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Synthesis of planar chiral [2.2]paracyclophanes by biotransformations: kinetic resolution of 4-formyl-[2.2]paracyclophane by asymmetric reduction

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Abstract: The synthesis of enantiomerically pure (S)-4-formyl-[2.2]paracyclophane **1** (>99% ee) and (R)-4-hydroxymethyl-[2.2]paracyclophane **2** (>78% ee) was achieved by bioreduction of (RS)-**1** with a yield of 49 and 34% respectively. From several microorganisms screened only a strain of the yeast *Saccharomyces cerevisiae* (DSM 11285) showed a stereospecific reduction of this planar chiral substrate (E>100). Despite the high enantiomeric ratio, it is necessary to maintain the conversion at almost 50% in order to obtain a high enantiomeric excess of both substrate and product of the reduction reaction. Tween 80 together with methanol was found to be the most suitable cosolvent mixture which enhances the solubility of the substrate and does not effect the biocatalyst. For the calculation of E the enantiomeric excesses of substrate and product were measured at various conversions by chiral gas chromatography. Commercially available alcohol dehydrogenases such as HLADH, YADH and TBADH were tested for the desired reaction too, but found to be completely inactive. © 1997 Elsevier Science Ltd. All rights reserved.

Introduction

Several attempts have been carried out to obtain enantiomerically pure planar chiral [2.2]paracyclophanes which have been used as chiral auxilaries in the asymmetric synthesis of ß-hydroxy-α-amino acids.[1] These attempts included diastereomeric resolution[1,2] and separation using chiral HPLC.[3] However the methods published so far are not generally applicable and enantiomerical excesses range from 85 to 99% ee while the yields are generally moderate to low excluding the isolation of preparative amounts of these compounds.

In contrast to central and axial chirality, planar chirality has not been found in nature so far. Nevertheless some isolated enzymes as well as microorganisms can act on planar chiral substrates and convert them to not only regio- but also enantioselective. For substituted ferrocenes and other metalloorganic planar chiral substrates, bioreductions of formyl-substituents catalyzed by alcoholdehydrogenases[4], hydrolysis of esters and esterification of alcohols using lipases and esterases[5] have been reported. On the other hand there have been only two attempts to synthesize enantiomerically pure [2.2]meta- and [2.2]paracyclophanes, respectively, using biotransformations.[6,7] Chiral discrimination was observed using both by microbial reduction and transesterification reactions but all attempts resulted only in low enantiomeric excess.

Because the use of chiral cyclophanes for enantioselective synthesis has been limited so far due to the limited access to optically active material, we started screening different microorganisms and commercially available alcohol dehydrogenases (ADH) for their reduction potential of (RS)-**1** (Scheme 1). The microorganisms selected are known for their potential in stereoselective reduction of ferrocene derivatives[8] and silicon containing ketones.[9] In order to minimize the influence of

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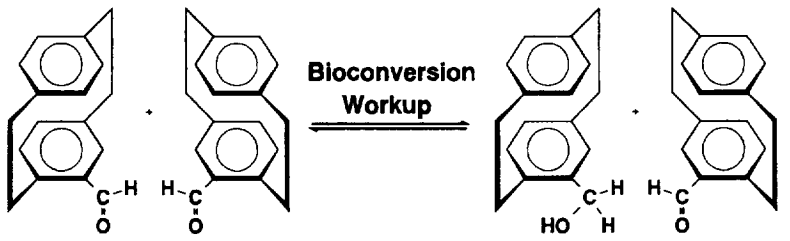

Scheme 1.

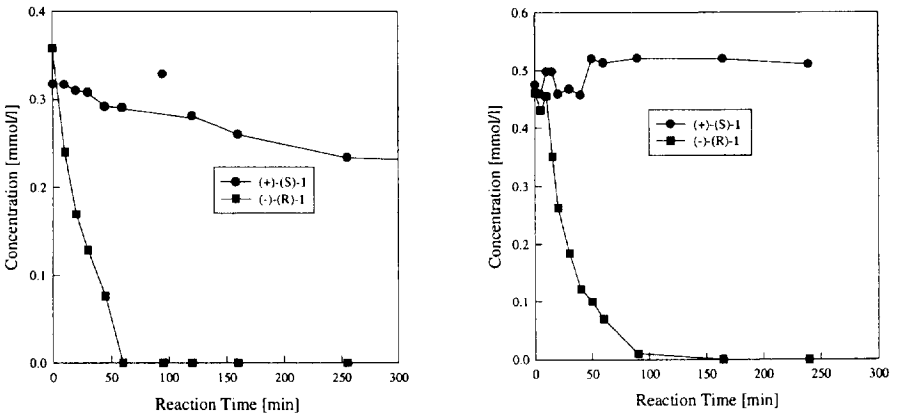

Figure 1. Comparison of the conversion of the enantiomers of 4-formyl-[2.2]paracyclophane **1** during the bioreduction using resting free cells of *Yarrowia lipolytica* DSM 1345 (left) and *Saccharomyces cerevisiae* DSM 11285 (right).

mass transfer limitations in multiphasic systems the reaction conditions have been optimized. For the quantitative analysis of substrate and product enantiomers a gas chromatographic method was developed according to König *et al.*[10] and the reaction kinetics were investigated.

Results and discussion

Screening for microorganisms with 4-formyl-[2.2]paracyclophane reducing activity

A total number of 10 microorganisms were evalutated for their ability to reduce (RS)-**1** to the corresponding alcohol (for the reaction see Scheme 1). TLC analyses of whole broth extracts indicated that all microorganisms investigated were able to catalyze the desired reaction to some extent. It was found that all reactions proceeded to more than 50% conversion indicating reactions which are not fully stereospecific. Therefore chiral GC of whole broth extracts was used to determine the enantiomeric excess of the substrate and the product during the reaction course. As shown for an example in Figure 1 enantiomeric excess of substrate **1** depends on the reaction time and on the microorganism used. In contrast to *Yarrowia lipolytica* DSM 1345, *Saccharomyces cerevisiae* DSM 11285 reduces the (S)-enantiomer of the substrate (S)-**1** only if the (R)-enantiomer has already reacted.

In Figure 2 the enantiomeric excess of the substrate is plotted versus the conversion for *Yarrowia lipolytica* DSM 1345 and *Saccharomyces cerevisiae* DSM 11285 as examples for all strains investigated. The data obtained were used for the calculation of the enantiomeric ratio E according to Chen and Sih[11] and the results are listed in Table 1.

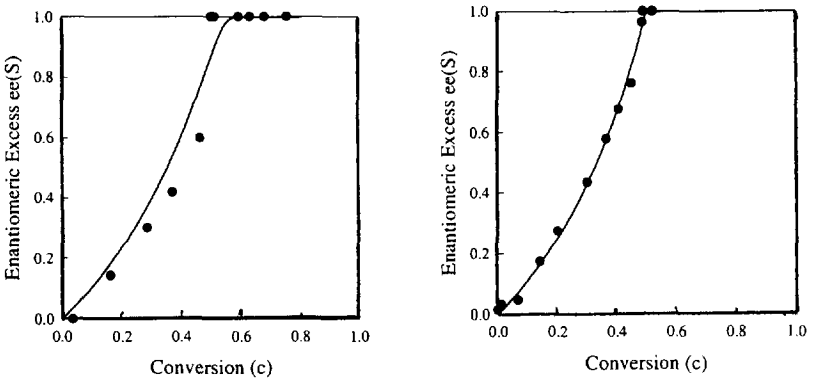

Figure 2. Enantiomeric excess of (S)-**1** versus conversion for the bioreduction of (RS)-4-formyl-[2.2]paracyclophane **1** using resting free cells of *Yarrowia lipolytica* DSM 1345 (left) and *Saccharomyces cerevisiae* DSM 11285 (right). Solid lines represent calculations using the model of Chen and Sih.[11]

Table 1. Screening for microorganisms with alcohol dehydrogenase activity for the reduction of 4-formyl-[2.2]paracyclophane **1**

Microorganism	ee(S) at 50% Conversion	Enantiomeric Ratio E
Saccharomyces cerevisiae DSM 11285	99%	> 100
Yarrowia lipolytica DSM 1345	87%	39.1
Cryptococcus humiculus DSM 70067	73%	13.9
Rhodotorula rubra IFO 889	56%	6.1
Rhodotorula mucilginosa ATCC 20129	47%	4.6
Candida boidinii DSM 70026	45%	4.2
Pichia jadinii DSM 2361	42%	3.7
Hanseniaspora osmophila DSM 2249	23%	1.9
Trigonopsis variabilis DSM 70714	18%	1.7
Rhodococcus erythropolis DSM 43066	63%	8.2

As can be seen from Table 1 *Saccharomyces cerevisiae* DSM 11285 is nearly stereospecific for the bioreduction of **2** and therefore is the most suitable biocatalyst for the desired reaction. Izumi and Hinata[7] investigated a *Saccharomyces cerevisiae* strain too but found only a low enantiomeric excess of the product. There are several possible reasons for this deviation. The most possible explanation relies on the time course of a kinetic resolution reaction. At low conversions only substrate with low enantiomeric excess is isolated (see Figure 2). On the other hand, it is possible to isolate the substrate with high enantiomeric excess even in the case of relatively low enantiomeric ratios E if the reaction is performed at a suitable conversion. For example an enantiomeric excess of 99% can be obtained using *Yarrowia lipolytica* DSM 1345 if one runs the reaction to 60% conversion. To obtain high enantiomeric excesses of both substrate and product one has to screen for a stereospecific biocatalyst. In a preparative scale bioconversion using *Saccharomyces cerevisiae* DSM 11285 the conversion (GC, TLC) was run to 51.4%, which resulted in 84 mg (S)-**1** (49% yield) with an ee of 99%.

However, it is possible that different commercially available strains of Baker's yeast supplied by yeast producing companies contain different kinds of enzymatic systems. Therefore we cultivated, isolated single colonies, and subcultured the *Saccharomyces cerevisiae* strain mentioned above. Its microbial determination was carried out at the DSM (Deutsche Sammlung für Mikroorganismen, German Collection of Microorganisms, Braunschweig, Germany).

Table 2. Reduction of selected aldehydes catalyzed by commercially available alcohol dehydrogenases

Substrate	Specific Activity of ADH of		
	Yeast (YADH)	Horse Liver (HLADH)	*Thermoanaerobium brockii* (TBADH)
acetaldehyde*	420 U/mg	950 U/mg	780 U/mg
acetaldehyde#	320 U/mg	350 U/mg	350 U/mg
4-formyl-[2.2]-paracyclophane+	0 U/mg	0 U/mg	0 U/mg

*0.1 M phosphate buffer, pH 6.5, 0.1 mM NADH, 0.5 mM acetaldehyde, 30°C

#0.1 M phosphate buffer with 3% (v/v) methanol and 3% (v/v) Tween 80, pH 6.5, 0.1 mM NADH, 0.5 mM acetaldehyde, 30°C

+0.1 M phosphate buffer with 3% (v/v) methanol and 3% (v/v) Tween 80, pH 6.5, 0.1 mM NADH, 0.3 mM 4-formyl-[2.2]paracyclophane, 30°C

Bioconversions using free enzymes

Commercially available enzymes (HLADH, YADH, TBADH) have been investigated for their reduction ability of (RS)-**1** but have been found to be inactive for this substrate (Table 2). Using acetaldehyde as a substrate it was proved, that the reaction conditions were suitable for an enzymatic reduction reaction.

Experimental

Chemicals, enzymes and cultivation media

Unless otherwise stated all chemicals used were of reagent grade and purchased from Fluka Chemie AG (Buchs, Switzerland). The substrate 4-formyl-[2.2]paracyclophane **1** used for the bioconversions was synthesized according to Rieche *et al.*[12] The product 4-hydroxymethyl-[2.2]paracyclophane was obtained by reduction with lithium aluminum hydride from 4-carboxy-[2.2]paracyclophane according to Laue.[13] *Thermoanaerobium brockii* ADH (EC 1.1.1.2, Lot. No. 329085-1 194) was purchased from Fluka Chemie AG (Buchs, Switzerland), Yeast ADH (EC 1.1.1.1, Lot. No. 83401322-451), and horse liver ADH (EC 1.1.1.1, Lot. No. 12164924-571) were purchased from Boehringer Mannheim GmbH (Mannheim, Germany). Glucose and peptone for the preparation of the cultivation media were purchased from Fluka Chemie AG (Buchs, Switzerland), yeast extract was purchased from Gibco Ltd. (Paisley, UK), and malt extract from Merck (Darmstadt, Germany).

The solution of salts and acids used in the procedures were prepared with deionized water. Low-pressure liquid chromatography was performed on Merck silica gel 60 (230–400 mesh).

Microorganisms

With the exception of *Saccharomyces cerevisiae* DSM 11285 which was isolated from a commercial sample, microorganisms employed in the screening were either obtained from the DSM, the IFO (Institute for Fermentation Osaka, Osaka, Japan), or the ATCC (American Type Culture Collection, Rockville, USA).

Cultivation

All microorganisms selected were cultivated in a complex medium containing 3 g/l yeast-extract, 3 g/l malt-extract, 5 g/l peptone and 10 g/l glucose. The glucose was sterilized as a 32% solution separately from the other medium components. Sterilization was carried out at 121°C for 20 minutes. After sterilization glucose was added to the medium under sterile conditions. A preculture was prepared by inoculation of 20 ml of the complex medium with fresh cells from an agar plate (swab of inoculation loop). Incubation was performed aerobically in a 100 ml Erlenmeyer shaking-flask, which was shaken on an orbital shaker operated at 100 rpm and 30°C for 40 h. 5 ml portions of the precultures were used to inoculate 200 ml portions of the medium in 1 l Erlenmeyer shaking-flasks. After incubation for 40

h at 30°C, the cells were harvested by centrifugation at 4500 rpm at 4°C for 45 minutes (Megafuge; Beckman, Germany) and gave a cell wet mass (cwm) of 15–70 g/l. The ratio 'cell wet mass'/'cell dry mass' of 7.5–15 was determined by drying a sample of wet cells at 105°C up to constant weight. The wet cells were used directly for bioconversions.

Bioconversion using resting free cells

Analytical scale

1.5 ml Tween 80 and 1.5 ml of a 8.8 mM solution of 4-formyl-[2.2]paracyclophane **1** (3.13 mg, 0.0132 mmol) in methanol were added to 22 ml of 0.1 M potassium phosphate buffer, pH 6,5 in a temperable stirred tank reactor (50 ml volume) at 30°C and stirred up to homogenity. The reaction was started by adding 25 ml of a prewarmed cell suspension (2 g cell wet mass in 25 ml of 0.1 M potassium phosphate buffer, pH 6.5 containing 20% glucose (w/v). Samples of 2 ml were taken in intervals of approximatly 10 min and extracted with 1 ml of ice-cooled dichloromethane. The emulsion formed was centrifugated for 10 min at 4500 rpm to separate the organic from the water phase. The organic phase was dried with sodium sulphate and analysed by GC and TLC.

Preparative scale

In a 2 l stirred tank reactor at 30°C, 15 g glucose, 45 ml Tween 80, and a solution of 170 mg of racemic 4-formyl-[2.2]paracyclophane (0.716 mmol) in 45 ml methanol were added to 660 ml of 0.1 M potassium phosphate buffer, pH 6.5 and homogenized. The reaction was started by addition of a prewarmed solution of 30 g *Saccharomyces cerevisiae* DSM 11285 in 750 ml 0.1 M potassium phosphate buffer, pH 6.5. Samples were taken, extracted as described above and analyzed by TLC and GC. After 210 min the reaction was stopped by the addition of 750 ml dichloromethane. The emulsion was centrifugated 20 min at 4°C and 4500 rpm to separate the organic from the water phase. The organic phase was dried with sodium sulphate and dichloromethane was evaporated. Tween 80 and the reaction products were separated by low-pressure liquid chromatography (ethyl acetate) resulting in 400 mg of crude product. Separation of substrate and product was performed by low-pressure liquid chromatography (dichloromethane) and yielded 84 mg (0.354 mmol, 49% yield) of enantiomerically pure (S)-4-formyl-[2.2]paracyclophane **1** (ee>99% (GC), $[\alpha]_D^{20}$=+121.6) and 58 mg (0.243 mmol, 34% yield) of (R)-4-hydroxymethyl-[2.2]paracyclophane **2** (ee>78% (GC), $[\alpha]_D^{20}$=− 55.8). The MS and NMR spectra of both samples were identical with those of the racemic samples.

Bioconversion using free enzymes

Enzyme solution

For the preparation of enzyme solutions of a suitable activity 10 µl of a suspension of yeast ADH (YADH) was diluted with 9990 µl 0.1 M potassium phosphate buffer, pH 6.5 (buffer A), 10 µl of a suspension of horse liver ADH (HLADH) was diluted with 990 µl buffer A and 0.1 mg of lyophilized *Thermoanaerobium brockii* ADH (TBADH) was diluted with 1 ml of buffer A and stored in ice.

NADH solution

A solution of 1 mM NADH in buffer A was prepared.

Substrate solution 1 (SL1)

A solution of 20 mM acetaldehyde in buffer A was prepared.

Cosolvent solution (CL)

1.5 ml Tween 80 and 1.5 ml methanol were added to 22 ml buffer A.

Substrate solution 2 (SL2)

1.5 ml Tween 80 and 1.5 ml of a solution of (RS)-**1** in methanol (8.8 mM) were added to 22 ml buffer A.

Bioconversion of acetaldehyde without cosolvents

In the cuvette of the spectrophotometer 25 µl SL1 (final concentration of acetaldehyde 0.5 mM), 855 µl buffer A, and 100 µl NADH (final concentration 0.1 mM) solution were mixed and the resulting solution prewarmed for 5 min at 30°C. The reaction was started with 20 µl of the enzyme solution.

Bioconversion of acetaldehyde using cosolvents

In the cuvette of the spectrophotometer 25 µl SL1 (final concentration 0.5 mM), 355 µl buffer A, 500 µl SL2 (final concentration of Tween 80 and methanol 3% each), and 100 µl NADH (final concentration 0.1 mM) solution were mixed and the resulting solution prewarmed for 5 min at 30°C. The reaction was started with 20 µl of the enzyme solution.

Bioconversion of 4-formyl-[2.2]paracyclophane 1 using cosolvents

In the cuvette of the spectrophotometer 380 µl buffer A and 500 µl SL2 (final concentration of Tween 80 3%, methanol 3%, and **1** 0.264 mM), and 100 µl NADH (final concentration 0.1 mM) solution were mixed and the resulting solution prewarmed for 5 min at 30°C. The reaction was started by adding 20 µl of the enzyme solution.

Analytical techniques

Assay of enzymatic activity

For bioconversions using free enzymes the decrease of NADH was measured at a wavelength of 340 nm using a Ultrospec III spectrophotometer (Pharmacia, Germany).

Thin layer chromatography

Silica gel 60 precoated plates without fluorescent indicator (20 x 20 cm, Merck, Germany) were predeveloped with diisopropylether to avoid pseudo peaks and subsequently dried. The extracted samples were spotted onto the plates using a TLC-sample applicator (model AS 30, Desaga, Heidelberg, Germany) and developed with dichloromethane. Quantitative analysis was performed with a TLC scanner (model scanner 3, Camag, Berlin, Germany) at 220 nm (R_f (**1**)=0.53; R_f (**2**)=0.22).

Chiral gas chromatography

For the separation of the enantiomers of both 4-formyl-[2.2]paracyclophane **1** and 4-hydroxymethyl-[2.2]paracyclophane **2** the method of König[10] was slightly modified. The GC system used (Hewlett Packard, Waldbronn, Germany) consisted of a gas chromatograph (model 5890 Series 2), equipped with an autosampler (modell 7673 B), an integrator (model 3396 II), a personal computer and the software 'Peak 96'. The separation was performed with 0.4 kPa helium as carrier gas at 170°C (isotherm) using a 15 m heptakis(2,3-di-*O*-methyl-6-*O*-dimethylthexylsilyl)-β-cyclodextrin (50% in polysiloxane OV 1701) column. Under these conditions separation factors α of the enantiomers were calculated to be 1.03 (**1**) and 1.07 (**2**) respectively. Retention times: S-**1**: 18.8 min; R-**1**: 19.4 min; and S-**2**: 37.2 min; R-**2**: 34.9 min.

Polarimetry

Optical rotations were determined with a polarimeter 241 from Perkin–Elmer (Überlingen, Germany).

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