, 95% ee [*R. oryzae* (72465)], and a 76% yield, 97% ee [*R. oryzae* (ATCC 9363)] with 97/3; 99/1 *dl*/*meso* ratio.

For the conversion of benzil to hydrobenzoin via benzoin, the enzyme system of the microorganisms must first realize selective reductions and isomerizations. It seems that many different enzymes of fungal strain participate in the selective conversion processes.

During the conversion of benzil to hydrobenzoin, the preferred substrate was benzil. Before the consumption of all benzil, only a small amount of hydrobenzoin formed.

Although the mechanism for deracemization is not clear at present, there are two possible paths for the asymmetrization of the substrates. One is the deracemization of the substrate via the formation of an ene-diol while the other is the enantioselective degradation of one enantiomer (Scheme 2). 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 smoothly and the enantiomeric excess of the product reached 97% after incubation. Moreover, upon changing the pH, the absolute configuration of benzoin also changed. 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 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, it is noteworthy that by only changing the pH of the medium a dramatic change in the enantioselectivity can be brought about. In all cases no reaction occurred in the absence of the biocatalyst.

3. Conclusion

In conclusion, we have found an enzyme system that gives both enantiomers of benzoin depending on the pH of the medium via a novel reduction of benzil and a deracemization reaction of benzoin. The reduction of benzoin isomers furnishes (*R*,*R*)-hydrobenzoin in high ee and good yield. This process can be performed under mild reaction conditions using an extremely simple procedure and provides 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.

4. Experimental

4.1. Materials and methods

NMR spectra were recorded on a Bruker DPX 400. Chemical shifts δ are reported in ppm relative to $CHCl_3$ (^{1}H: $\delta = 7.27$), $CDCl_3$ (^{13}C: $\delta = 77.0$) and CCl_4 (^{13}C: $\delta = 96.4$) as internal standards. Column chromatography was conducted on silica gel 60 (40–63 μm). TLC was carried out on aluminium sheets pre-coated with silica gel $60F_{254}$ (Merck), and the spots visualized with UV light ($\lambda = 254$ nm). Enantiomeric excesses were determined

by HPLC and LC–MS analysis using a Thermo Finnigan Surveyor equipped with an appropriate chiral phase column, as described in the footnotes of the tables. Optical rotations were measured with an Autopol IV automatic polarimeter.

4.2. General procedure for fungal bioconversion

Microorganisms were inoculated in a medium containing 0.2% ammonium sulfate, 0.065% monobasic potassium phosphate, 0.025% magnesium sulfate, 0.005% zinc sulfate, 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 the petri plates containing spores were rubbed with a sterile inoculation loop and then transferred to a 1 L sterile beaker containing a 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 distilled water and sterilized in autoclave for 15 min, 1 atm, 121 °C) and the organism grown in a rotary shaker at 35 °C for 2 days. After 2 days, benzyl or benzoin (2 mmol) dissolved in 5 mL DMSO was added (optimum pH of the reaction medium was found as 7.5–8.5). Conversions were monitored by TLC and LC–MS [equipped with a chiral column using authentic (*R*)- and (*S*)-benzoin, (*R*,*R*)-, (*S*,*S*)- and *meso*-hydrobenzoin as a reference]. The pH values of the reactions were adjusted using a 1 M NaOH solution and a lactic acid solution. After the reaction was complete, the fungal biomass was filtered off and the mixture was extracted three times with 200 mL of EtOAc. The combined organic layers were washed with brine and dried over $MgSO_4$. The crude product was purified by flash column chromatography (1:8 EtOAc–hexane for benzoin; 1:3 EtOAc–hexane for hydrobenzoin). All physical and chemical properties of benzoin and hydrobenzoin were identical with commercially available materials.

4.2.1. *R*,*R*-(+)-1,2-Diphenylethane-1,2-diol (*R*,*R*)-3. Colourless solid, mp: 147–149 °C (lit.[6c] 148–150 °C). Ee: 99%, $[\alpha]_D^{25} = +90.3$ (*c* 1, ethanol); lit.[6b] $[\alpha]_D^{25} = +91.6$ (*c* 1.05, ethanol) >99% ee.

4.2.2. (*R*)-(−)-2-Hydroxy-1,2-diphenylethan-1-one (*R*)-2. Colourless solid, mp: 132–134 °C [lit.[10c], mp 133–134 °C for (*R*)-enantiomer]; $[\alpha]_D^{25} = -114.5$ (*c* 1.5, CH_3COCH_3); {lit.[10c], $[\alpha]_D^{22} = -113.8$ (*c* 1.5, CH_3COCH_3)}.

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