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TETRAHEDRON: *ASYMMETRY*

Enzymatic kinetic resolution of ketorolac

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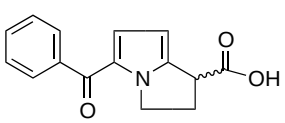
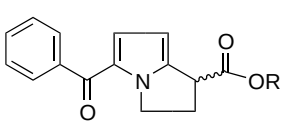
Abstract—Ketorolac **1** was resolved into each enantiomer by interesterification using lipase B from *Candida antarctica*. The acid reacted with various alcohols and the ester and acid were resolved up to >99% e.e. when reacted with octanol, which was the best result. To increase reactivity and enantioselectivity, the experimental conditions were adjusted by varying temperature, solvent, alcohols and reaction time. © 2001 Published by Elsevier Science Ltd.

1. Introduction

Ketorolac **1** (Toradol™), [(±)-5-benzoyl-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]pyrrole-1-carboxylic acid] is a non-steroidal anti-inflammatory drug (NSAID) with cyclooxygenase inhibitory activity.[1] It contains one stereogenic carbon center and is on the market as a racemic mixture. It is known that in several NSAIDs such as ibuprofen and naproxen one enantiomer differs in properties from the other.[2] In 1996, Mroszczak et al.[3] reported that its active enantiomer is the (−)- or (*S*)-form, while the (*R*)-form is inactive from the chiral kinetics and dynamics of ketorolac. In spite of its beneficial activity, it has some adverse effects such as gastrointestinal bleeding, renal impairment and platelet inhibition with altered haemostasis. One way to minimize these effects is to prescribe at the lowest dosage necessary.[4] If the active enantiomer of the racemate could be used, the dosage might be halved. Thus, it is important to resolve such compounds. Unfortunately, only a few results have been reported to date. One method involves resolution using hydrolase biocatalysts.[5] The results were excellent only when proteases were used. Herein, we report for the first time the resolutions of ketorolac via esterification using lipase B from *Candida antarctica*.



Ketorolac, (±)-**1**



(±)-**2**

2. Results and discussion

To resolve **1** using hydrolases, either hydrolase-catalyzed hydrolysis or esterification is suitable. At first, ketorolac butyl ester **3** was hydrolyzed as shown in Scheme 1. The results are shown in Table 1.

As reported previously,[5] the protease, *Streptomyces griseus*, hydrolyzed **3** in favor of pure (*S*)-acid, while *C. antarctica* lipase B favored the (*R*)-acid. The others resolved **3** enantioselectively. In this reaction, the faster reacting enantiomer was the (*R*)-isomer except when using *S. griseus*. Although the residual ester is quite pure using *S. griseus*, *C. antarctica* lipase B and *Mucor meihei*, the final step in the synthesis of ketorolac from the ester will induce racemization. Thus, it is important to obtain the acid in pure (*S*)-form. We therefore examined esterification as another method.

In search of screening esterifications it was most probable that **1** reacted with an alcohol such as 1-butanol using *C. antarctica* lipase B (Scheme 2). At first, we investigated the effect of the various alcohols on the enantioselectivity in the organic solvent. The results are shown in Table 2.

In this esterification, the straight chain alkanols interacted with acid **1** smoothly and enantioselectively ($E>200$), but the relatively hindered alcohols did not. The fast reacting enantiomers are (*R*)-form in both hydrolysis and esterification reactions. These results are concordant with the previous reports.[6–8] However, the stereospecificity is superior to them and among the various alcohols, the ethanol, 1-butanol, 1-octanol, and 1-tetradodecanol reactions are the best. Secondly, in

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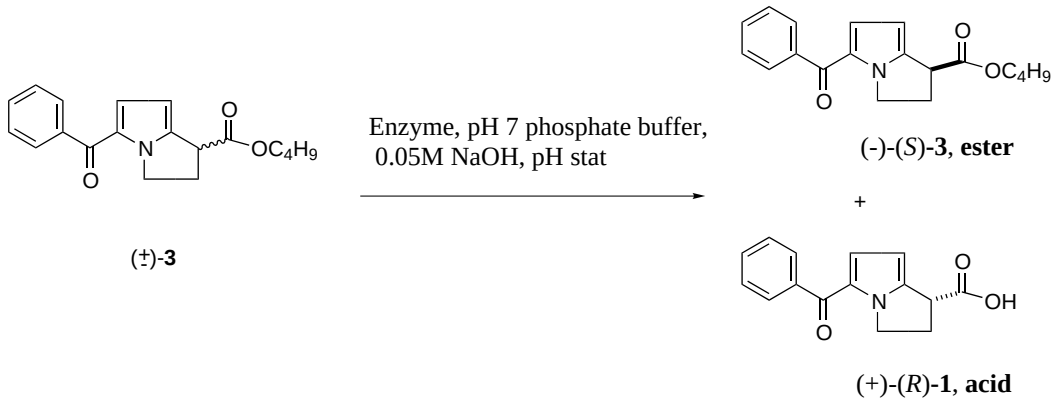
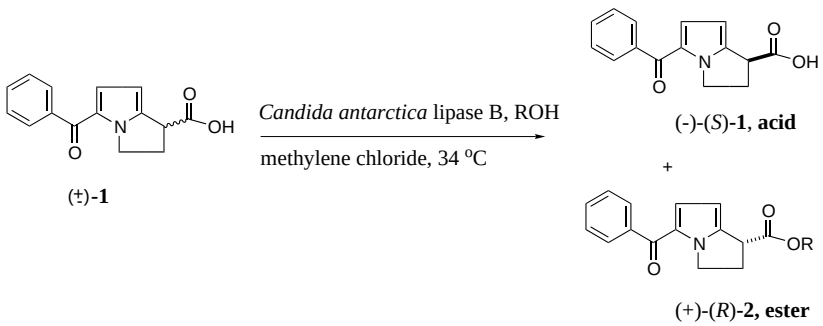


Scheme 1. Hydrolysis of ketorolac butyl ester **3**.

Table 1.

Hydrolase	Time (h)	Conversion (%)	Acid e.e. (%)	Ester e.e. (%)
C. rugosa lipase	7	47	91 (*R*)	–*
	28	84	–*	46 (*S*)
C. antarctica lipase B	0.5	19	100 (*R*)	–*
	3	60	–*	99 (*S*)
S. griseus	1.5	7	100 (*S*)	–*
	29	68	–*	98 (*R*)
A. saitoi	22	11	64 (*R*)	–*
	32	15	–*	1 (*S*)
M. meihei lipase	0.5	26	71 (*R*)	–*
	2.5	96	–*	92 (*S*)

* These values are very low.



Scheme 2. Esterification of racemic ketorolac **1** with various alcohols using CAL-B.

order to increase the enantioselectivity of the (*S*)-acid, the reaction conditions were monitored by changing solvent and temperature. In lipase-catalyzed esterifications, the reaction solvent is very important for increasing reactivity and stereoselectivity.[9–11] We studied the effect of organic solvents from non-polar to polar and showed conversion and enantioselectivity in Fig. 1 and Table 3.

As shown in Fig. 1, in this reaction, the conversion rate might be independent of the solvent polarity. In *n*-hexane, dichloromethane and diisopropyl ether, it had the same trend until 50% conversion. However, in tetrahydrofuran, acetonitrile, dimethylformamide and 1,2-dichloroethane, it was very low. In view of obtaining the pure (*S*)-acid, we are interested in the solvents such as dichloromethane and 1,2-dichloroethane because **1** was resolved in >99% e.e. $\{[\alpha]_D^{22}$ −154 (*c* 1.05, $CH_3OH)\}$.[5] This phenomenon cannot be fully explained at present, but might result from the formation of complex interactions.[11] Finally, in these chlorinated solvents, we controlled the reaction temperature to increase the reaction rate without the enzyme destabilization. To do this, we used 1,2-dichloroethane because of its high boiling point (83°C) and these results are shown in Fig. 2 and Table 4.

Table 2.

Alcohol	Reaction time (h)	Conversion (%)	Enantiomeric excess		*E*
			(% e.e.$_s$) (*S*)	(% e.e.$_p$) (*R*)	
Ethanol	43	43	52	99	>200
1-Butanol	42	53	90	98	>200
1-Hexanol	48	59	86	97	183
1-Octanol	52	58	93	96	167
1-Decanol	49	59	87	97	187
1-Tetradecanol	49	58	86	98	>200
2-Chloroethanol	25	2	47	86	21
Benzyl alcohol	25	1	80	71	14
iso-Propanol	116	5	25	89	21
iso-Butanol	49	56	76	97	151

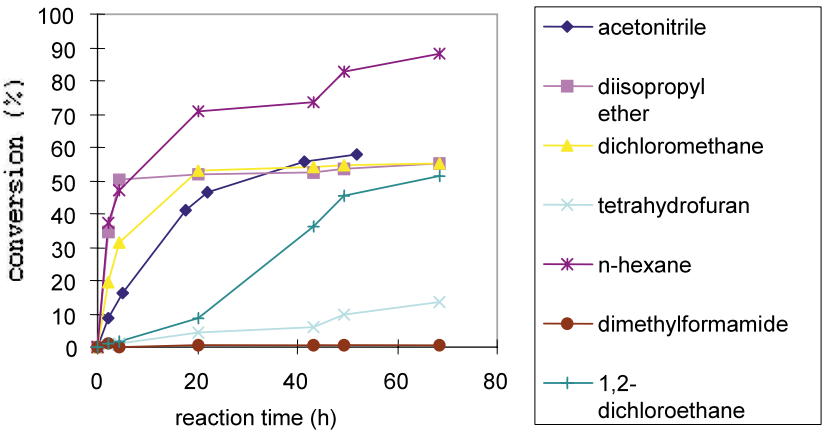


Figure 1. Solvent effect on the conversion in esterification of **1**.

Table 3.

Solvent	Reaction time (h)	Conversion (%)	Enantiomeric excess		*E*
			(% e.e.$_s$) (*S*)	(% e.e.$_p$) (*R*)	
Acetonitrile	52	58	93	96	167
Diisopropyl ether	5	50	87	99	>200
Dichloromethane	44	55	>99	>99	>200
Tetrahydrofuran	69	14	13	>99	>200
n-Hexane	3	37	22	99	>200
Dimethylformamide	3	1	–	–	–
1,2-Dichloroethane	69	50	>99	>99	>200

As anticipated, the reaction time required to reach around 50% conversion reduced with increasing reaction temperature. At 60°C, the reaction time required to obtain the pure (*S*)-isomer was one third of that observed at 34°C. Thus, we can select any reaction condition at either 34 or 60°C depending on time or energy considerations.

3. Conclusion

In summary, we resolved ketorolac [(±)-5-benzoyl-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]pyrrole-1-carboxylic acid], by esterification with various alcohols in organic solvents using *C. antarctica* lipase B. Among them, the resolving efficiency was very high in *n*-octanol. To check solvent effects, the acid **1** reacted with *n*-octanol in chlorinated solvents such as dichloromethane and 1,2-dichloromethane and each enantiomer was resolved in up to 99% e.e., respectively. To get pure (*S*)-acid, the temperature was acceptable from 34 to 60°C. These resolving conditions using *C. antarctica* lipase B will provide a useful guide for the other secondary chiral acids.

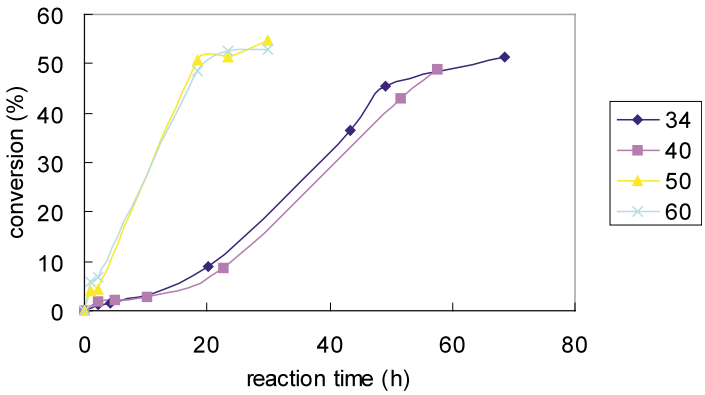


Figure 2.

Table 4.

Temperature (°C)	Reaction time (h)	Conversion (%)	Enantiomeric excess (% e.e.$_s$) (*S*)	(% e.e.$_p$) (*R*)	*E*
34	69	50	99	99	>200
40	58	49	85	98	>200
50	30	55	98	>99	>200
60	24	53	>99	98	>200

4. Experimental

4.1. General

The organic solvents, methanol, *n*-hexane and 2-propanol were all HPLC-grade and purchased from J. T. Baker. Trifluoroacetic acid (TFA) was obtained from Aldrich. *Candida rugosa* lipase, *S. griseus* and *Aspergillus saitoi* were obtained from Sigma Co. *Mucor miehei* was obtained from Fluka. *Candida antarctica* lipase B was obtained from NOVOZYME. The IR spectra were measured on a Perkin–Elmer 16FPC FT-IR grating infrared spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, at 300.13 and 75.46 MHz, respectively, on a Bruker AVANCE-300 spectrometer; chemical shifts being expressed in ppm with reference to Me_4Si, coupling constants (*J*) in Hz. Mass spectra were obtained on a Hewlett–Packard 5890 series II; a Hewlett–Packard 5972 series mass selective detector GC/MS spectrometer equipped with HP-5 cross-linked 5% phenyl methyl silicon fused silica capillary column (25 m×0.20 mm i.d.). The optical rotations were determined with an AUTOPOL III (Rudolph Research, Flanders, NJ) recording polarimeter in MeOH. A YOUNG-LIN high performance liquid chromatograph (Korea), equipped with a YOUNG-LIN M930 Pump; a YOUNG-LIN M720 absorbance detector were used. The computer programs used were Autochro 2.0 plus for chromatographic analysis. A chiralpak AD column (0.46 cmϕ×25 cm) packed with an amylose derivative coated on silica gel was used (Daicel Chemical Industries). TLC was carried out on Merck glass plates precoated with silica gel 60F-254. Column chromatography was performed by Merck 70–230 or 230–400 mesh silica gel. Enzymatic hydrolysis was carried out on 718 STAT Titrino (Metrohom, Switzerland).

4.2. 5-Benzoyl-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]pyrrole-1-carboxylic acid butyl ester

A solution of ketorolac[12] (100 mg, 0.392 mmol) in benzene (5 mL) was stirred at room temperature. 1-Butanol (35.9 μL, 3.92 mmol, 10 equiv.) and *p*-toluenesulfonic acid (7 mg, 0.0392 mmol, 0.1 equiv.) were added to the solution. The mixture was heated under reflux for 3 h then quenched with water. The product was extracted with ether, the organic layer was washed with saturated aqueous $NaHCO_3$ and brine. The extract was dried with anhydrous $MgSO_4$, filtered and the filtrate concentrated to an oil. The crude product was purified by column chromatography (*n*-hexane:ethyl acetate=10:1) to yield the ester as an oil (108 mg, 89%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, 3H, J=7.4), 1.37–1.44 (m, 2H), 1.64–1.69 (m, 2H), 2.77–2.94 (m, 2H), 4.05–4.20 (m, 2H), 4.47–4.58 (m, 2H), 6.11 (d, J=3.4, 1H), 6.83 (d, J=4.0), 7.43–7.54 (m, 3H), 7.82 (dd, J=6.9 Hz, J=1.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.071, 19.501, 31.003, 31.286, 43.069, 47.989, 65.751, 103.474, 125.358, 127.560, 128.530, 129.281, 131.756, 139.679, 142.945, 171.679, 185.359; IR (KBr) 2960, 1734, 1624, 1268, 1180 cm^{-1}; GC/MSD retention time (min) 13.76, *m*/*z* 51, 65, 77, 91, 105, 115, 123, 132, 140, 152, 167, 180, 191, 210 (100), 226, 234, 254, 268, 282, 294, 311(M$^+$).

4.3. Enzymatic hydrolysis of butyl ester of ketorolac

The butyl ester of ketorolac (40 mg, 0.16 mmol) was dissolved in phosphate buffer (7 mL, pH 7.0) and enzyme (0.2–2 mass equiv.) was added to the solution. A pH stat regulated the addition of aqueous 0.02 M NaOH solution at room temperature to maintain the pH at 7.0. A sample was taken for HPLC analysis

(chiralpak AD, *n*-hexane:IPA:TFA = 90:10:0.1 v/v%, 310 nm, 0.8 mL/min). The results are shown in Table 1.

4.4. Esterifications of ketolorac with alcohols using *C. antarctica* lipase B

Ketorolac (10 mg, 0.039 mmol) was dissolved in MeCN (5 mL) and alcohol (0.39 mmol, 10 equiv.), ground molecular sieves (4 Å, 30 mg) were added to the solution. CAL-B (10 mg, 1 mass equiv.) was added to the solution, and then mixture was shaken in incubator at 33.8°C. The mixture was taken periodically from the reactor and filtered and evaporated. The slurry was dissolved in 1 mL MeOH for HPLC analysis (chiralpak AD, *n*-hexane:*iso*-propanol:trifluoroacetic acid = 90:10:0.1 v/v%, 310 nm, 0.8 mL/min)

4.5. Lipase-catalyzed esterifications in different solvents

As described in Section 4.4, the same reaction as described above was performed in different alcohol solvents.

4.6. Lipase-catalyzed esterifications at different temperatures

As described in Section 4.4, the same reaction as described above was performed at different temperatures.

Acknowledgements

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