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The effect of temperature on the lipase-catalyzed asymmetric protonation of 1-acetoxy-2-methylcyclohexene giving (*R*)-2-methylcyclohexanone

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Abstract—Lipase-catalyzed enantioface-selective protonation of the enol acetate of 2-methylcyclohexanone was performed by using *Burkholderia cepacia* lipase immobilized on porous ceramic particles, 'Lipase PS-C II' (Amano Enzyme Inc.), in *i*-Pr_2O and ethanol, giving (*R*)-2-methylcyclohexanone with 77% ee at best with 82% conversion at 0 °C. The enantioselectivity (*E*-value) was found to be temperature dependent and significant in controlling the efficiency of the asymmetric protonation.

1. Introduction

Optically active α-substituted ketones are known to be important chiral intermediates prepared by a variety of synthetic methods.[1] Among them, chemical[2a–j,l] and biochemical[3a–i] enantioface-selective protonations of prochiral enol esters of ketones have been examined, although a method with both high enantioselectivity and operational simplicity is still being sought. Ohta et al.[3b] have reported a simple method for the enantioface-selective protonation via a biocatalytic hydrolysis of enol esters, where *Yamadazyma farinosa* (formally classified as *Pichia miso*, then *Pichia farinosa* IFO 19806) was reported to be the best biocatalyst. For example, the reaction of 1-acetoxy-2-methylcyclohexene **1** in buffer gave (*S*)-2-methylcyclohexanone (*S*)-**2** in 90% ee. On the other hand, Hirata et al.[3h] reported that an esterase (isolated from *Marchantia polymorpha*)-catalyzed asymmetric protonation of **1** in buffer also gave (*S*)-**2** in >99% ee. As seen in these examples, the screening of biocatalysts is essential in obtaining high enantiomeric excesses.

We recently proposed a 'low-temperature method' in the lipase-catalyzed resolution, which is highly effective and theoretically reliable for enhancing the enantioselectivity in the kinetic resolution of alcohols.[4a–e] Moreover, the low-temperature method used in combination with a porous ceramic (*Toyonite*)-immobilized lipase is effective for enhancing both the enantioselectivity and the reaction rate.[4c,d,e] The immobilization could prevent the lipase from aggregation, while the dispersed lipase molecules on the ceramic support seem to exert the intrinsic activity. The immobilized lipase used herein is commercially available as 'lipase PS-C II' (*Burkholderia cepacia*) from Amano Enzyme Inc.[5,6] Herein, we examine the applicability of the low-temperature method using lipase PS-C II to the asymmetric protonation of enol acetate **1**, and have found a correlation between *E*-value[7] and $1/T$. For example, 34% ee ($E = 2.0$) of product (*R*)-**2** at 60 °C was raised up to 77% ee ($E = 7.7$) at 0 °C, where the use of lipase PS-C II was found to have a crucial role in the rate enhancement. Here, commercially available lipase PS-C II was used exclusively instead of the biocatalysts reported by Ohta and co-workers[3a,b,d,e] and Hirata et al.[3h] because the purpose herein is to reveal the temperature effect on the enantioselectivity in the asymmetric protonation.

2. Results and discussion

We first attempted the asymmetric protonation of **1** with lipase PS (Celite-immobilized) in the presence of added

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H_2O (10 equiv) as a proton source in *i*-Pr_2O at various temperatures. As shown in Table 1 and Figure 1, the enantiomeric excesses slightly increased from 22% to 28% ee by lowering the temperature from 60 to 0 °C; however, the reaction rate (TTN/h:[4d,e] total turnover number per hour) was dramatically decreased from 1100 to 60. The enantiomeric excess was less than 30% ee in all cases, with the temperature effect not being obvious. Interestingly, the configuration is (*R*), which is the antipodal enantiomer of that obtained by using *Yamadazyma farinosa*[3a,b] or *Marchantia polymorpha*.[3h] Here, we replaced lipase PS with lipase PS-C II (Porous ceramic-immobilized), and found that the reaction was accelerated by more than 6 times, while the enantioselectivity also improved to 33% ee as shown in entry 4. This result encouraged us to use a bulkier proton source such as methanol and ethanol, which was expected to improve the enantioselectivity.

The reaction with lipase PS-C II in the presence of 10 equiv of methanol at 60 °C gave 91% conversion with 30% ee as shown in entry 5. Lowering the reaction temperature gradually increased the enantioselectivity, which improved up to 70% ee with >99% conversion at −10 °C. In these reactions, the rate acceleration by lipase PS-C II was apparently cancelled by using methanol, and it took as long as 65.7 h at −10 °C for completion of the reaction, although the amount of the lipase increased in the low temperature reactions. Thus, lowering the temperature further seemed to be impractical.

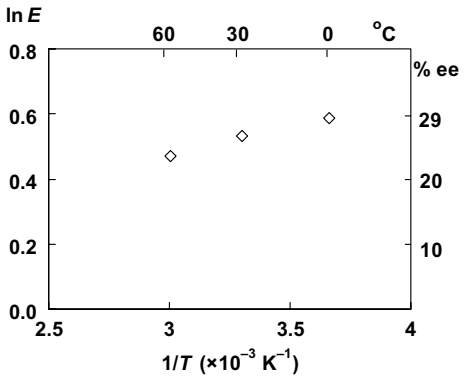
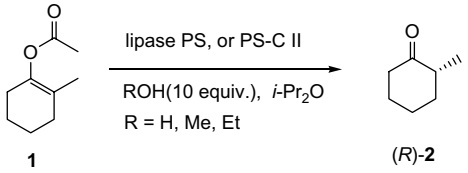


Figure 1. Correlation between ln *E* versus 1/*T* (Lipase PS on Celite, H_2O).

Further improvement of the enantioselectivity was observed by replacing methanol with ethanol, which attained 77% ee with 82% conversion at 0 °C (entry 14).[8] As shown in Figure 2, each plot of ln *E* versus 1/*T*[9] for methanol and ethanol showed that lowering the temperature increased the enantioselectivity, although, without obeying an exact linear correlation. Curiously, an increase of the *E*-value was not observed between 30 and 10 °C in both cases. This suggests a change of mechanism, although the details are presently unclear. The protons can be supplied from H_2O, methanol or ethanol, whose bulkiness may be important.[10]

Table 1. Asymmetric protonation with lipase PS or PS-C II



1 (*R*)-**2**

Conditions[a]	Entry	*T* (°C)	Lipase (mg)	Time (h)	Conv. (%)[b]	% Ee[c]	*E*	TTN/h[d]
PS[e]/H_2O	1	60	200	7.0	73	22	1.6	1100
	2	30	200	16.2	98	27	1.7	650
	3	0	200	46.7	26	28	1.8	60
PS-C II/H_2O	4	0	300	18.0	>99	33	2.0	390
PS-C II[f]/MeOH	5	60	300	6.8	91	30	1.9	960
	6	30	200	16.4	98	57	3.7	640
	7	10	200	26.5	88	54	3.4	350
	8	0	300	46.8	95	63	4.4	150
	9	−10	350	65.7	>99	70	5.7	90
PS-C II[f]/EtOH	10	60	300	8.7	98	34	2.0	810
	11	45	200	21.5	>99	47	2.8	490
	12	30	200	19.0	97	66	4.9	540
	13	10	200	28.0	84	67	5.1	320
	14	0	300	46.3	82	77	7.7	120
	15	−10	350	69.3	80	73	6.4	70

[a] Enol ester **1** (100 mg).
[b] Determined by GC.
[c] Determined by HPLC.
[d] Total turnover number per hour.
[e] Lipase PS immobilized on Celite containing ca 1.0 wt % of lipase.
[f] Lipase PS-C II immobilized on porous ceramic support (*Toyonite*).

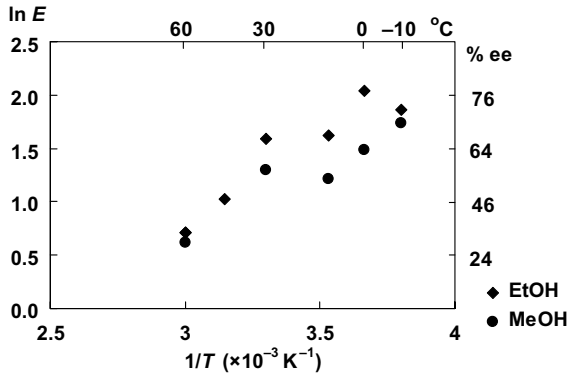


Figure 2. Correlation between ln E versus $1/T$ (Lipase PS on Toyonite, MeOH or EtOH).

3. Conclusion

We have reported the temperature effect on the enantioface-selective protonation in the lipase-catalyzed hydrolysis of prochiral enol ester of ketone, in which (*R*)-2-methylcyclohexanone was obtained at best in 77% ee with 82% conversion at 0 °C. The existence of the temperature effect is significant for the discussion of the mechanism and for fine-tuning the enantioselectivity. A combination of the low-temperature method and optimization of the enzyme is the next subject for improving further the asymmetric protonation.

4. Experimental

4.1. General

Silica gel column chromatography was performed using Fuji Silysia BW-127 ZH (100–270 mesh). Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254}. Lipase PS-C II and lipase PS were purchased from Amano Enzyme Inc. Diisopropyl ether was distilled from sodium before use. ^{1}H NMR spectra were measured at 200 MHz. Enantiomeric excesses were determined by HPLC analyses detected at 254 nm with a chiral column (*Chiralcel* OB-H, Daicel Chemical Industries). 1-Acetoxy-2-methylcyclohexene **1** was prepared by a reported procedure.[3b]

4.2. Typical procedure for lipase PS-C II-catalyzed asymmetric protonation in the presence of ethanol

A mixture of 1-acetoxy-2-methylcyclohexene **1**[3b] (100 mg, 0.65 mmol), lipase PS-C II (amount indicated in Table 1) and 5 mL of diisopropyl ether was placed in a test tube in a thermo-controlled bath. Ethanol (380 μL, 6.5 mmol) was quickly injected into the mixture through a syringe. The reaction progress was monitored by TLC (hexane/ethyl acetate = 4:1). The mixture was quickly filtered with suction to remove the lipase and the filtrate concentrated under reduced pressure. The conversion was analyzed by GC (PEG-3m, oven temp. 120 °C, injection temp. 200 °C), and the enantiomeric excess determined by HPLC [Chiralcel OB-H, hexane/*i*-PrOH = 100:1, flow rate = 0.5 mL/min, detection 254 nm, 17.5 min for (*S*)-**2**, 19.0 min for (*R*)-**2**]. The absolute configuration was determined to be (*R*) by comparison of the sign of the specific rotation {$[\alpha]_D = -6.0$ (*c* 0.5, CH_3OH), 54% ee} with that {$[\alpha]_D = +12.2$ (*c* 4, CH_3OH), 87% ee, (*S*)} already reported.[11] The retention times for (*S*)- and (*R*)-**2** were confirmed by using commercially available racemic **2**. In the reaction at 60 °C, the amount of lipase was also increased to shorten the reaction time, owing to the volatility of the solvent.

Acknowledgements

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$$\ln E = \ln(k_1/k_2) = \ln\frac{100+x}{100-x}$$

where k_1 is the reaction rate of the faster-reacting enantiomer, k_2 is the reaction rate of the slower-reacting enantiomer, and x is the enantiomeric purity (% ee) of the product.
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