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Enzymes as a tool for optical resolution of (±) α-methyl-4—(2-methylpropyl) benzeneacetic acid (ibuprofen)

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Abstract. Enzyme catalysed kinetic resolution of (±) α-methyl-4-(2-methylpropyl) benzene acetic acid (ibuprofen), esterified with several alcohols, in the presence of enzymes Candida Cylindracea Lipase (CCL), Mucor Mehei Lipase immobilised on support (Lipozyme) and Porcine Pancreatin Lipase (PPL) is reported.

Keywords. Ibuprofen; lipase; optical resolution.

1. Introduction

Ibuprofen, a non-steroidal anti-inflammatory agent, is one of the important drugs. It is currently marketed as a racemic mixture. Recent pharmacological studies have revealed that the *S*(+) isomer of ibuprofen is 165 times more active than the *R*(−) isomer (Hutt and Caldwell 1984; Rieu *et al* 1986). The process of inversion of *R*(−) to *S*(+) ibuprofen in the body takes considerable time. Hence, while its therapeutic effect is doubtful, it can also contribute to unavoidable side effects and toxicity.

S(+)-ibuprofen can be synthesized by different approaches like asymmetric synthesis or can be obtained from a racemic mixture by enzyme catalysed kinetic resolution (Chan 1993; Wu *et al* 1990; Kirchner *et al* 1985). The enzymatic kinetic resolutions of racemic compounds are well known in the literature (Kirchner *et al* 1985; Bevinakatti and Banerji 1988, 1991, 1992; Bevinakatti *et al* 1989) Enantioselective enzymatic hydrolysis of different esters of 2-arylalkanoic acids catalysed by Candida Cylindracea Lipase and Horse Liver Esterase (HLE) have already been reported (Ahmar *et al* 1989; Wu *et al* 1990). Enantioselective esterification of racemic ibuprofen in organic solvent as well as in water-in-oil microemulsion (AOT/isooctane) using lipase have been studied (Annika 1992; Hedstrom *et al* 1993).

In this communication, we report the enantioselective esterification of racemic ibuprofen with various alcohols, catalysed by different lipases in diisopropyl ether as a solvent. Anhydrous sodium sulphate was added in the medium to remove water formed during the course of the reaction.

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2. Results and discussion

Three enzymes were examined as catalysts for the esterification of ibuprofen (1). These were Porcine Pancreatic Lipase (PPL), Candida Cylindracea Lipase (CCL) and Mucor Mehei Lipase immobilised on support (Lipozyme). The reactions carried out are presented in scheme 1.

The results of the reactions carried out with CCL and lipozyme were quite encouraging. On the other hand, in case of PPL, the reaction did not show any signs of product even after 6-7 days and hence was abandoned from our studies.

In all the reactions, the pharmaceutically desired *S*(+)-isomer was found to be more active in forming the esters, thus enriching the substrate 1 (scheme 1) with *R*(−)-isomer. The ester so obtained can be hydrolysed to obtain the desired *S*(+)-isomer of ibuprofen.

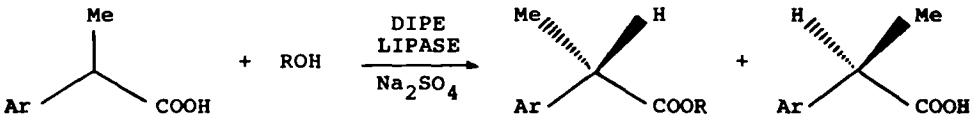
E, the enantiomeric ratio, indicates the efficiency of a particular reaction with respect to optical purity (Chen *et al* 1982). *E* values can be calculated for a reaction, knowing the percent conversion (*c*) and the percent enantiomeric excess of unreacted substrate (ee_s) using the equation:

$$E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]. \quad (1)$$

Table 1 shows, on basis of *E* values, that the reactions with CCL and lipozyme give the same results, *i.e.*, $E = 5$. Further studies were therefore restricted to only one of the enzymes, namely CCL.

To study the effect of carbon-chain length *i.e.* the number of carbon atoms in alcohol 2 on *E* values it was varied form a to c i.e. from alcohol having carbon number 4 (*n*-butanol) to carbon number 8 (*n*-octanol). On doing so, the *E* values were found to increase from 5 to 8, as shown in table 1. Knowing the *E* values for all the reactions it was possible to predict ee_s at various conversions based on calculations according to (1) (Chan *et al* 1982). The graph (figure 1) was thus computer generated. The effect of increase in carbon chain length of alcohol on ee_s can be observed in figure 1. The rate of reaction was also found to increase considerably.

In order to further enhance the optical purity of ibuprofen, the unreacted ibuprofen with optical purity 53 % *ee*, obtained from reaction no. 3, was recycled again with fresh enzyme. Further improvement in optical purity was observed as reported in table 2.



(±) S-(+) R-(-)

1 2 3 4

Ar = 4-isobutylphenyl

a. R = $CH_3(CH_2)_3$ (n-Bu)

b. R = $CH_3(CH_2)_5$ (n-Hx)

c. R = $CH_3(CH_2)_7$ (n-Oc)

Scheme 1.

Table 1. Lipase catalysed kinetic resolution of ibuprofen.

Reaction No.	Alcohol	Enzyme	Time (days)	%Conv.	$\%ee_s$[b] acid	E[c]	Isomer	$\%ee_p$[d] ester	Isomer
1	*n*-BuOH	CCL	21	44	43	5	*R*	52	*S*
2	*n*-HxOH	CCL	17	47	51	6	*R*	54	*S*
3	*n*-OcOH	CCL	9	45	53	8	*R*	56	*S*
4	*n*-BuOH[a]	CCL	10	26	20	5	*R*	58	*S*
5	*n*-BuOH	Lipo-zyme	13	55	56	5	*R*	45	*S*

a. Also used as solvent.

b. ee_s = enantiomeric excess of ibuprofen (unreacted) was determined by comparison with the reported specific rotation in literature $[\alpha]_d^{25} = +59$ ($c = 2$, EtOH) for the S-isomer.

c. $E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$; see Chen *et al* (1982)

d. ee_p = enantiomeric excess of esters (product) were determined by comparison with that of the chemically synthesized respective esters from optically active ibuprofen.

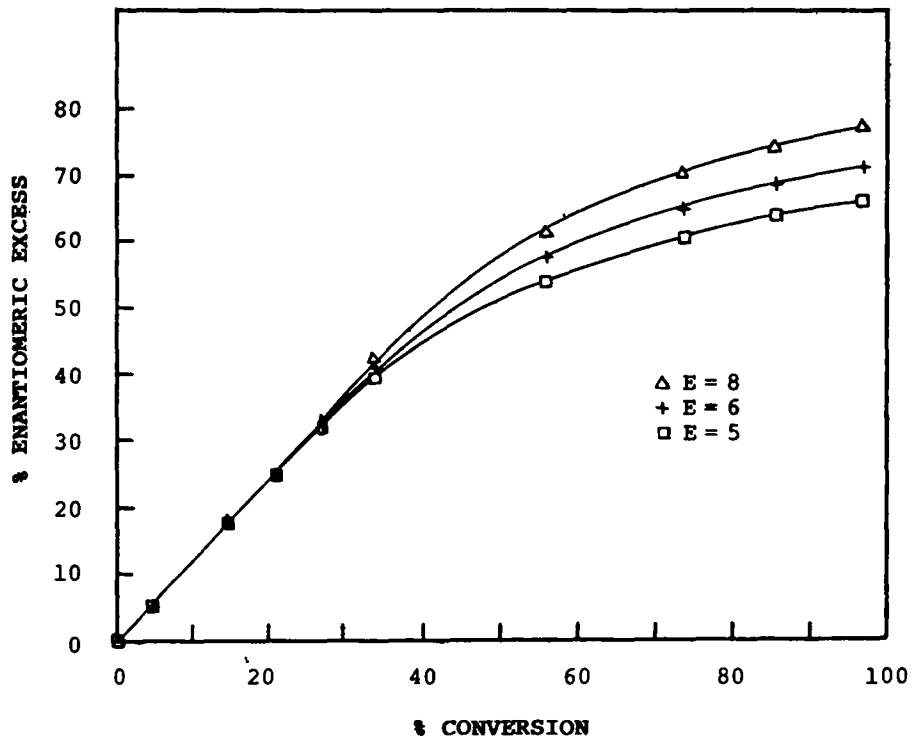


Figure 1. Plot of percent enantiomeric excess (ee_s) of unreacted ibuprofen as a function of the percent conversion (c) for various enantiomeric ratios (E).

Table 2. Recycling of ibuprofen for further enantiomeric enrichment.

Reaction no.	Alcohol	Enzyme	$\%ee_0^e$	Time (days)	%Conv.	$\%ee_s$	$\%ee_p$
6	*n*-BuOH	CCL	53(*R*)	20	26	70(*R*)	62(*S*)
7	*n*-BuOH	Lipozyme	53(*R*)	14	30	68(*R*)	58(*S*)
8	*n*-BuOH	CCL	52(*S*)	8	10	65(*R*)	78(*S*)

(*R*) and (*S*) are the optical isomers
e. ee_0 = initial enantiomeric excess.

Figure 2 was computer generated by us to draw a theoretical interrelationship between initial enantiomeric excess ee_0, percent conversion c and enantiomeric ratio E, using equations known in the literature (Chen *et al* 1982). With the help of figure 2, it was possible to predict the enantiomeric excess ee_s of unreacted ibuprofen for any percentage conversion.

Thus, for our chosen system with $E = 5$, $ee_0 = 53\%$ and $c = 26\%$, the expected ee_s would be 78% as against the found value of 73 % (table 2), thus indicating good agreement between the expected and experimental values. It would also be predicted

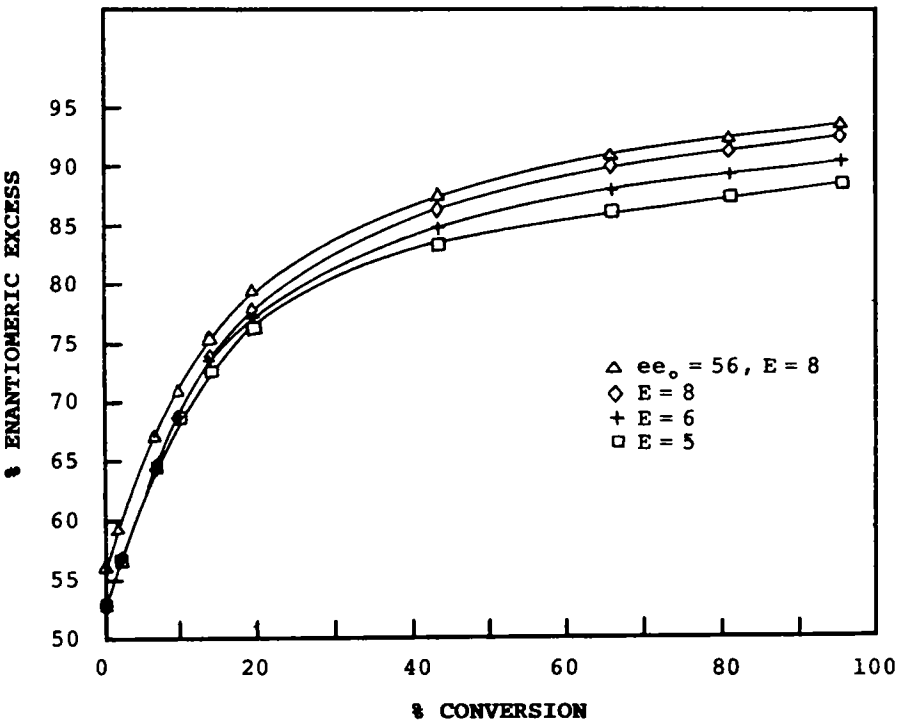


Figure 2. Computer generated plot showing the inter-relationships of the final percent enantiomeric excess (ee') of the unreacted ibuprofen, the percent conversion (c) and the enantiomeric ratio (E) when the initial percent enantiomeric excess (ee_0) was fixed to 53.

from figure 2 that ibuprofen with optical purity of about 99% *ee* could be obtained at conversion >90%. Finally, the enriched *S*(+) isomer of ibuprofen, generated by hydrolysis of the enantiomerically enriched ester from reaction no. 1, was resubjected to enzymatic esterification. *S*(+) isomer being more active further enantiomeric enrichment of ester, to the extent of 78% *ee* at 10% conversion, was observed (table 2).

3. Experimental

^{1}H NMR spectra were recorded on a Bruker 80 MHz FT instrument with TMS as an internal standard. Infrared spectra were obtained on a Perkin–Elmer 781 spectrophotometer. Optical rotations were measured on a JASCO DIP-140 polarimeter. GC analysis was carried out by using an HP 101 capillary column (methyl silicone) (25m × 0.2 μm thickness). Lipases CCL and PPL were purchased from Sigma. Lipozyme was purchased from Novo-Nordisk.

To a stirred mixture of 1 (1.5g, 7.2mmol), alcohol 2 a–c (7.2mmol) and diisopropyl ether (DIPE) (15ml), was added 1.5gm of the enzyme. Anhydrous sodium sulphate (500mg) was added to remove water generated as by-product in the medium. The reaction was monitored by TLC and GC. The reaction was stopped by filtration. The product was evaporated to dryness. Esters 3a–c and acid 1 (unreacted) were isolated by column chromatography on silica gel (finer than 200 mesh) using dichloromethane as the eluant. Various alcohols tried were *n*-butanol (*n*-BuOH), *n*-hexanol (*n*-HxOH) and *n*-octanol (*n*-OcOH). The enzymes used were PPL, CCL and lipozyme.

n-Butyl ester of ibuprofen, 3a: Obtained from reaction no.1 of table 1. It is a colourless liquid with specific rotation $[\alpha]_D^{25} + 33{\cdot}74$ ($C = 2$, EtOH). GC under isothermal condition at 220 °C shows a retention time $t_R = 5{\cdot}73$ min. IR, neat (cm^{-1}) 1740 (CO, ester). ^{1}H NMR ($CDCl_3$) 0·9 (*d*, 9H, $J = 6{\cdot}4$ Hz), 1·2–2 (*m*, 8H), 2·4 (*d*, 2H, $J = 7$ Hz), 3·6 (quartet, 1H, $J = 7{\cdot}1$ Hz), 4·1 (*t*, 2H, $J = 6{\cdot}4$ Hz), 7·1–7·3 (*m*, 4H, aromatic). ^{13}C NMR ($CDCl_3$) 13·6, 18·4, 19·0, 22·3, 30·1, 30·6, 45·1, 45·3, 64·5, 127·2, 129·2, 137·9, 140·4, 174·8. Found C, 77·92; H, 9·82%; $C_{17}H_{26}O_2$ requires C, 77·86; H, 9·92%.

n-Hexyl ester of ibuprofen, 3b: Obtained from reaction no. 2 of table 1. It is a colourless liquid with specific rotation $[\alpha]_D^{25} + 30{\cdot}33$ ($c = 2$, EtOH). GC under isothermal condition at 220 °C shows a retention time $t_R = 7.95$ min. IR, neat (cm^{-1}), 1740 (CO, ester). ^{1}H NMR ($CDCl_3$) 0·9 (*d*, 9H, $J = 6{\cdot}3$ Hz), 1·2–2 (*m*, 12H), 2·4 (*d*, 2H, $J = 7$ Hz), 3·6 (quartet, 1H, $J = 7{\cdot}1$ Hz), 4·1 (*t*, 2H, $J = 6{\cdot}3$ Hz), 7·1–7·3 (*m*, 4H, aromatic). ^{13}C NMR ($CDCl_3$) 13·7, 18·3, 22·3, 22·4, 25·6, 29·8, 30·3, 31·7, 45·1, 45·3, 65·0, 127·3, 129·1, 138·3, 140·2, 174·1. Found C, 78·78; H, 10·44%; $C_{19}H_{30}O_2$ requires C, 78·62; H, 10·34%.

n-octyl ester of ibuprofen, 3c: Obtained from reaction no.3 of table 1. It is a colourless liquid with specific rotation $[\alpha]_D^{25} + 24{\cdot}70$ ($c = 2$, EtOH). GC under isothermal condition at 220 °C shows retention time $t_R = 11{\cdot}5$ min. IR neat (cm^{-1}) 1740 (CO ester) ^{1}H NMR ($CDCl_3$) 0·9 (*d*, 9H, $J = 6{\cdot}2$ Hz), 1·2–2 (*m*, 16H), 2·4 (*d*, 2H, $J = 7{\cdot}0$ Hz), 3·6 (quartet, 1H, $J = 7{\cdot}2$ Hz), 4·1 (*t*, 2H, $J = 5{\cdot}5$ Hz), 6·9–7·2 (4H, aromatic). ^{13}C NMR ($CDCl_3$) 13·9, 18·3, 22·2, 22·5, 25·7, 28·5, 29·3, 30·0, 31·7, 32·7, 45·0, 45·1, 64·7, 127·0, 129·1, 137·8, 140·3, 174·0. Found C, 79·30; H, 10·70%; $C_{21}H_{34}O_2$ requires C, 79·25; H, 10·69%.

4. Conclusions

It can thus be concluded that (i) by increasing the carbon chain length of the alcohol it is

possible to effectively increase the E value in the kinetic resolution of ibuprofen using CCL. (ii) Further optical enrichment could be achieved by recycling the unreacted starting material or the product obtained from a reaction.

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