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An efficient preparative scale resolution of 3-phenylbutyric acid by lipase from *Burkholderia cepacia* (Chirazyme L1)

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Abstract

Lipase from *Burkholderia cepacia* (Chirazyme L1) catalysed the highly enantioselective hydrolysis of racemic methyl 3-phenylbutyrate to afford (*R*)-(−)-methyl 3-phenylbutyrate of >98% ee (E>50). The resolution was performed at 150 g scale yielding 68.7 g of (*R*)-(−)-methyl 3-phenylbutyrate (>98% ee, 92% yield on enantiomer) and (*S*)-(+)-3-phenylbutyric acid of 89% ee.

1. Introduction

β-Aryl carboxylic acids are valuable synthetic intermediates for the preparation of a range of compounds of biological interest. 3-Phenylbutyric acid was used in the synthesis of ar-juvabione, the aryl analogue of juvabione, a sesquiterpene possessing juvenile hormone activity.[1] (−)-Malyngolide, a δ-lactone antibiotic of algal origin, was prepared in enantiomerically pure form from (*R*)-(−)-3-phenylbutyric acid.[2] All four stereoisomers of β-methylphenylalanine have been prepared in high enantiomeric purity from (*R*)- and (*S*)-3-phenylbutyric acid.[3] β-Aryl carboxylic acids have also been used for the preparation of chiral 3-alkylindanones and their corresponding dihydrocoumarins.[4]

The preparation of enantiomerically enriched 3-phenylbutyric acid has been achieved using a number of approaches. The asymmetric hydrogenation of β-methylcinnamic acid using BINAP–ruthenium(II) complexes afforded (*S*)-(+)-3-phenylbutyric acid in 85% ee.[5] Alternatively, the asymmetric conjugate addition of organometallic reagents to chiral α,β-unsaturated amides,[4,6] esters[7] and vinyl sulfoximines[8] has yielded both enantiomers of 3-phenylbutyric acid in high enantiomeric excess. (*S*)-(+)-3-Phenylbutyric acid has also been obtained by enantioselective hydrolysis of racemic 3-phenylbutyronitrile by *Mycobacterium* strain A2777.[9]

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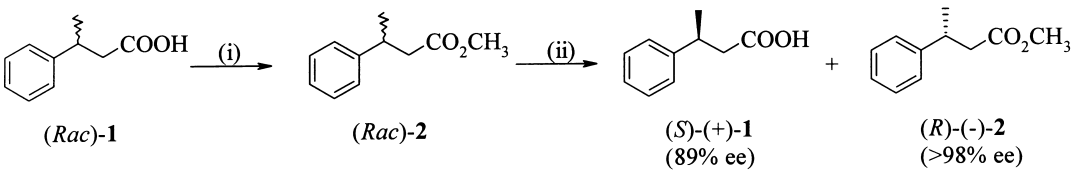
Here we describe the development of an efficient preparative scale resolution of 3-phenylbutyric acid **1** by lipase catalysed enantioselective hydrolysis of the corresponding methyl ester **2** (Scheme 1).



Scheme 1. Reagents: (i) methanol, cat. H_2SO_4, reflux, (ii) *Burkholderia cepacia* lipase (Chirazyme L1), 5 mM sodium phosphate buffer, pH 7.6, 25°C

2. Results and discussion

To identify enzymes catalysing the hydrolysis of (*rac*)-**2** a broad screen of over 60 commercially available lipases, esterases and proteases was performed using a simple protocol. Twenty of the lipases and esterases catalysed the hydrolysis of the substrate to varying extents, however none of the proteases were found to hydrolyse the substrate. Enzymes showing an appreciable degree of hydrolysis were investigated further in an autotitrator. Reactions were stopped at close to 50% conversion at which point unhydrolysed **2** was isolated. The enantioselectivity of each enzyme was calculated from the extent of conversion obtained by titration and the enantiomeric purity of unreacted **2** determined by chiral phase HPLC (Table 1).

Lipases from *Burkholderia cepacia*, *Pseudomonas* sp., *Pseudomonas fluorescens* and *Chromobacterium viscosum* all exhibited high enough enantioselectivity to be of practical use for the resolution of (*rac*)-**2**. However from a consideration of reaction rates and enzyme cost the lipase from *Burkholderia cepacia* was selected as the most appropriate for preparative scale use. Further developments focused on establishing the effect of enzyme concentration, substrate concentration and temperature on the performance of the resolution reaction.

In view of the fact that enzyme cost can be an important factor in the overall economics of a resolution process the effect of reducing the concentration of enzyme was studied. A series of resolution reactions were performed at a fixed (*rac*)-**2** concentration of 6.6 g L^{-1} and enzyme concentrations in the range 1.33

Table 1
Enantioselectivity of lipase and esterase catalysed hydrolysis of (*Rac*)-**2**

Entry	Enzyme	Time (min)	Conversion (%)	E.e.(%)-**2** (Configuration)	E^{10}
1	*Burkholderia cepacia* lipase[a]	230	54	>98 (*R*)	>50
2	*Pseudomonas* sp lipase[b]	1342	37	55 (*R*)	53
3	*Pseudomonas* sp lipase[c]	150	60	>98 (*R*)	>20
4	Porcine liver esterase (fraction 1)[d]	440	42	17 (*R*)	2
5	Porcine liver esterase (fraction 2)[e]	190	53	21 (*R*)	2
6	Porcine liver acetone powder[f]	300	64	20 (*R*)	1
7	*Pseudomonas fluorescens* lipase[g]	1090	56	>98 (*R*)	>34
8	*Chromobacterium viscosum* lipase[h]	1300	51	>98 (*R*)	>153
9	*Candida antarctica* lipase[i]	1240	62	18 (*R*)	2
10	*Aspergillus oryzae* lipase[j]	1300	77	30 (*R*)	2

[a]Chirazyme L1, [b]Chirazyme L4, [c]Chirazyme L6, [d]Chirazyme E1, [e]Chirazyme E2 (Chirazyme® Boehringer Mannheim), [f]Sigma, [g,h]Biocatalysts, [i]SP435, [j]SP524 (Novo Nordisk). Reactions were performed with 6.6 g L^{-1} (*Rac*)-**2** and 1.33 g L^{-1} enzyme (20% w/w wrt (*Rac*)-**2**) in 5mM sodium phosphate buffer, pH 7.6 at 25°C.

g L^{-1} to 0.166 g L^{-1}. A resolution time of approximately 22 h was observed at an enzyme concentration of 0.166 g L^{-1}, giving a substrate to catalyst ratio of 40:1 and an 8-fold reduction in enzyme usage. The efficiency of the resolution was further improved by increasing the concentration of (*rac*)-**2** from 6.6 to 50 g L^{-1}, a 7.5-fold increase in volume productivity. At a 40:1 substrate to catalyst ratio the resolution time was 21 h and the high degree of enantioselectivity was preserved.

In order to counteract the inevitable increase in reaction time resulting from a decrease in enzyme concentration the effect of temperature on the rate and enantioselectivity of the reaction was studied (Table 2).

Table 2
Effect of temperature on the rate and enantioselectivity of *Burkholderia cepacia* lipase catalysed resolution of (*Rac*)-**2**

Entry	Temperature (°C)	Initial rate of hydrolysis (μmoles min^{-1})	Conversion (%)	E.e (%)-**2** (Configuration)	Reaction time (h)
1	25	1.9	51	98 (*R*)	21
2	30	2.5	52	91 (*R*)	22
3	35	2.45	52	87 (*R*)	23

A 5°C rise in reaction temperature from 25 to 30°C produced an increase in initial rate of hydrolysis approximately in line with that expected from $Q_{10} \approx 2$. Although the initial rate of hydrolysis was seen to increase this was not reflected in a reduction in the overall resolution time. Indeed, a trend towards longer total resolution times at increasing temperature was apparent suggesting that the enzyme is not stable to elevated temperatures. The data obtained at 35°C further supports this view. Moreover, the increase in temperature led to a progressive decrease in the enantioselectivity of the resolution reflected in a decline in enantiomeric purity of unreacted **2** at equivalent extents of conversion. This can be rationalised on the basis of increased conformational flexibility of the enzyme at elevated temperatures allowing binding and hydrolysis of (*R*)-**2** by the enzyme.

The defined reaction conditions were applied on a preparative scale (150 g (*rac*)-**2**) allowing the isolation of 68.7 g of (*R*)-**2** (92% isolated yield on enantiomer) of >98% ee (Figs 1 and 2) and >98% chemical purity. The hydrolysis product, (*S*)-**1** was not quantitatively recovered from the resolution reaction however, isolation of a small sample followed by conversion to its methyl ester (*S*)-**2**, indicated it to be of 89% ee.

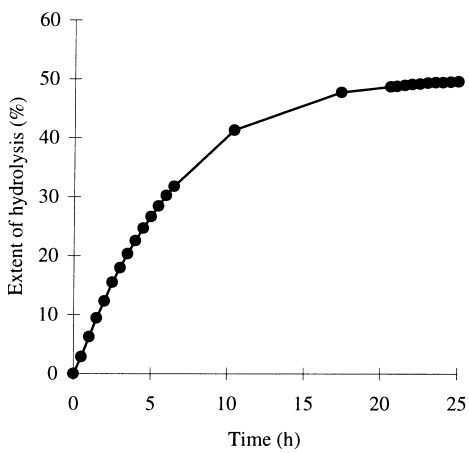
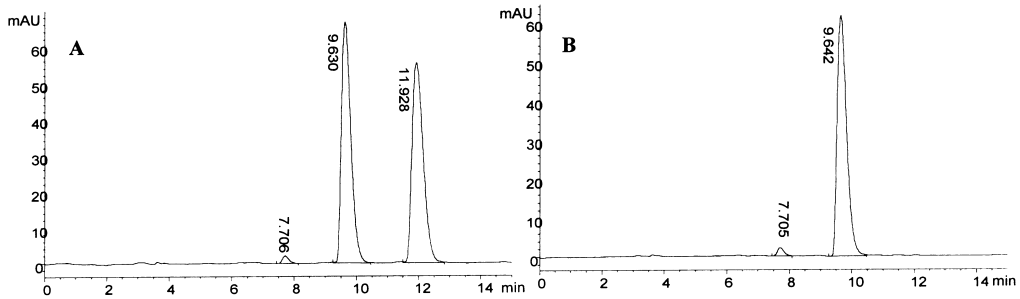


Fig. 1. Extent of hydrolysis of (*Rac*)-**2** by *Burkholderia cepacia* lipase as a function of time



Chromatogram A: (*Rac*)-**2**; Chromatogram B: (*R*)-**2**

Fig. 2. Chiral phase HPLC analysis of (*Rac*)-**2** and (*R*)-**2** obtained by lipase catalysed resolution

In conclusion, we have developed a simple and efficient resolution of 3-phenylbutyric acid using a commercially available lipase. The process has been operated on a preparative scale allowing access to both enantiomers in high enantiomeric purity. The high degree of enantioselectivity observed (E>50) indicates that the enantiomeric purity of the (*S*)-enantiomer could be significantly improved by terminating the reaction at approximately 45% conversion.

3. Experimental

^{1}H NMR spectra were recorded on a Jeol FX90Q spectrometer in $CDCl_3$. Optical rotations were measured on a POLAAR 20 automatic polarimeter (Optical Activity Limited). GC was performed on a Perkin–Elmer 8500GS system equipped with DB5 column (30 m×0.328 mm I.D. capillary column, J&W Scientific) using a helium carrier gas at 8 psi (split volume 53 ml min^{-1}) and a temperature gradient of 100°C rising to 200°C at a rate of 20°C min^{-1}. Retention times of **1** and **2** were 8.04 and 7.32 minutes respectively. Chiral phase HPLC was performed on a Hewlett Packard HP1050 system equipped with a Chiralcel OJ column (25 cm×4.6 mm I.D. analytical column and 5 cm×4.6 mm I.D. guard column, Daicel Industries Limited) eluted with hexane:2-propanol (90:10) at a flow rate of 1 ml min^{-1}. Compounds were detected by UV absorbance at 260 nm. Retention times of (*R*)- and (*S*)-**2** were 9.55 and 11.54 minutes respectively. TLC was performed on precoated silica gel plates (Merck) eluted with hexane:ethyl acetate (90:10). R_f values of **1** and **2** were 0.19 and 0.47 respectively.

*3.1. (*rac*)-Methyl 3-phenylbutyrate (2)*

(*rac*)-3-Phenylbutyric acid (180 g, 1.097 mol) was heated at reflux in methanol (500 ml) containing catalytic H_2SO_4. On completion of the reaction the solution was cooled and the methanol evaporated. The residue was taken up in heptane (250 ml) and washed successively with 10% sodium bicarbonate and water. The organic phase was dried over anhydrous sodium sulphate and evaporated to afford (*rac*)-**2** as a pale yellow oil (182.4 g, 93%) of >98% chemical purity (GC).

3.2. Enzyme screening

Screening reactions were performed in 5 ml of 50 mM sodium phosphate buffer, pH 7.6 containing (*rac*)-**2** (50mg) and enzyme (10 mg). Reactions were incubated at ambient temperature with stirring for 18 h. Hydrolysis of the substrate was detected by TLC.

3.3. Titration studies

Reaction profiles for the hydrolysis of (*rac*)-**2** were obtained using a DL-21 autotitrator (Mettler Toledo) using a pH stat function. Reactions were performed in 30 ml of 5 mM sodium phosphate buffer, pH 7.6 containing (*rac*)-**2** (0.2 g) at 25°C. Hydrolysis was initiated by the addition of enzyme (40 mg). The pH was maintained at 7.6 by automatic titration of 0.1 M NaOH solution. Reactions were stopped at approximately 50% hydrolysis and unreacted **2** extracted with ethyl acetate for analysis of enantiomeric purity.

3.4. Preparative scale resolution of (rac)-2 by Burkholderia cepacia lipase

Compound (*rac*)-**2** (150 g, 0.843 mol) and 5 mM sodium phosphate buffer, pH 7.6 (2.8 L) were placed in a temperature controlled reactor at 25°C equipped with pH control. The reaction was initiated by the addition of 3.75 g of enzyme dissolved in 200 ml of the above buffer. On completion unreacted **2** was isolated by extraction with 2×2 L of heptane. The pooled organic extract was dried over anhydrous sodium sulphate and evaporated to afford a slightly turbid pale yellow oil. The oil was taken up in 100 ml of heptane and passed through a bed of basic alumina to remove proteinaceous material followed by filtration through a 0.2 μ PTFE membrane. Removal of the solvent afforded (*R*)-**2** (68.7 g, 92% isolated yield on enantiomer) as a clear pale yellow oil in >98% ee, >98% chemical purity (GC) and $[\alpha]_D^{23}$ −41 (c=0.988, toluene), lit[11] $[\alpha]_D^{20}$ −44 (c=1, benzene).

Acidification of a sample of the aqueous phase with H_2SO_4 followed by extraction with ethyl acetate allowed the isolation of a sample of (*S*)-**1**. The sample was methylated following the general procedure described above to afford (*S*)-**2** as a pale yellow oil in 89% ee, >98% chemical purity (GC) and $[\alpha]_D^{23}$ +38.5 (c=1, toluene).

References

1. Kavitake, B. P.; Desai, U. V.; Desai, D. G.; Mane, R. B. *Indian J. Chem. Sect. B*. **1986**, 25B(2), 178–179.
2. Kogure, T.; Eliel, E. L. *J. Org. Chem*. **1984**, 49, 578–579.
3. Dharanipragada, R.; Nicolas, E.; Toth, G.; Hruby, V. J. *Tetrahedron Lett*. **1989**, 30, 6841–6844.
4. Stephan, E.; Rocher, R.; Aubouet, J.; Pourcelot, G.; Cresson, P. *Tetrahedron: Asymmetry* **1994**, 5, 41–44.
5. Ohta, T.; Takaya, H.; Kitamura, M.; Nagai, K.; Noyori, R. *J. Org. Chem*. **1987**, 52, 3176–3178.
6. Mukaiyama, T.; Iwasawa, N. *Chem. Lett*. **1981**, 913–916; Soai, K.; Machida, H.; Ookawa, A. *J. Chem. Soc., Chem. Commun*. **1985**, 469–470; Soai, K.; Ookawa, A. *J. Chem. Soc., Perkin Trans. 1* **1986**, 759–764; Soai, K.; Machida, H.; Yokota, N. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1909–1914; Palomo, C.; Berree, F.; Linden, A.; Villalgordo, J. *J. Chem. Soc., Chem. Commun*. **1994**, 1861–1862; Davies, S. G.; Sanganee, H. J. *Tetrahedron: Asymmetry*, **1995**, 6, 671–674.
7. Fang, C.; Ogawa, T.; Suemune, H.; Sakai, K. *Tetrahedron: Asymmetry* **1991**, 2, 389–398.
8. Pyne, S. G. *J. Org. Chem*. **1986**, 51, 81–87.
9. JP 05076390 (Asahi Chemical Industries Limited).
10. Chen, C.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc*. **1982**, 104, 7294–7299.
11. See Fluka Chemika catalogue, 1997–98.