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Kinetic resolution of racemic 2-amino-1-butanol with penicillin G acylase enzyme immobilised in gelatin gels

M.Adharvana Chari*[1], A.Jeevanram[2], K.Syamasundar[2]

[1]Catalysis Lab, Institute of Science & Technology, Jawaharalal Nehru Technological University, Kukatpally, Hyderabad-500072, (INDIA)

[2]Dept. of Pharmacy & Biotechnology, CMR College of Engg., Medchal, Hyderabad, (INDIA)

Tel : +91-40-23060056

E-mail : drmacj@yahoo.co.uk.

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ABSTRACT

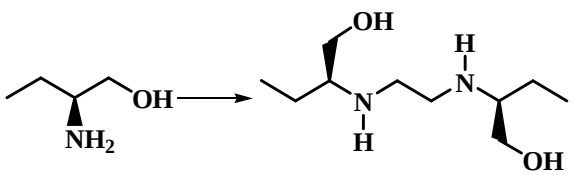
Racemic 2-amino-1-butanol has been resolved to obtain (S)-2-amino-1-butanol with >99% e.e. via enantio- selective hydrolysis of its N-phenyl acetyl derivative with penicillin G acylase immobilized on gelatin matrix.

KEYWORDS

Kinetic resolution
Racemic 2-amino-1-butanol;
Penicillin Gacylase;
Immobilization in gelatin matrix.

INTRODUCTION

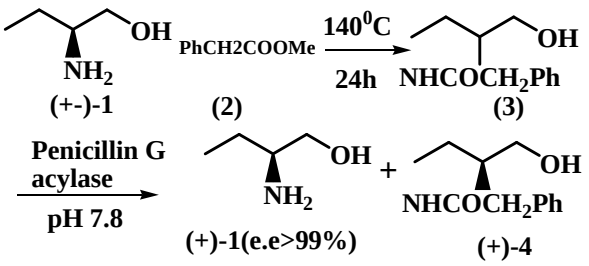
Enzymes are versatile biocatalysts and find increasing application in many areas, including organic syntheses. The major advantages of using enzymes in biocatalytic transformations are their chemo, regio and stereo specificity as well as the mild reaction conditions that can be used[1]. Resolution of racemic 2-amino-1-butanol is an industrially important process since the (S)-2-amino-1-butanol is used as an intermediate for the production of ethambutol and antibiotic for the treatment of tuberculosis[2](SCHEME 1).



SCHEME 1

Industrially, resolution of racemic 2-amino-1-butanol is carried out by fractional crystallization of the aminoalchol as a salt of an optically active acid such as mandelic, tartaric or glutamic acid from a solvent such as methanol or water[3]. The known enzymatic processes for resolution of racemic 2-amino-1-butanol are based on either an enantioselective hydrolysis of the ester function of an N,O-diacetyl derivative with an enzyme such as lipase[4-6] or hydrolysis of the *N*-benzoyl derivative with a fungus such as *Aspergillus oryzae* where the (S)-derivative is hydrolysed due to aminoacylase activity[7]. However, hydrolysis of the diacetyl derivative gives products which have to be separated by expensive chromatography, while the *A.oryzae* process is based on fermentation of the fungus and the enzyme is not readily available in the market.

Herein we report a simple methodology of obtaining enantiomerically pure(S)-2-amino-1-butanol on a multigram scale which is based on the priniciple of



SCHEME 2

enantioselective hydrolysis of an N-phenylacetyl derivative of racemic 2-amino-1-butanol with enzyme penicilline acylase (E.C. 3.5.1.11) immobilized on gelatin matrix[8]. The enzyme hydrolyses the (S)-enantiomer selectively to give(S)-2-amino-1-butanol with e.e.>99% (SCHEME 2). The amide substrate **(3)** is prepared by simple heating of methylphenyl acetate **(2)** with racemic 2-amino-1-butanol **(1)**; the enzyme is cheap, stable and commercially available in large quantities, recovery of the products is easy and ecofriendly.

RESULTS AND DISCUSSION

Penicillin G acylase[9] is used mainly for the production of 6-aminopenicillanic acid[10]. It is also useful for the production of semisynthetic antibiotics, resolution of alcohols[11], β-hydroxy-α-amino acids[12], β-amino acids[13], and for the deprotection of the phenylacetyl group in peptide synthesis[14]. Although Romeo et al. have reported that hydrolysis of N-phenyl acetyl 2-amino-1-butanol with penicillin G acylase proceeds with (S)-selectivity, the enantiomeric excess of the product at 50% hydrolysis stage was reported to be very low (e.e. 27%)[15]. During our investigations on the resolution of β-hydroxy-α-amino acids with penicillin acylase[12]. It was observed that the enantiomeric excess of the product was strongly dependent on the extent of hydrolysis and it was important to stop the reaction after 40% hydrolysis. Similar observation has been made by Giacomini and co-workers during the hydrolysis of racemic 3-amino-azetidin-2-one catalysed by penicillin G acylase[16]. It was thus necessary to determine the enanatiomeric excess of the product as a function conversion.Fig.1shows the results of these investigations it can be seen that the enantiomeric excess of (S)-2-amino-1-butanol is very high (e.e.>99%) up to 40% hydrolysis stage and then it drops it dramatically. When the reaction is allowed to continue further, the entire phenyl acetyl derivative is hydrolysed.

It is also worth mentioning that although the stereo chemical outcome of the reaction was not affected, the rates of reactions carried out in 0.05Mphosphate buffer (pH7.8) as generally reported in the literature were at least ten times slower than those carried out in distilled water with the pH being adjusted to 7.8 with 2N ammonia solution.

CONCULSION

The present work provides an excellent alternative to existing routes for resolution of racemic 2-amino-1-butanol. The enzyme penicillin Gacylase is commercially available in large quantities. The recycling of the lipases immobilized in gelatin matrix for several recycles has already been demonstrated and thus our procedure can be used for large scale preparations[17]. The product is obtained with high enantiomeric excess and the enzyme can be recycled several times. The overall process is ecofriendly.

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