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TETRAHEDRON: *ASYMMETRY*

Asymmetric benzoin reaction catalyzed by benzoylformate decarboxylase

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Abstract

Aromatic aldehydes are converted into benzoins by benzoylformate decarboxylase catalyzed C–C bond formation. The reaction affords (*R*)-benzoins with high enantiomeric excess and in good chemical yields. A broad range of aromatic aldehydes can be used as substrates in aqueous buffer or buffer/DMSO-solutions.

1. Introduction

Benzoin type 2-hydroxy ketones in general are important structural subunits in many biologically active compounds and are also important synthons for stereoselective syntheses.[1–5] The benzoin reaction,[6] one of the oldest C–C bond forming reactions in organic chemistry, has been developed for classical organic synthesis using non-chiral thiazolium[7–9] and for stereoselective synthesis using chiral triazolium salts[10,11] as catalyst. Some other chemical methods for the enantioselective synthesis of 2-hydroxy ketones, which are not based on C–C bond formation, have been evolved. Among them are the stereoselective oxidation of titanium enolates with dimethyldioxirane,[12] of silyl enol ethers with chiral salen complexes[13] or fructose derived dioxirane[14,15] and the stereoselective dihydroxylation of enolates with *N*-sulfonyloxaziridines.[16,17] As an alternative to chemical methods enantiomerically pure 2-hydroxy ketones are prepared enzymatically by reduction of the corresponding α-diketone with baker's yeast[18,19] or an enzymatic kinetic resolution of the racemate of either 2-peroxo-,[20] 2-hydroxy-[21] or 2-acetoxy ketones.[22,23]

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The enzymatically catalyzed reaction should be a versatile stereoselective alternative to the classical benzoin condensation.[6] Benzoylformate decarboxylase (BFD, E.C. 4.1.1.7) has been found in bacteria such as *Pseudomonas putida*, *Acinetobacter calcoaceticus* and *Pseudomonas aeruginosa*.[24–26]

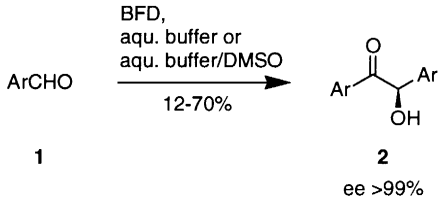
The enzyme is involved in the degradation of aromatic compounds of the mandelate catabolism. Only BFD from *P. putida* has been examined in detail so far. The coding gene was cloned[27] and the crystal structure published recently.[28] The main enzymatic reaction catalyzed by BFD is the non-oxidative decarboxylation of benzoylformate to benzaldehyde. BFD is a stable biocatalyst, which is available in large-scale quantities by fermentation of either *P. putida* or from a recombinant *E. coli*-strain carrying the BFD-gene.[29] BFD is the most active thiamine diphosphate (ThDP)-dependent 2-keto acid decarboxylase known so far which catalyzes both the decarboxylation (main reaction) and the carboligation (side reaction) yielding either (*R*)-benzoin or (*S*)-2-hydroxy propanone derivatives with high specific activities.[29]

The potential of BFD to catalyze C–C bond formation was first reported by Wilcocks and co-workers,[30] who observed the formation of (*S*)-2-hydroxy-1-phenyl propanone ((*S*)-2-HPP) if benzoylformate was decarboxylated in the presence of acetaldehyde using crude extracts of *P. putida*. Similar results were obtained using benzaldehyde and acetaldehyde for carboligation.[31] These results demonstrated that BFD has the potential to synthesize chiral 2-hydroxy ketones using aldehydes without a previous decarboxylation step. During this process (*R*)-benzoin is also obtained as a by-product in low yield.

We now present the first general synthesis of enantiomerically pure benzoin and substituted benzoin derivatives from aromatic aldehydes via BFD catalyzed C–C bond formation.

2. Results and discussion

In our ongoing research on the BFD catalyzed formation of (*S*)-2-HPP we found that the benzaldehyde/acetaldehyde ratio is very important for the product distribution. Low concentration of benzaldehyde in the presence of excess acetaldehyde results in the formation of highly enantiomerically enriched (*S*)-2-HPP.[29] By either increasing the benzaldehyde concentration or decreasing the acetaldehyde concentration we observed the formation of (*R*)-benzoin. Performing the carboligation with benzaldehyde as a sole substrate, (*R*)-benzoin is obtained in 20% yield and >99% ee. Optimization experiments *(*reaction time, amount of enzyme, cofactor, and reaction medium) resulted in high yield of (*R*)-benzoin with >99% ee using an aqueous buffer/DMSO-reaction system (Scheme 1).



Scheme 1.

Benzaldehyde was dissolved in a mixture of potassium phosphate buffer (50 mM, pH 7.0, containing 2.5 mM $MgSO_4$ and 0.15 mM ThDP) and 30% DMSO. After addition of BFD the reaction was accomplished for 48 h at room temperature and monitored by HPLC using a chiral stationary phase column with authentic samples as references. After work up (*R*)-benzoin (ee >99%) was obtained in 70% yield.

The reaction was carried out with different aromatic and heteroaromatic aldehydes and the corresponding benzoin derivatives were obtained in almost enantiomerically pure form as summarized in Table 1.

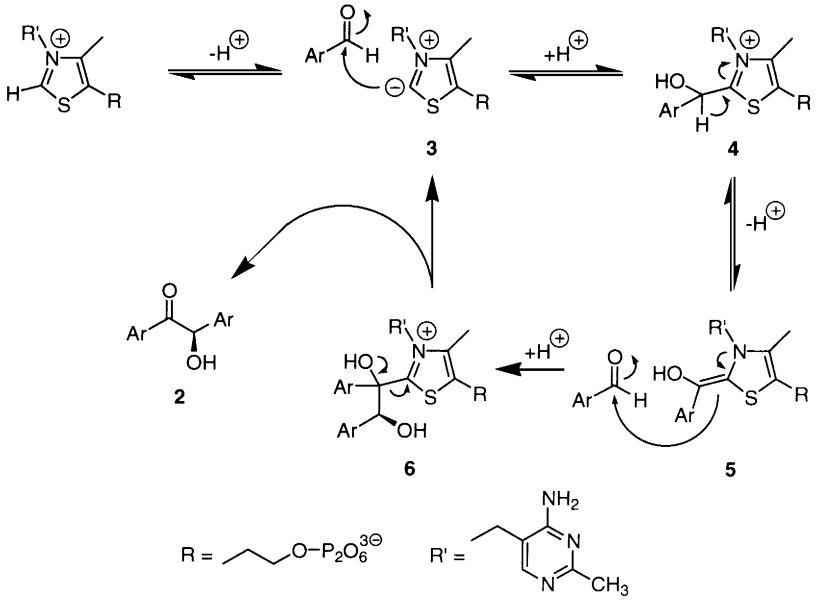
Table 1
Benzoins prepared by benzoylformate decarboxylase catalyzed enantioselective benzoin reaction

acyloins[a] **2**	Ar	yield (%)	ee (%)[b]	$[\alpha]_D^{20}$ (°) mp (°C)		config.[c]
a	Ph	70	>99	-114	(c = 1.5,CH_3COCH_3)[h] 135-136	*R*
b	3-$MeOC_6H_4$	18	>99	-157	(c = 1, MeOH)[i] 55-56	*R*
c	4-$MeOC_6H_4$	12	>99	-91	(c = 1.1, MeOH)[j] 110-112	*R*
d	4-MeC_6H_4	69	>99	-151	(c = 0.7, MeOH)[k] 89-90	*R*
e	2-FC_6H_4	68	>99	-266	(c = 0.5, MeOH)[l] 61-62	*R*
f	4-FC_6H_4	25	>99	-94	(c = 0.8, MeOH)[m] 81-82	*R*
g	4-ClC_6H_4	17	>99	-27	(c = 0.01, MeOH)[n] 86-87	*R*
h	4-BrC_6H_4	13	>99	-12	(c = 0.5, MeOH)[o] 94-96	*R*
i	2-furyl	62	94[d]	-22	(c = 0.01, MeOH)[p] 134-136	*R*
j	5-Me-2-furyl	50	96	-58	(c = 0.1, $CHCl_3$)[q] 92-94	*R*[e]
k	2-thiophenyl	65	95	-380	(c = 0.1, $CHCl_3$)[r] 106-107	*R*
l	2-Pyr.	70	94[f]	-29	(c = 0.01, $CHCl_3$)[s] 155-158	*R*
m	2-BrC_6H_4	<2[g]	n.d.			n.d.
n	2-CNC_6H_4	-	-			-
o	2-$MeOC_6H_4$	<2[g]	n.d.			n.d.

[a] The physical and spectroscopic data of the chiral and racemic compounds are in agreement with published data.
[b] Enantiomeric excess of benzoins **2** was determined by chiral phase HPLC analysis (Chiralpack AD column, UV detection at 254 nm, eluent: *i*-hexane/2-propanol = 9:1, flow 0.75 mL · min^{-1}, 20°C), using authentic homochiral and racemic compounds as references.
[c] Absolute configurations were determined by comparison of the specific rotations with literature values.
[d] The enantiomeric excess was measured immediately after work up procedure. After 24 h in solution the ee value decreased to 88%.
[e] The absolute configuration was assigned assuming a uniform reaction mechanism.
[f] The main part of the product exists in the enediol form. The optical rotation value was determined from the enediol-hydroxy ketone mixture immediately after work up procedure (GC-MS, ^{1}H-NMR, comparison of the product with an authentic sample by HPLC).
[g] Determined by GC-MS.
[h] (*R*)-**2a**: $[\alpha]_D^{20}$ = -115 (c = 1.5, CH_3COCH_3), mp: 134-136°C from commercially available compound.
[i] (*R*)-**2b**: $[\alpha]_D^{20}$ = -105.9 (c = 1, MeOH) for 66% ee,[11] mp: 55°C.[33]
[j] (*R*)-**2c**: $[\alpha]_D^{20}$ = -76.2 (c = 1, MeOH) for 86% ee,[11] mp: 110-112°C from commercially available compound.
[k] (*R*)-**2d**: $[\alpha]_D^{20}$ = -130.8 (c = 1, MeOH) for 82% ee,[11] mp: 89°C.[34]
[l] mp: 60-62°C.[35]
[m] (*R*)-**2f**: $[\alpha]_D^{20}$ = -41.2 (c = 1, MeOH) for 44% ee,[11] mp: 80-82°C[35]
[n] (*R*)-**2g**: $[\alpha]_D^{20}$ = -12.3 (c = 1, MeOH) for 29% ee,[11] mp: 87-88°C.[36]
[o] (*R*)-**2h**: $[\alpha]_D^{20}$ = -2.3 (c = 1, MeOH) for 20% ee,[11] mp : 94-95°C.[37]
[p] (*S*)-**2i**: $[\alpha]_D^{20}$ = +20.1 (c = 2, Et_2O),[38] mp: 134-137°C from commercially available compound.
[q] mp: 91.5-93°C.[39]
[r] mp: 107-108°C.[35]
[s] (*S*)-**2l**: $[\alpha]_D^{20}$ = +26.8 (c = 7, Et_2O),[38] mp: 156-160°C from commercially available compound.

Addition of DMSO increased the conversion rate, but had no effect on the enantiomeric excess of the products. Analysis of 2-hydroxy ketones was performed by HPLC on a chiral stationary phase column compared to racemic products, which were synthesized using classical chemical benzoin synthesis methodology.[32] The absolute configuration of the benzoin derivatives obtained was assigned to be (*R*) according to correlation of the specific rotation of benzoin with data from the literature (Table 1) and to HPLC data of commercially available enantiomers of benzoin.

As shown in Table 1, BFD accepts a broad range of different aromatic and heteroaromatic aldehydes, which are bound to ThDP (Scheme 2) prior to ligation to a second aldehyde molecule. All (*R*)-benzoin derivatives were obtained in high enantiomeric excess. The conversion rate was found to be a function of the aldehyde structure, being maximal with benzaldehyde. Substituents with a *+M*-effect decreased the conversion rate, whereas *ortho*-substituted benzaldehyde derivatives, except 2-fluorobenzaldehyde, were only poorly accepted (Table 1).



Scheme 2.

As shown in Scheme 2, enzyme-bound ThDP catalyzes the formation of benzoins starting from aldehydes. The mechanism was studied in detail with pyruvate decarboxylase, a ThDP-dependent enzyme similar to BFD, and it may be assumed that BFD operates with a comparable mechanism.[31] Catalysis involves initial deprotonation at C-2 to give the ylide **3**, followed by nucleophilic addition to the aldehyde (Scheme 2). The electron withdrawing thiazolium ring then allows deprotonation of C-2α of the adduct **4** resulting in the enamine ('active aldehyde') **5** which attacks the second aldehyde selectively from the less hindered side leading to an absolute configuration of (*R*) at the newly formed stereogenic center. Release of benzoin **2** from the adduct **6** regenerates the ylide and completes the catalytic cycle.

The ongoing research on BFD-catalyzed enantioselective formation of 2-hydroxy ketones shows that the binding of aromatic donor substrates to form the 'active aldehyde' **5** (Scheme 2) is preferred. Analysis of the three-dimensional X-ray crystallographic structure of BFD[28] suggests that this substrate's specificity may be due to stabilization by specific interactions in the active site of the enzyme.[29] In contrast to aliphatic acceptor aldehydes (like acetaldehyde) giving the corresponding (*S*)-2-HPP not enantiomerically pure (90–92% ee) with benzaldehyde as a donor, aromatic acceptor aldehydes (like benzaldehyde) should have only one possibility to fit into the cavity of the active site. Thus, the

carboligation must be a consequence of a selective attack of the active aldehyde to benzaldehyde leading to (*R*)-benzoins in enantiomerically pure form.

In summary, the method described here presents the first enzyme-catalyzed synthesis of enantiomerically pure (*R*)-benzoin derivatives in good yields. This work offers an attractive alternative synthesis of enantiomerically pure (*R*)-benzoins compared to other published methods.[7–17]

3. Experimental

Pure BFD was obtained as described elsewhere.[29] Preparative isolation of benzoins was carried out by column chromatography on silica gel 60 (mesh size 40–63 μm). Analysis of benzoins was performed using HPLC (HP series 1100, Hewlett Packard), fitted with a diode array detector, and equipped with a chiral phase column (Chiralpack AD, Daicel Ltd). Optical rotations were measured on a polarimeter 241 (Perkin–Elmer). NMR spectra were recorded on an AMX 300 (Bruker Physik AG, Germany). GC–MS spectra were determined on an HP 6890 series GC-system fitted with an HP 5973 mass selective detector (Hewlett Packard; column HP-5MS, 30 m×250 μm; T_{GC} (injector)=250°C, T_{MS} (ion source)=200°C, time programme (oven): $T_{0\ min}$=60°C, $T_{3\ min}$=60°C, $T_{14\ min}$=280°C (heating rate 20°C min^{-1}), $T_{19\ min}$=280°C).

*3.1. Representative example for the synthesis of benzoin derivatives: (*R*)-benzoin **2a***

Benzaldehyde (318 mg, 3 mmol) was dissolved in a mixture of DMSO (30 mL) and potassium phosphate buffer (100 mL, 50 mM, pH 7.0, containing $MgSO_4$ (2.5 mM) and ThDP (0.15 mM)). After addition of BFD (450 U related to decarboxylation of benzoylformate) the reaction was allowed to stand at room temperature for 48 h (the reaction was monitored by GC–MS and HPLC). The mixture was extracted with dichloromethane (250 mL), the organic layer washed with water (25 mL) and brine (25 mL) and dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by column chromatography (silica gel, dichloromethane) afforded 223 mg (70%) (*R*)-benzoin, ee >99%.

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