High Enantioselective Esterification of 2-Arylpropionic Acids Catalyzed by Immobilized Lipase from *Candida antarctica*: A Mechanistic Approach

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In order to study the mechanism of the enantioselective esterification of 2-arylpropionic acids catalyzed by lipases, a systematic study of the enzymatic activity of immobilized lipase from *Candida antarctica* (SP435A) in this reaction has been carried out. The main variables that have a positive effect on the reaction rate are temperature, amount of catalyst, reaction time, and an acid/alcohol molar ratio of 1:1. The enzyme is enantioselective in the esterification of *R*(−) acid. Therefore the *S*(+) form, with pharmacological activity, can be prepared by enantioselective esterification of the racemate at temperatures below 24 °C and at conversions greater than 50%. The racemic temperature in the esterification of (±)-ibuprofen is 65.4 °C. In the esterification of (±)-2-phenylpropionic acid, isooctane is the best solvent. The reactivity observed is (±) ketoprofen > (±)-2-phenylpropionic acid > (±)-ibuprofen > (±)-naproxen = (±)-flurbiprofen in isobutyl methyl ketone saturated with water, a solvent in which all these antiinflammatory drugs are soluble and the enzymatic derivative is active. A qualitative model of the active site of this immobilized enzyme is described.

Introduction

Lipases are stable in nonpolar solvents and have the remarkable ability of assuming a variety of conformations to accomodate substrates of varying sizes and stereochemical complexities. Moreover, lipase-catalyzed esterification reactions in organic solvents are often more enantioselective than the corresponding hydrolytic reactions in water.[1]

In recent years, lipases have been routinely used for the resolution of racemic mixtures of drugs[2] and agrochemicals through assymetric hydrolysis of the corresponding esters. There are references about enantioselective hydrolysis of racemic esters of naproxen catalyzed by native[3,4] or immobilized[5] lipase from *Candida cylindracea*. In organic media, enantioselective esterification of ibuprofen[6,7] and 2-chloropropionic acid (an intermediate of herbicide synthesis[8,9]), catalyzed by the native lipase from *C. cylindracea*, has also been reported. Nevertheless, systematic studies that deal with the influence of technical and structural variables on the enantioselectivity of immobilized enzymes are usually not reported in the organic chemistry literature.

The aim of this work has been the use of immobilized lipase-B from *Candida antarctica* in the enantioselective esterification of non-steroidal antiinflamatory drugs like ibuprofen, naproxen, and ketoprofen, in different organic solvents, and the analysis of all these topics by a systematic schedule. These drugs belong to the family of 2-arylpropionic acid derivatives whose activity is often related to the *S*(+)-enantiomer.[10]

Immobilized lipase-B from *C. antarctica* (SP435A, now Novozym 435) is a biocatalyst prepared by Novo Industries cheaply and with high thermostability. It has been employed in fat-modification,[11] selective esterification of glucosides,[12] and enantioselective transesterification of secondary alcohols.[13] In the present paper we show that this enzymatic derivative could be employed for the production of the *S*-form of the 2-aryl propionic acids, due to its *R*-stereoselectivity in esterification reactions in organic media.

Results and Discussion

Factorial Analysis. The first point to be analyzed was the nature of the main variables that affect the esterification yield. Factorial analysis was used as the experimental methodology.[14-16] The esterification of (*R,S*)-ibuprofen with 1-propanol was chosen as the reaction test. The selected response, *Y* (yield in ester), was

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Table 1. Variables and Levels in Factorial Design

variables	−1	0	1
X_A (μL of water)	0	150	300
X_B (°C)	24	37	50
X_C (rpm)	100	300	500
X_D (mg of SP435A)	100	300	500
X_E (molar ratio)	1:1	1:2	1:4
X_F (h)	3	5	7

Table 2. Factorial Design: Experimental Matrix

exp	X_A	X_B	X_C	X_D	X_E	X_F	Y (%)
1	−	−	−	−	−	−	7.4
2	+	−	−	−	+	−	1.8
3	−	+	−	−	+	+	26.4
4	+	+	−	−	−	+	80.3
5	−	−	+	−	+	+	3.0
6	+	−	+	−	−	+	16.6
7	−	+	+	−	−	−	43.9
8	+	+	+	−	+	−	16.6
9	−	−	−	+	−	+	49.5
10	+	−	−	+	+	+	22.0
11	−	+	−	+	+	−	49.0
12	+	+	−	+	−	−	84.4
13	−	−	+	+	+	−	11.2
14	+	−	+	+	−	−	34.5
15	−	+	+	+	−	+	91.0
16	+	+	+	+	+	+	71.2
17	0	0	0	0	0	0	42.4
18	0	0	0	0	0	0	40.5
19	0	0	0	0	0	0	43.7

Table 3. 2^6 Factorial Design: Statistical Analysis

number of experiments: 16
degrees of freedom: 15
results of statistical analysis:
$b_O = 38.1$ $b_{AB} = b_{CE} = 4.8$
$b_A = 5.7$ $b_{BE} = -8.3$
$b_B = 39.6$ $b_{BC} = b_{AE} = b_{DF} = -0.2$
$b_C = -4.1$ $b_{AD} = b_{EF} = -2.9$
$b_D = 27.1$ $b_{BD} = b_{CF} = 5.0$
$b_E = -25.8$ $b_{CD} = b_{BF} = 4.9$
$b_F = 13.9$ $b_{DE} = b_{AF} = -0.7$
significance test (Student's t):
centerpoint analysis
confidence level: 95%
$Y_m = 42.2$
$t_2 (\alpha = 0.05) = 4.303$
$S_x = 1.6$
confidence range = ±4.9

expressed by a polynomial function of six experimental variables:

$$Y = b_0 + \sum b_i x_i + \sum b_{ij} x_i x_j \qquad (1)$$

X_A = water amount (μL)

X_B = reaction temperature (°C)

X_C = stirring speed (rpm)

X_E = acid/alcohol molar ratio

X_D = catalyst amount (mg) X_F = reaction time (h)

The initial solvent volume was fixed at 10 mL of isooctane with a 66 mM concentration of (*R,S*) ibuprofen. The maximum and minimum levels of the variables are shown in Table 1. The experiments were randomly performed according to a 2^6 factorial design. The results obtained (entries 1−16) and the centerpoint (entries 17−19) are shown in Table 2. The statistical analysis of this factorial design can be summarized in Table 3.

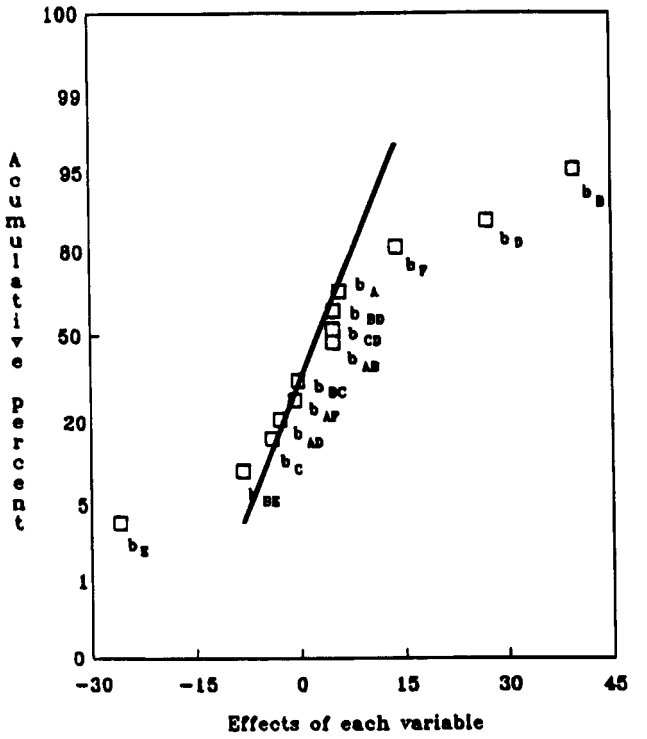

Figure 1. Error estimation: Daniel's method.

Daniel's method[17] was used as the significance test (Figure 1). In this methodology, the points that are not fitted to the statistical probability model are the effects that have influence in the esterification process. These effects are temperature ($b_B = 39.6$), catalyst amount ($b_D = 27.1$), acid/alcohol molar ratio ($b_E = -25.8$), reaction time ($b_F = 13.9$), and the combination of temperature and molar ratio ($b_B \times b_E = -8.3$).

Therefore we can conclude that the reponse equation is:

$$Y = 38.1 + 39.6X_B + 27.1X_D - 25.8X_E + 13.9X_F - 8.3X_BX_E \qquad (2)$$

The main variable is the reaction temperature. The interval considered was between 24 °C to 50 °C, lower than those described by Heldt−Hensen et al.[11] who found a temperature greater than 85 °C as optimum value for the interesterification of soybean oil with lauric acid, or by Björkling et al.[12a] who found 70 °C as the optimum temperature in the esterification of simple glucosides. In Figure 2, we show the temperature profile. The great influence of the temperature is demonstrated by the high slope of the curve in the interval of the factorial analysis (24−50 °C). The optimum temperature is 80 °C and the enzymatic derivative is deactivated at temperatures greater than 100 °C.

The influence of the amount of biocatalyst is shown at Figure 3. We can observe that amounts of enzymatic derivative greater than 500 mg should not be used because productivity (μmol of ibuprofen esterified per mg of biocatalyst per hour) diminishes, as can be observed in Table 4. When amounts of enzymatic derivative (SP435A) greater than 300 mg are used, both enantiomers are esterified because ester yields greater than 50% are achieved (Figure 3).

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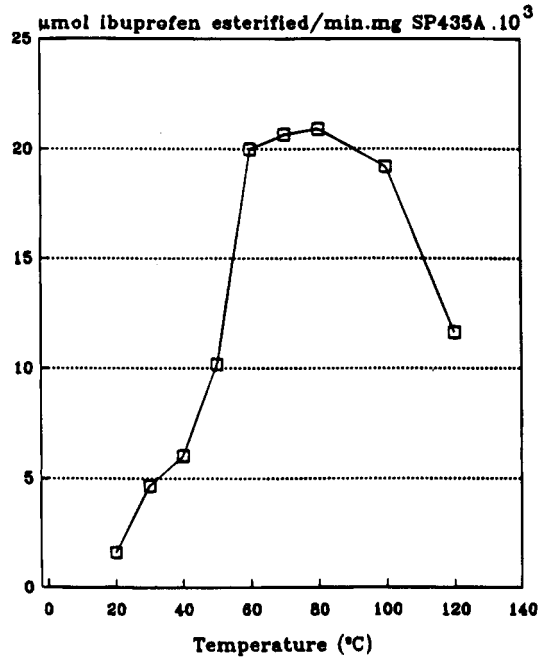

Figure 2. Influence of the temperature in the esterification of (±)-ibuprofen catalyzed by immobilized lipase from *C. antarctica* (SP435A) in isooctane. 66 mM (±)-ibuprofen + 66 mM 1-propanol in 10 mL of isooctane; 500 mg of SP435A; stirring speed = 300 rpm.

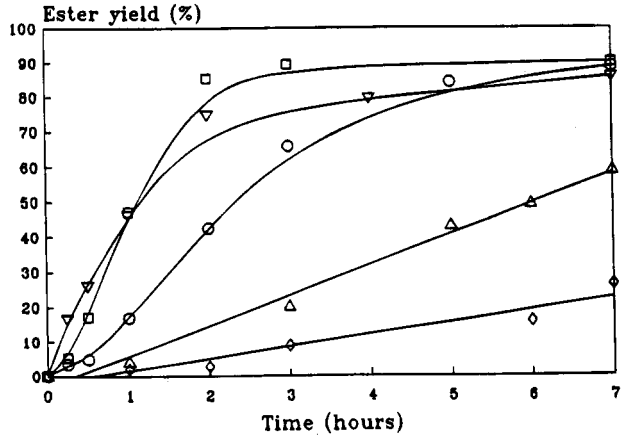

Figure 3. Influence of the amount of immobilized derivative SP435A in the esterification of (±)-ibuprofen with 1-propanol in isooctane. 66 mM ibuprofen + 66 mM propanol in 10 mL of isooctane; T^a = 50 °C; stirring speed = 300 rpm. (◇) 50 mg; (△) 100 mg; (○) 300 mg; (▽) 500 mg; (□) 750mg.

Table 4. Productivity of SP435A in the Esterification of (±)-Ibuprofen with 1-Propanol[a]

amount of derivative (mg)	ester yield (%) (t = 1 h)	μmol ibuprofen esterified/mg of SP435A/h
50	1.9	0.25
100	4.0	0.26
300	17.0	0.37
500	46.3	0.61
750	47.0	0.41

[a] See Figure 3 for conditions.

The negative effect of the acid/alcohol molar ratio (b_E = −25.8) (Figure 4) can be explained by taking into account that an excess of acid can diminish the microenviromental pH of the active site (maximum enzymatic activity of the lipase pH = 7.0[11]) and an excess of alcohol can denaturate the enzyme. The stripping of water

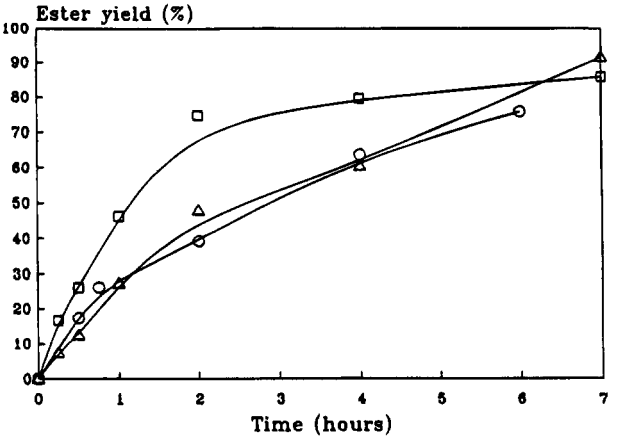

Figure 4. Influence of the acid/alcohol molar ratio in the esterification of (±)-ibuprofen with 1-propanol catalyzed by the immobilized lipase from *C. antarctica* (SP435A) in isooctane. 66 mM (±)-ibuprofen + 66 mM 1-propanol in 10 mL of isooctane; T^a = 50 °C; stirring speed = 300 rpm; 500 mg of SP435A. (□) 1:1; (△) 1:2; (○) 1:4.

produced by alcohols[18] and the partial substitution of water from the enzyme shield by water-mimicking additives such as ethylene glycol[19] are reported effects that alter the enzymatic activity. In addition, the denaturating effect of hydrophilic organic solvents on lipase from *C. cylindracea* has been described by Reslow et al.[20] and is related to the alteration of the water shield of the enzyme. An excess of either acid or alcohol diminishes the enzymatic activity, so that the optimum ratio is 1:1, as is recommended for esterification in the literature.[21,22]

The positive effect of the reaction time is evident. The very small effect observed for water content (0−300 μL) can be explained by the fact that the immobilized lipase has its water shield complete at this high water activity (a_w = 0.5 at 0.04 μL of water/mL of isooctane). Therefore, an increase in the amount of water added to the solution does not change the water shield of the lipase.

Finally, the negative effect of the interaction of the temperature and the acid/alcohol molar ratio (b_{BE} = −8.3) has not been described till now and could be explained as a negative effect due to the increase of two important variables which affect the stability of water/oil interface.

Influence of the Technical Variables on the Enantioselectivity of the Process. The enantioselectivity of immobilized lipase from *C. antarctica* (SP435A) was determined in the esterification of *R*(−)-ketoprofen and *S*(+)-ketoprofen with *n*-propanol. The results are shown in Figure 5. From these results we determined an enantiomeric ratio of E = 2.5 ± 0.1. This value is independent of the conversion. Therefore, the esterification process is stereoselective.[23]

The stereoselectivity of the lipase for the *R*(−) isomer is also observed in the esterification of (±)-ibuprofen. In Table 5 we show the enantiomeric excess of the remain-

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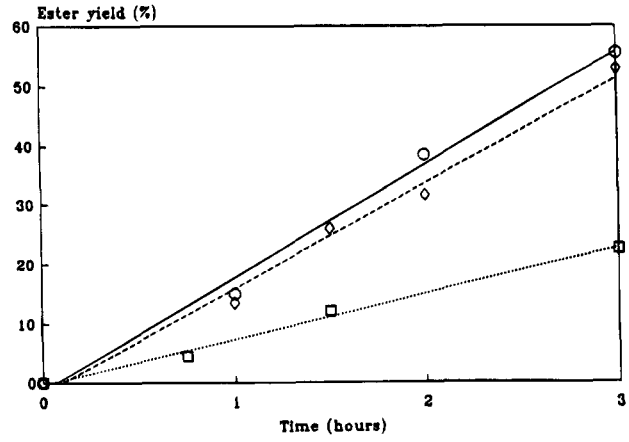

Figure 5. Esterification of ketoprofen with 1-propanol catalyzed by immobilized lipase from *C. antarctica* (SP435A). 66 mM acid + 66 mM 1-propanol in 10 mL of isobutyl methyl ketone; 300 mg of SP435A; T^a = 50 °C; stirring speed = 300 rpm. (○) (*R,S*)-ketoprofen, (◇) *R*(−)-ketoprofen, (□) *S*(+)-ketoprofen.

Table 5. Enantioselectivity of Immobilized Lipase from *C. antarctica* (SP435A) on the Esterification of (±)-Ibuprofen

entry	X_A[a]	X_B[b]	X_C[c]	X_D[d]	X_E[e]	X_F[f]	yield[g] (%)	ee[h] (%)	E[i]
1	150	37	300	300	1:2	5	42.2	20	2.1
2	0	50	500	100	1:1	3	43.9	6	1.2
3	0	24	100	500	1:1	7	49.6	27	2.2
4	0	50	100	500	1:4	3	49.0	9	1.3
5	300	24	500	500	1:1	3	34.5	29	4.4
6	300	50	500	500	1:4	7	71.2	31	1.6
7	300	50	100	500	1:1	3	84.4	49	1.7

[a] Microliters of water. [b] Temperature (°C). [c] Stirring speed (rpm). [d] Milligrams of SP435A. [e] Acid/alcohol molar ratio. [f] Reaction time (h). [g] Conversion to ester. [h] Enantiomeric excess (form *S*(+)) of the remaining acid. [i] Enantiomeric ratio: $E = \ln[(1 - c)(1 - ee_s)]/\ln[(1 - c)(1 + ee_s)]$.

ing acid obtained in different experimental conditions for the esterification of ibuprofen with propanol in isooctane. The initial concentration and solvent volume were fixed at 66 mM (±)-ibuprofen in 10 mL of isooctane. When the reaction yield is lower than 50%, a temperature increment diminishes E and ee (entries 2 and 4).

In Figure 6 we fit $-RT \ln E$ values with respect to $1/T$:

$$-RT \ln E = 198.8 - (67.3 \cdot 10^3)^1/_T; \; r = 0.83 \quad (3)$$

The racemic temperature T_R is defined as the temperature in which both enantiomers react at the same rate. At this temperature $E = 1$, and $RT \ln E = 0$. Therefore the racemic temperature is T_R = 65.4 °C for (±)-ibuprofen, higher than our working temperature,[24] so we can conclude that the greater the reaction temperature, the lower the enantiomeric ratio. This finding can be explained assuming that at high reaction temperature, the cavity of the active site of the lipase becomes greater and the enantioselectivity diminishes. This finding agrees with several recent papers reporting an inverse correlation between temperature and enantioselectivity for porcine pancreatic lipase and *Pseudomonas cepacia* lipase in the transesterification process,[25] and by other workers in esterification processes with different lipases.[26,27]

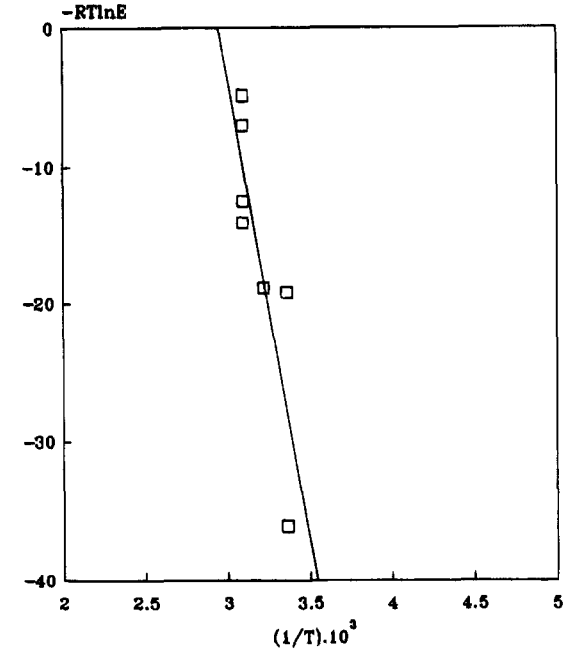

Figure 6. Racemic temperature for the esterification of (±)-ibuprofen with 1-propanol catalyzed by immobilized lipase from *C. antarctica* (SP435A).

The presence (entry 5) or absence (entry 3) of water at the same reaction temperature seems to be irrelevant in the enantiomeric excess of the remaining acid, compared with the effect of temperature. This finding agrees with the results reported by Carrea et al.[28] who observed no influence of water on the transesterification of (±)-sulcatol with vinyl acetate. On the other hand, when reaction yields are greater than 50%, the enantiomeric excess value of *S*(+)-acid increases. Therefore we can obtain pure *S*(+)-2-arylpropionic acids (with antiinflamatory activity) working at temperatures lower than 24 °C and at esterification yields greater than 50%.

Influence of the Nature of the Solvent. There are abundant references from the literature about microbial lipases employed in organic media.[1] Reactions of esterification and transesterification have been carried out with lipase from *C. cylindracea* in hexane,[29-31] cyclohexane,[32,33] and heptane.[34]

The effect of the nature of the organic solvent has been studied in the esterification rate of reactions catalyzed by immobilized lipase from *Mucor miehei* (Lipozyme).[35,36] We have studied the effect of different organic solvents on the esterification of (*R,S*)-2-phenylpropionic acid with

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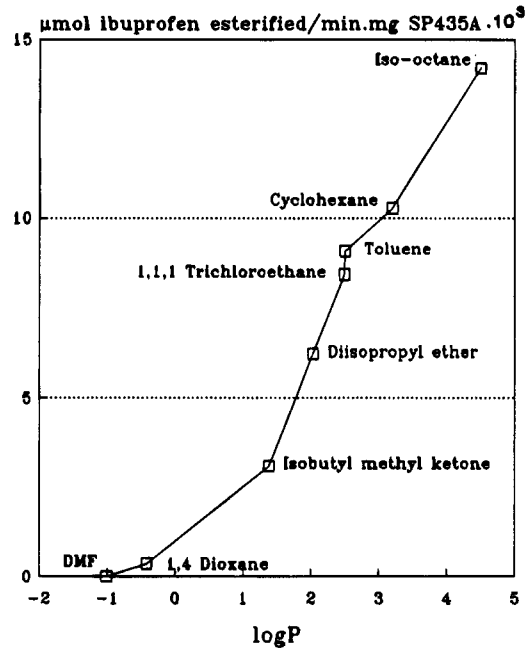


Figure 7. Influence of the nature of the solvent in the esterification of (±)-2-phenylpropionic acid catalyzed by immobilized lipase from *C. antarctica* (SP435A). 66 mM acid + 264 mM 1-propanol in 10 mL of solvent; T^a = 50 °C; 500 mg of SP435A, stirring speed = 300 rpm. Synthetic activity in the first hour of reaction.

1-propanol catalyzed by immobilized lipase from *C. antarctica*. In Figure 7, we show the specific enzymatic synthetic activity versus log *P*, a parameter which gives us an idea about the lipidic properties of the solvent.[37] High esterification rates were obtained with nonpolar solvents (whose log *P* > 2) whereas the activity was low in more hydrophilic and water-miscible solvents (log *P* < 2).

Similar results have been obtained using native lipase from *C. cylindracea* as a biocatalyst in the enantioselective esterification of (*R,S*)-ibuprofen[6] and (*R,S*)-2-chloropropionic acid[9] in organic media.

Influence of the Structural Variables. Influence of Alcohol Moiety. The ester formation catalyzed by microbial lipases is influenced by the alcohol moiety. Nonspecific lipases (from *C. cylindracea*, *C. antarctica*, etc.) catalyze the reaction both with primary and secondary alcohols, whereas specific lipases (from *M. miehei*, for instance) work preferably with primary alcohols. This fact has been checked in the synthesis of different esters of (*R,S*)-2-phenylpropionic acid. Addition of the same amount of immobilized lipase from *C. antarctica* (SP435A) resulted in total esterification of the racemic acid in 7 h with different primary alcohols (Figure 8). Ester synthesis was slower with the secondary alcohol (2-propanol), which also reached 100% ester conversion at 24 h (datum not shown). No reaction was observed with tertiary alcohols like *tert*-butyl alcohol. Mustranta[6] reported similar results in the esterification of (*R,S*)-ibuprofen catalyzed by native lipase from *C. cylindracea* in hexane, at 30 °C, with an acid/alcohol molar ratio of

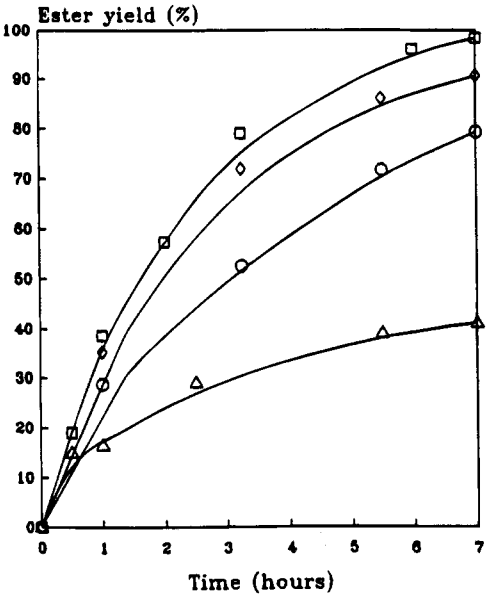
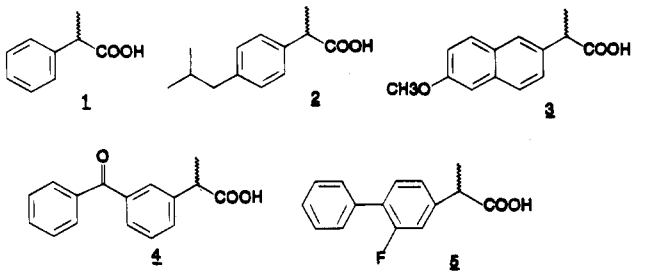


Figure 8. Influence of the alcohol moiety in the esterification of (±)-2-phenylpropionic acid catalyzed by immobilized lipase from *C. antarctica* (SP435A) in isooctane. 66 mM acid + 264 mM alcohol in 10 mL of isooctane; T^a = 50 °C; 500 mg of SP435A; stirring speed = 300 rpm. (□) 1-propanol; (◇) 1-octanol; (○) 1-butanol, and (△) 2-propanol.

Scheme 1



1:2 in which the esterification rate was larger with primary alcohols than with secondary ones.

Ester synthesis rate is also influenced by the length of the alcohol moiety. The highest ester conversion is obtained with 1-propanol, followed by 1-octanol and 1-butanol. Similar results were reported by Rao et al.[20] who employed these two latter alcohols in the esterification of palmitic acid catalyzed by lipase from *C. cylindracea* in reverse micelles. When they used isooctane as organic media, at 40 °C and with an acid/alcohol molar ratio of 1:1, the preference of lipase was toward long-chained alcohols (1-dodecanol and 1-octanol) rather than 1-butanol.

Influence of the acid moiety. The influence of the acid moiety has been analyzed using some commercial non-steroidal antiinflamatory drugs such as (±)-2-phenylpropionic acid (**1**), (±)-ibuprofen (**2**), (±)-naproxen (**3**), (±)-ketoprofen (**4**), and (±)-flurbiprofen (**5**) (Scheme 1) whose pharmacological properties are related to the *S*(+) enantiomer.[10]

The esterification of (±)-2-phenylpropionic acid and (±)-ibuprofen with *n*-propanol in isooctane shows that the acid with smaller molecular size gives better yields than the larger one (ibuprofen) (Figure 9). Nevertheless (±)-ketoprofen and (±)-naproxen are not soluble in isooctane, so that isobutyl methyl ketone (IBMK), which solubilizes

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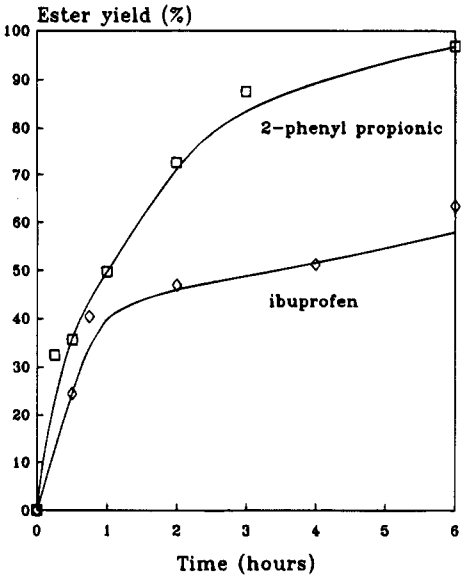

Figure 9. Influence of the structure of the acid in the esterification catalyzed by SP435A. 66 mM acid + 264 mM 2-propanol in 10 mL of isooctane; T^a = 50 °C; 500 mg of SP435A; stirring speed = 300 rpm.

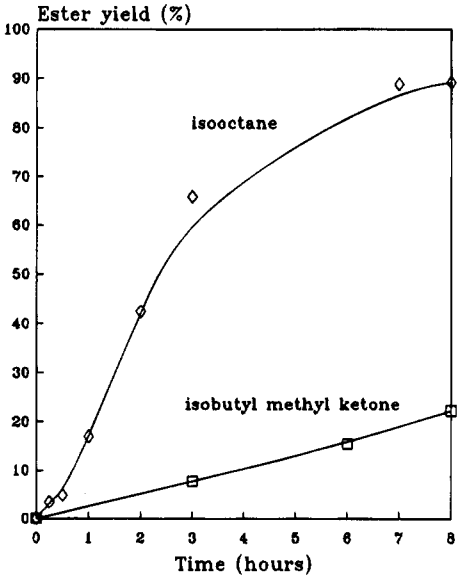

Figure 10. Influence of the solvent in the esterification of (±)-ibuprofen catalyzed by SP435A. 66 mM acid + 66 mM propanol in 10 mL of solvent; 300 mg of SP435A; T^a = 50 °C; stirring speed = 300 rpm.

all the products, was chosen as the solvent in spite of the poor esterification rates in solvents with low log P (Figures 7 and 10). The results obtained are shown at Figure 11. We can observe that (±)-2-phenylpropionic acid is a better substrate than (±)-ibuprofen in both isooctane (Figure 10) and isobutyl methyl ketone (Figure 11).

The enantiomeric ratio obtained at 47.6% yield (t = 24 h) in isobutyl methyl ketone was E = 2.49, greater than the E values obtained in isooctane at similar yields (E = 1.2 and 1.3; see Table 5). The increase of the enantioselectivity value can be related to the hydrophilic/hydrophobic characteristics of the solvents (log P = 1.38 for IBMK and log P = 4.50 for isooctane). It is well documented that the nature of the organic solvent and the presence of water-mimicking solvent changes the

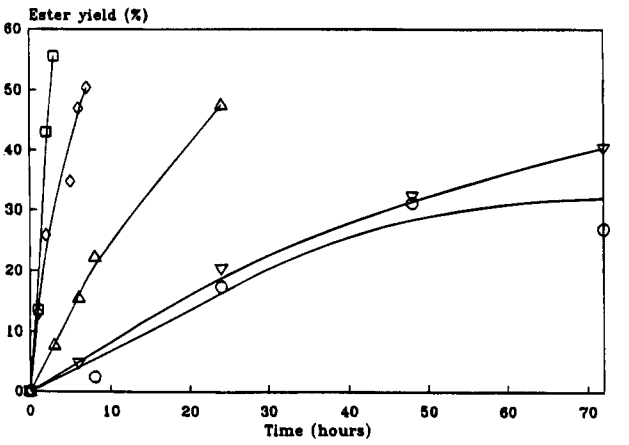

Figure 11. Esterification of 2-arylpropionic acids catalyzed by immobilized lipase from *C. antarctica* (SP435A). 66 mM acid + 66 mM 1-propanol in 10 mL of isobutyl methyl ketone, 300 mg of SP435A, T^a = 50 °C, stirring speed = 300 rpm. (□) ketoprofen, (◇) 2-phenylpropionic acid, (△) ibuprofen, (▽) flurbiprofen, (○) naproxen.

Table 6. Enantiomeric Ratio Obtained in the Esterification of 2-Arylpropionic Acids Catalyzed by SP453A in IBMK[a]

substrate	ester yield (%)	E
(±)-ketoprofen	55.6	2.6
(±)-2-phenylpropionic acid	43.8	2.6
(±)-ibuprofen	47.6	2.5
(±)-flurbiprofen	40.4	2.0
(±)-naproxen	31.2	1.3

[a] See Figure 11 for conditions.

enantioselectivity of lipases.[7,19,38] All these effects have been explained by Klibanov et al.,[38] according to the amount of water which remains near the enzyme molecules. Similarly, Högberg et al.[39] and Hedström et al.[7] showed that an increase of a_w increases the enantioselectivity of *C. cylindracea* lipase toward the $S(+)$ enantiomer in the esterification of alkanoic acids and ibuprofen. Taking into account that the experiments in Figure 10 were carried out with wet solvents, the esterification in IBMK (with lower log P) was performed at a higher concentration of water than the reaction in isooctane. This fact produces an increase in the enzymatic rate on the $R(-)$ isomer, the most reactive for *C. antarctica* lipase and, as a consequence, the E value increases.

The enantiomeric ratio is very similar for all the acids approaching the 50% of ester yield (Table 6). The lower value of the enantiomeric ratio for naproxen can be related to the low yield obtained in the reaction. Due to the fact that the $R(-)$ isomer is more reactive than $S(+)$ isomer (Figure 5), when high esterification yields are achieved, the percentage of $R(-)$ form diminishes in the remaining acid and the E value rises.

The reactivity observed, (±)-ketoprofen > (±)-2-phenylpropionic acid > (±)-ibuprofen > (±)-flurbiprofen = (±)-naproxen, must be related to sterical factors. Sih et al.[2] observed this variation in the hydrolysis of methyl esters of these acids catalyzed by native lipase from *C. cylindracea*: (±)-2-phenylpropionate > (±)-ketoprofenate > (±)-ibuprofenate > (±)-naproxenate ≈ (±)-flurbiprofenate.

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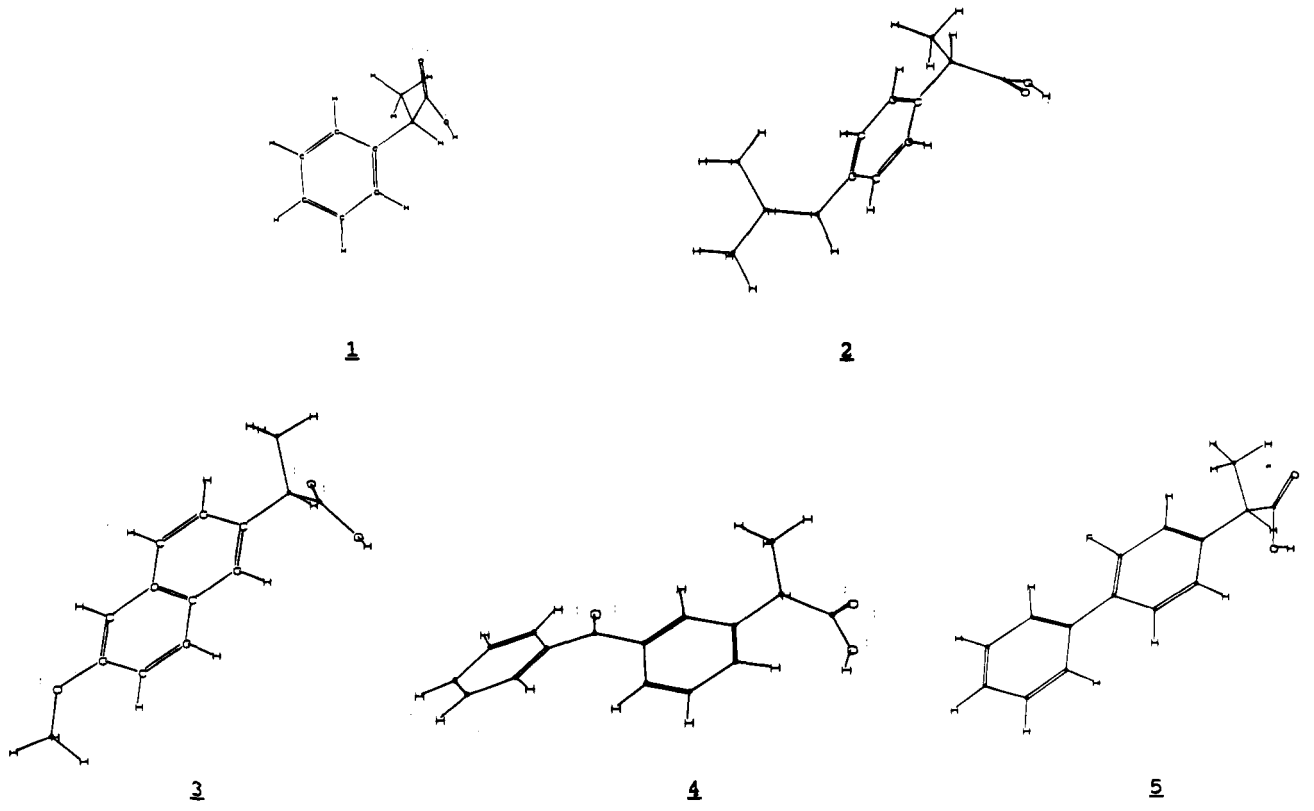
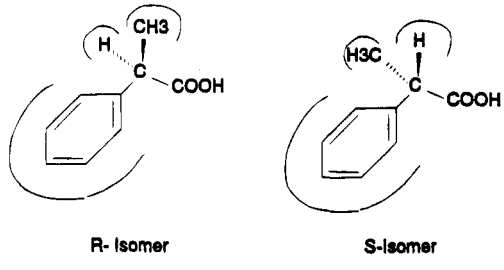
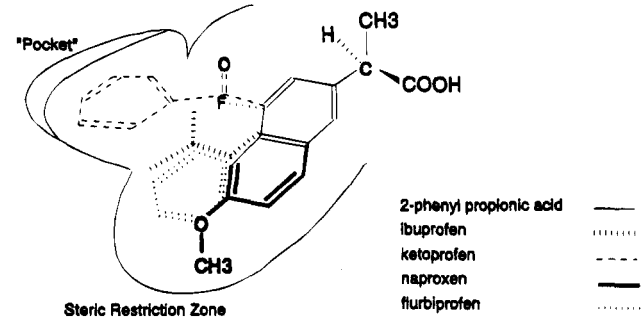


Figure 12. Minimum energy conformer of $R(-)$-2-arylpropionic acids. **1**: 2-phenylpropionic acid; **2**: ibuprofen; **3**: naproxen; **4**: ketoprofen, and **5**: flurbiprofen.

Scheme 2



Scheme 3



In order to increase our knowledge of the enzyme–substrate interaction from *C. antarctica*, a molecular mechanic analysis was carried out employing PCMODEL program.[40] The minimum energy conformers of the $R(-)$ acids are shown in Figure 12. According to the classic acyl-binding model of lipases,[41] and the *R*-stereoselectivity of lipase from *C. antarctica*, we can conclude there is a certain steric restriction in the small subsite ("S") to accept the methyl group instead of hydrogen; it is not a very important restriction due to the small size of the methyl group (Scheme 2).

The dimensions of the large subsite ("L") of the active site of immobilized lipase from *C. antarctica* have not been described to our knowledge. The good yields in esterification obtained with ketoprofen (a twisted molecule according to the MM analysis) and the poor yields obtained with naproxen (a rigid molecule with different directional steric parameters) permits us to describe, at a qualitative level, a "pocket" that can accept the molecules of $R(-)$-ketoprofen and $R(-)$-ibuprofen (Figure 12) and a "steric restriction zone" in which the naphthalene ring of $R(-)$-naproxen fits with difficulty (Scheme 3).

Experimental Section

General. Immobilized lipase-B from *C. antarctica* (SP435A) was a gift from Novo Industries (Denmark). The activity of the enzyme preparation used in the experiments was 10.200 PLU/g (*P*ropyl *L*aurate *U*nits). Racemic 2-phenylpropionic acid was purchased from Fluka (Buchs, Switzerland). Racemic ibuprofen and flurbiprofen were gifts from Boots Pharmaceuticals (Nottingham, U.K.). Racemic and pure enantiomers of ketoprofen were kindly given by Laboratorios Menarini S.A. (Badalona, Spain). Racemic naproxen, formerly *R,S*, was a gift from Syntex Research (Palo Alto, California). The alcohols used were from Sigma (St. Louis, Missouri) and the organic solvents (with analytical grade) were from Merck (Germany).

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General Procedure for Esterification. The reaction mixture was composed of organic solvent (10 mL), racemic 2-arylpropionic acid (66 mM), and the alcohol (66–264 mM). The reaction was started by adding different amounts of the immobilized lipase to the solution. The reactions were carried out at a fixed temperature by shaking in 25 mL flasks for a specified time. Then 100 μL of the solution was added to 1.4 mL of the same organic solvent to be analyzed by gas chromatography for conversion to the ester.

Gas Chromatography Analysis. Gas chromatography was performed in a Shimadzu GC-14A gas chromatograph equipped with FID detector, a split injector (1:2), and a SPB-1 sulfur column (15m × 0.32 mm). Injector temperature was 300 °C and the detector temperature 350 °C; carrier gas was nitrogen. Different conditions for quantitative analysis were used depending on the compounds: for 2-phenylpropionic acid, a column temperature of 180 °C and a N_2 stream of 3 mL/min; for ibuprofen, a column temperature of 180 °C and a N_2 stream of 12 mL/min; for naproxen, ketoprofen, and flurbiprofen, a column temperature of 170 °C and a N_2 stream of 30 mL/min. An external standard method was employed to quantify the remnant acid and the formed ester.

General Procedure for the Enantiomeric Excess Determination. Once the reactions were terminated, the immobilized enzyme was filtered and the organic solvent evaporated to dryness in a Buchi concentrator–evaporator. The residue was diluted in a 25 mL of water and the solution acidified with H_2SO_4. Ester and acid were extracted with diethyl ether (3 × 25 mL). The organic phase was placed in a clean glass and a new extraction was carried out with NaOH 0.1 N (3 × 25 mL). The aqueous phase was acidified with HCl and then, a third extraction was performed with diethyl ether (3 × 25 mL). The remaining organic phase was evaporated to dryness. A solution 0.053 M of the obtained residue (acid) and 0.027 M of (*R,R*)-1,2-diphenyl-1,2-diaminoethane in 0.9 mL of $CDCl_3$ (deutered chloroform) gave the diasteroisomeric salt complexes that allowed the direct ^{1}H-NMR determination (Brucker 250) of the enantiomeric purity as described by Fullwood et al.[42]

Acknowledgment. This work was supported by a grant from C. I. C.Y.T. (Ministerio de Educación y Ciencia, Spain), Project PB090-010.

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