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The enantiomer ratio strongly depends on the alkyl part of the acyl donor in transesterification with lipase B from *Candida antarctica*.

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Abstract: Three secondary alcohols, 1-phenoxy-, 1-phenylmethoxy- and 1-(2-phenylethoxy)-2-propanol, have been resolved by transesterification with the acyl donors 2-chloroethyl butanoate, 2,2,2-trichloroethyl butanoate, vinyl butanoate and butanoic anhydride using lipase B from *Candida antarctica* as catalyst in hexane. The enantiomer ratio *E*, which was calculated on the basis of a ping-pong bi-bi mechanism, was highest when 2-chloroethyl butanoate was used as acyl donor, however the reaction was reversible. It was shown that the *E*-value increased when the amount of 2,2,2-trichloroethyl butanoate was reduced. It is also indicated that butanoic anhydride and vinyl butanoate make the lipase less specific probably by acylation. Copyright © 1996 Elsevier Science Ltd

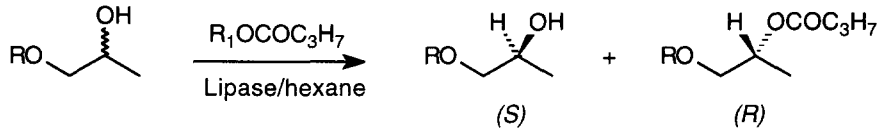
INTRODUCTION

Results obtained by hydrolysis of *n*-alkanoic esters with chiral alkyl parts indicate that butanoates give the highest enantiomer ratios.[2] Moreover, transesterifications of cyclopentenyl esters using vinyl esters as the acylating agents, highest *E*-values were obtained using butanoates.[3] Transesterifications using acyl donors with a stereocentre in the acyl part will proceed via two diastereomeric acyl enzymes. Thus the structure of the acyl donor obviously will influence the stereochemical result of the reaction. However, there is no obvious reason why the alkyl part of the acyl donor should influence the enantiospecificity of a resolution since the reaction will proceed via the same acyl enzyme. Previous reports indicate that 2,2,2-trifluoroethyl butanoate was a more specific acyl donor than butanoates with other alkyl parts in transesterifications of 2-octanol catalysed by PPL.[4] 2-Octanol has also been resolved using different esters of octanoic acid as acyl donors.[5] Sulcatol has been resolved in diethyl ether with PPL using 2,2,2-trifluoroethyl laurate, other acyl groups were less favourable.[6]

Resolution of chiral glycerol derivatives have due to their potential as chiral building blocks, been much in focus.[7] We have previously concentrated on hydrolysis for this purpose[8,9] and have had excellent results using cosolvents.[10]

RESULTS AND DISCUSSION

During our search for optimized transesterification conditions of 1-phenoxy-, 1-phenylmethoxy- and 1-(2-phenylethoxy)-2-propanol, we screened four different acyldonors, 2-chloroethyl butanoate, 2,2,2-trichloroethyl butanoate, butanoic anhydride and vinyl butanoate, all forming the same acyl enzyme. For the transesterifications of substrates **1-3** with these acyl donors (Scheme 1) we have calculated enantiomer ratios, *E* and equilibrium constants, K_{eq} on the basis of ping-pong bi-bi kinetics[11](Table 1).



Scheme 1: R= Ph (**1**), CH_2Ph (**2**), CH_2CH_2Ph (**3**): R_1= -CH_2CH_2Cl, -CH_2CCl_3, -COC_3H_7, -$CH{=}CH_2$

Table 1. Transesterifications of **1**, **2** and **3** with different acyl donors, 5 times excess of acyl donor.

	1		**2**		**3**	
Acyldonor	*E*	K_{eq}	*E*	K_{eq}	*E*	K_{eq}
2-chloroethyl butanoate	139	0.32	22	0.37	319	0.16
2,2,2-trichloroethyl butanoate	26	**	3	**	26	4.3
butanoic anhydride	8	*	2	*	25	*
vinyl butanoate	4	*	1	*	7	*

* K_{eq} > 20, ** unreliable values

In Figure 1 are shown the generated curves for **1** and **3**. The reactions with 2-chloroethyl butanoate are very enantiospecific, but they are reversible. Resolutions with the other acyl donors give quasi irreversible reactions, but lower the *E*-values.

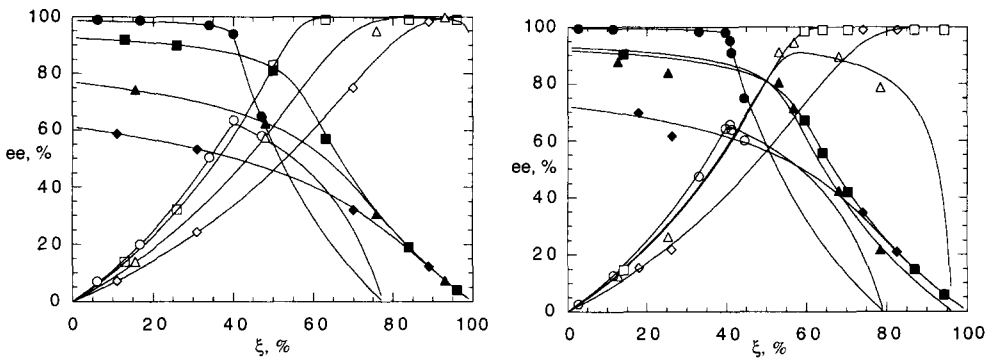


Figure 1. Transesterification of **1**(left) and **3**(right) with different acyldonors, 2-chloroethyl butanoate(O), 2,2,2-trichloroethyl butanoate(□), butanoic anhydride(Δ), and vinyl butanoate.(◊) Filled symbols represent product, open symbols remaining substrate.

Modelling studies[12] of lipase B with related secondary substrates ($RCH_2CHOHCH_2R'$) have shown that the large substituents [R=-$O(CH_2)_nPh$] were directed out of the enzyme. For the good fitting enantiomer the smaller group (R'=-OCH_3) was placed in a pocket near the catalytic site of the enzyme. Therefore it is a bit surprising that the enantiomer ratio was also dependent on the length of the large group. When this group was -Ph(n=0) or -CH_2CH_2Ph(n=2) the *E*-values were >100, while for -CH_2Ph(n=1) it was lower (*E*=22).[8] When R= -Cl a similar trend was observed.[9]

In order to deduce what caused the difference in enantiospecificity between the two chloro activated acyl donors, two sets of experiments were carried out. Firstly transesterifications were performed in the presence of the alcohols liberated from the acyl donor, secondly with varying amounts of acyldonor. The first set of results are presented in Table 2. Different amounts of 2-chloroethanol were added to transesterifications of **1** with 2,2,2-trichloroethyl butanoate and similarly different amounts of 2,2,2-trichloroethanol were used in transesterifications with 2-chloroethyl butanoate as acyl donor.

Addition of chloroalcohols had only a small positive effect on *E* for reactions with both acyldonors. Therefore one may conclude it is not likely that the liberated chloroalcohols are responsible for, at least all of the difference in enantiospecificity between the acyl donors. The values of the equilibrium constants were not included in Table 2 since there are several equilibria involved, and the present version of the program (E&K Calculator) is not able to handle this situation.

Table 2. Transesterification of **1** with 2,2,2-trichloroethyl butanoate in the presence of 2-chloroethanol (left) and with 2-chloroethyl butanoate in the presence of 2,2,2-chloroethanol (right, and fig. 2), 5 times excess of acyl donor.

2-Chloroethanol: Substrate	*E*	2,2,2-Trichloroethanol: Substrate	*E*
0	26	0	130
0.3:1	24	0.3:1	177
1:1	49	1:1	240

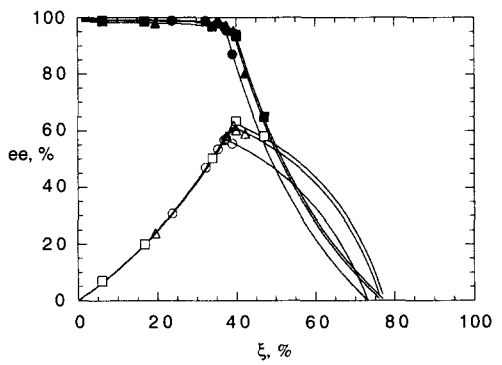


Figure 2. Transesterification of **1** using 2-chloroethyl butanoate in the presence of varying relative concentrations of 2,2,2-trichloroethanol, 0(□), 0.3:1(Δ), 1:1(O), filled symbols represent product, open symbols remaining substrate.

In the second set of experiments transesterifications of **1** were performed with each acyl donor at different concentrations. The results are presented in Tables 3-6. A change of concentration of

2-chloroethyl butanoate had no clear effect on the enantiospecificity.[11] However, reduced concentration of 2,2,2-trichloroethyl butanoate lead to an increase of the enantiomer ratio.(Table 3) This observation indicates that in this case the acyl donor itself is responsible for the difference in E and not the alcohols liberated from the acyl donor in the reaction. However, a low concentration of acyl donor leads to an unfavourable equilibrium position.(Fig. 3)

Table 3. Transesterification of **1** with different concentrations of 2,2,2-trichloroethyl butanoate.

Excess of acyl donor	E	K_{eq}
5	26	**
1	113	4.6
0.6	219	5.0

** unreliable value

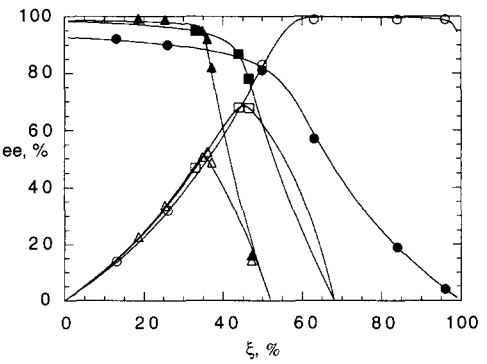


Figure 3. Transesterification of **1** with different concentrations of 2,2,2-trichloroethyl butanoate, 5(O), 1(□) and 0.5(Δ) times excess, filled symbols represent product, open symbols remaining substrate.

Table 4. Transesterification of **1** with different concentrations of butanoic anhydride.

Excess of butanoic anhydride	E	K_{eq}
5	8	**
2	11	*
1.1	9.8	17.8

* $K_{eq} > 20$, ** unreliable value

When the concentration of butanoic anhydride was reduced, the results at first indicated that the E-value was not affected. However, the experimental values of ee_s and ee_p did not fit the curves corresponding to these E-values. Therefore calculation of E by the minimization procedure was performed separately for the first and the second half of experimental values of ee_s and ee_p.(Table 5) The results indicate that the enantiospecificity of the enzyme changed during the reaction. The reason for this seems to be that the relative preference of the enzyme is changed during the reaction. This effect may be due to butanoic acid which is liberated as a

coproduct. Low concentrations of butanoic anhydride will cause lower reaction rate, which in turn will give the butanoic acid more time to modify the enzyme. However, it is clear that in reactions at reduced concentrations of butanoic anhydride, the product can be isolated at low conversions in high enantiomeric yield.

Transesterifications at different concentration of vinyl butanoate showed that reduced concentrations gave higher *E*. The concentration of vinyl butanoate can be reduced considerably without affecting the yield. The results indicate that the acyl donor itself is the reason for the difference in enantiospecificity. There are different ways to explain this observation One is that the acyl donor may function as a cosolvent and change the conformation of the lipase. When 10 times excess of acyl donor was used, its volume corresponds to 6% of the total reaction volume. Such small concentrations of cosolvent do not usually affect changes in the protein conformation,[10] hence the difference in enantiospecificity is probably not due to this effect. A more likely explanation is that the acyl donor modifies the enzyme chemically by acylating amino acid side chains. The fact that the more activated acyl donors give lowest *E*, is an indication of this.

Table 5. Transesterification of **1** with butanoic anhydride as acyl donor.

Excess of butanoic anhydride	# of measure-ments	ξ, %	*E*
2	1-3 out of 6	9.6- 45.2	30
2	4-6 out of 6	59.1-90.7	5
1.1	1-3 out of 7	8.5-34.3	22
1.1	4-7 out of 7	52.7-79.0	7

Table 6. Transesterification of **1** with different concentrations of vinyl butanoate.

Excess of vinyl butanoate	*E*	K_{eq}
5	4	**
3	7	**
1.1	9	10.8

** unreliable values

Conclusion: For production of enantiomerically pure alcohols **1-3** with lipase B from *Candida antarctica* hydrolysis of the corresponding butanoates may be the method of choice since they give extremely high *E*-values.[13] This method may be suitable for production of both unreacted ester and product since enantiomer ratio is so high. If transesterification is preferred for any reason, choice of acyl donor is very important. 2-Chloroethyl butanoate gives the highest enantiomer ratio, however, the reversibility of the reaction must be taken into account. This is clearly evident from Figure 1. A large excess of acyl donor may be helpful, but it remains to see if the lipase is harmed.

The problem with reversibility may be circumvented by using 2,2,2-trichloroethyl butanoate as acyl donor when the substrates are **1** or **3**. By stopping the reaction at 60 % conversion the substrate may be isolated in high enantiomeric purity. (Fig. 1) Another way of performing

resolutions with this acyl donor is by adding the acyl donor as the reaction proceeds. In this way the reacting species that affects the enantiospecificity negatively may be held at a constant low level. For substrate **2** only 2-chloroethyl butanoate gave an acceptable *E*-value.

EXPERIMENTAL

General: Chiral analyses and determination of absolute configurations have been described earlier.[13,14]

Transesterifications: To hexane (3 mL), was added substrate (20 mg) and acyl donor. The reaction was started by adding lipase (20 mg) to the reaction mixture. The reactions were performed in a shaker incubator at 30 °C. The samples were filtered to remove the immobilised enzyme before analysis. Analysis gave ee_s and ee_p-values and from these ξ was determined. $[\xi=ee_s/(ee_s + ee_p)]$

Calculation of E and K_{eq} was based on ping-pong bi-bi kinetics and performed with a computer program described earlier.[11] The program uses 4-6 pairs of ee_s/ee_p measurements, and calculates *E* by mimimization using a Power Macintosh. In situations where the reactions showed no decline in the ee_s curves, reliable K_{eq}-values could not be obtained. In control experiments under the same reaction conditions without enzyme, no acylation was observed.

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