Pergamon

Tetrahedron: Asymmetry Vol. 6, No. 9, pp. 2287-2290, 1995
Elsevier Science Ltd
Printed in Great Britain
0957-4166/95 $9.50+0.00

0957-4166(95)00304-5

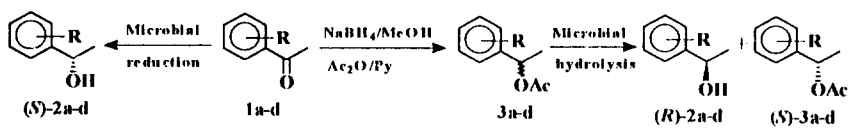
Biotransformations with *Rhizopus arrhizus*: Preparation of the Enantiomers of 1-Phenylethanol and 1-(*o*-, *m*- and *p*-Methoxyphenyl)ethanols

Neeta A. Salvi, Prashant N. Patil, Sanninje R. Udupa* and Asoke Banerji

Microbial Chemistry Section, Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085, India.

Abstract : The fungus *Rhizopus arrhizus* has been used for the reduction of acetophenone and its *o*-, *m*- and *p*-methoxy derivatives **1a-d** to the corresponding (*S*)-(-)-alcohols. Their racemic acetates were also hydrolyzed to (*R*)-(+)-alcohols. The analysis of products revealed that maximum enantioselectivity (78-88 %ee) could be obtained using *m*-methoxy acetophenone as substrate.

The preparation of homochiral compounds is one of the major thrust in organic synthesis. Applications of biological systems in the synthesis of homochiral compounds are gaining increasing importance because of their simplicity and stereospecificity. The alkylaryl carbinols are useful intermediates in the synthesis of agrochemicals and pharmaceuticals[1]. Asymmetric reduction of prochiral ketones and stereoselective hydrolysis of racemic acetates are the main routes to the synthesis of homochiral carbinols. During our investigations on microbial transformations, we have observed that the mould, *Rhizopus arrhizus*, brings about these types of reactions on a variety of substrates[2]. As an extension of the work, the biotransformation of acetophenone, its methoxy derivatives and racemic acetates to chiral carbinols have been studied. (**Scheme**).



(*S*)-2a-d 1a-d 3a-d (*R*)-2a-d (*S*)-3a-d

R:
a = H; **b** = *o*-methoxy; **c** = *m*-methoxy; **d** = *p*-methoxy

Scheme : Biotransformation using *Rhizopus arrhizus*

Asymmetric reduction of 1a-d : In order to optimize the time for the microbial reduction, experiments were carried out with **1a** for different periods of time intervals. Fermentation for 7 days was found to yield the best results. Therefore 1-phenylalkanones **1a-d** were incubated with *R. arrhizus* in modified Czepak Dox medium[3] for 7 days. At the end of fermentation the transformation products **2a-d** were isolated and characterized. All these alcohols were found to have negative specific rotations.

Determination of absolute configuration and enantiomeric excesses of 2a-d : The absolute cofigurations and enantiomeric excesses of alcohols **2a-d** were determined by GC analysis and ^{1}H NMR studies of their corresponding MTPA esters. These esters were prepared according to the literature procedure[4]. The enantiomers of the MTPA esters resolved well in GC. It has been reported that the retention times of the (*R*)-enantiomers are lower than those of (*S*)-enantiomers[5,6]. Also, the signals for methoxy group of MTPA portion in the racemic MTPA esters appeared at ~ δ 3.47 for (*R*) and ~ δ 3.56 for (*S*)-isomers in ^{1}H NMR spectra[7]. The ^{1}H NMR data of the esters of the alcohols obtained from microbial reduction of **1a-d** showed a prominent signal for the (*S*)-enantiomer. The enantiomeric excesses were determined from the integral ratios. The results obtained from GC as well as ^{1}H NMR were comparable. From **Table 1** it can be seen that of various isomers, *m*-methoxy acetophenone **1c** was found to be the best substrate for asymmetric reduction (e.e 78.3 %, ($[\alpha]^{26}$ -36.66, c, 1.162, $CHCl_3$). Based on GLC profile, NMR data and negative specific rotations the alcohols obtained from microbial reduction were assigned (*S*)-configurations.

TABLE 1 : Asymmetric Reduction of 1a-d using *R. arrhizus*

Substrate	Product	%Yield[a]	%ee[b]	Config.
1a	2a	33.2	71.5	S
1b	2b	62.3	10.2	S
1c	2c	82.6	78.3	S
1d	2d	36.8	32.3	S

TABLE 2 : Asymmetric Hydrolysis of 3a-d using *R. arrhizus*

Substrate	Product	%Yield[a]	%ee[b]	Config.
3a	2a	44.0	76.7	R
3b	2b	73.4	5.8	R
3c	2c	55.5	89.0	R
3d	2d	81.8	35.5	R

a After purification by silica gel chromatography,
b Determined by GC analysis and ^{1}H NMR study of MTPA esters

Enzymatic resolution of (±) acetates 3a-d : From the preliminary experiments, the optimum period for incubaton for asymmetric hydrolysis was found to be 3 days. Therefore the enzymatic hydrolysis of **3a-d** was carried out for 3 days of incubation and at the end of fermentation the product alcohols were isolated and characterized. These alcohols **2a-d** were found to have positive specific rotations while unchanged acetates showed negative rotations. On the basis of GC analysis and ^{1}H NMR studies of MTPA esters, these alcohols were assigned the (*R*)-configurations. In the control experiments, **3b** and **3d** underwent hydrolysis to the extent of 27 % and 30 % respectively, while **3c** was resistant to hydrolysis. Therefore, the enantiomeric excesses in the cases of **3b** and **3d** were poor although the chemical yields were high (73.4 % and 81.8 % respectively) (**Table 2**). However, **3c** yielded (*R*)-(+)-alcohol in 89.0 % e.e and chemical yield of 55.5%.

In conclusion, the biotransformation using fungus *Rhizopus arrhizus* provides an inexpensive complementary method for asymmetric reduction as well as asymmetric hydrolysis of alkylaryl ketones and their corresponding acetates respectively. Of the various acetophenones, *m*-methoxy acetophenone gave the the best results.

EXPERIMENTAL

^{1}H NMR spectra were recorded on Varian EM 360 L (60 MHz) and Bruker 200 AC FT NMR spetrometers using TMS as the internal standared. IR spectra were recorded on a Perkin Elmer IR spectrophotometer model 783. Optical rotations were measured on Jasco 360 digital polarimeter. MS spectra were obtained on Shimadzu Gas chromatography QP 1000. GLC analysis were carried out on a Shimadzu Gas-Chromatograph GC-16A, FID detector.

Chemicals: Compounds **1a-d** were obtained from BDH (UK). The racemic ethyl acetates **3a-d** were prepared by the reduction of the corresponding ketones with $NaBH_4$ followed by the acetylation using acetic anhydride and pyridine. (*R*)-(+)-α-Methoxy-α-trifluoromethyl phenyl acetic acid (MTPA) was the product of Aldrich. MTPA chloride and its corresponding esters of compounds **2a-d** were synthesized according to literature procedures.

Microorganisms: The fungus *Rhizopus arrhizus* was obtained from National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India. The fungus from PDA slants was cultivated on 150 ml sterilized modified Czepak Dox medium in 500 ml Erlenmeyer flasks at room temparature on a rotary shaker (150 r.p.m. Remi, Bombay).

Fermentation and Product Analysis: The substrates **1a-d** and **3a-d** were added to a 72 h grown culture and shaken for desired periods. Substrate and organism controls were also run simultaniously. At the end of fermentation, the mycelial mass was filtered from the culture medium. The filtrate was extracted with $CHCl_3$ (3 x 50 ml), washed with water (2 x 20 ml) and dried (anhy. Na_2SO_4). The solvent was removed under reduced pressure and the oily residue was obtained as a filtrate extract. The mycelial mass was washed with acetone (3 x 50 ml) and acetone was removed. An oily residue obtained was then taken into water and extracted with ethyl acetate (3 x 50 ml), washed (H_2O), dried (anhy. Na_2SO_4) and evaporated to give an oily mycelial extract. Control experiments were also extracted in similar way. The transformed products were isolated and purified by preparative TLC (Silica gel G, E. Merck, India, 10 % EtOAc / Pet. Ether). The products were characterized by IR, ^{1}H NMR, MS and optical rotations.

Separation by gas chromatography: Product analysis of MTPA esters was performed on capillary column (50 x 0.25 ID) packed with OV-17. The temparature of the injector and detector was set at 250°. Detection was carried out with a flame ionization detector (FID) using nitrogen as carrier gas. GC was performed isothermically at temperatures indicated in parenthesis and retention times for the MTPA esters are as follows : (*R*) 1-phenylethanol : 25.9 min, (*S*) 1-phenylethanol: 26.8 min (150° C); (*R*) 1-(*o*-methoxyphenyl)ethanol: 28.5 min, (*S*) 1-(o-methoxyphenyl)ethanol: 29.4 min (160° C); (*R*) 1-(*m*-methoxyphenyl)ethanol:19.6 min, (*S*) 1-(*m*-methoxyphenyl)ethanol: 20.2 min (180° C); (*R*) 1-(*p*-methoxyphenyl)ethanol: 22.2 min, (*S*) 1-(*p*-methoxyphenyl)ethanol: 22.6 min (180° C).

References:

1. Ito, Y.; Hayashi, T. *Japan Kakai Tokyo, Kho Japan*, 02,264,736; [*Chem. Abstr.* **1991**, *114*, 142856e].
2. Patil, P. N.; Salvi, N. A.; Udupa, S. R.; Banerji, A. 4th National Symposium on Bio-organic Chemistry, **1992**, Bhabha Atomic Research Centre, Bombay (India) p. 47.
3. Prema, B. R.; Bhattacharya, P. K. *Appl. Microbiology*, **1962**, *10*, 524-528.
4. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.*, **1973**, *95*, 512-519.
5. Peters, J.; Zelinski, T.; Minuth, T.; Kula, M. *Tetrahedron Asymmetry*, **1993**, *4*, 1683-1692.
6. Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P.; Poli, S. *Tetrahedron Asymmetry*, **1993**, *4*, 1607-1612.
7. Brown, S. M.; Davies, S. G.; de Sousa, J. A. A. *Tetrahedron Asymmetry*, **1993**, *4*, 813-822.

(*Received in UK* 21 *July* 1995)