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Lipase Catalysed Enantioselective Amidation of α-phenylethylamine

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Abstract: Some commercial lipases were screened for enantioselective resolution of (R, S) α-phenylethylamine. Ethyl acetate was used as acyl donor and the reaction medium. The results were interpreted by comparing specific rotations with optically pure α-phenylethyl acetamide. R amide was the major enantiomer produced in all cases except Pseudomonas fluroscens. Kinetic resolution was further improved for lipases from Candida antarctica Lipase B (L2, chirazyme, Roche) and from *Pseudomonas cepacia* (PS-C and PS-D, Amano. Japan) when ethoxy ethyl acetate was used as acylating agent, enantiopure R amide was obtained. The solvent in which the reaction is carried out plays an important role in reaction. Immobilized enzyme showed better yields and selectivity.

Key words: Aminolysis, enantioselectivity, lipase, α-phenylethylamine, *Pseudomonas cepacia*

INTRODUCTION

Optically active amines are important compounds in organic synthesis as they form key intermediates in multistep reactions such as drug intermediates. Numerous reports exist on their production by resolution of their racemic form using lipases. Amides too have importance in peptide and lactam synthesis (Li and Kanerva, 2005; Yang, 2006). Lipases have been extensively used in organic chemists as chemo-, regio- and stereoselective reactions such as hydrolysis, esterification, transesteification and aminolysis (Faber, 1997; Wong and Whitesides, 1994). Microbial lipases have also been used to resolve a wide variety of racemates (Pallavicini *et al*., 1994; Sanchez *et al*., 1997; Goswami *et al*., 2005; Kasture *et al*., 2005).

Numerous reports on resolution of primary and secondary alcohols by esterification reactions catalyzed by *Pseudomonas cepacia* lipases are available in literature (Kanji *et al*., 1997; Mezzetti *et al*., 2003). However enantioselective resolution of amines by aminolysis of esters has been sparsely studied. Phenylethyl amine is known to be a basic compound required for many drugs. Lipase catalyzed acylation has proved very useful in the resolution of racemic amines and aminolysis of racemic esters (Gonzalez-Sabin *et al*., 2002; Ohrner *et al*., 1996; Castro *et al*., 2000). *Candida antarctica* B has been reported to resolve racemic phenylethyl amine (Reetz and Schimossek, 1996; Wagegg *et al*., 1998). The aim of this study was to screen various commercially available lipases for their potential chiral selectivity of this compound.

MATERIALS AND METHODS

Enzymes

Lipases-L-1 (*Candida cylindracea*-CCL also known as *Candida rugosa)*, L-4 (*P. seudomonas cepacia*), L-17 (CAL B)), L-20 (*Alcaligenes*) and L-21 (*Pseudomonas fluroscens*) were obtained from Europa Bioproducts; lipases- Chirazymes L2 (CAL B) was from Roche and *P. cepacia* PS, PS-C, PS-D from Amano, Japan.

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Table 1: Resolution of (R, S) α -phenylethylamine, by lipases using ethyl acetate as acylating agent and solvent at 28°C

Entry No.	Lipase	Reaction time (h)	Amide yield (%)	Sp. rotation $[\alpha]_D$	Optical purity E (%)
1	L-1, CCL	96	46.10	0.00	0.00
2	L-17, CAL B	48	34.00	+10.43	7.78
3	L2, CAL B	24	60.00	+130.00	99.90
5	L10 *Alcaligenes*	96	27.00	+22.40	16.72
6	L-21, *P. fluroscens*	96	34.12	-8.40	6.28
7	L-4, *P. cepacia*	48	47.15	+7.24	5.40
8	PS, *P. cepacia*	96	40.00	+4.82	3.59
9	PS-C, *P. cepacia*	96	59.42	+112.44	83.58
10	PS-D, *P. cepacia*	96	67.87	+67.27	50.28
11	PS-D, *P. cepacia**	128	13.80	+57.30	42.76

* Reaction in hexane using ethyl acetate as acylating agent

Chemicals

Racemic α-phenylethylamine was kind gift from Alkyl Amines Chemical Ltd. Pune, India. Standard S (-) Phenylethylamine was purchased from Hi media Laboratories Ltd., Mumbai, India. Ethyl acetate used was of HPLC grade; ethoxyethyl acetate and solvents were from SD Fine Chemicals Ltd. Mumbai.

Lipase Catalyzed Reactions

In 15 mL dry pure ethyl acetate racemic amine (3.0 mmol) and Lipases (100 mg) was added. The reaction mixture was shaken at 28°C and 222 rpm on orbital shaker (Table 1). Enzyme was separated from solvent. Solvent evaporated and residue obtained was acidified with 3 N H_2SO_4 (20 mL). Amide (as a white solid) was extracted with dichloromethane (3×15 mL). Aqueous phase was made with solid NaOH and amine was extracted with dichloromethane (4×20 mL). All yields were calculated taking into account the percentage of conversion.

When ethoxyethyl acetate was used as the acyl donor, MTBE (methyl tributyl ether) or diethyl ether was the reaction solvent.

Enantiopure phenylethyl acetamide, required as standard for specific rotation, was prepared chemically from S (-) phenyl amine and acetic anhydride and extracted as above.

Analysis

The progress of the reaction was monitored on HP 5800 gas chromatography. The column used was packed OV-17, with temperature programming 100-200°C, rate 40°C min. carrier gas was nitrogen, flow rate 30 mL min^{-1}. A standard graph of α-phenlyethyl acetamide was constructed of known concentrations (0.1-1%). Fixed volume of the reaction mixture was injected and the yield calculated on the basis of the standard graph.

Optical rotations were recorded on a Jasco Dip-181 polarimeter using a sodium lamp. Optical purity was determined by comparing the specific rotation to the chemically prepared optically pure S (-) α-phenylethyl acetamide that was (-) 134.

RESULTS AND DISCUSSION

The resolution of α-phenylethyl amine was carried out by lipases under simple and mild reaction conditions, using ethyl acetate as the acyl donor and solvent. Figure 1 outlines the synthesis of chorally pure amine from the racemic mixture, wherein one isomer is used for aminolys and the other remains unreacted.

Of the nine commercial lipases tried, three enzymes gave conversions in the range of 60-70%. On the basis of optical rotation values of phenylethyl acetamide produced, three lipases viz. *Candida antarctica* (from Roche) and Amano PS-C and PS-D showed good selectivity and were hence selected for acetylation with another acyl donor. In spite of 60% yield the rotation of amide

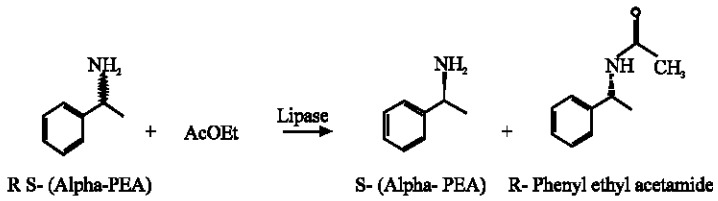

Fig. 1: Amidation of phenylethylamine using ethyl acetate as acylating agent and reaction medium

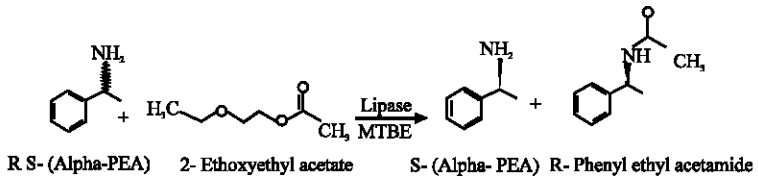

Fig. 2: Amidation of phenylethylamine with ethoxy ethyl acetate as acylating agent

by *Candida antarctica* was maximum thereby indicating the dynamic resolution of (RS) phenylethylamine. The R amide was the preferred enantiomer formed in all cases, except *Pseudomonas fluroscens*, which showed preference for S amide (entry 6).

Effect of Acylating Agent

Acylating agents are known to influence the kinetic resolution of lipase-catalyzed reactions. Consequently reactions of lipases by *Candida antarctica* (Roche), Amano P-SC and Amano P-SD that gave best results with ethyl acetate were repeated using 2-ethoxy ethyl acetate as acetylating agent, reaction Fig. 2, results are presented in Table 2. A change in the acetylating agent improved the optical purity of amide formed by PS-C from 83.58 to 99.9% and by PS-D from 50.20 to 99.9%. (Ohrner *et al*., 1996) resolved phenyl ethylamine, by amidation of ethyl octonate using CAL B (Novozyme 435™) as catalyst, the reaction was carried out at 39°C under reduced pressure, R amide was the major amide formed. Castro *et al*. (2000) studied the potential of a number of commercial lipases for amidation reactions they also resolved (R, S) phenyl ethylamine by aminolysis of ethyl butyrate.

Effect of Solvent

The solvent used for carrying out reaction plays an important role in the output of the reaction. Thus screening of different solvents as reaction medium for amidation phenyl ethylamine with 2-ethoxy ethyl acetate by lipases PS-C and PS-D indicated diethyl ether and Methyl Tributyl Ether (MTBE) gave best yields whereas in hexane, a non-polar solvent no reaction was observed.

Effect of Immobilization

CAL B (Roche) which was in immobilized form gave good yield 60 and 100% optical purity, this reaction was earlier reported by (Reetz *et al*., 2006) whereas the same lipase in the form of oily liquid (Europa products) gave optically purity of 7.55 only. Similar results were obtained for amides,

Table 2: Enantioselective resolution of (R, S) α-phenlyethylamine using ethoxyethyl acetate as acyl donor at 28°C in MTBE solvent

Lipase	Reaction time (h)	Amide yield (%)	Optical purity E (%)
L2 CAL B	96	40	99.9
P. cepacia P-SC	144	20	99.9
P. cepacia P-SD	120	25	99.9

catalyzed by Amano lipase, PS-C and PS-D which were immobilized on ceramic particles and diatornite, respectively, showed optical purity of 83.58 and 50.20%, whereas lipase PS lyophilized powder form had only slight optical purity of 3.59% (entries 8-10).

CONCLUSIONS

In our screening study we were able to identify lipases from P. *seudomonas cepacia*, Amano PS-D and PS-C, which gave good enantioselectivity of 99.9%, R - α-phenyethyl acetamide at 28°C when ethoxyethyl acetate was used as the acyl donor. This is the first report, to the best of our knowledge, where *P. cepacia* has been successfully used for complete resolution of an amine.

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