Monatshefte für Chemie 131, 633–638 (2000)

Monatshefte für Chemie
Chemical Monthly

Lipase-Catalyzed Resolution of Ibuprofen

Erik Henke, Sascha Schuster, Hong Yang, and **Uwe T. Bornscheuer***

Institut für Chemie und Biochemie, Abt. Technische Chemie und Biotechnologie, Universität Greifswald, D-17487 Greifswald, Germany

Summary. The resolution of ibuprofen by transesterification of its corresponding vinylester using lipase B from *Candida antarctica* is described. Compared to transesterification or hydrolysis of the ibuprofen ethyl ester ($E < 2$, 28–48 h), the reaction with vinylesters occurred significantly faster (1.5–5 h) and with considerably higher enantioselectivity ($E = 8$–39).

Keywords. Enantioselectivity; *Candida antarctica* B; Ibuprofen; Lipase; Organic solvent; Vinylester.

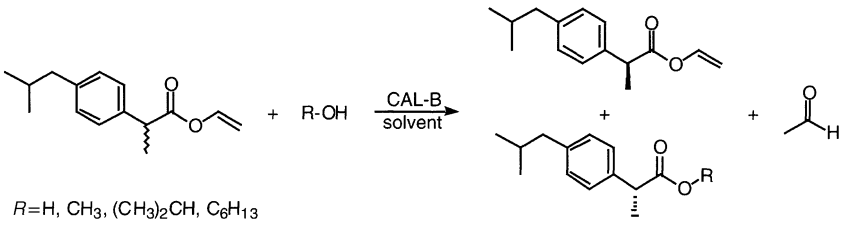
Introduction

2-Arylpropionic acids (profens) are known as a major group of nonsteroidal anti-inflammatory drugs (NSAID) used in the treatment of arthritis and related diseases [1, 2]. *In vitro* tests have shown that the anti-prostaglandin synthetase activity of profens resides almost exclusively in the (+)-(*S*)-enantiomers, yet all profens except naproxen are marketed as racemates [3]. In the case of ibuprofen (2-(4-isobutylphenyl)-propionic acid) it has been shown that the (*S*)-enantiomer is about 160 times biologically more active than its (*R*)-counterpart [4].

Lipases (EC 3.1.1.3) are very suitable enzymes for organic syntheses because they accept a wide range of non-natural substrates, are stable and active in organic solvents, do not require cofactors, and are readily available from several (micro)organisms in sufficient quantities. So far, the resolution of >1000 substances has been described, and it turned out that enantioselectivity toward secondary alcohols is usually very high. In contrast, primary alcohols as well as carboxylic acids are often difficult to resolve [5].

Several groups already have investigated the kinetic resolution of NSAID-precursors, but as outlined above, the enantioselectivity E [6] was only moderate in most cases due to the low enantioselectivity observed in the lipase-catalyzed hydrolysis of racemic carboxylic acid derivatives (*e.g.* a methyl ester) of NSAIDs. In contrary, esterification of the racemic carboxylic acids with simple alcohols (*e.g.* methanol) is hampered by an unfavourable equilibrium leading to long reaction times and low conversions. For instance, esterification of (*R,S*)-ibuprofen with *n*-propanol using lipase from *Candida rugosa* proceeded with $E = 3$ in isooctane.

* Corresponding author



Scheme 1

In the presence of microemulsion, enantioselectivity was much higher ($E = 150$), but 10 days reaction time were necessary to achieve ~30% conversion [7]. A similar reaction in supercritical carbon dioxide as solvent gave an *E*-value of *ca*. 6 at < 20% conversion [8]. In another example, two subsequent kinetic resolutions using lipase B from *Candida antarctica* (CAL-B) have led to the isolation of (*S*)-ibuprofen with 85% *ee* after the first resolution and 97.5% *ee* after the second step, which corresponds to an *E*-value of 17–20. The overall yield was 15% at a total reaction time of 72 h [9].

Recently, we have shown that the resolution of carboxylic acids can be efficiently performed by employing the corresponding vinyl esters, which are transesterified with a suitable alcohol by the aid of lipase B from *Candida antarctica* (CAL-B) [10]. This approach not only lead to significantly enhanced reaction rates as compared to the ethyl esters, but also enantioselectivity increased dramatically from *e.g.* $E = 6.5$ to $E > 100$ in the resolution of 2-phenylbutyric acid derivatives at reasonable reaction times. Moreover, this method also enabled an efficient synthesis of optically pure diastereomeric esters [11]. In both cases, high reaction rates stem from the fact that the vinyl alcohol generated during the transesterification tautomerizes to acetaldehyde, thus making the reaction irreversible. Preliminary computer-aided molecular modeling based on the structure of CAL-B suggests that the increase in enantioselectivity is due to a favorable interaction of the vinyl ester compared to ethyl ester in the binding pocket of CAL-B (data not shown). In this paper, we describe the application of this method for the resolution of ibuprofen (Scheme 1).

Results and Discussion

Initially, we have investigated the ability of three commonly-used lipases (from *Candida antarctica* (CAL-B), *Pseudomonas cepacia* (PCL), and *Candida rugosa* (CRL)) to resolve racemic ethyl or vinyl esters of ibuprofen either in a hydrolytic reaction or by transesterification in toluene as solvent.

For a fast and accurate determination of activity and enantioselectivity and the rapid evaluation of enzymes and reaction conditions, we used a reaction setup with 2 cm^3 reaction vials thermostated in a thermoshaker, which enabled us to use small amounts of substrates (100 µmol/reaction). Samples were analyzed by gas chromatography on a column coated with a chiral carrier, allowing the determination of enantiomeric excess of substrate and product as well as conversion in a single run.

PCL showed no activity towards (*R,S*)-ibuprofen vinyl ester (**1**) or (*R,S*)-ibuprofen ethyl ester (**2**), whereas CRL was only modestly active and enantiose-

Table 1. Resolution of ibuprofen vinyl ester (**1**) or ibuprofen ethyl ester (**2**) using CAL-B

Substrate	Nucleophile[a]	Reaction time (h)	Conversion (%)	Enantiomeric ($\%ee_S$)[b]	excess ($\%ee_P$)	E[c]
1	Methanol	2	52	68 (75)	69	12
1	2-Propanol	5	50	65 (63)	63	8
1	*n*-Hexanol	1.5	57	99 (99)	75	39
2	Methanol	28	33	8 (10)	21	<2
1	Water	11.5	86	99 (99)	16	12
2	Water	48	49	15 (16)	17	<2

[a] For reaction conditions with different nucleophiles see experimental section; [b](*S*)-configuration, values calculated from ee_p and conversion are given in parentheses; [c]calculated according to Ref. [6] using the program available at *http://www-orgc.tu-graz.ac.at*

lective ($E\sim5$) in hydrolysis and also did not catalyze transesterifications, presumably because a non-immobilized preparation was used. In accordance with our previous results [10], CAL-B showed activity in both reactions (Table 1, Scheme 1). However, it is obvious from Table 1 that the reaction rate as well as the enantioselectivity differed considerably. The highest enantioselectivity ($E = 39$) and shortest reaction time (1.5 h) were observed for the transesterification of vinyl ester **1** with *n*-hexanol. The use of other alcohols as nucleophiles still proceeded much faster than hydrolysis, but *E* dropped to values between 8 and 12. In contrast, resolution of the ethyl ester gave almost racemic products at *E*-values below 2 independent whether transesterification with methanol or hydrolysis were performed. Thus, the only method to obtain highly optically pure ibuprofens is by using vinyl esters. This is also exemplified by the time course for the resolution using vinyl ester **1** (Fig. 1) or ethyl ester **2** (Fig. 2). In both reactions, the remaining ester had (*S*)-configuration.

Because the higher enantioselectivity was also observed in the hydrolysis of the vinyl ester, the vinyl group must be responsible for a more stereoselective binding

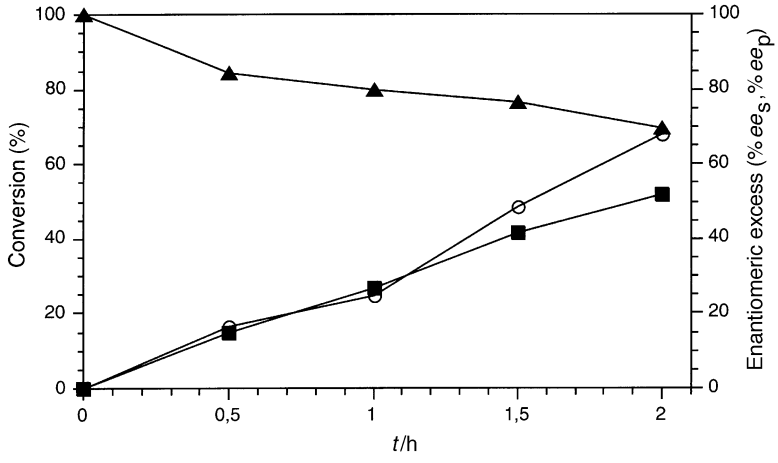


Fig. 1. Transesterification of ibuprofen vinyl ester (**1**) with methanol; ■: conversion, ○: enantiomeric excess of vinyl ester (ee_S), ▲: enantiomeric excess methyl ester (ee_P)

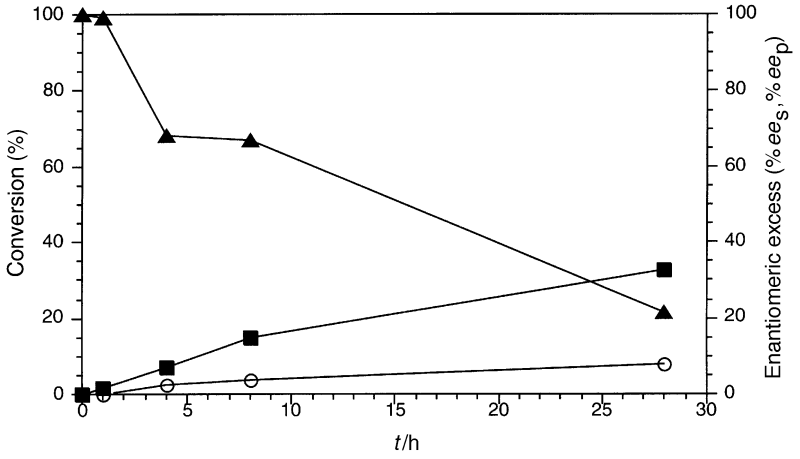


Fig. 2. Transesterification of ibuprofen ethyl ester (**2**) with methanol; ■: conversion, ○ enantiomeric excess ethylester (ee_S); ▲: enantiomeric excess methyl ester (ee_P)

of the substrate within the active site of CAL-B compared to the ethyl group. Although both molecules differ only slightly in terms of size, the vinyl group might lead to a better binding due to the presence of the double bond, *i.e.* by forming an additional hydrogen bond, thus causing a more favoured conformation of the vinyl ester. In addition, *E* increased with increasing chain length of the alcohol used, suggesting that the binding of the nucleophile also contributes to enantioselectivity.

Experimental

^{1}H NMR spectra were recorded at 250.1 and 500.1 MHz, ^{13}C NMR spectra at 62.9 and 125.7 MHz using Bruker NMR spectrometer in $CDCl_3$ with tetramethylsilane as internal standard. Gas chromatographic analyses were conducted on a Finnigan gas chromatograph equipped with a flame ionization detector using a *heptakis*-(2,3-di-O-methyl-6-O-pentyl)-β-cyclodextrin column (25 m × 0.25 mm, Prof. *W. A. König*, University of Hamburg, Germany). Immobilized lipase B from *Candida antarctica* (Chirazyme L-2, c.-f., C2, lyo.; 5000 U/g) and lipase from *Pseudomonas cepacia* (Chirazyme L-1, c.-f., lyo.; 5000 U/g) were donated by Roche Diagnostics, Penzberg, Germany. Lipase from *Candida rugosa* (Amano AY) was donated by Amano Inc., Nagoya, Japan. All chemicals were purchased from Fluka-Sigma-Aldrich, Deisenhofen, Germany. The results of elemental analyses agreed favourably with the calculated values.

(R,S)-Ibuprofen vinyl ester (**1**, $C_{15}H_{20}O_2$)

In 90 cm^3 vinylacetate (1.05 mol), 2 g ibuprofen (9.7 mmol), 348 mg palladium(II)acetate (1.55 mmol), and 54 mg potassium hydroxide (0.97 mmol) were dissolved. After refluxing the mixture for 15 h, the solids were filtered off and the residue was washed with 20 cm^3 vinyl acetate. The solvent was removed under vacuum before the remaining crude product was purified by flash column chromatography (silica gel, petrolether:diethylether = 20:1).

Yield: 2.10 g ibuprofen vinyl ester (7.48 mmol, 80%); ^{1}H NMR (500.1 MHz, $CDCl_3$), $\delta = 0.89$ (6H, d, $J = 6.5$), 1.52 (3H, d, $J = 7.1$), 1.81–1.87 (1H, m), 2.44 (2H, d, $J = 7.2$), 3.75 (1H, q, $J = 7.1$), 4.54 (1H, dd, $J = 1.6$, $J = 6.3$), 4.86 (1H, dd, $J = 1.6$, $J = 14.6$), 7.09–7.24 (4H, m) ppm; ^{13}C NMR (125.8 MHz, $CDCl_3$): $\delta = 18.50$, 22.46, 30.24, 44.96, 45.11, 97.92, 127.27, 129.51, 136.97, 140.89, 141.50, 171.90 ppm.

(R,S)-Ibuprofen ethyl ester (**2**; $C_{15}H_{18}O_2$)

In a 100 cm^3 round bottomed flask, 1 g (*R,S*)-ibuprofen, (4.88 mmol) were dissolved in 30 cm^3 ethanol. After addition of 50 mm^3 H_2SO_4 (0.98 mmol) the mixture was refluxed on an oil bath for 22 h. Ethanol was removed by distillation using a 20 cm vigreux column. The residual volume of approximately 10 cm^3 was dissolved in 50 cm^3 diethyl ether and washed with 100 cm^3 saturated sodium hydrogen carbonate solution. The aqueous layer was extracted twice with 30 cm^3 diethyl ether. The organic layers were combined, washed with 50 cm^3 water, and dried over anhydrous sodium sulfate. Solvent removal under vacuum yielded 1 g of colorless product (4.28 mmol, 87%) which needed no further purification (purity >99% as determined by GC and NMR analysis).

^{1}H NMR (500.1 MHz, $CDCl_3$): $\delta = 0.89$ (6H, d, $J = 6.5$), 1.20 (3H, d, $J = 7.2$), 1.48 (3H, d, $J = 7.1$), 1.86 (1H, m), 2.44 (2H, d, $J = 7.2$), 3.67 (1H, q, $J = 7.2$), 4.06–4.17 (2H, m), 7.09 (2H, d, $J = 8.1$), 7.15 (2H, d, $J = 8.1$) ppm; ^{13}C NMR (125.8 MHz, $CDCl_3$): $\delta = 14.53$, 19.02, 22.80, 30.59, 45.44, 45.56, 61.05, 127.52, 129.69, 138.28, 140.85, 175.19 ppm.

General procedure for the enzymatic transesterification of ibuprofen vinyl ester (**1**) *and ibuprofen ethyl ester* (**2**)

To 1 cm^3 of a solution of 100 m*M* **1** and 100 m*M* alcohol (500 m*M* alcohol in case of methanol and **2**) in dry toluene, 10 mg of the lipase preparation were added in a 2 cm^3 reaction vial and incubated at 40°C in a thermoshaker. Samples of 10 mm^3 were withdrawn from the reaction mixture, diluted with 1 cm^3 of toluene, and the lipase was removed by centrifugation. The diluted sample was analyzed directly by gas chromatography on a chiral column.

General procedure for the enzymatic hydrolysis of **1** *and* **2**

To 200 mm^3 of a solution of 500 m*M* **1** or **2** in toluene, 10 mg of the lipase preparation and 800 mm^3 sodium phosphate buffer (50 m*M*, $pH = 7.5$) were added in a 2 cm^3 reaction vial and incubated at 40°C in a thermoshaker. Samples of 5 mm^3 were withdrawn from the organic layer, diluted in 1 cm^3 of toluene, and the lipase was removed by centrifugation. The diluted sample was analyzed directly by gas chromatography on a chiral column.

Lipase-catalyzed synthesis of ibuprofen methyl ester

Racemic ibuprofen vinyl ester (**1,** 209 mg, 0.90 mmol) and 300 mm^3 methanol (240 mg, 7.5 mmol) were dissolved in 8 cm^3 toluene, and the mixture was heated to 40°C. The reaction was started by addition of 416 mg lipase CAL-B. After 5 h the immobilized lipase was filtered off, and the solvent was removed under vacuum. An aliquot of the residue was used to determine the conversion (40.5%) by gas chromatography. The remaining mixture of product and non-converted substrate was separated by flash column chromatography (silica gel, petrolether:diethyl ether = 35:1).

Yield: 64 mg ibuprofen methyl ester (0.29 mmol, 32%); $ee_p = 76\%$ *ee* (GC-analysis); $[\alpha]^{25}_{589nm} = -50.2°$ (corresponding to 77% ee_P; $CDCl_3$; $c = 6.800$; Ref. [13]: $[\alpha]^{25}_{589nm} = 64.6°$ ($CDCl_3$) for (*S*)-Ibuprofen methyl ester); 98 mg ibuprofen vinyl ester (0.42 mmol, 47%) $ee_S = 52\%$ *ee* (calculated from ee_p and conversion); $[\alpha]^{25}_{589nm} = +28.6°$ ($CDCl_3$, $c = 4.900$); $E = 12$.

Ibuprofen methyl ester: ^{1}H NMR (250.1 MHz, $CDCl_3$): $\delta = 0.82$ (6H, d, J = 6.6 Hz), 1.41 Hz (3H, d, $J = 7.2$ Hz), 1.77 (1H, septet, $J = 6.8$), 2.37 (2H, d, $J = 7.2$ Hz), 3.58 (3H, s), 3.70 (1H, q, $J = 7.2$ Hz), 7.00–7.18 (4H, m) ppm; ^{13}C NMR (62.9 MHz, $CDCl_3$), $\delta = 18.70$, 22.47, 30.25, 45.10, 45.11, 52.05, 127.20, 129.43, 137.82, 140.63, 175.30 ppm. NMR data of isolated vinyl ester matched with those of the racemic compound **1** as given above.

Acknowledgements

The authors are grateful for a grant by the German Research Foundation (DFG, Bonn, Germany), financial support by the *Fonds der Chemischen Industrie* (Frankfurt, Germany), and a postdoctoral stipend for Dr. *Hong Yang* from the *Alexander v. Humboldt Foundation* (Bonn, Germany). We also thank Prof. *W. A. König* (University of Hamburg, Germany) for providing the column for chiral analysis by gas chromatography.

References

[1] Adams SS (1992) J Clin Pharmacol **32**: 317
[2] Davies EF, Avery GS (1971) Drugs **2**: 416
[3] Mayer JM (1990) Acta Pharm Nord **2**: 197
[4] Adams SS, Bresloff P, Mason GC (1976) J Pharm Pharmacol **28**: 256
[5] Bornscheuer UT, Kazlauskas RJ (1999) Hydrolases in Organic Synthesis – Regio- and Stereoselective Biotransformations. Wiley-VCH, Weinheim
[6] Chen CS, Fujimoto Y, Girdaukas G, Sih CJ (1982) J Am Chem Soc **104**: 7294
[7] Hedström G, Backlund M, Slotte JP (1993) Biotechnol Bioeng **42**: 618
[8] Rantakylä M, Aaltonen O (1994) Biotechnol Lett **16**: 825
[9] Trani M, Ducret A, Pepin P, Lortie R (1995) Biotechnol Lett **17**: 1095
[10] Yang H, Henke E, Bornscheuer UT (1999) J Org Chem **64**: 1709
[11] Yang H, Henke E, Bornscheuer UT (1999) Tetrahedron Asymmetry **10**: 957
[12] Lobell M, Schneider MP (1994) Synthesis 375
[13] Ghislandi V, Manna A, Azzolina O, Gazzaniga A, Vercesi D (1982) Farmaco **37**: 81

Received December 20, 1999. Accepted January 12, 2000