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Enzymatic resolution of 2-aryloxy-1-propanols via lipase-catalyzed enantioselective acylation using acid anhydrides as acyl donors

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Abstract

Pseudomonas sp. lipase-catalyzed enantioselective acylation procedure using acid anhydrides as acyl donors was exploited for the resolution of 2-aryloxy-1-propanols carrying different substituents on the benzene ring. These primary alcohols, which belong to primary alcohols with an oxygen atom at the stereocenter, were resolved generally with moderate to good enantioselectivity (*E* of up to 55) through the acylation with hexanoic anhydride in diisopropyl ether at 25 °C in a short reaction time. With the alcohol substrate, which gave a low enantioselectivity in the acylation at ordinary temperature, the selectivity proved to be enhanced by conducting the reaction at low temperature (−10 °C). By this acylation procedure employing the acid anhydride, enantiomerically pure (*R*)-2-phenoxy-1-propanol was prepared in a gram-scale reaction.

Keywords: Resolution; 2-Aryloxy-1-propanols; Enantioselective acylation; Acid anhydrides; *Pseudomonas* sp. lipase; Low temperature

1. Introduction

Lipases from a variety of sources have been utilized for producing chiral compounds through resolution or desymmetrization [1–3]. The most common substrates for lipases are secondary alcohols and their derivatives. Compared with a great number of reports on the resolution of secondary alcohols using lipases, successful examples have been far less accumulated with primary alcohols. This must be ascribed to the fact that the chiral center is parted from the reaction site by at least one methylene group in primary alcohols. It has been reported that most lipases show low enantioselectivity toward primary alcohols but only lipases from *Pseudomonas cepacia* and porcine pancreas resolve these substrates with moderate to high enantioselectivity [3]. Moreover, for the lipase-catalyzed resolution of secondary alcohols, a simple rule based on the size of the substituents was proposed which could predict the favored enantiomer [4]. Although a similar rule was later introduced for the *P. cepacia* lipase-catalyzed reactions of primary alcohols, it is not reliable for primary alcohols with an oxygen atom at the stereocenter [5]. Accordingly, it is still a challenge to explore a practically potent procedure for the resolution of primary alcohols of this kind. We have already reported the resolution of 2-aryloxy-1-propanols through the *Pseudomonas* sp. lipase-catalyzed enantioselective acylation using vinyl butanoate as an acyl donor [6]. During the course of this investigation, we found that the enzymatic acylation of the parent 2-phenoxy-1-propanol proceeded more smoothly with a higher enantioselectivity by employing butanoic anhydride instead of the vinyl ester as the acyl donor. This prompted us to investigate the usefulness of acid anhydrides as acyl donors for the resolution of 2-aryloxy-1-propanols.

Homochiral alcohols are versatile building blocks for the synthesis of a wide variety of biologically active compounds and functional materials such as liquid crystals. Among them, 2-aryloxy-1-propanols are useful chiral starting materials for the synthesis of a sorbinil homologue [7] and of juvenile hormone analogues (juvenoids) of the 2-(4-hydroxybenzyl)-1-cyclohexanone series [8].

2. Experimental

2.1. General

^{1}H NMR spectra were recorded at 300 MHz on a Varian Unity 300 spectrometer using $CDCl_3$ as a solvent with

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TMS as an internal standard. Optical rotations were measured using a JASCO DIP-4 digital polarimeter. All organic solvents were distilled following standard protocols and dried over molecular sieves prior to use. *Pseudomonas* sp. lipase (lipase AK) was supplied by Amano Pharmaceutical Co. and had a specific activity of 40 U/mg solid (pH 7.0) (one Amano unit is defined as the enzyme quantity which hydrolyzes olive oil to produce 1 μmol of fatty acids per minute at 37 °C, according to the supplier). Racemic 2-aryloxy-1-propanols were those prepared by the reduction of the corresponding 2-aryloxypropanoic acids [9,10] using borane–tetrahydrofuran complex [6].

2.2. Preparation of hexanoates of 2-aryloxy-1-propanols

Authentic samples of the hexanoates of racemic 2-aryloxy-1-propanols were prepared by the reaction of each 2-aryoxy-1-propanol with hexanoyl chloride and purified by preparative TLC. The hexanoylation of the 4-trifluoromethyl derivative **1i** is described as a typical example. To a stirred solution of **1i** (320 mg, 1.5 mmol) and pyridine (180 μl, 2.3 mmol) in dry $CHCl_3$ (3 ml) was added dropwise from a syringe a solution of hexanoyl chloride (270 μl, 2.0 mmol) in $CHCl_3$ (0.6 ml) under ice-cooling, and the mixture was stirred at ambient temperature overnight. 1-(2-Aminoethyl)piperazine (40 μl, 0.3 mmol) was added and the mixture stirred for 30 min. Ether was added and the yellowish precipitates were filtered off, and then the filtrate was washed successively with 1 M HCl, water, 1 M aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. After evaporation of the solvent in vacuo, the residual oil was purified by preparative TLC on silica gel (Wakogel B-5F) using ether–hexane (1:9, v/v) as an eluent, to give the hexanoate of **1i** as a colorless oil (310 mg, 67%). The physical and ^{1}H NMR data of the hexanoates of **1** thus prepared are compiled in Table 1. Those for the hexanoate of **1a** have been described elsewhere [6].

2.3. HPLC analyses

Acylation reactions were monitored by chiral HPLC on a Chiralpak AS column (4.6 mm i.d. × 250 mm) or a Chiralpak AD column (4.6 mm i.d. × 250 mm) (Daicel Chemical Industries) using hexane–2-propanol (e.g. 95:5, v/v) as an eluent. The liquid chromatograph employed was a Shimadzu LC-10AS instrument, equipped with a Rheodyne 7725i sample injector and a Shimadzu SPD-10A variable wavelength UV monitor. A Shimadzu C-R6A data processor was used for data acquisition and processing. In general, the enantiomers of alkanoates of 2-aryloxy-1-propanols were not separated on either column satisfactorily enough for the accurate determination of the enantiomeric excess (e.e.) value, while the corresponding alcohols were separated well on either of the columns by choosing an appropriate proportion of hexane/2-propanol for each compound. The separation of the enantiomers of **1h** and **1i** is shown in Table 2.

The peak(s) for an alkanoate and the peaks for the parent alcohol were separated on the chiral columns well enough for the determination of the extent of conversion.

Table 1
Physical and ^{1}H NMR data of the hexanoates of 2-aryloxy-1-propanols (**1**)

Hexanoate of	TLC, R_f[a]	^{1}H NMR δ_H ($CDCl_3$)
1b	0.38	0.88 (3H, t, J = 6.9 Hz, $CH_2C\underline{H}_3$), 1.22–1.36 (4H, m, $C\underline{H}_2C\underline{H}_2CH_3$), 1.31 (3H, d, J = 6.3 Hz, $CHC\underline{H}_3$), 1.60 (2H, quint-like, J = 7.5 Hz, $C\underline{H}_2CH_2CO$), 2.30 (2H, t, J = 7.5 Hz, CH_2CO), 4.12–4.27 (2H, d of ABq, J = 11.4, 6.3 and 4.5 Hz, OCH_2), 4.50 (1H, d of quint, J = 6.3 and 4.5 Hz, CH), 6.84–7.01 (4H, m, Ar)
1c	0.32	0.87 (3H, t, J = 6.9 Hz, $CH_2C\underline{H}_3$), 1.22–1.36 (4H, m, $C\underline{H}_2C\underline{H}_2CH_3$), 1.32 (3H, d, J = 6.0 Hz, $CHC\underline{H}_3$), 1.60 (2H, quint-like, J = 7.5 Hz, $C\underline{H}_2CH_2CO$), 2.30 (2H, t, J = 7.5 Hz, CH_2CO), 4.12–4.28 (2H, d of ABq, J = 11.7, 6.3 and 4.2 Hz, OCH_2), 4.55 (1H, d of quint, J = 6.3 and 4.2 Hz, CH), 6.83–7.26 (4H, m, Ar)
1d	0.24	0.87 (3H, t, J = 7.0 Hz, $CH_2C\underline{H}_3$), 1.22–1.35 (4H, m, $C\underline{H}_2C\underline{H}_2CH_3$), 1.39 (3H, d, J = 6.3 Hz, $CHC\underline{H}_3$), 1.60 (2H, quint-like, J = 7.5 Hz, $C\underline{H}_2CH_2CO$), 2.30 (2H, t, J = 7.5 Hz, CH_2CO), 4.18–4.33 (2H, d of ABq, J = 11.7, 6.3 and 4.5 Hz, OCH_2), 4.60 (1H, d of quint, J = 6.3 and 4.5 Hz, CH), 6.89–7.38 (4H, m, Ar)
1e	0.23	0.88 (3H, t, J = 6.9 Hz, $CH_2C\underline{H}_3$), 1.22–1.36 (4H, m, $C\underline{H}_2C\underline{H}_2CH_3$), 1.37 (3H, d, J = 6.3 Hz, $CHC\underline{H}_3$), 1.59 (2H, quint-like, J = 7.5 Hz, $C\underline{H}_2CH_2CO$), 2.30 (2H, t, J = 7.5 Hz, CH_2CO), 4.17–4.31 (2H, d of ABq, J = 11.7, 6.3 and 4.2 Hz, OCH_2), 4.56 (1H, d of quint, J = 6.3 and 4.2 Hz, CH), 6.94 (1H, d, J = 9.0 Hz, Ar), 7.17 (1H, dd, J = 9.0 and 2.4 Hz, Ar), 7.37 (1H, d, J = 2.4 Hz, Ar)
1f	0.26	0.87 (3H, t, J = 7.0 Hz, $CH_2CH_2C\underline{H}_3$), 1.21 (3H, t, J = 7.7 Hz, $ArCH_2C\underline{H}_3$), 1.24–1.36 (4H, m, $C\underline{H}_2C\underline{H}_2CH_3$), 1.32 (3H, d, J = 6.3 Hz, $CHC\underline{H}_3$), 1.60 (2H, quint-like, J = 7.5 Hz, $C\underline{H}_2CH_2CO$), 2.30 (2H, t, J = 7.5 Hz, CH_2CO), 2.59 (2H, q, J = 7.5 Hz, $ArC\underline{H}_2$), 4.12–4.28 (2H, d of ABq, J = 11.4, 6.3 and 4.5Hz, OCH_2), 4.56 (1H, d of quint, J = 6.3 and 4.5 Hz, CH), 6.83–7.12 (4H, m, Ar)
1g	0.33	0.87 (3H, t, J = 6.9 Hz, $CH_2C\underline{H}_3$), 1.22 (6H, d, J = 6.9 Hz, $CH(C\underline{H}_3)_2$), 1.23–1.33 (4H, m, $C\underline{H}_2C\underline{H}_2CH_3$), 1.32 (3H, d, J = 6.3 Hz, $CHC\underline{H}_3$), 1.60 (2H, quint-like, J = 7.5 Hz, $C\underline{H}_2CH_2CO$), 2.30 (2H, t, J = 7.5 Hz, CH_2CO), 2.85 (1H, sept, J = 6.9 Hz, $C\underline{H}(CH_3)_2$), 4.12–4.28 (2H, d of ABq, J = 11.4, 6.3 and 4.2 Hz, OCH_2), 4.56 (1H, d of quint, J = 6.3 and 4.2 Hz, CH), 6.84–7.14 (4H, m, Ar)
1h	0.67	0.88 (3H, t, J = 7.1 Hz, $CH_2C\underline{H}_3$), 1.22–1.37 (4H, m, $C\underline{H}_2C\underline{H}_2CH_3$), 1.30 (3H, d, J = 6.3 Hz, $CHC\underline{H}_3$), 1.61 (2H, quint-like, J = 7.5 Hz, $C\underline{H}_2CH_2CO$), 2.31 (2H, t, J = 7.5 Hz, CH_2CO), 3.77 (3H, s, OCH_3), 4.12–4.26 (2H, d of ABq, J = 11.4, 6.3 and 4.2 Hz, OCH_2), 4.46 (1H, d of quint, J = 6.3 and 4.2 Hz, CH), 6.79–6.91 (4H, m, Ar)
1i	0.64	0.86 (3H, t, J = 6.9 Hz, $CH_2C\underline{H}_3$), 1.22–1.36 (4H, m, $C\underline{H}_2C\underline{H}_2CH_3$), 1.36 (3H, d, J = 6.3 Hz, $CHC\underline{H}_3$), 1.59 (2H, quint-like, J = 7.5 Hz, $C\underline{H}_2CH_2CO$), 2.29 (2H, t, J = 7.5 Hz, CH_2CO), 4.15–4.31 (2H, d of ABq, J = 11.7, 6.3 and 4.2 Hz, OCH_2), 4.68 (1H, d of quint, J = 6.3 and 4.2 Hz, CH), 6.97 (2H, d, J = 8.4 Hz, Ar), 7.54 (2H, d, J = 8.4 Hz, Ar)

[a] Solvent: ether–hexane (1:9, v/v).

Table 2
HPLC separation of the enantiomers of 2-aryloxy-1-propanols (**1**)[a]

Compound	k'_1[b]	k'_2[b]	α[c]
1h	3.18	4.18	1.31
1i	1.71	2.96	1.73

[a] HPLC conditions: column, Chiralpak AD; mobile phase, hexane–2-propanol (95:5, v/v); flow rate, 1.0 ml min^{-1}; column temperature, 30 °C; detection, UV at 254 nm.
[b] Capacity factor: $k' = (t_R - t_0)/t_0$, where t_0 denotes the void time, which was determined using 1,3,5-tri-*t*-butylbenzene. The suffixes 1 and 2 denote the faster and slower eluting enantiomers, respectively.
[c] Separation factor: $\alpha = k'_2/k'_1$.

2.4. General procedure for the lipase-catalyzed enantioselective acylation of 2-aryloxy-1-propanols

A solution of the 2-aryloxy-1-propanol (0.3 mmol) and the acid anhydride (0.9 mmol) in dry diisopropyl ether (0.8 ml) was stirred with the lipase preparation (3 mg) in a thermostated bath. The monitoring of the reaction and the determination of the e.e. value of the remaining alcohol substrate were conducted simultaneously by HPLC analysis on the chiral columns mentioned above. Aliquots (ca. 10 μl) of the reaction mixture were withdrawn at frequent intervals, diluted with diisopropyl ether (100 μl), filtered through a PTFE membrane filter and then injected onto the column.

Blank experiments were conducted employing each alcohol substrate in the same manner as above without the addition of the lipase, which showed no occurrence of the non-enzymatic acylation over the period of 1 h.

The enantiomeric ratio, *E* [11], was calculated using $E = \ln[(1-c)(1-\text{e.e.}_S)]/\ln[(1-c)(1+\text{e.e.}_S)]$ where *c* and e.e._S represent the extent of conversion and the enantiomeric excess of the remaining alcohol substrate, respectively. Each acylation reaction was carried out at least in duplicate to determine the *E*-value, and the typical deviation from the mean did not exceed 8%.

In all the cases, the preferential acylation of the (*S*)-form of the alcohols was confirmed by comparison with the authentic samples, i.e. (*S*)- or (*R*)-rich 2-aryloxy-1-propanols prepared through the reduction of the corresponding (*S*)- or (*R*)-rich 2-phenoxypropanoic acids, if available [9,10], on HPLC or suggested from the regularity of elution order (*S* before *R*) of the enantiomers on HPLC.

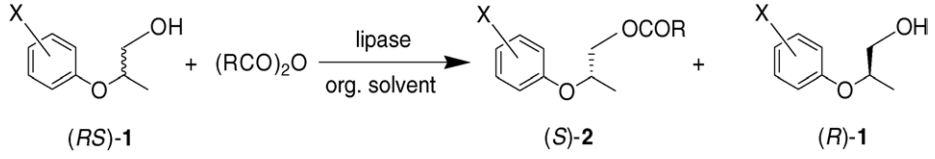
2.5. Gram-scale resolution of 2-phenoxy-1-propanol (*1a*)

The racemic alcohol **1a** (1.37 g, 9.0 mmol) was dissolved in dry diisopropyl ether (20 ml), followed by the addition of hexanoic anhydride (6.2 ml, 26.8 mmol) and then the lipase preparation (90 mg). The reaction mixture was stirred at 25 °C. The reaction was terminated after 45 min (57% conversion) by removing the enzyme powder by filtration. The enzyme was washed with ether. Evaporation of the solvent in vacuo from the combined filtrate and washing afforded a yellowish oil, from which the products were isolated by column chromatography on silica gel (Wakogel C-300) using ether–hexane (1:20, v/v) as an eluent, to give initially the hexanoate of (*S*)-**1a** as a colorless oil (1.18 g, 52% yield). Further elution afforded (*R*)-**1a** as a colorless oil (0.55 g, 40% yield); $[\alpha]_D^{25}$ −39.2° (*c* 1.0, MeOH); >99% e.e. by HPLC. Lit. [12], $[\alpha]_D^{20}$ −38.0° (*c* 0.9, MeOH). ^{1}H NMR δ_H ($CDCl_3$) 1.27 (3H, d, J = 6.3 Hz, CH_3), 2.12 (1H, br s, OH), 3.67–3.80 (2H, d of ABq, J = 11.4, 6.3 and 3.9 Hz, CH_2), 4.50 (1H, d of quint, J = 6.3 and 3.9 Hz, CH), 6.90–7.33 (5H, m, Ph).

3. Results and discussion

Acid anhydrides are known to catalyze a complete irreversible acylation of alcohols like enol esters such as the most commonly used vinyl ester [2]. The major possible disadvantages of acid anhydrides are the decrease of enantioselectivity of the reaction caused by the release of carboxylic acid [13] and the occurrence of non-enzymatic reaction. Fortunately, the enzyme of interest in the present study, *Pseudomonas* sp. lipase (Amano AK), is known to be insensitive to the presence of the liberated acid [14] unlike some lipases such as *Candida rugosa* lipase which requires protection, e.g. by the addition of a weak inorganic or organic base as an acid scavenger [13]. On the other hand, uncatalyzed acylation with the acid anhydride is likely to occur in polar solvents. In the solvent of choice in the present study, diisopropyl ether [15,16], we found no occurrence of the non-enzymatic acylation (vide infra).

Initially, the effect of different acid anhydrides as acyl donors on the acylation of the parent 2-phenoxy-1-propanol (**1a**) was investigated in diisopropyl ether (Scheme 1). The reaction was monitored (conversion and enantiomeric excess values) by HPLC on a chiral column. Since the lipase-catalyzed enantioselective acylation of a racemic alcohol proceeds via the acyl-enzyme intermediate, the nature of the acyl donor, i.e. an acid anhydride or acid vinyl ester, should affect the rate of formation of acyl-enzyme intermediate and/or the stability of the preceding enzyme–substrate (ES) complex. The enantiodiscrimination of the alcohol substrate should occur during the subsequent alcoholysis of the intermediate. The results obtained employing different anhydrides are shown in Table 3. In the last column of the table are included also the *E*-values [11] observed in the



Scheme 1. *Pseudomonas* sp. lipase-catalyzed enantioselective acylation of racemic 2-aryloxy-1-propanols [(*RS*)-**1**] with acid anhydrides. See Table 3 for the alkyl chain R and Table 4 for the substituent X.

Table 3
Pseudomonas sp. lipase-catalyzed acylation of (*RS*)-**1a** with different acid anhydrides as acyl donors in diisopropyl ether[a]

R of acid anhydride	Conversion (%)	Time (min)	e.e.$_S$[b] (%)	*E*	cf. *E*[c]
CH_3	53	30	96	48	42
n-C_3H_7	54	30	97	43	35
i-C_3H_7	47	120	71	19	
n-C_5H_{11}	48	20	83	49	36
n-C_7H_{15}	50	10	85	33	28

[a] Conditions: 0.3 mmol of (*RS*)-**1a**, 0.9 mmol of an acid anhydride and 3 mg of *Pseudomonas* sp. lipase in 0.8 ml of diisopropyl ether at 25 °C.
[b] Enantiomeric excess of the remaining alcohol.
[c] *Pseudomonas* sp. lipase-catalyzed acylation of (*RS*)-**1a** with the corresponding vinyl alkanoates in diisopropyl ether [6].

Table 4
Pseudomonas sp. lipase-catalyzed acylation of 2-aryloxy-1-propanols [(*RS*)-**1**] with hexanoic anhydride as the acyl donor in diisopropyl ether[a]

Compound	X	Conversion (%)	Time (min)	e.e.$_S$[b] (%)	*E*	cf. *E*[c]
1a	H	48	20	83	49	35
1a[d]		49	120	88	66	
1b	4-F	51	30	92	53	34
1c	2-Cl	49	30	84	39	48
1d	4-Cl	47	30	81	55	58
1e	2,4-Cl_2	51	60	67	9.1	19
1e[d]		47	165	71	19	
1f	4-Et	55	15	50	3.8	18
1g	4-Pr*i*	50	30	80	22	15
1h	4-MeO	48	20	78	28	
1i	4-CF_3	50	40	84	30	

[a] Conditions: 0.3 mmol of (*RS*)-**1**, 0.9 mmol of hexanoic anhydride and 3 mg of *Pseudomonas* sp. lipase in 0.8 ml of diisopropyl ether at 25 °C.
[b] Enantiomeric excess of the remaining alcohol.
[c] *Pseudomonas* sp. lipase-catalyzed acylation of (*RS*)-**1** with vinyl butanoate in diisopropyl ether [6].
[d] Reactions were conducted at −10 °C.

acylation of **1a** with the corresponding vinyl alkanoates in diisopropyl ether mediated by the same lipase [6]. The use of the acid anhydride resulted in a faster reaction and at the same time, a more or less increased enantioselectivity than that of the corresponding vinyl ester in each case.

It is reasonable to anticipate that the enantioselectivity as well as the rate of acylation must be affected by the fatty acid moiety of the acyl donor. In fact, the chain length of the acid moiety of the acid anhydride produced a considerable effect on the conversion rate: there was a tendency that acid anhydrides carrying a longer alkyl chain served as better acyl donors than those carrying a shorter one. This tendency can be attributable to the lipase's high affinity toward long-chain acyl groups. On the other hand, the change of enantioselectivity with the length of the alkyl chain (R in Scheme 1) from C_1 to C_5 was not very profound (*E* = 43–49). With 2-methylpropanoic anhydride (isobutyric anhydride) the reaction was retarded to a great extent probably because of the steric bulk of the isopropyl group, which must be also responsible for the deteriorated enantioselectivity (*E* = 19). With octanoic anhydride the enantioselectivity decreased considerably (*E* = 33), although the reaction was the fastest. As the reaction was reasonably fast and highly enantioselective with hexanoic anhydride, this anhydride was chosen throughout the following experiments. Another reason for choosing hexanoic anhydride was that it can avoid the handling of the stinking butanoic acid liberated during the reaction if butanoic anhydride is used.

Next, the resolution of a number of aryloxy-1-propanols (**1**) carrying different substituents on the benzene ring was examined through the acylation with hexanoic anhydride mediated by *Pseudomonas* sp. lipase in diisopropyl ether. The results are shown in Table 4. Blank experiments conducted with each alcohol substrate showed no occurrence of the non-enzymatic acylation over the period of at least 1 h. In the last column of the table are included also the *E*-values observed in the acylation of **1** with vinyl butanoate in diisopropyl ether mediated by the same lipase [6]. As can be seen from the table, the benzene ring substituent had a significant effect on the enantioselectivity of the reaction. Generally speaking, the effect of substituent on enantioselectivity observed here resembles that observed in the acylation of **1** with vinyl butanoate as the acyl donor. When scrutinized, however, the enantioselectivity was ameliorated in some cases by using the acid anhydride like in the acylation of **1a** mentioned at the beginning, while in other cases it was deteriorated. The moderate *E*-values observed with the 2,4-dichloro derivative **1e** and the 4-ethyl derivative **1f** using vinyl butanoate became low in the present case. This is probably attributable to the difference in the acyl-enzyme intermediates formed, for the acylation of **1e** with vinyl hexanoate also gave a low *E*-value of 11 (42% conversion, 4 h, 53% e.e.$_S$).

Therefore, we examined next the possibility of improving the enantioselectivity by lowering temperature in the acylation of **1e** with hexanoic anhydride (*E* = 9.1 at 25 °C). It is widely believed that in enzymatic reactions, as in the conventional chemical reactions, lower temperatures lead to higher enantioselectivity [17]; this has been supported by a number of experimental results [18,19] including those reported from our laboratory [6,20]. A rational understanding of such a temperature effect on enantioselectivity has been proposed [17,21]. In the present case also, the enantioselectivity was found to enhance with decreasing temperature (−10 to 40 °C), although the reaction was retarded considerably. Moreover, when $-RT\ln E$ obtained from the *E*-value at each temperature was plotted against the absolute temperature (*T*), an approximately straight line was obtained, although the slope was rather small (Fig. 1). The existence of a linear relationship between $-RT\ln E$ and *T* indicates that the temperature effect on the enantioselectivity is governed by the equation, $-RT\ln E = \Delta\Delta G^{\ddagger} = \Delta\Delta H^{\ddagger} - T\Delta\Delta S^{\ddagger}$, and the enzyme retains its active conformation within the temperature range examined. Thus, when the reaction temperature was lowered to −10 °C, an enhanced *E* value of 19 was obtained (Table 4). The improvement of enantioselectivity at low temperature was observed also with the parent alcohol substrate **1a** (*E* = 49 at 25 °C to *E* = 66 at −10 °C).

In all the cases mentioned above, the preferential acylation of the (*S*)-form of the alcohols yielding the (*S*)-hexanoates (**2**) was confirmed by comparison with the authentic samples, if available [9,10], on HPLC or suggested from the regularity of

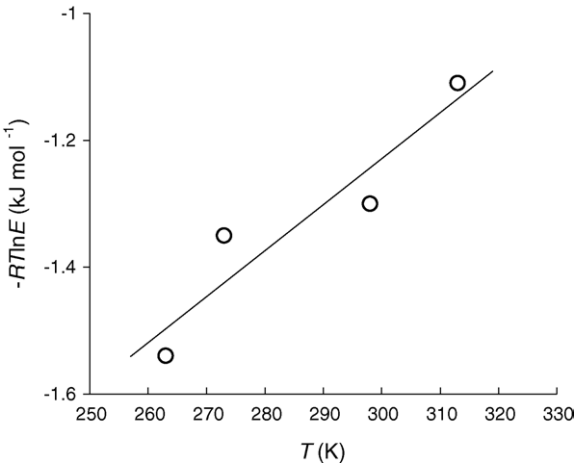


Fig. 1. Influence of temperature (T) on the difference in the activation free energy ($\Delta\Delta G^{\ddagger} = -RT\ln E$) between the enantiomers for the *Pseudomonas* sp. lipase-catalyzed acylation of racemic 2-(2,4-dichlorophenoxy)-1-propanol [(*RS*)-**1e**] with hexanoic anhydride in diisopropyl ether.

elution order of the enantiomers on HPLC. The stereochemical preference observed here is the same as that obtained using vinyl butanoate as the acyl donor and is contrary to that predicted by the empirical rule for the *P. cepacia* lipase-catalyzed reactions of primary alcohols without an oxygen atom at the stereocenter [5].

Furthermore, a gram-scale resolution of the parent alcohol substrate **1a** was achieved by the *Pseudomonas* sp. lipase-catalyzed acylation using hexanoic anhydride as the acyl donor in diisopropyl ether. After 45 min of incubation (57% conversion), (*R*)-**1a** with >99% e.e. was isolated as the remaining alcohol substrate through column chromatography on silica gel. Thus, the enantiomerically pure alcohol was obtained through such a short-time reaction.

4. Conclusion

Pseudomonas sp. lipase-catalyzed enantioselective acylation procedure using acid anhydrides as acyl donors was exploited for the resolution of 2-aryloxy-1-propanols which belong to primary alcohols with an oxygen atom at the stereocenter. The change of the acyl donor from the vinyl ester to the corresponding acid anhydride resulted in a faster reaction. The non-enzymatic acylation was not observed by the use of acid anhydrides. Thus, the primary alcohols carrying different substituents on the benzene ring were resolved generally with moderate to good enantioselectivity through acylation with hexanoic anhydride. In the present case also, low temperature proved to enhance the enantioselectivity. The results obtained here, together with those reported before, demonstrate that *Pseudomonas* lipases are useful enzymes for the resolution of primary alcohols and that acid anhydrides can be utilized as acyl donors besides the conventional vinyl esters.

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