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Kinetic resolution of *rac*-alkyl alcohols *via* lipase-catalyzed enantioselective acylation using succinic anhydride as acylating agent

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ABSTRACT

With succinic anhydride as acylating agent, three commercial lipases – *Candida antarctica* lipase B (CALB), *Pseudomonas cepacia* lipase and *Pseudomonas fluorescens* lipase – were employed in the kinetic resolution of a series of *rac*-alkyl alcohols: 2-butanol, 2-pentanol, 2-hexanol, 2-heptanol, 2-octanol, 3-hexanol, 3-methyl-2-butanol, 6-methyl-5-heptene-2-ol, 3-methyl-2-cyclohexene-1-ol and 2-methyl-1-pentanol. The most effective tested enzyme, immobilized CALB, was able to resolve most of the alcohols with high enantioselectivity, even higher (with enantiomeric ratios up to 115 and 91, for 3-hexanol and 3-methyl-2-butanol, respectively) than when vinyl acetate was used as the acylating agent. More importantly, the unreacted alcohol and the monoester succinate produced could be easily separated by a simple aqueous base-organic solvent liquid–liquid extraction. Using succinic anhydride as acylating agent and CALB, enantiomerically pure (*S*)-2-pentanol with 99% *ee* and (*R*)-2-pentanol with 95% *ee* were prepared in gram-scale reactions.

1. Introduction

Lipase-catalyzed kinetic resolution has proved to be an efficient technique for the preparation of optically enriched compounds [1]. For instance, chiral alkyl alcohols, which are important organic synthetic intermediates, have been obtained through lipase-catalyzed resolution of their racemates *via* esterification, transesterification and hydrolysis of the esters. The acylating agents employed in lipase-catalyzed resolution of *rac*-alcohols substantially affect the enantiomeric ratios (*E* values), the ease of separating the products from the unreacted substrates, and thus the cost-effectiveness of the process [2,3]. Enol esters have been the most widely used acylating agents for resolving *rac*-alcohols to date, due to their high reactivity, irreversibility and enantioselectivity [4–6]. However, the only published procedure for separating the unreacted alcohols and the esters produced when enol esters are used as acylating agents is silica-gel column chromatography, which consumes large quantities of organic solvents. A potential solution is to use cyclic anhydrides as acylating agents [7]. The acid-esters produced can then be easily separated from the unreacted alcohols by simple aqueous base-organic solvent liquid–liquid extraction [3]. More importantly, alcohols of higher optical purity can be obtained since they are not produced as by-products of the reactions [8]. However, to date this technique has mainly been applied in the resolution of *rac*-alcohols bearing bulky and electrophilic groups, such as phenyl or nitro [3,7–14]. To the best of our knowledge, no previous investigations have been published on the resolution of *rac*-alkyl alcohols using cyclic anhydrides as acylating agents and liquid–liquid extraction as a separation method, except for a single report on the resolution of *rac*-lavandulol [15].

In the present study, three commercial lipases – *Candida antarctica* lipase B, *Pseudomonas cepacia* lipase and *Pseudomonas fluorescens* lipase – were employed in the kinetic resolution of a series of *rac*-alkyl alcohols with succinic anhydride as acylating agent, and a simple aqueous base-organic solvent liquid–liquid extraction was used to separate the products from the substrates. Effects of vinyl acetate and succinic anhydride on the enantioselectivity of *C. antarctica* lipase B were also investigated. Furthermore, gram-scale resolution of *rac*-2-pentanol was performed to verify the practicality of the procedure.

2. Materials and methods

2.1. Materials

C. antarctica lipase B (CALB) immobilized on macroporous acrylic resin was purchased from Novo Nordisk. Lipases from *P. cepacia* (PCL) and *P. fluorescens* (PFL) were kindly donated by Amano Enzyme Inc., Japan. The *rac*-alkyl alcohols (Scheme 1), namely: 2-butanol **1**, 2-pentanol **2**, 2-hexanol **3**, 2-heptanol **4**, 2-octanol **5**,

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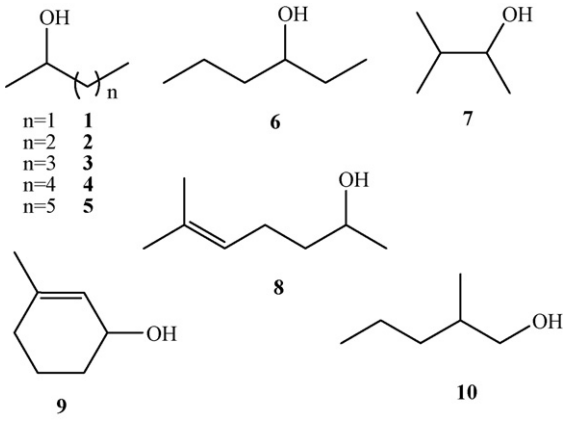


Scheme 1. *rac*-Alkyl alcohols used in the lipase-catalyzed kinetic resolution studies.

3-hexanol **6**, 3-methyl-2-butanol **7**, 6-methyl-5-heptene-2-ol **8**, 3-methyl-2-cyclohexene-1-ol **9** and 2-methyl-1-pentanol **10**, were purchased from Aldrich. Succinic anhydride, 99% purity, was purchased from Aldrich and used without further purification. Other chemicals were of analytical grade and used without further purification, unless otherwise stated.

2.2. General procedure for lipase-catalyzed acylation with succinic anhydride

Lipase-catalyzed acylation of *rac*-alcohols with succinic anhydride as acylating agent was performed as shown in Scheme 2. In a typical experiment, *rac*-alcohol (1 mmol) and succinic anhydride (1 mmol) were mixed in 5 ml of diethyl ether in a 20 ml reaction vessel. The reaction was initiated by the addition of lipase (50 mg of PCL, or 20 mg of either PFL or CALB). The reaction mixture was shaken at 150 rpm at 25 °C for 48 h. After removal of the lipase by filtration, the filtrate was shaken with 1 M Na_2CO_3 solution, and the unreacted alcohol and the monoester succinate produced were separated by aqueous base-organic solvent liquid–liquid extraction. The unreacted enantiomer was obtained from the organic layer, and the aqueous phase was treated by adding 1 M NaOH solution to obtain the other enantiomer. The enantiomeric excess values of the unreacted substrate (ee_s, %) and the other enantiomer (ee_p, %) obtained through saponification were measured by gas chromatography (GC), as described below.

2.3. General procedure for CALB-catalyzed acylation with vinyl acetate

CALB-catalyzed acylation of the same set of *rac*-alcohols was performed with vinyl acetate as acylating agent, as shown in Scheme 3. In each case, the *rac*-alcohol (1 mmol) and vinyl acetate (1 mmol) were mixed in 5 ml of diethyl ether in a 20 ml reaction vessel. The reaction was initiated by the addition of CALB (5 mg), the reaction mixture was shaken at 150 rpm at 25 °C for 4 h, the reaction was then terminated by removing the lipase by filtration, and the filtrate was concentrated under reduced pressure. The enantiomeric excess (*ee*) values of the unreacted alcohol and the ester produced were measured by GC, as described below.

2.4. Enantioselective GC analysis

Enantioselective GC analysis was performed using an Agilent 6890N instrument equipped with flame ionization detector (FID) and an HP-chiral (30 m × 0.25 mm) or chiraldex G-TA (30 m × 0.25 mm) chiral capillary column. The analytical conditions were: injector temp., 250 °C; FID temp., 250 °C; oven temp., varying from 40 to 80 °C, depending on the analyzed compounds, to separate the substrates and products. N_2 was used as the carrier gas at a velocity of 39 cm s^{-1}. The *E* value was calculated from the ee_s and ee_p values at a certain conversion (*c*, %) using the following equation [16]:

$$E = \frac{\ln[(1-c)(1-ee_s)]}{\ln[(1-c)(1+ee_s)]}, \quad c = \frac{ee_s}{ee_s + ee_p}$$

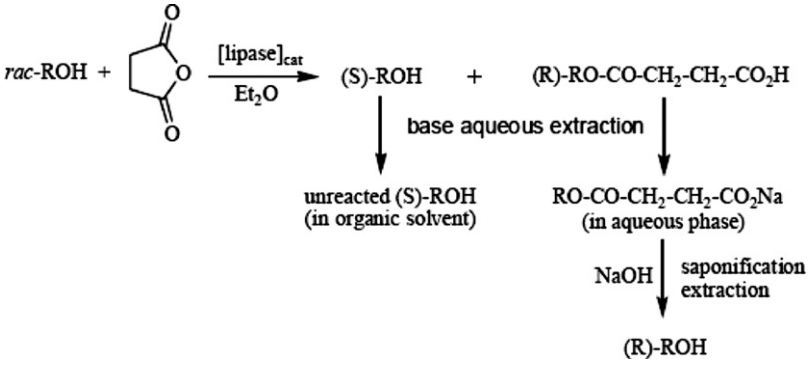
2.5. Gram-scale resolution of rac-2-pentanol **2**

To verify the practicality of the procedure, it was used to acylate *rac*-2-pentanol **2** with succinic anhydride at gram-scale. The reaction mixture was composed of 50 mmol *rac*-2-pentanol **2**, 50 mmol equivalent of succinic anhydride, 70 ml diethyl ether and 150 mg CALB. The reaction was carried out at 25 °C with shaking at 150 rpm for 48 h. The *E* values, optical purity and yields of (*S*)-2-pentanol and (*R*)-2-pentanol obtained were then determined.

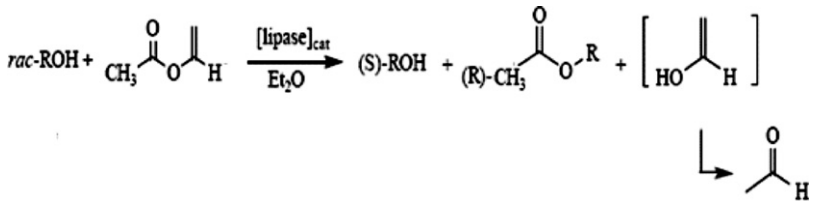
3. Results and discussion

Lipase-catalyzed transesterification with enol esters as acylating agents has been shown to be an effective method for preparing chiral alcohols [4–6]. However, this method has a major drawback, since lengthy silica-gel column chromatography with large quantities of organic solvents is required to separate the products from the substrates. In contrast, using cyclic succinic anhydride as an acylating agent as described here, one enantiomer of the alcohol could be converted into the monoester succinate, which could be easily separated from the unreacted alcohol by aqueous base-organic solvent liquid–liquid extraction.

Using succinic anhydride as acylating agent, we investigated the kinetic resolution of *rac*-alkyl alcohols catalyzed by three different



Scheme 2. Lipase-catalyzed acylation with succinic anhydride as acylating agent.



Scheme 3. Lipase-catalyzed acylation with vinyl acetate as acylating agent.

Table 1
Conversions, enantiomeric excess values and *E* values obtained from the CALB-catalyzed acylation of alcohols **1**–**10** with succinic anhydride as acylating agent

Substrate	Conversion (%)	Unreacted alcohol, ee_s (%)	Alcohol from ester saponification, ee_p (%)	*E*
1	67	75 (*S*)	37 (*R*)	5
2	52	>99 (*S*)	92 (*R*)	205
3	49	95 (*S*)	98 (*R*)	240
4	49	95 (*S*)	97 (*R*)	230
5	49	94 (*S*)	97 (*R*)	220
6	49	91 (*S*)	95 (*R*)	115
7	40	63 (*S*)	96 (*R*)	91
8	35	52 (*S*)	97 (*R*)	128
9	43	49 (*S*)	65 (*R*)	8
10	42	34 (*R*)	47 (*S*)	4

Table 2
Conversions, enantiomeric excess values and *E* values obtained from the PCL-catalyzed acylation of alcohols **1**–**10** with succinic anhydride as acylating agent

Substrate	Conversion (%)	Unreacted alcohol, ee_s (%)	Alcohol from ester saponification, ee_p (%)	*E*
1	31	3 (*S*)	5 (*R*)	<2
2	41	48 (*S*)	68 (*R*)	9
3	29	36 (*S*)	90 (*R*)	26
4	26	30 (*S*)	85 (*R*)	17
5	30	37 (*S*)	88 (*R*)	22
6	24	20 (*S*)	64 (*R*)	6
7	30	42 (*S*)	>98 (*R*)	173
8	18	20 (*S*)	90 (*R*)	23
9	15	12 (*S*)	70 (*R*)	6
10	76	98 (*R*)	31 (*S*)	8

lipases: CALB, PCL and PFL. As shown in Table 1, CALB exhibited viable enantioselectivity towards alcohols **2**–**8** ($E > 90$). (*R*)-alcohols were obtained through ester saponification, therefore they were of high optical purity (*ee* > 92%). Compared with CALB, PCL and PFL displayed lower enantioselectivity towards most of the alcohols, as shown in Tables 2 and 3 ($E < 30$). In addition, the optical purities of alcohols obtained through ester saponification were lower than those obtained from CALB-catalyzed acylation. It has been postulated that the enantioselectivity of these reactions is likely to be reduced by the release of carboxylic acid [14,17]. Therefore, the results could be attributed to the insensitivity of CALB to the presence of the liberated acid. Surprisingly, PCL showed excellent enantioselectivity ($E = 173$) for the resolution of alcohol **7**, much higher than that shown by CALB ($E = 91$). The (*R*)-alcohol **7** it generated was also of high optical purity, with an *ee* value exceeding 98%. None of the lipases employed showed sufficiently strong enantioselectivity ($E < 20$) in acylation with succinic anhydride for practical, cost-effective resolution of alcohols **1**, **9** and **10**.

Most of the above results were consistent with Kazlauskas' empirical rule, which yields predictions that *E* values should be high for *sec*-alcohols when substituents at the stereocenter differ greatly in size [18]. For alcohols **1** and **9**, there is little, and virtually no, difference in the size of the substituents at the stereocenter, respectively. Thus, the low enantioselectivity obtained for these alcohols was consistent with expectations. The high *E* values obtained for alcohols **2**–**8** were also consistent with the greater

Table 3
Conversions, enantiomeric excess values and *E* values obtained from the PFL-catalyzed acylation of alcohols **1**–**10** with succinic anhydride as acylating agent

Substrate	Conversion (%)	Unreacted alcohol, ee_s (%)	Alcohol from ester saponification, ee_p (%)	*E*
1	52	11 (*S*)	10 (*R*)	<2
2	27	25 (*S*)	69 (*R*)	7
3	31	38 (*S*)	86 (*R*)	20
4	32	37 (*S*)	79 (*R*)	12
5	41	32 (*S*)	46 (*R*)	4
6	22	21 (*S*)	75 (*R*)	9
7	9	9 (*S*)	92 (*R*)	26
8	20	23 (*S*)	92 (*R*)	30
9	50	70 (*S*)	71 (*R*)	12
10	71	61 (*R*)	25 (*S*)	3

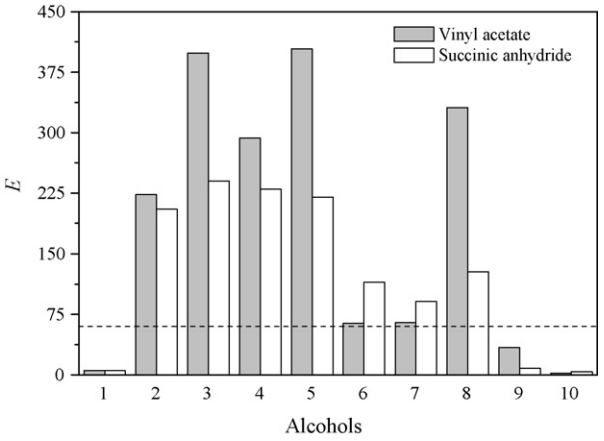


Fig. 1. Comparison of *E* values obtained from the CALB-catalyzed kinetic resolution of alcohols **1**–**10** with vinyl acetate and succinic anhydride as acylating agents. The dashed line indicates an *E* value of 60.

difference in the relative size of the substituents at their stereocenters. Interestingly, however, the *E* values were all high (>200), and similar, for alcohols **2**–**5**, all of which have the same medium-sized group (methyl) and a larger group (propyl to hexyl) at their stereocenters. The results indicate that the interactions between the lipases and isomers are quite complex, and the shape of the binding pockets might influence the enzymes' enantioselectivity as well as difference in the size of the substituents at the stereocenter. In support of this hypothesis, alcohol **6** has a similar difference in size of substituents (methylene group) to alcohol **1** at the stereocenter, but enantioselectivity was greater for alcohol **6**. Alcohols **7** and **8** were resolved with lower enantioselectivity, probably due to the substituents in the large group. These results provided further support for the above assumption. Generally, compared with enzymatic resolution of *sec*-alcohols, the kinetic resolution of racemates of primary alcohols was difficult to achieve due to the lower enantioselectivity of lipases towards primary alcohols [1]. In our study, lower enantioselectivity was obtained for the resolution of primary alcohol **10**.

To assess the effects of acylating agents on the enantioselectivity of CALB, CALB-catalyzed resolution of *rac*-alcohols using succinic anhydride and vinyl acetate as acylating agents was compared. As shown in Fig. 1, both of the acylating agents had similar influences on the CALB-catalyzed resolution of *rac*-alkyl alcohols. CALB exhibited viable enantioselectivity towards alcohols **2**–**8** ($E > 60$), and lower *E* values for alcohols **1**, **9** and **10** ($E < 34$), regardless of whether succinic anhydride or vinyl acetate was used as the acylating agent. It was noteworthy that the *E* values obtained with succinic anhydride were higher than those obtained with vinyl acetate for alcohols **6** and **7**. The CALB/succinic anhydride system resolved the alcohols **6** ($E = 115$) and **7** ($E = 91$) with higher enantioselectivity than the CALB/vinyl acetate system (*E* ca. 64).

To verify the practicality of the protocol, CALB-catalyzed acylation of *rac*-2-pentanol **2** with succinic anhydride at gram-scale was performed in diethyl ether at 25 °C for 48 h. (*S*)-2-Pentanol and (*R*)-2-pentanol were obtained with an *E* value of 205, in quantities of 2.0 g (yield 45%, 99% *ee*) and 1.8 g (yield 40%, 95% *ee*), respectively, following the procedure in Scheme 2, due to the easy separation of even small amounts of unreacted alcohol from the large quantities of monoester succinate produced. In contrast, using succinic anhydride as acylating agent in the solvent-free system, and preparing the product by distillation under vacuum, Patel and co-workers obtained (*S*)-2-pentanol at 36% yield (98.8–99.3% *ee*) with an *E* value of 48 [19]. Compared with their procedure, the method presented here has several advantages: (1) (*S*)-2-pentanol was obtained in a higher yield (45%); (2) higher enantioselectivity was obtained ($E = 205$); (3) (*R*)-2-pentanol was prepared by saponification and extraction, while in their procedure it was prepared by hydrolysis of racemic 2-pentyl acetate; and (4) the method for separating the products and substrates was more straightforward. The high enantioselectivity, simple separation procedure, high yields and optical purity of the product obtained using succinic anhydride as acylating agent in the manner described here suggests that the procedure may be very useful for preparing enantiomerically pure alcohols.

4. Conclusions

In conclusion, a straightforward method using succinic anhydride as acylating agent and simple liquid–liquid extraction was successfully used for the lipase-catalyzed kinetic resolution of *rac*-alkyl alcohols. Good enantioselectivity was achieved for most of the selected *rac*-alkyl alcohols, especially 3-hexanol and 3-methyl-2-butanol. Given the simple separation of the unreacted substrate and the generated product by extraction, the procedure is expected to be an effective tool for preparing enantiomerically pure alcohols at an industrial scale.

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