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TETRAHEDRON: *ASYMMETRY*

Resolution of racemic 2-amino-1-butanol with immobilised penicillin G acylase†

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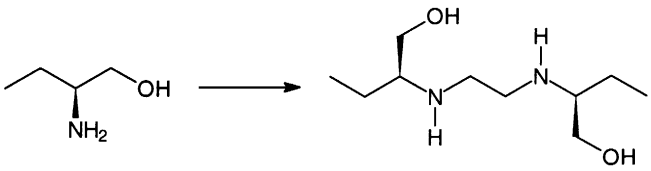
Abstract

Racemic 2-amino-1-butanol has been resolved to obtain (*S*)-2-amino-1-butanol with >99% e.e. via enantioselective hydrolysis of its *N*-phenylacetyl derivative with penicillin G acylase immobilised on Eupergit C.

1. Introduction

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, an antibiotic for the treatment of tuberculosis[1] (Scheme 1).



Scheme 1.

Industrially, resolution of racemic 2-amino-1-butanol is carried out by fractional crystallisation of the aminoalcohol as a salt of an optically active acid such as mandelic, tartaric or glutamic acid from a solvent such as methanol or water.[2] 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[3–5] or hydrolysis of the *N*-benzoyl derivative with a fungus such as *Aspergillus*

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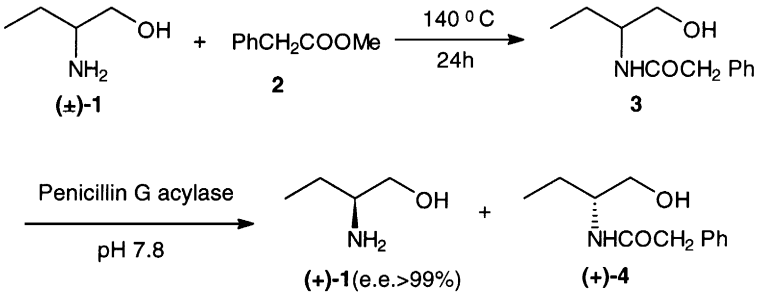
oryzae where the (*S*)-derivative is hydrolysed due to aminoacylase activity.[6] However, hydrolysis of the diacetyl derivative gives products which have to be separated by expensive chromatography, while the *A. oryzae* process is based on fermentation of the fungus and the enzyme is not readily available 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 principle of enantioselective hydrolysis of an *N*-phenylacetyl derivative of racemic 2-amino-1-butanol with enzyme penicillin acylase (E.C. 3.5.1.11) immobilised on Eupergit C.[7] 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 the overall process produces very little effluent.



Scheme 2.

2. Results and discussion

Penicillin G acylase[8] is used mainly for the production of 6-aminopenicillanic acid.[9] It is also useful for the production of semisynthetic antibiotics,[10] 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*-phenylacetyl 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 enantiomeric excess of the product as a function of conversion. Fig. 1 shows 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 dramatically. When the reaction is allowed to continue further, all of the phenylacetyl derivative is hydrolysed.

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

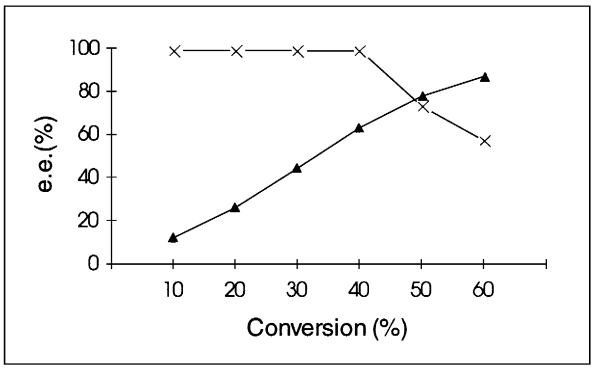


Fig. 1. Enantioselective hydrolysis of racemic 2-[*N*-(phenylacetyl)amino]-1-butanol with immobilised penicillin G acylase

2.1. Effect of substrate concentration on reaction rate

The results in Fig. 1 are somewhat puzzling. According to the theoretical calculation of enzyme-catalysed kinetic resolution, the e.e. of the product at 50% conversion should be greater than 95% when the reaction at 40% conversion affords the product in >99% e.e. (enantioselectivity E >100).[17,18] In our experiments we have obtained the product with a much lower e.e. of 73%. To find out the reasons for this deviation, the following experiments were conducted with enantiomerically pure (*R*)- and (*S*)-amides separately. In the first experiment the substrate concentration varied from 0.5 to 6% but the enzyme:substrate ratio (w:w) was held constant at 1:5. It was observed that the (*S*)-amide hydrolysed 42–45 times faster than the (*R*)-amide in all the cases, indicating that the enantioselectivity of the enzyme-catalysed reaction E is about 44.

In the second experiment, the substrate concentration was increased from 0.5 to 6% but the enzyme concentration was held constant at 1 g/100 ml. Here, very interesting results were obtained (Table 1).

Table 1
Effect of substrate concentration on the hydrolysis of optically pure (*S*)- and (*R*)-2-[*N*-(phenylacetyl)-amino]-1-butanol with immobilised penicillin G acylase

[Amide], M; (%, w/w)	Reaction rate[a] for (*S*)-amide mmol/h/g	Reaction rate for (*R*)-amide mmol/h/g	Rate Ratio
0.024 (0.5)	10.3	0.24	42.9
0.048 (1.0)	14.8	0.35	42.3
0.097 (2.0)	24.2	0.57	42.5
0.145 (3.0)	30.6	0.68	45.0
0.193 (4.0)	34.7	0.76	45.6
0.242 (5.0)	34.3	0.78	43.9
0.290 (6.0)	34.5	0.77	44.8

[a] The reactions were carried out with 100 ml reaction mixture and analysed by HPLC. Product concentration is based on amount of phenylacetic acid produced due to hydrolysis. Rates are given per gram of immobilised enzyme preparation.

It is clear from the data in Table 1 that the apparent K_m value ($K_{m,app}$) for the reaction with immobilised penicillin G acylase is quite high (~0.05 M, 1% solution) for both the enantiomers, and the reaction takes place at V_{max} only when the substrate concentration is 4% and above. This explains the low enantioselectivity at 50% conversion. When the reaction crosses 40% conversion stage, the overall concentration of the (*S*)-enantiomer in the solution falls to 1% (in 10 g of amide the actual amount

of (*S*)-amide is 5 g out of which 4 g of amide has hydrolysed), where the rate of hydrolysis of the (*S*)-enantiomer is 2.3 times slower than that at 5% concentration while the rate of hydrolysis of the (*R*)-enantiomer remains unchanged. In other words, the value of enantioselectivity E falls to 19 from a value of 44 and it is expected that the e.e. of the product would be about 75%. Using experimental values of ee_s and ee_p at 50% conversion stage we obtain E=15 by Sih's method[17] which is in reasonable agreement with the data in Table 1. The situation gets worse at 60% conversion stage (concentration of (*S*)-amide falls presumably below 0.9%). The rate ratio further decreases which is again in good agreement with the E value calculated from the experimental e.e. at 60% conversion (E=10).

With the rate ratio of 44, one would expect a maximum e.e. of 95–96% for the hydrolysis product.[18] The observed e.e. of >99% for the (*S*)-2-amino-1-butanol could be due to other factors such as inhibition of the reaction of (*R*)-amide by (*S*)-amide, or by the product (*S*)-aminoalcohol.

In a typical experiment, an aqueous solution containing the substrate **3** (103 g, 0.5 mol) in water (1000 ml) is hydrolysed with the immobilised enzyme (35 g, 150 units/g) in a stirred tank reactor at pH 7.8 (adjusted with 2N ammonia solution) at room temperature. The reactants are stirred at 75–80 rpm and the reaction is monitored by HPLC analysis of the reaction mixture following the formation of phenylacetic acid. The reaction is stopped at 40% hydrolysis stage (30 min) and the reactants are removed from the enzyme by filtration. The filtrate is first extracted with ethyl acetate to recover unreacted amide, acidified with 4N HCl and again extracted with ethyl acetate to recover phenylacetic acid, and (*S*)-2-amino-1-butanol is recovered from the aqueous solution by distillation. Pure (*S*)-2-amino-1-butanol is obtained as a colourless liquid with >99% e.e. in 70% theoretical yield. The enantiomeric excess of the product was measured by determination of its specific rotation and by chiral HPLC analysis of its phenylacetyl derivative.

3. Conclusion

The present methodology provides an excellent alternative to existing routes for resolution of racemic 2-amino-1-butanol. The enzyme penicillin G acylase is commercially available in large quantities, the product is obtained with high enantiomeric excess and the enzyme can be recycled several times. The overall process is eco-friendly since it produces very little effluent.

4. Experimental

Penicillin G acylase was obtained from Kopran Ltd, Mumbai, India. All other reagents were A.R. grade obtained from SD Fine Chem, India. NMR spectra were recorded on a Varian FT-200 MHz (Gemini) using tetramethylsilane (TMS) as the internal standard. Infrared spectra were scanned on a Perkin–Elmer 1310 spectrophotometer with sodium chloride optics. Elemental analyses were carried out on a Vario EL, Elementar, Germany. HPLC analyses were carried out on a Hewlett Packard HP1090 unit with diode array detector and HP ChemStation.

*4.1. Methyl phenylacetate **2***

Phenylacetic acid (136 g, 1 mol) and methanol (500 ml) were refluxed in a round-bottomed flask in the presence of ion-exchange resin (Amberlyst 15, Fluka AG, Switzerland; 10 g) for 12 h. The conversion of the acid to ester was followed by TLC. When all the acid was converted to ester, the resin was filtered

and solvent was removed by rotavapor distillation. The residue was then distilled under vacuum to obtain methyl phenylacetate (145 g, 95% yield). ^{1}H NMR ($CDCl_3$) δ 7.32 (m, 5H), 3.71 (s, 3H), 3.65 (s, 2H).

4.2. (RS)-2-[N-(Phenylacetyl)amino]-1-butanol **3**

Methyl phenylacetate **2** (150 g, 1 mol) and racemic 2-amino-1-butanol **1** (89 g, 1 mol) were heated in a round-bottomed flask at 140°C for 24 h. The residue was then recrystallised from chloroform–hexane. The product 2-[*N*-(phenylacetyl)]-1-butanol **3** was obtained as a white powder (200 g, 96%; mp 65.2°C). IR ($CHCl_3$): 3475, 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$+DMSO-d_6): δ 0.85 (t, 3H), 1.3–1.6 (m, 2H), 3.0 (br s, 1H), 3.4–3.6 (m, 4H), 3.8 (m, 1H), 5.7 (br s, 1H), 7.3 (m, 5H). ^{13}C NMR ($CDCl_3$+DMSO-d_6, 200 MHz): δ 10.03, 23.49, 42.89, 52.45, 63.31, 126.14, 127.95, 128.55, 135.47, 170.59. Anal. calcd for $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76; found C, 69.32; H, 8.13; N, 6.53.

4.3. Enzymatic hydrolysis

The racemic 2-[*N*-(phenylacetyl)amino]-1-butanol **3** (103 g, 0.5 mol) was dissolved in 1 l distilled water by warming, the pH was adjusted to 7.8 with 2N ammonia and immobilised penicillin G acylase enzyme (35 g, 150 units/g) was added. The reaction mixture was stirred at 75–80 rpm with an overhead stirrer at 30°C. The conversion during the hydrolysis was followed by HPLC. When the hydrolysis was 40% complete (35 min), the enzyme was filtered from the reaction mixture, washed with water and recycled several times without loss of activity over one week.

4.4. Recovery of (S)-2-amino-1-butanol **1**

Unreacted amide was extracted with ethyl acetate (3×250 ml) and the reaction mixture was then acidified to pH 2.5 with 4N HCl. Phenylacetic acid formed during the reaction was extracted with ethyl acetate (3×250 ml). The aqueous solution was concentrated by distillation of water at atmospheric pressure and residual water was removed by azeotropic distillation with benzene. Finally, the residue was treated with solid KOH until the crude product separates out, which was then distilled under vacuum to yield (*S*)-2-amino-1-butanol **1** (15.5 g, 70%). $[\alpha]_D^{25}$ +10 (neat) [lit.[19] $[\alpha]_D^{25}$ +9.8 (neat)]; e.e. >99%, determined by chiral HPLC of its *N*-phenylacetyl derivative; $[\alpha]_D^{25}=-36.4$ (*c* 4, methanol); lit.[15] $[\alpha]_D^{25}=-34$ (*c* 4, methanol).

4.5. Recovery of (R)-2-[N-(phenylacetyl)amino]-1-butanol **4**

The ethyl acetate layer was washed with 10% Na_2CO_3 solution to remove traces of phenylacetic acid. The organic layer was dried over magnesium sulphate and evaporated to collect unreacted (*R*)-*N*-phenylacetyl 2-amino-1-butanol **4** (60.5 g, 98%); e.e. 70%. $[\alpha]_D^{25}=+25.4$ (*c* 4, methanol).

4.6. Determination of course of hydrolysis

Hydrolysis of the amide **1** was followed by reverse phase HPLC. Column: C8, 5×250 mm, Chrompack, The Netherlands; detection wavelength: 254 nm; mobile phase: 30% acetonitrile in water containing 1 ml of 70% perchloric acid in 1 l; flow rate: 1 ml/min; retention times: 2-[*N*-(phenylacetyl)amino]-1-butanol 5.1 min; phenylacetic acid 5.7 min.

*4.7. Determination of optical purity of 2-amino-1-butanol **1***

Enantiomeric excess of the aminoalcohol was determined by HPLC analysis of the phenylacetyl derivative using a chiral HPLC column (Chiralcel OJ, 5×250 mm, Daicel Chemical Industries Ltd, Japan). Detection wavelength: 254 nm; mobile phase: 10% isopropanol–90% hexane; flow rate: 0.4 ml/min; retention times: *S* 14.2, *R* 20.1 min.

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