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Resolution of 1-phenoxy-, 1-phenylmethoxy- and 1-(2-phenylethoxy)-2-propanol and their butanoates by hydrolysis with lipase B from *Candida antarctica*

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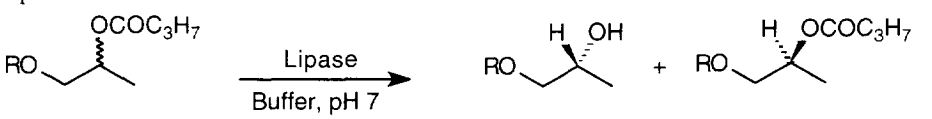
Abstract: Resolutions of butanoic esters of 1-phenoxy-2-propanol, 1-phenylmethoxy-2-propanol and 1-(2-phenylethoxy)-2-propanol have been studied with four different lipases as catalysts. Using lipase B from *Candida antarctica* very high enantiomer ratios were obtained. These substrate-lipase pairs represent an excellent way of getting enantiomerically pure protected 1,2-propanediols in high chemical yield.

INTRODUCTION

Although having the disadvantage of only 50% theoretical yield, lipase catalysed resolution is one of the most powerful methods for production of enantiomerically pure compounds. This relies on the often high degree of enantiospecificity obtained in such resolutions. As more knowledge about enzymes and their interaction with substrates, products, added substances (water, inhibitors etc.) and the bulk solvent is gathered, the choice of catalyst will be more a matter of literature search, modelling and intellectual work, than time consuming trial and error experiments.



(Rac) (**1a-c**) *(R)* (**2a-c**) *(S)* (**1a-c**)

Scheme 1: R= -Ph (**a**), R= -CH_2Ph (**b**), R= -CH_2CH_2Ph (**c**)

As a continuation of previous work on the resolution of various derivatives of glycerol using lipase B from *Candida antarctica* as catalyst[2-5] we also wanted to find ways of producing both enantiomers of protected 1,2-propanediols (Scheme 1). Related 1,2-diols have been resolved by others.[6-11] Modelling studies of lipase B with related substrates[12] indicated that the chosen substrates would be excellent for performing resolutions. For the good fitting enantiomer a slight collision of -CH_2OCH_3 with tryptophane 104 was observed, and we anticipated that reducing the size of this group to -CH_3 would increase the enantiomer ratio of the resolution.

Enantiomerically pure C-3 compounds are of tremendous importance as synthons.[13] 1,2-Propanediols have less value as a starting synthon, however, they have been useful in synthesis of drugs with anti viral activity[14-16] and cardiovascular drugs.[17] Enantiomerically pure 1,2-propandiols can also be used for production of epoxypropane, as part of enantiomerically pure crown ethers,[18] and may be used as part of chiral polymers,

chromatography columns etc. These alcohols could also be useful as derivatising agents for chiral acids for analyses of enantiomeric excess on achiral columns or by NMR.

RESULTS AND DISCUSSION

The presence of the protecting groups suit two purposes. Firstly they affect the enzyme in the chiral recognition process.[19-20] Secondly the protecting groups may also be important in terms of regioselectivity for further reaction of the synthons. The faster reacting enantiomer was the *(R)*-enantiomer as predicted by the model proposed by Kazlauskaz,[21] and as observed in resolution of similar substrates.[3-5,22-25] Recent studies of resolution of long chain 2-alkanols and 1,1,1-trifluro-2-alkanols do not correspond with the mentioned model.[26] This is probably caused by special packing of the long alkyl chains. The conversion was measured both with pH-stat and using $\xi = ee_s/ee_s+ee_p$. The use of pH-stat was however inaccurate, and the latter method was preferred. The *E*-values were determined both by the method of Rakels *et al.*[27] and by two data based programs.[28-29] In the tables below are shown the *E*-values derived from minimisation of several points. The enantiomeric excess data are only given for the experimental points nearest 50% conversion.

For substrate **1a-b** different lipases were tried, but their use were hampered by different factors. PPL showed only modest specificity for resolution of **1a** (*E*=7), and for **1b** there was hardly any detectable selectivity at all. In resolution of **1a** the lipase form *Rhizomucor miehei* showed high selectivity at the start of the reaction, but it was impossible to reach a reasonable chemical yield and enantiomeric purity of the substrate because of low reaction rate and a decline of specificity as time passed. The lipase did probably not "survive" the stirring conditions of the resolution.[30]

Table 1. Lipase catalysed resolution of **1a**

Lipase	*E*	ee_p	ee_s	ζ, %	Time
Amano PS	>900	98	99	50	8 h.
Rhizomucor miehei	-	99	47	31	4 days
Rhizomucor miehei (same as above)	-	84	61	42	6 days
PPL	7	63	46	42	3 days

Table 2. Lipase catalysed resolution of **1b**

Lipase	*E*	ee_p	ee_s	ζ, %	Time
Amano PS	164	95	97	50	24 h
Rhizomucor miehei	-	80	10	11	6 h
Rhizomucor miehei (same as above)	-	48	17	25	1 day
PPL	None	7	6	49.5	6 h

The lipase from *Pseudomonas cepacia* (Amano PS) showed excellent enantiospecificity in resolution of the substrates **1a-b**. The *E*-value was >900 for R=Ph and >100 for R=CH_2Ph, but the

reaction rate was significantly lower than for lipase B from *Candida antarctica*. The best results for the resolution of **1a-c** were obtained with lipase B from *Candida antarctica* as the catalyst. In Table 3 the results from the three resolutions are presented. As can be seen the enantiomeric excess of the product and starting material was excellent at 50% conversion in all cases, leading to almost 50% yield of each enantiomer which is rare in such resolutions.

Table 3. Resolution of propanediols **1a-c** with lipase B as catalyst

Substrate, R:	ee_s %	ee_p %	ζ, %	*E*
-Ph	99	99	50	>900
$-CH_2Ph$	97	97	50	>200
$-CH_2CH_2Ph$	97	98	50	>600

In figure 1 enantiomeric excesses of product and substrate are plotted against conversion for resolution of the butanoate of 2-phenoxy-2-propanol (**1a**). As can be seen no points are present beyond 50% conversion.

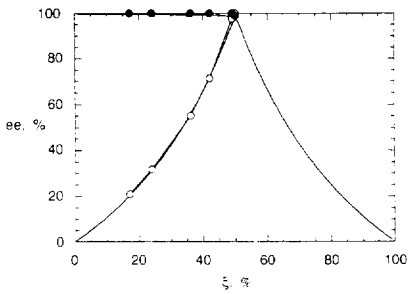


Figure 1. Resolution of racemic 2-phenoxy-2-propyl butanoate (**1a**). Enantiomeric excess of product and substrate plotted *vs.* conversion, ξ.

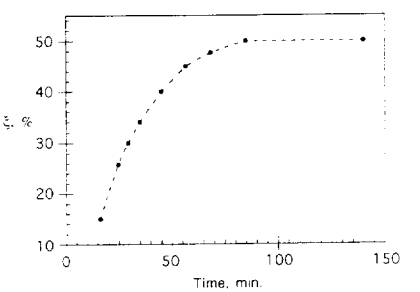


Figure 2. Hydrolysis of racemic 2-phenoxy-2-propyl butanoate (**1a**). Conversion is plotted *vs.* time. Conditions: **1a** (0.5 mmol), phosphate buffer 0.05 M (35 mL, pH 7.0), immobilized lipase B (3 mg).

This is another characteristic of these resolutions. After the good fitting enantiomer had reacted no further reaction took place. This effect is visualised in figure 2. In this resolution 50% conversion was reached after 84 min., during the next hour no further reaction was detected. For resolution of **1b** the reaction seemed to stop before 51% conversion was reached. The bad fitting enantiomer *(S)* probably function as a dead-end inhibitor.[31] This is very convenient since it is not necessary to monitor the reaction in order not to get contamination of the undesired enantiomer. This was demonstrated by performing semi quantitative resolution with substrates **1a-c**. Without checking the ee versus conversion, esters and alcohols could be obtained in the same enantiomeric purity as in the small scale experiments just by allowing the reactions to stir over night. The reaction rate until 50% conversion was also good. Without taking mass transfer limitations into account, **1a** reacted approximately 45 times faster than the butanoate of 1-phenoxy-3-methoxy-2-propanol (E=22).[3]

EXPERIMENTAL

Enzymes. Lipase B from *Candida antarctica* and *Rhizomucor miehei* were gifts from Novo-Nordisk A/S. Lipase Amano PS (*Pseudomonas cepacia*) was a gift from Amano Enzyme Europe Ltd. PPL type II was purchased from Sigma(Sigma L-3126). Lipase B (EC 3.1.1.3) from *Candida antarctica* (SP 435) Novo-Nordisk, immobilised on Lewatit, had specific activity of 19000 PLU/g.

Analytical methods. Chiral analyses were performed using a Varian 3400 gas chromatography with a CP-Chiracil-DEX-CB from Chrompack. 1-phenoxy-2-propanol **2a** and its butanoate **1a** were analysed without derivatisation in the same run, temp. prog. 100-142 °C, 1°/min. Chromatographic parameters for **2a** were: R_{t1}: 18.99 min *(S)*, R_{t2}:19.61 min *(R)*, R_S 1.36. For the butanoate **1a:** R_{t1}: 38.04 *(S)*, R_{t2}: 38.93 *(R)*, R_S 2.95. 1-Phenylmethoxy-2-propanol **2b** and 1-(2-phenylethoxy)-2-propanol **2c** were analysed as their acetates, in the same run as their butanoates **1b-c**, temp. prog. for **1b** and **2b** 120-150 °C, 1°C/min., for **1c** and **2c** 125-155 °C, 1 °C/min. Chromatographic parameters for **2b** were: R_{t1}: 16.56 min. *(S)*, R_{t2}:17.70 min. *(R)*, R_S 3.93. For the butanoate **1b** R_{t1}: 27.61 *(S)*, R_{t2}: 28.22 *(R)*, RS: 2.04. For **2c**: R_{t1}: 16.59 min. *(S)*, R_{t2}:17.37 min. *(R)*, R_S 2.60. For the butanoate **1c** R_{t1}: 26.12 *(S)*, R_{t2}: 26.54 *(R)*, R_S 1.55.

Alcohols ***2a-c*** were prepared in a two step sequence. In the first step glycidyl ethers were synthesized according to Takano *et al.*,[32] with prolonged reaction time (18-24 h) and a slight (1.3 times) excess of epichlorohydrin. The products were isolated by bulb to bulb distillation *in vacuo*, yield: 72-85%. In the second step the glycidyl ethers were added to a solution of anhydr. THF cooled in an ice bath. To this stirred solution excess $LiAlH_4$ was added in portions, and the reaction was stirred over night at room temp. The reaction was quenched with a saturated solution of NH_4Cl. THF was removed *in vacuo*, and the residue extracted with Et_2O, and washed with NaCl soln. and water. After drying over anhydr. $MgSO_4$, and concentration of the organic fraction, the crude product was bulb to bulb distilled *in vacuo*, yield: 85-90%.

Preparation of butanoates **1a-c** *from alcohols* **2a-c** have been described earlier.[3]

NMR experiments were preformed using Bruker DPX 300 and 400. Chemical shifts are given in ppm rel. to TMS and coupling constants in Hz. Both one dimentional ^1H, ^{13}C, and 2D ^1H-^1H and ^1H-^{13}C COSY experiments were performed.

1-Phenoxy-2-propanol **2a**: ^1H-NMR: 6.85-7.35 (m, aromatic), 2.50 (OH), $ABMX_2$ system for $-CH_2CH(OH)CH_3$. 3.92 (1H), 3.78 (1H), 4.19 (1H), 1.28 (3H). J_{AB} = 9.3 Hz, J_{AM} = 3.4 Hz, J_{BM} = 8.0 Hz and J_{XM} = 6.4 Hz. ^{13}C-NMR: 19.0 (q), 66.1 (d), 73.1 (t), 114.5 (2d), 120.9 (d), 129.5 (2d), 158.6 (s).

Butanoate of 1-Phenoxy-2-propanol **1a**. ^1H-NMR: 6.88-7.30 (m, aromatic), $ABMX_3$ spektrum for $-CH_2CH(OCOC_3H_7)CH_3$, 4.01 (1H), 3.97 (1H), 5.25 (1H), 1.35 (3H). J_{AB}=9.8 Hz, J_{BM}=4.6 Hz, J_{AM}=5.9 Hz og J_{MX}=6.3 Hz. For $-OCOC_3H_7$, 2.29 (t, 2H), 1.67 (m, 2H), 0.95 (t, 3H). ^{13}C-NMR: 13.6 (q), 16.8 (q), 18.5 (t), 36.4 (t), 68.6 (d), 69.9 (d), 114.6 (2d), 120.9 (d), 129.5 (2d), 158.6 (s), 173.1 (s).

1-Phenylmethoxy-2-propanol **2b**. ^1H-NMR: 7.25-7.40 (m, aromatic), 4.56 (2H, benzylic), 2.40 (1H, -OH), $ABMX_3$ for $-CH_2CH(OH)CH_3$: 3.47 (1H), 3.29 (1H), 4.00 (1H), 1.15 (3H). J_{AB} = 9.3 Hz, J_{AM} = 3.4 Hz, J_{BM} = 7.3 Hz og J_{XM} = 6.6 Hz. ^{13}C-NMR: 19.0 (q), 66.2 (d), 73.1 (t), 75.9 (t),127.6 (d), 127.7 (2d), 128.3 (2d), 138.1 (s).

Butanoate of 1-Phenylmethoxy-2-propanol **1b**. ^1H-NMR: 7.20-7.37 (m, aromatic), $ABMX_3$ system for $-CH_2CH(OCOC_3H_7)CH_3$: 3.51 (1H), 3.47 (1H), 5.13 (1H), 1.24 (3H). J_{AB}=10.5 Hz, J_{AM}=5.9

Hz, J_{BM}=4.4 Hz and J_{XM}=6.4 Hz. AB system for benzyl, 4.57 (1H), 4.53 (1H), J_{AB}=12.2 Hz. For -$OCOC_3H_7$, 2.29 (t, 2H), 1.66 (m, 2H), 0.95 (t, 3H). ^{13}C-NMR: 13.6 (q), 16.7 (q), 18.5 (t), 36.4 (t), 69.0 (d), 72.5 (t), 73.0 (t), 127.5 (2d), 127.6 (d), 128.3 (2d), 138.2 (s), 172.9 (s).

1-(2-Phenylethoxy)-2-propanol **2c**. ^{1}H-NMR: 7.19-7.33 (m, aromatic), 2.29 (OH), $ABMX_3$-system for -$CH_2CH(OH)CH_3$: 3.45 (1H), 3.22 (1H), 3.94 (1H) and 1.12 (3H). J_{AB}=9.4 Hz, J_{AM}=3.0 Hz, J_{BM}=8.3 Hz and J_{XM}=6.4 Hz. ABX_2-system for -OCH_2CH_2Ph: 3.72 (1H), 3.68 (1H) og 2.90 (2H). J_{AB}=9.4 Hz, J_{AX}=7.1 Hz and J_{BX}=7.0 Hz. ^{13}C-NMR: 19.2 (q), 36.7 (t), 66.7 (d), 72.6 (t), 76.9 (t), 126.7 (d), 128.8 (2d), 129.3 (2d), 139.3 (s).

Butanoate of 1-(2-Phenylethoxy)-2-propanol **1c**. ^{1}H-NMR: 7.16-7.30, (m, aromatic). ABX_2-system for -OCH_2CH_2Ph: 3.69 (1H), 3.63 (1H), 2.87 (2H), J_{AB}= 9.4 Hz, J_{AX}= 6.7 Hz og J_{BX}= 7.0 Hz. $ABMX_3$-system for -$CH_2CH(OCOC_3H_7)CH_3$: 3.48 (1H), 3.43 (1H), 5.07 (1H), 1.20 (3H), J_{AB}= 10.5 Hz, J_{AM}= 5.8 Hz, J_{BM}= 4.5 Hz og J_{XM}= 6.6 Hz. For -$OCOC_3H_7$: 2.26 (t, 2H), 1.63 (m, 2H) og 0.94 (t, 3H). ^{13}C-NMR: 14.0 (q), 17.1 (q), 18.9 (t), 36.7 (t), 36.8 (t), 69.4 (d), 72.6 (t), 73.7 (t), 126.6 (d), 128.7 (2d), 129.3 (2d), 139.0 (s), 173.5 (s).

Absolute configurations: The configuration of **2a** was verified by comparison with earlier work,[33] **2b** was compared with the *(S)*-enantiomer synthesised from enantiomerically pure *(R)*-epichlorohydrin(Daiso Co. Ltd., Osaka, Japan), and **2c** was identified by comparison with the elution order of **2a** and **2b**. The three *(S)*-butanoates *(S)*-**1a-c** were hydrolysed to yield the corresponding *(S)*-alcohols. Specific rotations (Optical Activity Ltd. AA-10, Automatic polarimeter). *(R)*-alcohols: **2a** $[\alpha]_D^{25}$ = +2.8 (c 1.00, EtOH), $[\alpha]_D^{22}$ = -23.0 (c 2.44, $CHCl_3$), **2b** $[\alpha]_D^{22}$ = -10.8 (c 2.66, $CHCl_3$), **2c** $[\alpha]_D^{22}$ = -15.6 (c 3.04, $CHCl_3$), *(S)*-alcohols **a** $[\alpha]_D^{25}$ = -2.7 (c 1.00, EtOH), $[\alpha]_D^{22}$ = +20.2 (c 1.70, $CHCl_3$), **b** $[\alpha]_D^{22}$ = +11.0 (c 3.18, $CHCl_3$) and **c** $[\alpha]_D^{22}$ = +13.2 (c 3.04., $CHCl_3$).

General experimental procedure enzymatic hydrolysis with lipase B. The butanoates **1a-c** (0.5 mmol) were suspended in 0.05 M phosphate buffer (35 mL, pH 7.0). The lipase (10 mg) was added and the reaction stirred at room temp. The enzymatic hydrolysis was monitored by a pH-stat consisting of a Radiometer PHM 64 pH meter and a Metrohm Dosimat pump. Addition of 0.1N NaOH solution was controlled by a Copam PC. Samples were withdrawn for each 10% conversion, The hydrolysis was stopped by repeated extraction with Et_2O, and the reaction mixture analysed by the above mentioned methods.

Reactions with the other lipases were performed by suspending substrate (0.25 mmol) in reaction vials with 0.05 M phosphate buffer (15 mL, pH 7.0). The reactions were started by adding lipase: Amano PS (5 mg), *Rhizomucor miehei* (30 mg), and PPL (40 mg). The reactions were stirred in room temp. without pH-stat control.

Typical procedure for semi preparative resolution with lipase B. The butanoates **1a-c** (11.3 mmol) were suspended in a beaker with 0.1 M phosphate buffer(400 mL, pH 7.0). Lipase (50 mg) was added and the reaction stirred at room temp. over night without pH-stat control. The hydrolysis was stopped by repeated extraction with Et_2O, and the reaction mixture analysed by the above mentioned methods. Analysis showed that the reaction had reached 50% conversion.

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