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Resolution of methylarylmethanols via oxidation with *Nocardia corallina*

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Abstract—Ten different racemates of methylarylmethanols were subjected to whole cells of *Nocardia corallina* B-276 to give, via enantioselective oxidations, the corresponding ketone and the enantiomerically enriched secondary alcohol in moderate yields and excellent e.e.s. The configuration of the resulting alcohol is (*R*). This procedure permits a very good separation of the racemates of *meta*- and *para*-monosubstituted methylarylmethanols. © 2001 Published by Elsevier Science Ltd.

1. Introduction

Biotransformations of alcohols to ketones by isolated enzymes and whole cell systems in asymmetric synthesis are well established. However, there are fewer reports of enantioselective enzymatic oxidation of alcohols than the enantioselective reduction of ketones.[1] *Corynebacterium equi*,[2] *Rhodococcus erythropilis*,[3] Baker's yeast,[4] *Bacillus stearothermophilus*,[5] and *Yarrowia lipolytica*[6] are microorganisms used in the enantioselective oxidation of *sec*-alcohols, producing variable yields of the corresponding ketone and an enantiomerically enriched alcohol. Microbial resolutions of racemic secondary carbinols provide approaches to enantiomerically pure chiral alcohols, which are interesting chiral building blocks for the synthesis of natural products, pharmaceutical, and agricultural chemicals.[6,7]

Recently, we reported that *Nocardia corallina* B-276 oxidises aldehydes, allylic and benzylic alcohols to carboxylic acids or ketones, respectively.[8,9] Then, we extended our studies to the enantioselective oxidation of racemic benzhydrols[10] as a useful procedure for preparation of *meta*- and *para*-monosubstituted optically active diaryl carbinols in good yields and high e.e.s.

These applications of this microorganism in organic chemistry encouraged us to extend the study to methylarylmethanols and explore the effect of the structure in microbial oxidation reactions, to determine the scope and limitations of this methodology. Racemic methylarylmethanols have been resolved in variable yields through different approaches including dynamic kinetic resolution with enzymes coupled with ruthenium-catalysed racemization,[11] asymmetric acylation with twisted amides possessing axial chirality,[12] by acylation with planar-chiral derivative of DMAP,[13] asymmetric oxygenation catalysed by chiral ruthenium porphyrin,[14] by microbial deracemization with *Geotrichum candidum*,[7] or combined microbial oxidation/reduction with *Bacillus stearothermophilus* and *Yarrowia lipolytica*.[5]

In asymmetric synthesis use of a microbial process with whole cells would avoid the use of a chiral ligand, which has to be readily available or easy recycled to be successfully exploited.[15] Herein, we report an alternative approach to resolve racemic methylarylmethanols via oxidation with *Nocardia corallina* B-276.

2. Results and discussion

When the racemate of methylphenylmethanol, **1**, was subjected to *Nocardia corallina* B-276 for 25 hours, we observed an enantioselective oxidation of the (*S*)-alcohol (Scheme 1; Table 1, entry 1), the unreacted alcohol obtained showed >99% e.e.

A remarkable steric effect was observed for the *ortho*-substituted methylarylmethanols (entries 2, 3, 12 and 13); even with extended reaction times, only conversions of 2–7% to the ketones was observed and low e.e.s

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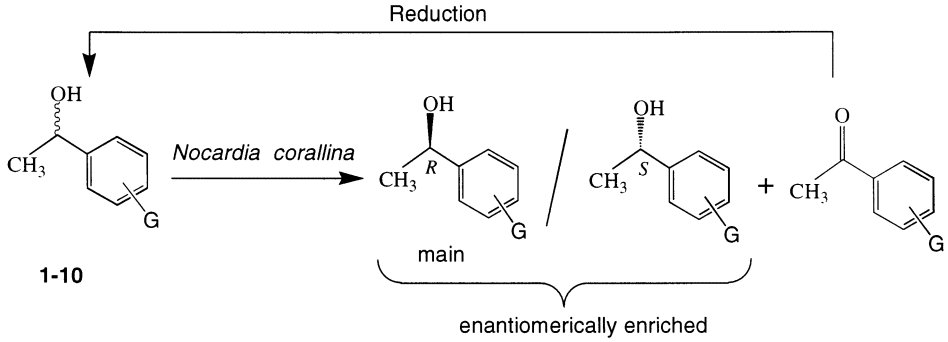

Scheme 1.

Table 1. Enantioselective oxidation of methlarylmethanols

Entry	Alcohol	G	Reaction time (h)	Ketone/unreacted alcohol	E.e. % (conf.)[d]
1	**1**	H	25	72/28[a]	>99[a] (*R*)[16]
2	**2**	2-Br	25	2/98[a]	4[a] (*R*)[16]
3	**2**	2-Br	120	5/95[a]	5[a] (*R*)[16]
4	**3**	3-Br	4	40/60[b]	38[b] (*R*)[16]
5	**3**	3-Br	25	63/37[a]	>99[a] (*R*)[16]
6	**4**	3-Cl	4	43/57[a]	22[a] (*R*)[16]
7	**4**	3-Cl	25	67/33[a]	>99[b] (*R*)[16]
8	**5**	4-Br	25	47/53[b]	72[b] (*R*)[17]
9	**6**	4-*i*-C_3H_7	25	52/48[b]	91[b] (*R*)[e]
10	**7**	4-OCH_3	24	50/50[b]	>99[b] (*R*)[17]
11	**8**	3-OC_6H_5	25	64/36[c]	>99[c]
12	**9**	2,3-diOCH_3	25	4/96[a]	3[a] (*R*)[e]
13	**9**	2,3-diOCH_3	48	7/93[a]	4[a] (*R*)[e]
14	**10**	3,4-diOCH_3	25	61/39[b]	>99[b] (*R*)[e]

[a] Ratio and enantiomeric excess determined by GC on Chiraldex B-PH column.
[b] Ratio and enantiomeric excess determined by GC on Chiraldex G-TA column.
[c] Ratio and enantiomeric excess determined by ^{1}H NMR, the e.e. by the α-CH signal in the spectrum of the alcohols using (+)-[Eu(hfc)$_3$] as a chiral shift reagent.
[d] Assignment of absolute configuration of the unreacted alcohol was carried out by comparison of the sign of the specific rotation with literature data.
[e] Assignment of absolute configuration of the unreacted alcohol was based on the assumption that *R*-enantiomers of *sec*-alcohols of this kind are eluted before *S*-enantiomers on Chiraldex B-PH and G-TA columns.

were measured. In contrast, the *meta*- and *para*-substituted compounds showed the largest enantioselectivity (entries 5, 7, 9, 10, 11 and 14), with e.e. values of >99% in some cases.

Interestingly, the bulkier *meta*-phenoxy group (**8**, entry 11) gave the enantiomerically enriched alcohol after 25 hours in >99% e.e., similar to entries 5 and 7 with halogen substituents (Br and Cl). It seems that the *meta*-substituent did not show any electronic effect in this biotransformation. For the *para*-substituted substrates **5**, **6**, **7** (entries 8, 9 and 10), we obtained high yields and excellent selectivity, but slightly lower enantioselectivity than in our previous study with *para*-substituted benzhydrols.[10] These results demonstrate the difficulty in predicting the steric requirements of the enzymes involved in this biotransformation. However, some generalities apply, i.e.:

(a) *sec*-alcohols, with *meta*- and *para*-substituted patterns are transformed to the corresponding ketones by *Nocardia corallina* B-276.

(b) *ortho*-substituted *sec*-alcohols are not transformed.

(c) The transformation seems to be independent of electronic effects of the aromatic ring substituent.

With respect to the configuration of the enriched alcohol obtained by this procedure, comparison of the sign of the specific rotation with literature data[16,17] allowed assignment of the (*R*)-configuration to the alcohols **1**, **2**, **3**, **4**, **5** and **7**. The assignment of (*R*)-configuration to the alcohols **6**, **9** and **10** was based on the assumption that the (*R*)-enantiomer elutes before the (*S*)-enantiomer from Chiraldex B-PH and G-TA columns. Brunner[18] used a similar argument using a Chirasil-L-Val column[19] with the urethane derivatives of *sec*-alcohols. These results are consistent with our previous observations for the enantioselective oxidation with *Nocardia corallina* B-276.[10]

3. Conclusion

We have extended this microbial resolution methodology using *Nocardia corallina* to afford methylaryl-

methanols in moderate chemical yields, which are unoptimized with excellent e.e.s. However, the *ortho*-derivatives were almost inert to these conditions, perhaps due to steric hindrance. This behaviour is similar to the microbial deracemization of similar alcohols with *Geotrichum candidum*.[14] *Nocardia corallina* B-276 oxidises allylic and benzylic alcohols, in contrast to *Yarrowia lipolytica*, which is not able to oxidise methylphenylmethanol.[6]

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 carried out in an orbital shaker; broth compositions. 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 methylarylmethanols and ketones were identified by their infrared spectra (Perkin–Elmer Paragon 1000) as liquid films or KBr discs, hydrogen nuclear magnetic resonance (^{1}H NMR) (Brücker 500 MHz) and by TLC on silica gel 60 GF_{254} Merck, and comparative analysis with authentic samples. The specific rotations were determined in a Perkin–Elmer 341 polarimeter. The GC analysis was performed on a Hewlett Packard 6890 gas chromatograph equipped with a flame ionization detector and a Chiraldex column B-PH (30 m) or G-TA (30 m).

4.2. General procedure for biotransformations

4.2.1. Preculture I. A 125 mL Erlenmeyer flask containing sterile culture medium (50 mL) was inoculated from an agar plate (3 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 (0.6 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 methylarylmethanols were extracted with ethyl acetate (4×25 mL). The products were compared with authentic samples by ^{1}H NMR, IR and TLC.

4.3. Determination of the enantiomeric excess for the methylarylmethanols

(a) By GC. All e.e.s were determined by comparing the GC data of the chiral products with those of the corresponding racemic alcohols on the indicated Chiraldex column.

Methylphenylmethanol, 1: B-PH column, 95°C, N_2, 1.0 mL/min. Racemic alcohols, $t_R = 33.52$ min, $t_S = 34.21$ min. Product from the enantioselective oxidation gave $t_R = 33.56$ min, with >99% e.e.

Methyl-(2-bromophenyl)methanol, 2: B-PH column, 130°C, N_2, 0.6 mL/min. Racemic alcohols, $t_R = 9.85$ min, $t_S = 10.76$ min. Product enrichment from the enantioselective oxidation gave $t_R = 9.92$ min and $t_S = 10.89$ min with 4% e.e.

Methyl-(3-bromophenyl)methanol, 3: B-PH column, 130°C, N_2, 0.8 mL/min. Racemic alcohols, $t_R = 55.65$ min, $t_S = 56.30$ min. Product from the enantioselective oxidation gave $t_R = 54.72$ min, with >99% e.e.

Methyl-(3-chlorophenyl)methanol, 4: B-PH column, 120°C, N_2, 1.0 mL/min. Racemic alcohols, $t_R = 40.62$ min, $t_S = 41.18$ min. Product from the enantioselective oxidation gave $t_R = 39.21$ min, with >99% e.e.

Methyl-(4-bromophenyl)methanol, 5: G-TA column, 100°C, N_2, 0.6 mL/min. Racemic alcohols, $t_R = 26.22$ min, $t_S = 28.93$ min. Product enrichment from the enantioselective oxidation gave $t_R = 26.53$ min and $t_S = 30.59$ min with 72% e.e.

Methyl-(4-isopropylphenyl)methanol, 6: G-TA column, 90°C, N_2, 0.5 mL/min. Racemic alcohols, $t_R = 21.44$ min, $t_S = 22.28$ min. Product enrichment from the enantioselective oxidation gave $t_R = 21.11$ min and $t_S = 22.79$ min with 91% e.e.

Methyl-(4-methoxyphenyl)methanol, 7: G-TA column, 90°C, N_2, 0.6 mL/min. Racemic alcohols, $t_R = 36.60$ min, $t_S = 37.44$ min. Product from the enantioselective oxidation gave $t_R = 34.35$ min, with >99% e.e.

Methyl-(2,3-dimethoxyphenyl)methanol, 9: B-PH column, 110°C, N_2, 0.6 mL/min. Racemic alcohols, $t_R = 41.71$ min, $t_S = 42.54$ min. Product enrichment from the enantioselective oxidation gave $t_R = 42.46$ min and $t_S = 43.18$ min with 3% e.e.

Methyl-(3,4-dimethoxyphenyl)methanol, 10: G-TA column, 110°C, N_2, 0.5 mL/min. Racemic alcohols, $t_R = 47.71$ min, $t_S = 49.27$ min. Product from the enantioselective oxidation gave $t_R = 48.07$ min, with >99% e.e.

(b) By ^{1}H NMR. It was determined by comparing the ^{1}H NMR data of the chiral product 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.

Methyl-(3-phenoxyphenyl)methanol, 8: α-CH signal at 4.83 ppm, the signal shifted to 6.20 and 6.14 ppm. The product had >99% e.e.

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