CONVENIENT PREPARATION OF (*S*)-FENOPROFEN BY BIOCATALYSED IRREVERSIBLE ESTERIFICATION

R. Morrone*, N. D'Antona and G. Nicolosi
CNR – Istituto di Chimica Biomolecolare, Via Del Santuario 110,
95028 Valverde, Catania, Italy.
E-mail: raffaele.morrone@icb.cnr.it

ABSTRACT

*Immobilised lipase B from Candida antarctica catalyzes the enantioselective esterification of rac-fenoprofen, (±)-**1**. The use of orthoformates as alcohol donors permitted to synthesize the ester in irreversible manner, therefore improving the performance of the enantioselective esterification. This convenient procedure permitted to prepare (S)-fenoprofen in good yield and in high optical purity.*

***Keywords**: Lipase, Irreversible esterification, Orthoformates, Fenoprofen, NSAID*

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are used for the treatment of inflammation and pain in various rheumatic and musculoskeletal disorders. One of the most important classes of NSAIDs are the "profens", derivatives of 2-arylpropionic acid.[1–3] This compounds are used in clinical since more than 30 years and, although clinical studies have fully showed that mainly the S-enantiomer act as anti-inflammatory, they are for the most part marketed nowadays as racemic mixture. For this reason, several investigations have been carried out to obtain enantiomerically pure (*S*)-2-arylpropanoic acids. Among the developed procedures, the biocatalytic separation of the racemate has often furnished satisfactory results and the use of lipases in the resolution of chiral acids has provided good results both by hydrolysis of the preformed esters and by direct esterification in non-acqueous medium.[4-6] In this context resolutions of naproxen,[7–9] ibuprofen,[10,11] ketoprofen,[12,13] flurbiprofen,[14] suprofen[15] and indoprofen[16] have been reported.

In this paper, we describe the preparation of enantiopure S-fenoprofen, realized through kinetic resolution of *rac*-fenoprofen catalysed by lipase B from *Candida antarctica* (Novozym 435), using an irreversible process of esterification that exploits orthoformates as alcohol donor.

EXPERIMENTAL

Commercially available chemicals were used without further purification unless otherwise stated. Novozym 435 was a gift from Novo Nordisk. *rac*-Fenoprofen calcium salt hydrate was purchased from Sigma. Trimethyl-, triethyl-, tripropyl- and tributyl orthoformate were purchased from Fluka. Solvents used were HPLC grade. The conversion values and enantiomeric excesses were determined by HPLC equipped by chiral column (Chirex (R) NGLY-DNB; 250 x 4.6 mm) using as mobile phase 0.02 M ammonium acetate in methanol. NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz for hydrogen and at 100.62 MHz for carbon. Optical rotation was measured with a Dip-370 polarimeter from Jasco.

(±)-2-(3-Phenoxyphenyl)propionic acid; (±)-1

The *rac*-fenoprofen [(±)-2-(3-Phenoxyphenyl)propionic acid] was prepared as follows: 2 g of *rac*-fenoprofen calcium salt hydrate was dissolved in 100 ml of water and the solution was treated with a solution 0.5 N of sulphuric acid until the pH value was 3.0. The insoluble fenoprofen was extracted with tert-butylmethylether (3 x 50 ml). The combined organic extracts were washed with water until neutral and dried with Na_2SO_4. After evaporation of solvent 1.8 g of (±)-**1** was obtained. ^{1}H NMR was in accord to literature data[17].

General procedure for esterification of (±)-1

A solution of (±)-**1** (10 mg, 0.041 mmol) in the solvent of choice (1 ml, Table 1) was added with Novozym 435 (10 mg) and nucleophile (0.123 mmol). The mixture was incubated at 45 °C and shaken at 300 rpm. Aliquots of 10 μl were taken periodically and analysed by chiral HPLC.

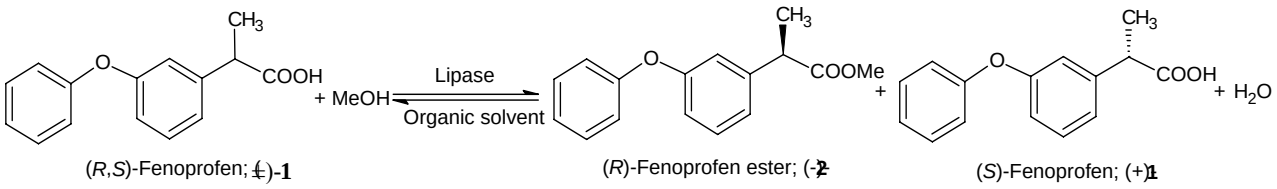
(S)-(+)-2-(3-Phenoxyphenyl)propionic acid, (+)-(S)-1

Novozym 435 (1 g), tributyl orthoformate (2.87 g, 12.39 mmol) and n-butanol (3.2 mg, 0.0413 mmol) were added to a solution of (±)-**1** (1 g, 4.13 mmol) in methyl cyclohexane (100 ml). The reaction mixture was shaken (300 rpm) at 45 °C until 78 % of conversion value (28 h) then the reaction was stopped filtering off the lipase. The methylcyclohexane was extracted with saturated aqueous $NaHCO_3$ (3 x 50 ml). The organic phase was dried over Na_2SO_4 anhydrous and then evaporated in vacuo to afford (*R*)-(-)-fenoprofen butyl ester (920 mg, 75% yield, ee = 27%). The aqueous phase was acidified with 0.5 N H_2SO_4 and extracted with methylcyclohexane (3 x 50 ml). The organic phase was washed until neutral, dried over Na_2SO_4 anhydrous and evaporated in vacuo to yield (+)-(*S*)-**1** (210 mg, 21% yield, ee = 96%); $[\alpha]_D$ = + 44.2 (c=1, $CHCl_3$) [lit.[18] $[\alpha]_D$ = + 46.0 (c=1, $CHCl_3$)].

RESULTS AND DISCUSSION

Selection of lipase

As preliminary investigation, (±)-**1** was subjected to direct esterification in organic solvent using methanol as nucleophile, in presence of lipases from Novozym 435, *Candida cylindracea, Psudomonas cepacea, Rhizomucor javanicus, Mucor miehei, Mucor javanicus, Aspergillus niger,* and porcine pancreatic.



Scheme-1 – Esterification of *rac*-fenoprofen catalysed by lipase in organic solvent

Of all lipases investigated only Novozym 435 and lipase from *Mucor miehei* were able to catalyse the reaction, showing both to possess the same R-stereopreference (Figure 1). Conversely different reaction rates were evidenced, the first furnishing a conversion value of 36% in two hours, while the second only 3% in eight hours. At the light of these results the further experiments were performed using Novozym 435 as biocatalyst.

Solvent effect

Literature data report that the nature of the solvent is one of the parameters that affects the performance of a lipase acting in non conventional media. In general it has been observed that lipophilic solvents, characterized by high log P_{oct} value (the partition coefficient of a given substance in the octanol/water two-phase system), permit a better activity and are less harmfull to the enzyme. Therefore in order to find

out the best enantioselectivity (E) for Novozym 435 different experiments of esterification of (±)-**1** were carried out in four solvents ranging in a 1,5−4.5 log P_{oct} value.

Table-1. Esterification of (±)-**1** with Novozym 435 in different solvents

Solvent	log P_{oct}	Time (h)	Conv. (%)	ee $_{est}$	ee $_{ac}$	E
Diisopropyl ether	1.5	5	35	52	28	4
Hexane	3.5	2	36	53	30	4
Methyl cyclohexane	3.6	2	31	67	30	7
Isooctane	4.5	1	22	62	17	5

As reported in table 1 the reaction in diisopropyl ether occurs at lower rate, in opposition the hydrophobic isooctane permitted to obtain the higher conversion value (22% in 1 hour) but with an enantioselectivity value of 5. The reactions carried out in hexane and methylcyclohexane show comparable reaction rate, but the second furnishes the higher enantioselectivity, with a value of 7, and therefore was chosen as solvent for further experiments.

Nucleophile effect

It is well known that the nucleophile agent has, generally, significant effects on the activity and enantioselectivity of a lipase operating in organic solvent; therefore the influence of four straight-chain C_{1-4} primary aliphatic alcohols on the esterification of *rac*-fenoprofen in methylcyclohexane was investigated.

Table-2. Activity and enantioselectivity of Novozym 435 in the esterification of (±)-**1** in methylcyclohexane with different alcohols

Alcohol	Time (h)	Conv. (%)	ee $_{est}$	ee $_{ac}$	E
Methanol	4	47	54	48	5
Ethanol	4	28	48	19	3
Propanol	4	30	54	23	4
Butanol	4	13	80	12	10

Methanol permitted to obtain, as shown in table 2, the highest conversion value followed by propanol, ethanol and butanol thus indicating an inverse correlation between esterification rate and chain length of the alcohol. In term of enantioselectivity butanol showed the best value, but no linear correlation between the enantioselectivity values and nature of the alcohols was recorded.

Irreversible esterification

In order to improve the performance of the enantioselective esterification of (±)-**1** catalysed by Novozym 435 a further investigation was considered. Due to the R stereopreference observed for Novozyme 435, in theory, the pure S-enantiomer could be obtained as unreacted substrate by prolonging the reaction beyond 50% of conversion until a value depending from the lipase enantioselectivity. With this intent a esterification reaction of (±)-**1** with butanol was protracted for 64 hours but, as reported in figure 1, without practical advantage because long incubation time has as consequence a decreased enantiomeric excess of both enantioforms. This result is consistent considering that, due to the reversible nature of the process, the gradual-production of water in the ester formation has as effect the faster hydrolysis of the higher-rate esterified enantiomer (R) and consequentely a loss of ee is observed both for acid and ester species.

Therefore the removing of the water produced during the esterification process permits to overcome this drawback.

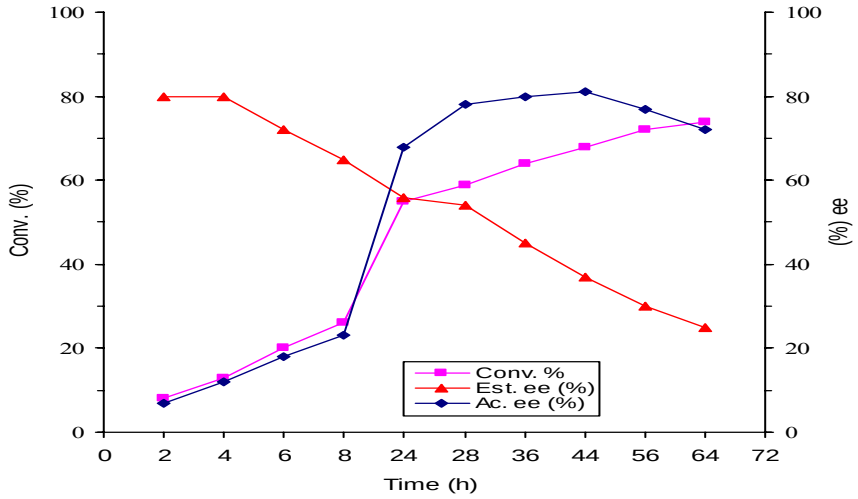


Figure-1. Profile of the reaction of esterification of (±)-**1** by Novozyme 435 when butanol was used as nucleophile

It is known that orthoformates at low pH values undergo hydrolysis giving two molecules of alcohol and, as reported by us in a previous work[19], they can be efficiently exploited as alcohol donors in esterification of acids catalysed by lipases. In figure 2 is reported the general reaction scheme, accordingly the water produced in the initial esterification step is used to hydrolyse the orthoformate with a double advantage: consume of the water and at the same time progression of the esterification by the produced alcohol. For this reason the esterification of (±)-**1** by Novozym 435 was performed in methylcyclohexane using four different orthoformates (Table 3).

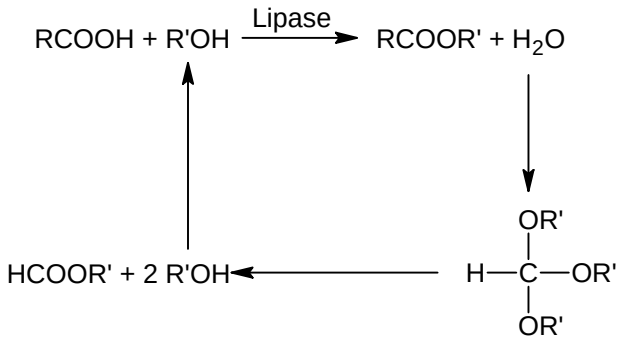


Figure-2. Scheme of irreversible biocatalysed esterification using orthoformates as alcohol donors.

All the considered orthoesters allowed to exceed, even if with different reaction times, the 50% conversion value.
The esterification realised by tributyl orthoformate as alcohol donor, although have the lower reaction rate, shows the best result in terms of enantioselectivity therefore it was used to carry out a preparation of (*S*)-fenoprofen.

Table-3: Activity and enantioselectivity of Novozym 435 in the esterification of (±)-**1** in methylcyclohexane with different orthoformates

Alcohol donor	Time (h)	Conv. (%)	ee $_{est}$	ee $_{ac}$	E
Trimethyl orthoformate	4	67	38	78	5
Triethyl orthoformate	4	50	46	46	4
Tripropyl orthoformate	8	58	43	60	4
Tributyl orthoformate	24	69	41	87	6

Preparative esterification
A gram-scale preparation of (S)-fenoprofen was performed by esterification in methylcyclohexane of (±)-**1** in presence of Novozym 435 using tributyl orthoformate as nucleophile; a small quantity of butanol was added to start the reaction. The course of this reaction is depicted in figure 3. In the conditions adopted after 28 h a conversion value of 78% was measured and the desired (*S*)-fenoprofen with ee 96% was obtained. Selective extraction of the acid, followed by crystallization, furnished (*S*)-fenoprofen in 44 % yield.

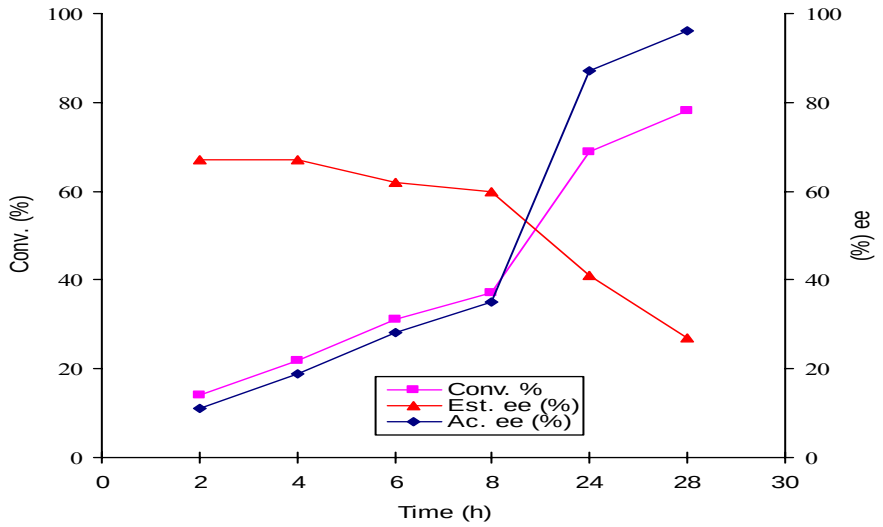


Figure-3 – Course of the irreversible biocatalysed esterification reaction of fenoprofen when tributyl orthoformate was used as alcohol donor.

CONCLUSIONS

The results obtained by this work show that in presence of an irreversible methodology it is possible to obtain the resolution of *rac*-fenoprofen even in the presence of biocatalysts with a low enantioselectivity. This goal can be reached using orthoformates as alcohol donors in direct esterification reactions of acids catalysed by lipase. Furthermore the easy recovery of the (S)-fenoprofen from reaction mixture, the possibility to recycle the ester after racemisation and the absence of by-products given the soft conditions of reaction make this procedure suitable for industrial purposes.

REFERENCES

1. T.Y. Shen, Angew. Chem., *Int. Ed. Engl.*, **11**, 460 (1972).
2. C.Giordano, G.Castaldi, F.Uggeri, *Angew. Chem., Int. Ed. Engl.*, **23**, 413 (1984).
3. H.R.Sonawane, N.S.Bellur, J.R.Ahuja, D.G.Kulkarni, *Tetrahedron*: *Asymmetry*, **3**, 163 (1992).
4. R.D.Schmid, R.Verger, *Angew. Chem. Int. Ed. Engl.*, **37**, 1608 (1998).
5. P.Villeneuve, J.M.Muderhwa, J.Graille, M.J.Haas, *J. Mol. Cat. B Enzym.*, **9**, 113 (2000).
6. M.T.Reetz, *Curr. Opin. Chem. Biol.*, **6**, 145 (2002).
7. E.Battistelli, D.Bianchi, P.Cesti, C.Pina, *Biotechnol. Bioeng.*, **38**, 659 (1991).
8. S.W.Tsai, H.J.Wei, *Enzyme Microb. Technol.*, **16**, 328 (1994).
9. S.W.Tsai, H.J.Wei, *Biotechnol. Bioeng.*, **43**, 64 (1994).
10. A.Mustranta, *Appl. Microbiol. Biotechnol*., **38**, 61 (1992).
11. K.Won, J.Hong, K.Kim, S.Moon, *Process Biochemistry*, **41**, 264 (2006).
12. A.Palomer, M.Cabrè, J.Ginestra, D.Mauleòn, G.Carganico, *Chirality,* **5**, 320 (1993).
13. A.L.Ong, A.H.Kamaruddin, S.Bhatia, *Process Biochemistry*, **40**, 3526 (2005).
14. R.Morrone, G.Nicolosi, A.Patti, M.Piattelli, *Tetrahdron: Asymmetry*, **6**, 1773 (1995).
15. P.Mertoli, G.Nicolosi, A.Patti, M.Piattelli, *Chirality*, **8**, 377 (1996).
16. R.Morrone, G.Nicolosi, M.Piattelli, *Chirality,* **10**, 321 (1998).
17. J.Damodar, S.R.K.Mohan, S.R.Jayarama Reddy, *Electrochem. Commun.* **3**, 762, (2001).
18. W.S.Marshal (Eli Lilly and Co.) Patent FR 2015728.
19. R.Morrone, M.Piattelli, G.Nicolosi, *Eur. J. Org. Chem.*, 1441 (2001).

(Received: 28 May 2008 Accepted: 30 August 2008 RJC-194)