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Highly Enantioselective Acylation of *rac*-Alkyl Lactates Using *Candida antarctica* Lipase B

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Abstract:

By using *Candida antarctica* lipase B under mild conditions, the highly enantioselective acylation of alkyl (*R*)-lactate from racemic mixture with vinyl alkanoate has been accomplished. In this research effects of the organic solvent, the alkyl chain length of the alkyl lactates and of the vinyl alkanoates, and the reaction temperature on the enantiomeric excess as well as the reaction rate, were investigated. In all cases, only alkyl (*R*)-lactate was stereoselectively acylated at >99.5% ee. The lipase-catalyzed acylation rate of the alkyl lactates was affected by the nature of the organic solvents, but showed no correlation to log *P* of the solvent. The lipase-catalyzed acylation rate of the alkyl lactates was enhanced by increasing the chain length of the vinyl alkanoate from acetyl to butanoyl and by raising the reaction temperature to 65 °C. Finally, the lipase-catalyzed acylation and subsequent vacuum distillation successfully provided both butyl (*R*)-*O*-butanoyllactate and butyl (*S*)-lactate in excellent yields (48%) and enantioselectivities (>99.5% ee) on a large scale. It is expected that the present method will prove to be more efficient in achieving the chiral resolution of racemic alkyl lactate than other conventional methods in terms of environmental friendliness and simplicity.

Introduction

Lactic acid (2-hydroxypropionic acid) is a naturally occurring organic acid and one of the simplest optically active compounds having (*R*)- and (*S*)-optical isomers as enantiomers. Optically pure (*R*)- and (*S*)-lactic acid derivatives are used as a starting material for chiral drugs[1] and chiral polylactic acids.[2] Whereas (*S*)-lactic acid is commercially produced today through the microbial fermentation of glucose, (*R*)-lactic acid is difficult to obtain. There are two major methods to produce (*R*)-lactic acid production: kinetic resolution of a racemic mixture[3] and the direct cultivation of microorganisms producing (*R*)-lactic acid.[4]

Due to their excellent chiral recognition, lipases have long been used to achieve kinetic resolution of chiral alcohol or carboxylic acid. Lactic acid possesses both a chiral alcohol and a chiral carboxylic acid group, which can be resolved by lipases. Several attempts to resolve racemic lactic acid derivatives with lipases have been reported,[5,6] but the substrate concentration and purifying process were not good enough for industrial applications.

In the present research, we describe the enantioselective acylation of racemic alkyl lactates with vinyl alkanoates using Novozym 435, which is an immobilized form of lipase B from *Candida antarctica* (CALB) (Scheme 1). CALB was used for the enantioselective acylation of lactic acid with vinyl acetate in *tert*-butyl methyl ether but was found not to be efficient in resolving lactic acid.[5] Instead of lactic acid, we used alkyl lactates as an acyl acceptor. The effects of the organic solvent, alkyl chain length of the alkyl lactates and vinyl alkanoates, and the reaction temperature were investigated briefly, and the large-scale enzymatic stereoselective acylation of butyl lactate was achieved without the use of organic solvents.

Results and Discussion

First, as it is well-known that enzyme properties such as activity and enantioselectivity can depend on the nature of organic solvent in which the reaction is carried out, the effects of the organic solvent were investigated. Among the many physical parameters of organic solvents, log *P* value, which is the logarithm of the partition coefficient of the solvent between octanol and water, has been used for correlating enzyme properties of a solvent nature.[7,8] The lipase-catalyzed acylation of ethyl lactate (R^1 = ethyl) with vinyl propionate or butanoate (R^2 = ethyl or propyl) was conducted in a variety of organic solvents at 25 °C. The conversion and enantiomeric excess of **(*S*)-1** and **(*R*)-2** are shown at a reaction time of 26 h, in Table 1. Log *P* values of the organic solvents used, which were calculated using a hydrophobic fragmental method developed by Rekker and de Kort,[9] are also described in ascending order in Table 1. Only the ethyl (*R*)-lactates were stereoselectively acylated by the Novozym 435 in all the tested solvents. This is in accord with the empirical rule established by Kazlauskas et al..[6] This empirical rule predicts which enantiomer will react the fastest by comparing the size of the substituents at the chiral center of secondary alcohols. The enantioselectivity was found to

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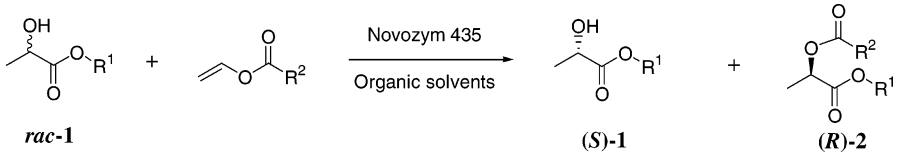
Scheme 1

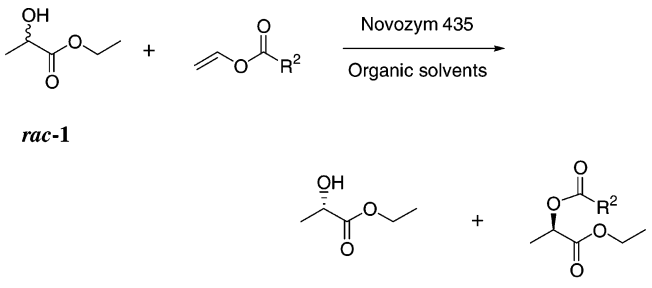


rac-**1** (*S*)-**1** (*R*)-**2**

$R^1 = CH_3, CH_3CH_2, CH_3CH_2CH_2CH_2$

$R^2 = CH_3, CH_3CH_2, CH_3CH_2CH_2$

Table 1. **Effects of the organic solvent on the conversion and enantiomeric excess**



rac-**1** (*S*)-**1** (*R*)-**2**

organic solvent	log *P*[a]	R^2	conversion[b] (%)	ee (%) (*S*)-**1**	ee (%) (*R*)-**2**
1,4-dioxane	−1.1	ethyl	43.5	77	>99.5
		propyl	45.7	84	>99.5
acetonitrile	−0.33	ethyl	37.6	60	>99.5
		propyl	47.0	89	>99.5
tetrahydrofuran	0.49	ethyl	38.8	63	>99.5
		propyl	44.2	79	>99.5
ethyl ether	0.85	ethyl	46.7	88	>99.5
		propyl	50.0	100	>99.5
tert-butyl methyl ether	1.4	ethyl	39.3	65	>99.5
		propyl	47.8	92	>99.5
dichloromethane	1.5	ethyl	30.2	43	>99.5
		propyl	44.3	80	>99.5
isopropyl ether	1.9	ethyl	47.0	89	>99.5
		propyl	50.0	100	>99.5
toluene	2.5	ethyl	45.0	82	>99.5
		propyl	49.9	100	>99.5
carbon tetrachloride	3.0	ethyl	39.9	66	>99.5
		propyl	48.7	95	>99.5
hexane	3.5	ethyl	43.2	76	>99.5
		propyl	49.9	99	>99.5

[a] Log *P* values were calculated using a hydrophobic fragmental method developed by Rekker and de Kort. [b] Conversion of the acylation reaction of ethyl lactate at 25 °C for 26 h.

be extremely high *E* (>200)[10] irrespective of the nature of the organic solvent and of the acyl donor.

The general guidelines for the use of enzymes in organic solvents usually recommend using nonpolar (log *P* > 2) solvents in order to ensure high activity and stability.[8] However, CALB displays a rather unique activity and stability in polar organic solvents as well as nonpolar ones.[11−13] The reaction conversion rate in hydrophilic 1,4-dioxane (log *P* = −1.1) was as high as in hydrophobic hexane (log *P* = 3.5), and no correlation was found between log *P* and the reaction conversion rate. It was revealed that, in all the solvents used, when vinyl butanoate was employed as the acyl donor, higher reaction conversion was achieved than when vinyl propionate was used. The effects of the alkyl chain length of the vinyl alkanoate will be examined and explained in detail below.

We also investigated the effects of the alkyl chain length in the alkyl lactates and vinyl alkanoates and the reaction temperature. Alkyl lactates with three different chain lengths (methyl, ethyl, or butyl) were enzymatically acylated with vinyl acetate, propionate, or butanoate at various temperatures in diisopropyl ether. Table 2 shows that ee values of **(*S*)-1** and **(*R*)-2** were >99.5% ee in all cases. The structure of substrates (acyl donor and acyl acceptor) and reaction temperature can generally affect the enantioselectivity. *E* can be higher for the longer acyl groups, larger substituent of *sec*-alcohol, and lower temperature.[13] However, in the present tested range, the alkyl chain length of the alkyl lactates and vinyl alkanoates, and the reaction temperature had little effect on the enantioselectivity. By contrast, the reaction rate was dependent on the chain length of the vinyl alkanoate and the reaction temperature. Figure 1 demonstrates the effect of the length of the alkyl lactate and vinyl alkanoate on the reaction time at the point of 50% conversion at 50 °C. Increasing the length of R^1 in the alkyl lactate had a little effect on the reaction rate, but increasing the length of R^2 in the vinyl alkanoate significantly increased the reaction rate. This may be due to the substrate specificity of CALB. Although CALB has a much broader specificity towards straight-chain fatty acids than other lipases, the conversion of fatty acid esterification with octanol in hexane increased when the chain length of the fatty acid was increased to the level of a butanoic acid.[14] A study on CALB specificity using several ethyl esters of different acyl chain lengths in cyclohexane also showed that lipase activity increased with increasing the length from C_2 to C_6.[15] Since the elevation of the reaction temperature results in an increase in the reaction rate, the reaction temperature should be as high as possible

(10) Enatiomeric ratio (E) = $\ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$ where c is conversion and ee_p is enantiomeric excess of product. In all cases, **(*S*)-2** peaks were not detected by GC ($ee_p = 1$) such that E becomes infinity. However, it should be pointed out that values of E > 200 cannot be accurately determined due to the inaccuracies emerging from the determination of the enantiomeric excess by GC or HPLC because in this range even a very small variation of ee causes a significant change in the numerical value of E. In this sense, we expressed E > 200 when ee_p was >99.5% ee.

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Table 2. **Effects of the alkyl chain length in alkyl lactate and vinyl alkanoate and of reaction temperature on the reaction rate and enantioselectivity**

				% ee	
R^1	R^2	temp (°C)	time[a] (h)	**(*S*)-1**	**(*R*)-2**
methyl	methyl	50	8	>99.5	>99.5
		65	4	>99.5	>99.5
methyl	ethyl	50	6	>99.5	>99.5
		65	3	>99.5	>99.5
methyl	propyl	50	3	>99.5	>99.5
		65	2	>99.5	>99.5
ethyl	methyl	25	72	>99.5	>99.5
		35	32	>99.5	>99.5
		50	15	>99.5	>99.5
		65	8	>99.5	>99.5
ethyl	ethyl	25	32	>99.5	>99.5
		35	20	>99.5	>99.5
		50	8	>99.5	>99.5
		65	6	>99.5	>99.5
ethyl	propyl	25	24	>99.5	>99.5
		35	12	>99.5	>99.5
		50	4	>99.5	>99.5
		65	3	>99.5	>99.5
butyl	methyl	50	14	>99.5	>99.5
		65	5	>99.5	>99.5
butyl	ethyl	50	6	>99.5	>99.5
		65	4	>99.5	>99.5
butyl	propyl	50	4	>99.5	>99.5
		65	3	>99.5	>99.5

[a] The time required to reach 50% conversion.

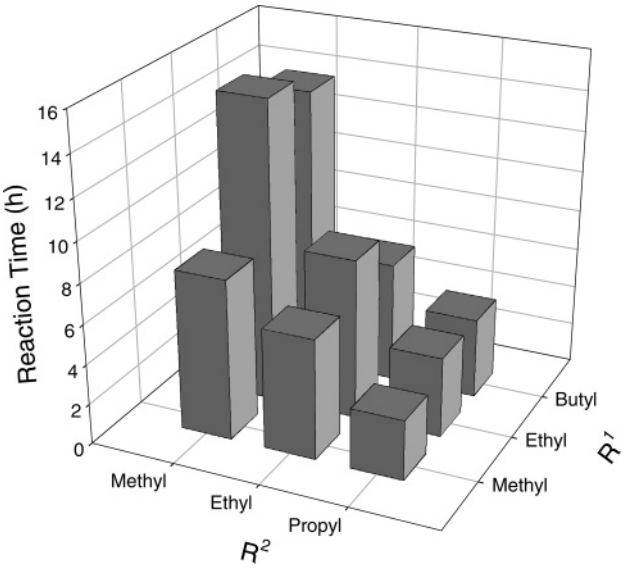

Figure 1. **Effect of the chain length in the alkyl lactate and vinyl alkanoate on the reaction time at 50 °C. The data in figure are the part of those shown in Table 2.**

to ensure the proper reaction rate. In this respect, Novozym 435 is very appropriate, because immobilized CALB is known to be highly thermostable and can be used in continuous operation at 60−80 °C without any significant loss in activity for extended periods of time.[11] We also found that an increase in reaction temperature to 65 °C decreased the reaction time required to reach 50% conversion without any activity loss (Table 2). Raising the temperature by 10 °C reduced the reaction time by approximately half.

Table 3. **Effects of concentration on the reaction rate and enantioselectivity[a]**

butyl lactate (mmol)	vinyl butanoate (mmol)	Novozym 435 (mg)	solvent (mL)	time[b] (h)	ee (%) **(*S*)-1**	ee (%) **(*R*)-2**
1	2	2	3	25	>99.5	>99.5
5	10	10	2	14	>99.5	>99.5
5	10	10	1	10	>99.5	>99.5
10	20	20	0	9	>99.5	>99.5

[a] The lipase-catalyzed acylation reaction was carried out in isopropyl ether at 65 °C. [b] The time required to reach 50% conversion.

Even though an enzymatic catalysis in nonaqueous solvents has many advantages, it would be more technologically attractive to perform enzymatic reactions in a mixture of the substrates themselves without the use of bulk solvents.[16] This approach, if feasible, can combine the advantages of nonaqueous enzymology with high levels of productivity. Vinyl alkanoate is often used as both a solvent and an acylating agent in enzymatic esterification reactions.[17] In the present study, a solvent-free reaction system was attempted. Increasing the substrate concentration and decreasing the amount of solvent, we conducted the lipase-catalyzed acylation reactions at 65 °C, when the ratio of butyl lactate (mmol) and vinyl butanoate (mmol) to the enzyme (mg) was fixed at 1:2:2. In Table 3, the reaction time at the point of 50% conversion and the ee values of **(*S*)-1** and **(*R*)-2** are shown. As shown in Table 3, the more concentrated the substrate was, the more accelerated the enzymatic acylation rate was without any stereoselectivity loss. This result means that a significant amount of racemic alkyl lactate can be resolved effectively in a small reactor with >99.5% optical purity even without the use of bulk organic solvents.

Last, we attempted a scale-up experiment in order to explore the possibility of the enzymatic chiral resolution of racemic alkyl lactate. The enantioselective butanoylation of racemic butyl lactate with vinyl butanoate was carried out in the presence of Novozym 435 at 65 °C on a large scale. Following the disappearance of the butyl (*R*)-lactate (19 h) in the GC, filtration and vacuum evaporation were used to remove the enzyme and remaining vinyl butanoate from the reaction medium, respectively. A subsequent vacuum distillation of the resultant reaction mixture offered butyl (*S*)-lactate and butyl (*R*)-*O*-butanoyllactate.[18] The yields and enantiomeric purities of the these compounds were 48% and >99.5% ee, respectively. The used Novozym 435 was reused three times with less than 10% loss of activity per cycle while

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(18) Butyl (*S*)-lactate: Retention time 10.51 min; $[\alpha]^{25}_D = -8.2°$ ($c = 0.02$, chloroform); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.29−4.24 (m, 1H), 4.18 (dt, 2H, $J = 3.0$ Hz, $J = 6.7$ Hz), 2.80 (d, 1H, $J = 5.3$ Hz), 1.70−1.60 (d, 2H), 1.44 (d, 3H, $J = 6.6$ Hz), 1.41−1.26 (m, 2H), 0.95 (t, 3H, $J = 7.5$ Hz). Butyl (*R*)-butanoyllactate: Retention time 21.29 min; $[\alpha]^{25}_D = +40.2°$ ($c = 0.02$, chloroform); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.08 (q, 1H, $J = 7.2$ Hz), 4.15 (dt, 2H, $J = 1.9$ Hz, $J = 6.7$ Hz), 2.37 (dt, 1H, $J = 2.7$ Hz, $J = 7.5$ Hz), 1.73−1.60 (m, 4H), 1.48 (d, 3H, $J = 6.9$ Hz), 1.39−1.35 (m, 2H), 0.98 (t, 3H, $J = 7.2$ Hz), 0.93 (t, 3H, $J = 7.5$ Hz).

enantioselectivity was not influenced. In this manner, the enantioselective acylation of alkyl (*R*)-lactate from racemic alkyl lactate was successfully performed on a large scale without the use of any organic solvents.

Conclusion

This experiment marks for the first time that the enzymatic kinetic resolution of racemic alkyl lactate for the production of alkyl (*S*)-lactate and alkyl ester of (*R*)-lactyl alkanoate has been successfully performed through enantioselective acylation of alkyl lactate with vinyl alkanoate using CALB. The effects of the organic solvent, alkyl chain length of the alkyl lactates and vinyl alkanoates, and the temperature on the enantiomeric excess and reaction rate were all investigated. In all cases, only the alkyl (*R*)-lactate was highly stereoselectively acylated at >99.5% ee ($E > 200$). Organic solvents affected the reaction rate, which showed no correlation with log *P* of the organic solvent. Increasing the chain length of the alkyl lactate had little effect on the reaction rate, but changing the acyl group of the vinyl alkanoate from acetyl to butanoyl increased the reaction rate significantly. Raising the reaction temperature to 65 °C also reduced the reaction time. The reaction proceeded efficiently even without the use of bulk organic solvents. Finally, a scale-up experiment revealed that butyl (*R*)-*O*-butanoyllactate and butyl (*S*)-lactate were successfully obtained in excellent yields (48%s) and enantiomeric purities (>99.5% ee). It is expected that the present method will prove to be more efficient in achieving the chiral resolution of racemic alkyl lactate than other conventional methods in terms of environmental friendliness and simplicity of process.

Experimental Section

All chemicals and solvents were purchased from commercial suppliers and used without prior purification. Novozym 435 is a generous gift from Novozymes A/S, Denmark. ^{1}H NMR spectra were recorded on a Bruker 300 spectrometer. The optical rotation was determined with Autopol III (Rudolph Research). Enantiomeric excess and the conversion of alkyl lactate were determined by GC analysis on a CycloSil-B chiral column (Agilent, Palo Alto, CA) with a flame ionization detector. The column temperature was maintained at 100 °C for 5 min and then increased to 200 °C at 10 °C/min. The injector and detector temperatures were both set at 230 °C. Nitrogen was supplied as a carrier gas.

Typical Procedure for the Enzymatic Acylation of Racemic Alkyl Lactates with Vinyl Alkanoates. Unless otherwise mentioned, the reaction was carried out as follows: A solution of racemic alkyl lactates (0.3 mmol) and vinyl alkanoates (0.6 mmol) in organic solvents (3 mL) was incubated at a reaction temperature. The reaction was started by the addition of Novozym 435 (10 mg). The resultant reaction mixture was shaken at 200 rpm. The progress of the reaction was monitored by GC analysis.

Enzymatic Resolution of *rac*-Butyl Lactate on a Large Scale. To the solution of racemic butyl lactate (73.1 g, 0.5 mol) and vinyl butanoate (114.1 g, 1 mol), 200 mg of Novozym 435 were added at 65 °C. The resultant reaction mixture was agitated with a mechanical stirrer at 50 rpm, and then the acetaldehyde was distilled out. The progress of the reaction was monitored by GC analysis. After complete butanoylation of butyl (*R*)-lactate, Novozym 435 was removed by filtration and the excess of vinyl butanoate was evaporated under reduced pressure. Vacuum distillation of the residue at 20 mmHg gave 35.1 g (yield 48%) of the butyl (*S*)-lactate (80−82 °C) and the 51.9 g (yield 48%) of the butyl (*R*)-*O*-butanoyllactate (114−117 °C).[18]

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