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Enantioselective oxidation of diaryl carbinols by *Nocardia corallina* B-276

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Abstract

Whole cells of *Nocardia corallina* B-276 oxidized diaryl carbinols enantioselectively to give ketones in moderate yields, and some of the unreacted alcohols showed high ee's. This asymmetric oxidation is a simple and efficient method for preparing *meta*- and *para*-monosubstituted optically active diaryl carbinols from the racemates. The *para*-substituted chiral alcohols have an *R* configuration. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Chiral diaryl carbinols are useful intermediates for the preparation of biologically active compounds,[1–3] but are difficult to obtain in an enantiopure form by chemical resolution of their acid phthalates with chiral bases,[4] complexation with brucine[5] or by asymmetric catalysis.[6] The asymmetric reduction of substituted diaryl ketones is difficult because prochiral diaryl ketones usually contain two sterically and electronically similar aryl groups.[6] However, a very efficient method of asymmetric hydrogenation of *ortho*-substituted diaryl ketones using a chiral Ru(II) complex was recently reported[7] without the formation of substituted diphenylmethane, a usual over-reduction product.

In contrast to the large number of published highly enantioselective alkyl additions, only a handful of reports about the catalytic high enantioselective addition of organometallic aryl reagents to aromatic aldehydes have appeared.[6] Enzymatic process could be an alternative method; however, attempts to achieve kinetic resolution of simple alcohol by oxidation have been relatively unsuccessful on the large scale, because in the oxidative process the enzyme requires cofactors and is particularly sensitive to product inhibition.[8] Recently nonenzymatic methods have also been extensively explored.[9] These facts turned our attention to microbial oxidations, because a process with whole cells would avoid this kind of problem.

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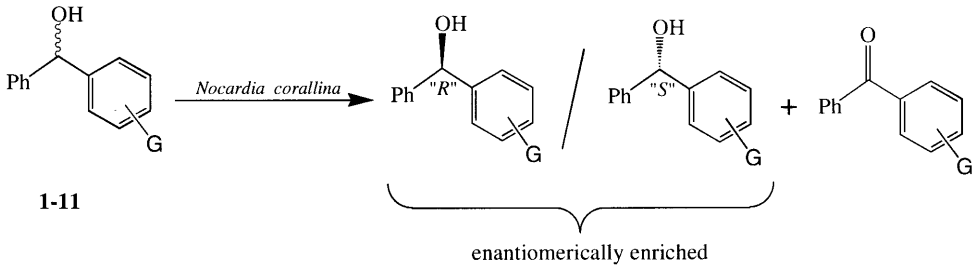
Recently, we reported the oxidation of aldehydes, and allylic and benzylic alcohols to produce carboxylic acids or ketones by *Nocardia corallina* B-276.[10,11] Due to our interest on the application of this microorganism in organic chemistry, we decided to study the microbial oxidation of benzhydrols.

2. Results and discussion

First, we selected a model compound to adjust the biotransformation conditions on a 0.5 mmol scale. The bioreaction of benzhydrol, **1**, was allowed to proceed to about 50% conversion (27 h, 28–30°C), then the crude mixture was analyzed. A preparative experiment with benzhydrol, **1**, under the optimal reaction conditions produced benzophenone and recovered benzhydrol in 40% and 37% yields, respectively. In this way, it was demonstrated that *Nocardia corallina* B-276 has the ability to oxidize diaryl methanols, with potential application for the kinetic resolution of these kinds of secondary alcohols (Scheme 1).



Scheme 1.

Ten substrates were selected for this study, and the results are shown in Table 1. It is worth mentioning that the reaction conditions have not been optimized for each particular case.

All substrates were oxidized, with the exception of substrate **11** which produced only 2% ketone after 27 h by GC analysis. The *para*-substituted substrates **2**, **5**, **6**, **7** and **9** gave the highest ee's for this enantioselective oxidation, in most cases with >99% ee. It is worth noting that these compounds are important chiral synthons.[12]

A very fast oxidation of compound **4** (entry 5) was observed and consequently it was necessary to stop the biotransformation after 8 h, in order to have approximately 50% conversion (entry 6). This reaction showed an excellent enantioselectivity, giving the unreacted alcohol **4** in >99% ee. In contrast to **8**, another *meta*-substituted substrate (entry 10), with the bulkier phenoxy group, gave the alcohol in only 46% ee, at a lower rate.

Interestingly, the *ortho*-substituted substrate **3** (entries 3 and 4) seemed to present a significant steric hindrance, because even after 120 h of reaction, only 19% conversion was observed without any enantioselectivity; similar to the observed behavior of **11**.

Regarding the stereochemistry of the reaction, comparison of specific rotations of **2**, $[\alpha]_D = -14.7$ (*c* 1.0, $CHCl_3$); **3**, $[\alpha]_D = +0.51$ (*c* 1.2, $CHCl_3$); **5**, $[\alpha]_D = +5.54$ (*c* 0.16, C_6H_6) and **7**, $[\alpha]_D = +10.7$ (*c* 0.10, C_6H_6) with the literature values: **2**, $[\alpha]_D = -6.0$ (*c* 0.91, $CHCl_3$) 41% ee, *R*;[6] **3**, $[\alpha]_D = -41.9$ (*c* 1.19, $CHCl_3$) 96% ee, *S*;[7] **5**, $[\alpha]_D = -10.3$ (*c* 5.0, C_6H_6) >99% ee, *S*;[4] and **7**, $[\alpha]_D = +7.94$ (*c* 1.63, C_6H_6) 54% ee, *R*,[6] respectively, allowed us to assign the '*R*' configuration to the alcohols produced by this procedure.

Table 1
Kinetic resolution of chiral diaryl carbinols

Entry	Alcohol	G	Reaction time (h)	Ketone/unreacted alcohol[a]	Ee (%) (conf.)[b]
2	**2**	4-Cl	27	53/47	>99[c] (*R*)[6]
3	**3**	2-Br	27	12/88	0.12[c] (*R*)[7]
4	**3**	2-Br	120	19/81	N.d.[d]
5	**4**	3-Br	27	100/0	–
6	**4**	3-Br	8	43/57	>99[c]
7	**5**	4-CH_3	27	41/59	78[c] (*R*)[4]
8	**6**	4-*i*-C_3H_7	33	52/48	>99[e] (*R*)[h]
9	**7**	4-OCH_3	27	63/57	>99[c] (*R*)[6]
10	**8**	3-OC_6H_5	33	66/34	46[c]
11	**9**	4-Br	27	52/48	>99[f] (*R*)[h]
12	**10**	3,4-diOCH_3	27	59/41	>99[c]
13	**11**	2,3-diOCH_3	27	2/98[g]	20[c]

[a] Ratio determined by ^{1}H NMR (500 MHz).
[b] Assignment of absolute configuration of the unreacted alcohol was done by comparison of the sign of the specific rotation with literature data.
[c] Enantiomeric excess determined by the α-C$\underline{H}$ signal in the ^{1}H NMR spectrum of the alcohols using (+)-[Eu(hfc)$_3$] as a chiral shift reagent.
[d] N.d.: not determined.
[e] Enantiomeric excess determined by HPLC on a Chiralcel-OD column.
[f] Enantiomeric excess determined by HPLC of the corresponding acetates on a Chiralcel-OD column.
[g] Ratio determined by GC on Chiraldex-BPH column.
[h] By optical rotation correlation.[13]

According to the correlation proposed by Mosher,[13] the *para*-substituted (*S*)-benzhydrols with a deactivating group (Br or Cl) are dextrorotatory, and in our case we obtained levorotatory values for **2** and **9** which permit us to assign to them the *R* configuration. On the other hand, *para*-substituted (*S*)-benzhydrols with activating groups (CH_3 or OCH_3) are levorotatory. We observed for **5**, **6** and **7** dextrorotatory values, and using the same correlation we propose an *R* configuration for **6**, which is not reported in the literature.

Unfortunately, the lack of reported absolute configurations for **4**, **8**, **10** and **11** do not allow us to make a generalization about the stereochemistry of the benzhydrols obtained by this biotransformation.

3. Conclusion

We have found a microbiological asymmetric oxidation of chiral diaryl carbinols, providing moderate chemical yields, which is unoptimized, and high enantiomeric excesses. It is also important to mention that, although the ability of *Nocardia corallina* to oxidize alkenes to optically active 1,2-epoxyalkenes is well documented,[14] this is the first microbial resolution of a chiral compound by enantioselective oxidation with *Nocardia corallina* B-276.

4. Experimental

4.1. Materials and methods

Organism and growth. *Nocardia corallina* B-276 (ATCC 31338) was grown at 28–30°C on agar plates (3 g beef extract/l; 5 g peptone/l; 15 g agar/l). Incubation of liquid cultures was done in an orbital shaker; the broth composition was: solution A: 0.05 g $FeSO_4 \cdot 7H_2O$/l; 1.74 g K_2HPO_4/l; 2 g $(NH_4)_2SO_4$/l; 1 g yeast extract/l; solution B: 1.5 g $MgSO_4$/l; solution C: 2 g glucose/l; each solution was sterilized separately and later combined, and the pH adjusted to 8.0 (±0.5). All substrates were prepared by conventional methods or purchased from Aldrich, Sigma or Janssen. The diaryl carbinols and ketones were identified by their infrared spectra (Perkin–Elmer Paragon 1600) as liquid films or KBr discs, hydrogen nuclear magnetic resonance (^{1}H NMR) (Bruker 500 MHz) and by TLC on silica gel 60 GF_{254} Merck, and comparative analysis with authentic samples. The specific rotations were measured in a Perkin–Elmer 341 polarimeter with the indicated solvent. The GC analysis was performed on a Hewlett–Packard HP 6890 series gas chromatograph, equipped with a flame ionization detector and a Chiraldex column B-PH (30 m) column. The HPLC analysis was performed on a Hewlett–Packard HP 1050 series liquid chromatograph, equipped with a UV detector and a Chiralcel OD column.

4.2. General procedure for biotransformation

4.2.1. Preculture I

A 125 ml Erlenmeyer flask containing 50 ml of sterile culture medium was inoculated from an agar plate (three days old) and incubated at 28–30°C on an orbital shaker (200 rpm) for 20–24 h.

4.2.2. Preculture II

The content of preculture I flask was aseptically poured into a 250 ml Erlenmeyer flask containing 100 ml of fresh sterile culture medium. The flask was incubated at 28–30°C on an orbital shaker (200 rpm) for 24 h.

4.2.3. Biotransformation

Under aseptic conditions the substrate (0.5 mmol) was added to the flask containing preculture II using 1 ml of *N*,*N*-dimethylformamide, followed by the addition of *n*-octane (10 ml). The mixture (161 ml final volume) was incubated at 28–30°C on an orbital shaker (200 rpm). The progress of the biotransformation was monitored by TLC and stopped at the time indicated in Table 1, then it was saturated with NaCl and filtered through Celite; the ketones and diaryl carbinols were extracted with ethyl acetate (4×25 ml). The products were compared with authentic samples by ^{1}H NMR, IR and TLC.

Specific rotations of the recovered chiral alcohols of the enantioselective oxidation:

(*R*)-(4-Chlorophenyl)phenylmethanol 2: $[\alpha]_D = -14.7$ (*c* 1.0, $CHCl_3$) [lit.[6] $[\alpha]_D = -6.0$ (*c* 0.91, $CHCl_3$) 41% ee, *R*].

(*R*)-(2-Bromophenyl)phenylmethanol 3: $[\alpha]_D = +0.51$ (*c* 1.2, $CHCl_3$) [lit.[7] $[\alpha]_D = -41.9$ (*c* 1.19, $CHCl_3$) 96% ee, *S*].

(3-Bromophenyl)phenylmethanol 4: $[\alpha]_D = -4.29$ (*c* 0.26, CH_3OH).

(*R*)-(4-Methylphenyl)phenylmethanol 5: $[\alpha]_D = +5.54$ (*c* 0.16, C_6H_6) [lit.[4] $[\alpha]_D = -10.3$ (*c* 5.0, C_6H_6) >99% ee, *S*].

(*R*)-(4-Isopropylphenyl)phenylmethanol 6: $[\alpha]_D = +5.03$ (*c* 0.06, C_6H_6).

(*R*)-(4-Methoxyphenyl)phenylmethanol 7: $[\alpha]_D = +10.7$ (*c* 0.10, C_6H_6) [lit.[6] $[\alpha]_D = +7.94$ (*c* 1.63, C_6H_6) 54% ee, *R*].

(3-Phenoxyphenyl)phenylmethanol 8: $[\alpha]_D = -14.13$ (*c* 0.06, C_6H_6).

(*R*)-(4-Bromophenyl)phenylmethanol 9: $[\alpha]_D = -8.37$ (*c* 0.11, C_6H_6).

(3,4-Dimethoxyphenyl)phenylmethanol 10: $[\alpha]_D = +20.85$ (*c* 0.51, C_6H_6).

(2,3-Dimethoxyphenyl)phenylmethanol 11: $[\alpha]_D = +0.91$ (*c* 1.7, C_6H_6).

4.3. Determination of the enantiomeric excess for the chiral diaryl carbinols

(a) By HPLC. All ee's were determined by comparing the HPLC data of the chiral products with those of the corresponding racemic alcohols on a: Chiracel OD column, hexanes/2-propanol as eluent, at 250 nm.

(4-Isopropylphenyl)phenylmethanol 6: Racemic alcohols, $t_S = 16.72$ min, $t_R = 18.12$ min (hexanes/2-propanol = 95:5 at 0.8 ml/min). The product of the enantioselective oxidation gave $t_R = 18.13$ min, with >99% ee.

(4-Bromophenyl)phenylmethanol 9: The ee was measured by analyzing its acetate by HPLC. The racemic acetates had the following retention times: $t_S = 29.10$ min, $t_R = 31.11$ min (hexanes/2-propanol = 99:1 at 0.4 ml/min). The acetate, from the product of the enantioselective oxidation gave, $t_R = 32.10$ min, with >99% ee.

(b) By ^{1}H NMR. All ee's were determined by comparing the ^{1}H NMR data of the chiral products with those of the corresponding racemic alcohols in $CDCl_3$, using the α-C<u>H</u> signal in the spectra and (+)-[Eu(hfc)$_3$] as a chiral shift reagent.

(4-Chlorophenyl)phenylmethanol 2: α-C<u>H</u> signal in 5.77 ppm, the signal shifted to 9.23 and 9.19 ppm and refined by deconvolution procedure. The product had >99% ee.

(3-Bromophenyl)phenylmethanol 4: α-C<u>H</u> signal in 5.72 ppm, the signal shifted to 6.64 and 6.66 ppm and refined by deconvolution procedure. The product had >99% ee.

(4-Methylphenyl)phenylmethanol 5: α-C<u>H</u> signal in 5.79 ppm, the signal shifted to 8.00 and 7.96 ppm and refined by deconvolution procedure. The product had 78% ee.

(4-Methoxyphenyl)phenylmethanol 7: α-C<u>H</u> signal in 5.78 ppm, the signal shifted to 6.68 and 6.64 ppm and refined by deconvolution procedure. The product had >99% ee.

(3-Phenoxyphenyl)phenylmethanol 8: α-C<u>H</u> signal in 5.78 ppm, the signal shifted to 6.90 and 6.88 ppm and refined by deconvolution procedure. The product had 46% ee.

(2-Bromophenyl)phenylmethanol 3: α-C<u>H</u> signal in 6.19 ppm, the signal shifted to 6.77 and 6.71 ppm. The product had 0.12% ee.

(3,4-Dimethoxyphenyl)phenylmethanol 10: α-C<u>H</u> signal in 5.78 ppm, the signal shifted to 7.85 and 7.80 ppm and refined by deconvolution procedure. The product had >99% ee.

(2,3-Dimethoxyphenyl)phenylmethanol 11: α-C<u>H</u> signal in 6.00 ppm, the signal shifted to 9.19 and 9.01 ppm and refined by deconvolution procedure. The product had 20% ee.

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