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# Effects of Medium and of Reaction Conditions on the Enantioselectivity of Lipases in Organic Solvents and Possible Rationales

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**ABSTRACT.** The nature of organic solvents markedly influenced the enantioselectivity, expressed as the enantiomeric ratio, of porcine pancreatic lipase and of Lipase PS in transesterification between trifluoroethyl butanoate and the two unrelated alcohols (±)-sulcatol (1) and (±)-3-bromo-5-hydroxymethyl isoxazoline (2). However, there was no correlation between enantioselectivity and such physicochemical characteristics of the solvent as hydrophobicity and dielectric constant. A rationale based on the formation of solvent-enzyme complexes is proposed to explain the results. Enzyme enantioselectivity was also affected by temperature but not by the chain length of the acylating agent nor by the removal of water from the reaction medium by molecular sieves. The effects of these parameters on transesterification rates were also investigated.

## INTRODUCTION

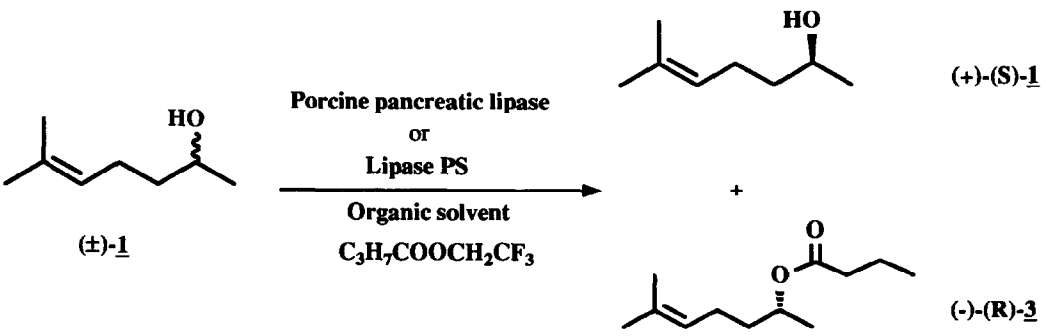
Biocatalysis in organic media is becoming increasingly popular among chemists and several systems (biphasic systems, reverse micelles and monophasic systems) and classes of enzymes (oxidoreductases, hydrolases and isomerases) have been successfully employed for synthetic purposes.[1] In this context, especially widespread are the hydrolase catalyzed esterifications and transesterifications in nearly anhydrous organic solvents for regioselective acylation of polyhydroxylated natural compounds[2] and for resolution of racemic alcohols and acids.[1f,3] However, it is only recently that the effects of the nature of organic solvents on the regio-[4] and enantioselectivity[5] of hydrolases have started to be investigated.

Klibanov and coworkers [5a-c] and Parida and Dordick[5d] have reported marked variations in enzyme enantioselectivity as a function of the solvent employed. However, no rationale of general validity and predictive value has been presented so far. In fact, for the protease subtilisin Carlsberg there is an inverse

correlation between selectivity and solvent hydrophobicity with N-acetylalanine chloroethyl ester as the substrate,[5a] an inverse correlation between selectivity and dielectric constant of the solvent with *sec*-phenethyl alcohol,[5c] and no correlation with either hydrophobicity or dielectric constant with chiral amines.[5b] With *Candida cylindracea* lipase and 2-hydroxy acids, instead, enantioselectivity increases with increasing solvent hydrophobicity.[5d]

In the present study, we have investigated the effects of solvents on the enantioselectivity and activity of Lipase PS and porcine pancreatic lipase, using as the substrates two unrelated model compounds, namely , the secondary alcohol sulcatol (6-methyl-5-hepten-2-ol) (1) (Scheme I)

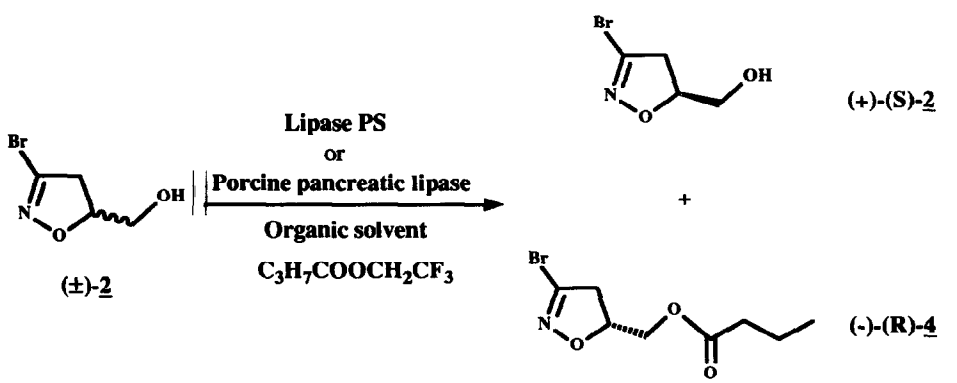
**SCHEME I**




and the primary alcohol 3-bromo-5-hydroxymethyl isoxazoline (2) (Scheme II).

We have also studied the effects of the chain length of the acylating agents, the temperature and removal of water on the enantioselectivity and activity of the two lipases.

**SCHEME II**



## RESULTS AND DISCUSSION

**Effects of Organic solvents.** Lipase PS and porcine pancreatic lipase are among the enzymes most widely used for the resolution of racemic alcohols and acids.[3] In this study, each of the two lipases was employed in several organic solvents for the resolution of (±)-1 (Scheme I), a pheromone of an ambrosia beetle,[6] and (±)-2 (Scheme II), a precursor of a muscarinic agonist,[7] using trifluroethyl butyrate as the acylating agent . With these two substrates both enzymes showed a preference for the (R)-configuration .

As an index of enantioselectivity we used the enantiomeric ratio E, which can be calculated from the degrees of conversion and the correspondent enantiomeric excess (ee) values of either the product or the substrate by the equations developed by Chen et al.[3d] These equations, because of the reversible nature of the transesterification reactions in organic solvents, also take into account the parameter K, which is the ratio between the concentrations of the substrate and product at equilibrium. Under our experimental conditions, the degrees of conversion were, at equilibrium, between 95 and 97 %.

The ee values were determined by chiral GLC for 1 and by chiral HPLC for 2 . These methods were very effective and easy to use and the enantiomers of the substrates and ester products were base-line separated in the same run without any prior derivatization (Figure 1).

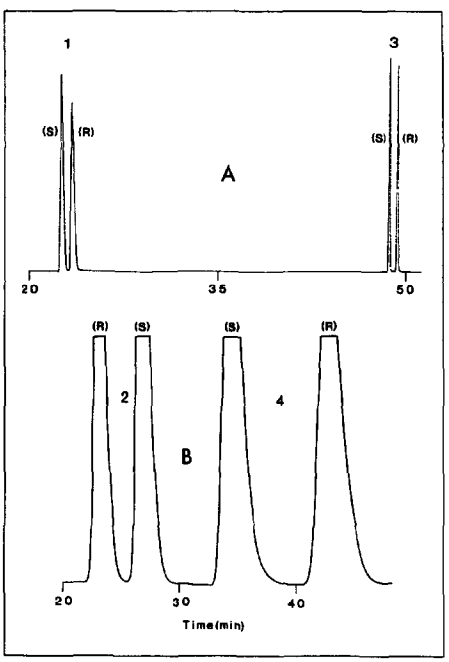



**Figure 1.** Chiral GLC of (±)-sulcatol (1) and (±)-sulcatol butyrate (3) on CP-Cyclodextrin-β-2,3,6-M-19 (A),and chiral HPLC of (±)-3-bromo-5-hydroxymethyl isoxazoline (2) and (±)-3-bromo-5-hydroxymethyl isoxazoline butyrate (4) on Chiracel OB (B). Note, in the case of 2, the inversion in the elution order of the product ((S) elutes before (R)) compared to the substrate ((R) elutes before (S)).

The effects of the nature of the organic solvents on the enantioselectivity of crude porcine pancreatic lipase with (±)-**1** and (±)-**2** as the substrates are shown in Table I.

**Table I.** Effects of Organic Solvents on the Enantioselectivity (Enantiomeric Ratio, E) and Transesterification Rate of Porcine Pancreatic Lipase with (±)-**1** and (±)-**2** as the Substrates.

| | | | (±)-**1**[c] | | (±)-**2**[d] | |
|---|---|---|---|---|---|---|
| solvent | log P[a] | $\epsilon$[b] | E[e] | initial rate[f] (μmol/h) | E[e] | initial rate[f] (μmol/h) |
| dioxane | -1.1 | 2.2 | 42 (50)[g] | 6.2 (0.5)[g] | | |
| acetone | -0.2 | 20.6 | 34 | 3.0 | 1.6 | 2.4 |
| tetrahydrofuran | 0.5 | 7.6 | 40 | 4.8 | | |
| 3-pentanone | 0.8 | 17.0 | 22 (25) | 4.7 (0.4) | 2.4 | 5.3 |
| *t*-amylalcohol | 1.4 | 5.8 | 20 (24) | 2.0 (0.2) | 1.3 | 5.6 |
| 3-methyl-3-pentanol | 2.0 | 4.3 | 17 (22) | 1.6 (0.2) | 1.6 | 6.0 |
| benzene | 2.0 | 2.3 | 62 (71) | 10.7 (1.4) | | |
| toluene | 2.5 | 2.4 | 39 (42) | 8.6 (1.3) | 2.0 | 7.9 |
| dibutyl ether | 2.9 | 1.1 | 20 | 11.4 | | |
| cyclohexane | 3.1 | 2.0 | 26 (32) | 9.7 (2.0) | | |
| dodecane | 6.6 | | 23 (28) | 6.7 (1.1) | | |

[a]Log P values were calculated according to Rekker, R. F.; De Kort, H. M. Eur. J. Med. Chem. Therapeut. **1979**, 14, 479-88. [b]Dielectric constant ($\epsilon$) values were taken from: Reichardt, C. Solvents and Solvent Effects in Organic Chemistry, 2nd ed.; VCH: Weinheim, Germany, 1988, pp.408-10. [c]To 1 ml of organic solvent containing (±)-**1** (79 mM) and trifluoroethyl butyrate (480 mM), 50 mg of enzyme and 100 mg of molecular sieves were added, and the suspension was shaken in an orbital shaker at 250 rpm, at 45 °C. The degree of conversion and the optical purity of the ester product were determined at different times by GLC. [d]To 1 ml of organic solvent containing (±)-**2** (28 mM) and trifluoroethyl butyrate (56 mM), 50 mg of enzyme and 100 mg of molecular sieves were added, and the suspension was shaken at 250 rpm, at 45 °C. The degree of conversion and the optical purity of the product were determined at different times by HPLC. [e]E values were calculated from the degree of conversion and the ee of the product according to Chen et al.[3d] Each value was the average of 3-5 E values calculated for conversions ranging from 10 to 50 %. [f]No appreciable conversion was observed in the absence of the enzyme. [g]The results obtained with immobilized purified pancreas lipase (50 mg of enzyme-matrix complex) are reported in parentheses.

It can be seen that the enantioselectivity was markedly influenced by the solvent, but no correlation with either the hydrophobicity (log P) or dielectric constant of the medium was observed.[8] In

the case of (±)-2, the differences in E values are small in absolute terms, but quite significant in relative terms. In fact, in terms of the ee values of the product (4), the differences as a function of the solvent are great and easily measurable (Figure 2A). Thus, at low degrees of conversion, the ee values are about 40 % in pentanone (E=2.4), 20 % in 3-methyl-3-pentanol (E=1.6) and 10 % in *tert*-amyl alcohol (E=1.3).

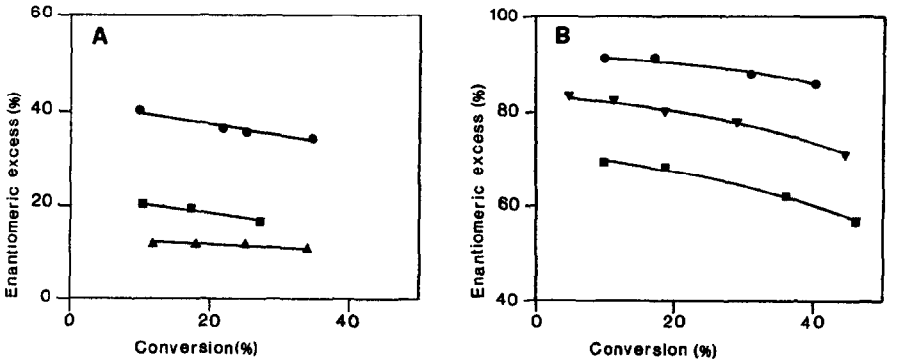



Figure 2A. Porcine pancreatic lipase catalyzed transesterificatioon of (±)-2 with trifluoroethyl butyrate. The ee values of 2-butyrate obtained in 3-pentanone (●) (average E value 2.4), 3-methyl-3-pentanol (■) (average E value 1.6), and tert-amyl alcohol (▲) (average E value 1.3) were plotted against the degree of conversion. The curves were computer generated from the average E values.[3d] **Figure 2B.** Lipase PS catalyzed transesterification of (±)-2 with trifluoroethyl butyrate. The ee values of 2-butyrate obtained in 3-pentanone (●) (average E value 25), tetrahydrofuran (▼) (average E value 11), and 3-methyl-3-pentanol (■) (average E value 6) were plotted against the degree of conversion. The curves were computer generated from the average E values.[3d]

Analogously, the enantioselectivity of Lipase PS with (±)-1 and (±)-2 changed notably as a function of the organic medium but, once again, without any correlation with the hydrophobicity or dielectric constant of the solvent (Table II).[9] As an example, Figure 2B shows the ee values of the ester product (4) obtained in the resolution of (±)-2 in three different solvents. The values were about 90 % in 3-pentanone (E=25), 80 % in tetrahydrofuran (E=11) and 70 % in 3-methyl-3-pentanol (E=6).

The transesterification rates of the two lipases with (±)-1 and (±)-2 also change markedly by changing the solvent, but in this case too, there was little or no correlation with the physicochemical characteristics of the solvents (Table I and II). However, it should be emphasized that these rates were not determined at saturating concentrations of substrate, and therefore they could reflect variations in $K_m$ values (see below).

It should be emphasized that crude catalyst preparations can contain enzymes with different or even opposite enantioselectivity. However, Table I shows that very similar E values (and identical trend) were obtained with the crude porcine pancreatic lipase (53 units/mg), which is known to contain esterase activities,[3c] and with the purified one (20500 units/mg; see data in parentheses). This strongly suggests that lipase only acts on (±)-1 and that the effects of solvents on E do not reflect variations in the relative activities of the enzymes present in the crude material. Furthermore, the reaction rates do not change or

slightly increase on increasing the chain length of the acylating agent (see next section) and this is typical of lipases and not of esterases. Table II shows that also crude Lipase PS (20 units/mg) and purified Lipo Protein Lipase (800 units/mg; see data in parentheses) gave practically identical E values as a function of the different solvents. Regarding Lipase PS, it should be mentioned that it contains only one lipase, and that the esterase activity is negligible.[10]

**Table II.** Effects of Organic Solvents on the Enantioselectivity (Enantiomeric Ratio, E) and Transesterification Rate of Lipase PS with (±)-**1** and (±)-**2** as the Substrates

| | | | (±)-**1**[a] | | (±)-**2**[b] | |
|---|---|---|---|---|---|---|
| | | | | initial rate | | initial rate |
| solvent | logP | $\epsilon$ | E[c] | (μmol/h) | E[c] | (μmol/h) |
| dioxane | -1.1 | 2.2 | 23 (22)[d] | 1.6 (2.9)[d] | 11 | 3.6 |
| acetone | -0.2 | 20.6 | 40 (42) | 2.4 (3.1) | 23 | 1.2 |
| tetrahydrofuran | 0.5 | 7.6 | 27 | 2.0 | 11 | 7.2 |
| 3-pentanone | 0.8 | 17.0 | 47 (45) | 3.8 (10.6) | 25 | 15.0 |
| *t*-amylalcohol | 1.4 | 5.8 | 20 (18) | 3.4 (10.1) | 8 | 40.2 |
| 3-methyl-3-pentanol | 2.0 | 4.3 | 16 | 5.1 | 6 | 30.0 |
| benzene | 2.0 | 2.3 | 32 | 4.8 | 10 | 28.8 |
| toluene | 2.5 | 2.4 | 34 (37) | 6.2 (14.2) | 16 | 16.2 |
| dibutyl ether | 2.9 | 1.1 | 22 | 6.6 | 7 | 25.8 |
| cyclohexane | 3.1 | 2.0 | 13 (14) | 5.0 (14.0) | | |
| dodecane | 6.6 | | 21 | 4.7 | | |

[a]To 1 ml of organic solvent containing (±)-**1** (79 mM) and trifluoroethyl butyrate (180 mM), 50 mg of enzyme and 100 mg of molecular sieves were added, and the suspension was shaken at 250 rpm, at 45 °C. The degree of conversion and the optical purity of the product were determined as described in the legend to Table I. [b]To 1 of ml organic solvent containing (±)-**2** (28 mM) and trifluoroethyl butyrate (56 mM), 3 mg of enzyme and 100 mg of molecular sieves were added, and the suspension was shaken at 250 rpm, at 45 °C. The degree of conversion and the optical purity of the product were determined as described in the legend to Table I. [c]See footnote e to Table I. [d]The results obtained with immobilized Lypo Protein Lipase are reported in parentheses. To 1 ml of organic solvent containing (±)-**1** (126 mM) and vinyl acetate (150 mM), 10 mg of enzyme-matrix complex were added, and the suspension was shaken at 250 rpm, at 45°C.

The enantioselectivity of porcine pancreatic lipase with sulcatol was also determined by another approach. The specificity constants ($V_{max}/K_m$) of the enzyme for the two enantiomers of **1** were measured and then the E values obtained from their ratios.[11] There were still variations of enantioselectivity as a function of the nature of the solvent (Table III, see 3-methyl-3-pentanol and

toluene), though less evident than those found by the previously described method (Table I). Table III also shows the effects of the solvents on the $V_{max}$ and $K_m$ values for the two enantiomers. It can be seen that $V_{max}$ values were moderately affected by the solvent, with the exception of 3-methyl-3-pentanol, whereas $K_m$ values dramatically increased with increasing solvent polarity.[12] Regarding the origin of enantioselectivity the data in Table III indicate that the main contribution results from differences in $V_{max}$ (R-sulcatol 8-11 times faster than S-sulcatol) rather than in $K_m$ values ($K_m$ for R-sulcatol 2-3 times lower than $K_m$ for S-sulcatol) for the two enantiomers.

**Table III.** Enantiomeric Ratio, Expressed as $(V_{max}/K_m)_R/(V_{max}/K_m)_S$, of Porcine Pancreatic Lipase in Various Solvents with (R)- and (S)-**1** as the Substrates[a]

| solvent | $(V_{max}/K_m)_R$ / $(V_{max}/K_m)_S$ | $V_{max}$(μmol/h) (R)-**1** | $V_{max}$(μmol/h) (S)-**1** | $K_m$ (mM) (R)-**1** | $K_m$ (mM) (S)-**1** |
|---|---|---|---|---|---|
| 3-pentanone | 23 | 39.0 | 4.5 | 217.0 | 568.3 |
| 3-methyl-3-pentanol | 18 | 10.4 | 1.3 | 311.2 | 696.5 |
| toluene | 34 | 40.1 | 3.5 | 47.6 | 141.4 |
| cyclohexane | 26 | 52.7 | 4.9 | 21.6 | 52.8 |
| dodecane | 24 | 40.0 | 4.6 | 11.0 | 30.5 |

[a]To 1 ml of organic solvent containing (-)-(R)-**1** (6-300 mM) or (+)-(S)-**1** (13-650 mM) and trifluoroethyl butyrate (480 mM), 50 mg of enzyme and 100 mg of molecular sieves were added, and the suspension was shaken in an orbital shaker at 250 rpm, at 45 °C. Periodically, aliquots (5-6 per experiment) were withdrawn and assayed for product formation by GLC. $V_{max}$ and $K_m$ values were obtained from the initial rate measurements using ENZFITTER (Leatherbarrow, R. J. Enzfitter a Non-Linear Regression Data Analysis Program for I.B.M. P.C., Elsevier Biosoft, Cambridge 1987). The $K_m$ values for trifluoroethyl butyrate were also determined and they were found to be poorly affected by the solvent (from 10 to 18 mM with (R)-**1** and from 21 to 39 mM with (S)-**1**).

The determination of enantioselectivity by measurement of specificity constants was quite informative but also rather complex and laborious and, therefore, it was not applied to **2** and to Lipase PS. In addition, at least in our hands, it was not so highly reliable as the method based on the determination of ee values. In this latter case, in fact, both the degree of conversion and ee, from which the E value was obtained, were determined in a single chromatographic run without any prior manipulation. It should also be emphasized that possible inaccuracies in time measurements and in enzyme and reagent concentrations, as well as variations in shaking rates and catalyst dispersion in the reaction medium did not affect the determination of E, because the equations utilized[3d] are not dependent on these parameters.

**Effects of temperature, chain length of the acylating agent, and removal of water by molecular sieves.** The effects of temperature on the enantioselectivity of porcine pancreatic lipase with (±)-1, and of lipase PS with (±)-2 were investigated over the range 22-65 °C. Table IV shows that for both enzymes there were significant decreases in E values with increase of temperature. This finding is in line with recent papers reporting an inverse correlation between temperature and enantioselectivity for alcohol dehydrogenases from *T. brockii*[13a] and *T. ethanolicus*,[13b] pig liver esterase[13c], *Candida cylindracea* lipase,[13d] *Pseudomonas cepacia* lipase[13e],and also with Otto's theoretical predictions.[13f] With porcine pancreatic lipase the reaction rate increased as a function of temperature, whereas with Lipase PS the rate at 65 °C was lower than at 45 °C (Table IV), probably because of inactivating effects.

**Table IV.** Effect of Temperature on the Enantioselectivity and Transesterification Rate of Porcine Pancreatic Lipase with (±)-1, and of Lipase PS with (±)-2

| | porcine pancreatic lipase[a] | | Lipase PS[b] | |
|---|---|---|---|---|
| temperature (°C) | E[c] | rate (μmol/h) | E[c] | rate (μmol/h) |
| 22 | 27 | 6.0 | 32 | 7.4 |
| 45 | 23 | 6.7 | 25 | 15.0 |
| 65 | 17 | 8.3[d] | 17 | 7.9[d] |

[a]Conditions as described in the legend to Table I, with dodecane as the solvent. [b]Conditions as described in the legend to Table II, with 3-pentanone as the solvent. [c]See footnote *e* to Table I. [d]Also at 65 °C, no appreciable conversion was observed in the absence of the enzyme.

The chain length of the acylating agent (trifluoroethyl butyrate, hexanoate, octanoate, dodecanoate and hexadecanoate) did not significantly affect the enantioselectivities of either porcine pancreatic lipase (E values ranging from 23 to 27 with 1 as the substrate and dodecane as the solvent) nor Lipase PS (E values from 24 to 28 with 2 as the substrate and 3-pentanone as the solvent). This result was to some extent unexpected because there are several reports about the dependence of the enantioselectivity of some lipases on the nature of the acylating agents.[3d,3e,14] In particular, our data do not confirm the marked increase of selectivity reported for porcine pancreatic lipase with (±)-1 when using trifluoroethyl dodecanoate (E equal to 100 instead of 19-27 with shorter-chain acylating agents and even with trichloroethyl dodecanoate).[14d] Also, the reaction rates were similar for all the esters tested, with relative rates ranging from 75 to 100 for porcine pancreatic lipase and from 83 to 100 for Lipase PS.

The addition of molecular sieves that remove water from the reaction medium markedly increased the transesterification rates, especially in the case of hydrophobic solvents. Thus, with porcine pancreatic

lipase, the rates increased about 4 times with toluene, cyclohexane and dodecane whereas small increases or none were obtained with 3-pentanone and 3-methyl-3-pentanol (Table V). Similar behavior was observed for Lipase PS, with 1.15-, 1.5-, and 10- fold increases with dioxane, 3-pentanone and toluene (Table V).

**Table V.** Effects of Molecular Sieves on the Transesterification Rate of Porcine Pancreatic Lipase and Lipase PS with (±)-**1**

| | rate (μmol/h) | | | |
|---|---|---|---|---|
| | porcine pancreatic lipase | | Lipase PS | |
| solvent | control | molec. sieves[a] | control | molec. sieves[b] |
| dioxane | - | - | 1.4 | 1.6 |
| 3-pentanone | 2.6 | 4.7 | 2.5 | 3.8 |
| 3-methyl-3-pentanol | 1.5 | 1.6 | - | - |
| toluene | 1.9 | 8.6 | 0.6 | 6.2 |
| cyclohexane | 2.6 | 9.7 | - | - |
| dodecane | 1.7 | 6.7 | - | - |

[a]Data taken from Table I. [b]Data taken from Table II. Control experiments were carried out under identical conditions but in the absence of molecular sieves.

These results are in agreement with previous data reported by us[3e] and by others[15] describing increasing rates of esterification or transesterification catalyzed by porcine pancreatic lipase and Lipases PS as water content was lowered. For esterification reactions, this phenomenon was attributed by Yamane et al.[15] to the decrease in the rate of the reversible reaction, i.e., ester hydrolysis. That explanation should also be valid for transesterification reactions since, for them too, water hydrolyzes the ester product. The differences in the increase caused by molecular sieves for the various solvents could reside in the different capacities of the solvents to strip water from the enzymes. In a hydrophobic medium, enzymes would be surrounded by high concentrations of water,[16] which would hasten the hydrolysis of the ester product as it formed. Instead, in hydrophilic solvents, water would be mainly dissolved in the medium and, therefore, its concentration around the enzymes low. As a consequence, water removal by molecular sieves has marked effects on transesterification rates only in hydrophobic solvents.

The enantioselelectivity of the two enzymes did not change significantly when molecular sieves were omitted.

## CONCLUSION

The present study unambiguously demonstrates that the nature of organic solvents influences the enantioselectivity of Lipase PS and porcine pancreatic lipase with sulcatol and 3-bromo-5-hydroxymethyl isoxazoline as the substrates. Since it is likely that such a behavior also occurs with other compounds, these results are of notable practical interest because the two lipases are widely used in organic solvents for racemate resolution.[1f,3] The effects on enantioselectivity, however, not only cannot be predicted on the basis of the physicochemical properties of the solvents but they also change, for a given substrate, by changing the enzyme and, for a given enzyme, by changing the substrate (see Table I and II and note 9). Therefore, rationales based on differences of partitioning of the water associated with the binding site (correlated with solvent hydrophobicity),[5a] or on differences of enzyme flexibility (correlated with the dielectric constant of the solvent)[5c] are not compatible with our results, and other explanations have to be found.

We could speculate that one or more molecules of solvent interact with the enzyme in or near the substrate binding site and, depending on their structure (bulkyness, chain ramification, rigidity, etc.), interfere with the association or transformation of one enantiomer more than with the other, thus affecting enantioselectivity. Therefore, according to this hypothesis, it would be the solvent-enzyme complex that dictates enantioselectivity. The formation of such a complex requires that in organic media, as in aqueous media in which there is bulk water and water tightly bound to the enzyme,[17] there is bulk solvent and bound solvent. This model, however, cannot be of predictive value (at least in the absence of the tridimensional structure of the enzymes and of challenging modelling) because of the great number (equal to the number of usable solvents) of possible solvent-enzyme complexes and because each complex might behave differently depending on the nature of the substrate.

It is also of remarkable practical importance that for both lipases there is an inverse correlation between temperature and enantioselectivity, and that removal of water from the reaction medium by molecular sieves markedly increases transesterification rates in hydrophobic solvents.

## EXPERIMENTAL SECTION

**Materials.** Lipases from porcine pancreas (Type II, 53 units/mg solid using olive oil; Type VI-S, purified, 20500 units/mg solid using olive oil) were purchased from Sigma, and Lipase from *Pseudomonas* species (Lipase PS, 30 units/mg solid using sesame oil; Lipo Protein Lipase, LPL, 800 units/mg solid using sesame oil) from Amano. Crude porcine pancreatic lipase and Lipase PS were used directly without any pretreatment, whereas purified porcine pancreatic lipase and Lipo Protein Lipase were immobilized (see below). (±)-Sulcatol (1) was purchased from Aldrich and (±)-3-bromo-5-hydroxymethyl isoxazoline (2) was synthesized according to De Amici et al.[7] (+)-(S)-Sulcatol was prepared by reducing 6-methyl-5-hepten-2-one with alcohol dehydrogenase from *T.brockii* as described by Belan et al[18] ((S)-1 > 99 % by chiral GLC).(-)-(R)-Sulcatol was obtained by resolving the racemic mixture with porcine pancreatic lipase following the method described by Belan et al[18] modified by using trifluoroethyl- instead of trichloroethyl butyrate, benzene instead of ether, and by performing the transesterification in the presence of molecular sieves ((R)-1 96 % by chiral GLC). Trifluoroethyl esters were preparaed as described elsewhere.[19] Organic solvents were dried over 3 A° molecular sieves.

**Enzyme immobilization.** Purified porcine pancreatic lipase and Lipo Protein Lipase had to be immobilized before use because, in organic solvents, these enzyme powders stuck to reactor walls maintaining very poor activity. Purified porcine pancreatic lipase (5 mg) or Lipo Protein Lipase (300 mg) were dissolved in 0.1 M potassium phosphate buffer pH 7 (6 ml) and added to Hyflo-Super Cell (2 g). The amount of the water solutions added were such to completely swell the matrix. The hydrated enzyme-support mixtures were finally dried over vacuum pump (20 h, 0.02 mbar) and the water content (about 2 %, w/w) determined by the optimized Fischer method.[16]

**Transesterification reactions.** In a typical experiment, a powdered enzyme sample and 100 mg molecular sieves were placed in a 3-ml vial, followed by the addition of 1 ml of solvent containing trifluoroethyl butyrate and the substrate ((±)-1 or (±)-2). Then the reaction mixture was shaken in an orbital shaker at 250 rpm, 45 °C. Periodically, aliquots were withdrawn and assayed by chiral GLC or HPLC as described below.

**Determination of enantiomeric excess and degree of conversion.** Both the degree of conversion of (±)-sulcatol into the correspondent ester and the ee of either the ester product or the remaining substrate were determined by chiral GLC with a CP-Cyclodextrin-β-2,3,6-M-19 column (50 m, 0.25 mm ID, Chrompack) under the following conditios: oven temperature from 90 °C (initial time 25 min) to 130 °C with a heating rate of 1.5 °C/min; $H_2$ as carrier gas. When trifluoroethyl butyrate was used as the acylating agent (the great majority of experiments), E values were calculated from the ee values of the product. In the study on the effect of the chain length of the acylating agent on enantioselectivity, E values were calculated from the ee values of the remaining substrate because the enantiomers of ester products other than butyrate were not satisfactorily resolved by chiral GLC (see Figure 1A showing the

separation of sulcatol and sulcatol butyrate enantiomers).

The degree of conversion of (±)-3-bromo-5-hydroxymethyl isoxazoline and the ee of either the ester product or the remaining substrate were determined by chiral HPLC with a Chiracel OB column (4.6 x 250 mm, Baker) under the following conditions: flow rate 0.5 ml/min; eluent *n*-hexane/*n*-propanol 4.5:1, reading at 215 nm. When trifuoroethyl butyrate was used as the acylating agent, E values were calculated from the ee values of the product. In the study on the effect of the chain length of the acylating agent, E values were calculated from the ee values of the remaining substrate because of the unsatisfactory separation of ester products other than butyrate (see Figure 1B showing the separation of the enantiomers of 2 and 2-butyrate).

The equilibrium constant K was determined on the fast-reacting species as described by Chen et al.[3d]

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(8) Also Fitzpatrick and Klibanov (ref. 5c) did not find any correlation between the nature of the solvent and the enantioselectivity of porcine pancreatic lipase with *sec*-phenethyl alcohol.

(9) The enantioselectivity of Lipase PS with *trans*-sobrerol was 520, 212, 178, 142 and 69 in *t*-amyl alcohol, 3-pentanone, dioxane, acetone and tetrahydrofuran, respectively (Bovara, R.; Carrea, G.; Ferrara, L.; Riva, S. *Tetrahedron Asymmetry* **1991**, *2*, 931-8).

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