

# Esterifications of 1- and *rac*-2-Octanols With Selected Acids and Acid Derivatives Using Lipases

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Several aliphatic acids and their ethyl, isopropenyl and 2,2,2-trichloroethyl esters were allowed to react with 1- and 2-octanols catalyzed by commercial lipase preparations of porcine pancreas and the fungi *Candida rugosa*, *Aspergillus niger* and *Mucor miehei*.

Comparisons of reactivity of the acids and esters were made in common organic solvents using the primary alcohol. Reactions of octanoic acid and its esters with 2-octanol in hexane allowed an evaluation of stereoselectivity of the lipases with different substrates that carried the same (octanoyl) residue. A partial resolution of *rac*-2-hexadecanol with *A. niger* lipase is described, and the utility of lipase selectivities (stereo, positional, fatty acid and ester) is discussed with reference to the data presented.

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Fatty acid selectivities of commercial and purified microbial lipases continue to receive much attention (1,2). Such information would be useful for projected industrial reactions such as the synthesis of fatty acid esters and transformations of natural triglycerides (3-6). The effect of the alcohol or other residue attached to a given acyl substrate on lipase preferences for fatty acids has been studied less frequently, although the accumulated evidence (7-10) indicates that acylation of the enzyme may be promoted, and/or acylation may be made irreversible, by suitable choice of acylating agent. Moreover, the large number of lipase-mediated kinetic resolutions of alcohols (7) and the oft documented triglyceride position preferences of lipases (1) demonstrate the importance of the structure of the alcohol component in reactions that are catalyzed by these enzymes.

We have screened separately and compared several acid derivatives of acetic, octanoic and oleic acids in reactions with 1- and 2-octanol, in order to further assess the potential importance of the nonacyl portion of the substrate in lipase reactions. In the process, we found that a lipase of *Aspergillus niger* (Amano-Lipase K) also is useful for resolving secondary alcohols. In addition, we observed that the degree of stereobias exhibited by lipases can be affected sometimes by the nature of the group associated with the acid residue in the starting material. Lipase selectivities based on the data reported here are evaluated for utility in kinetic resolution of enantiomeric alcohols and in selective transesterification of primary alcohols.

## MATERIALS AND METHODS

Gas liquid chromatography (GLC) was performed with a Shimadzu GC-Mini 2 instrument (Columbia, MD) using

an SPB-1 column (0.25 mm i.d.  $\times$  30 m) fitted with a flame ionization detector and operated at column temperatures indicated herein, with a 50:1 split ratio and with He carrier gas. Chromatograms were recorded with either a Perkin-Elmer Model 023 Recorder (Norwalk, CT) or a Hewlett-Packard 3390 Recording Integrator (Avondale, PA). Infrared spectra (IR) were obtained on a Perkin-Elmer 1310 Spectrophotometer (3% solutions in  $\text{CCl}_4$ ). Nuclear magnetic resonance spectra (NMR) were obtained in  $\text{CDCl}_3$  using a JEOL JNM-GX 400 FT NMR spectrometer (Piscataway, NJ). Mass spectra (MS) were recorded with a Hewlett-Packard 5995 GC-MS System interfaced with an OV-1 column (0.25 mm i.d.  $\times$  12 m). Optical rotations were obtained using a Perkin-Elmer 141 Polarimeter.

Acetic acid was Baker Analyzed Reagent (Baker, Phillipsburg, NJ), all other organic acids were Aldrich Reagent Grade (Aldrich, Milwaukee, WI) and were employed without purification. Hydrocarbon standards for GLC were purchased and used as received, also. Ethyl and 2,2,2-trichloroethyl esters were synthesized in the usual manner from the alcohols and acids (Fisher esterification) or acid halides (triethylamine,  $\text{CH}_2\text{Cl}_2$ ). They were distilled and characterized by IR, MS and GLC. Isopropenyl esters were prepared in slightly better yield, and more conveniently than previously reported (11), and an example is given as well. Solvents were purchased "distilled in glass," or were reagent grade and were distilled before use (acetone distilled from  $\text{KMnO}_4$  and 1,2-dimethoxyethane distilled from lithium aluminum hydride). Diethyl ether was Analyzed Reagent-Anhydrous (Baker) and was used directly. The enzyme preparations by biological source (commercial source; company trade name; initial rate assay on olive oil emulsions given in  $\mu\text{mol}$  free fatty acid released,  $\text{min}^{-1}$ ,  $\text{mg}^{-1}$ ) were *C. rugosa* (Enzeco, none, 9.82) (Enzyme Development Corp.; New York, NY), *A. niger* (Amano; Lipase K; 11.2) (Amano, Troy, VA), *M. miehei* (NOVO; Lipase-3A; 5.33) (NOVO, Wilton, CT) and Porcine pancreatic lipase (Sigma; none; 15.6) (Sigma, St. Louis, MO).

**Immobilization of lipases.** Lipase-3A is on a resin (proprietary), as purchased. For ease in use, greater stability and potential for recovery, the other two fungal lipases were adsorbed onto Duolite, an ion-exchange resin available from Rohm and Haas Co. (Philadelphia, PA). Duolite (50 g) was washed sequentially with  $2 \times 100$  ml of methanol, and then  $2 \times 100$  ml of .05 N phosphate buffer at pH 7.0. The material was then swirled in 100 ml of the same buffer containing 5 g of the commercial lipase powder (30°C, 16 hr). The mixture was suction filtered and washed with  $2 \times 100$  ml of reagent acetone. The granular product was dried under vacuum at 25°C.

Activities of the lipases in organic solvent before and after adsorption were evaluated as follows: 5 ml of wet hexane containing 0.50 mmol of 1-octanol and 0.25 mmol of octadecane, and 5 ml of wet hexane containing 0.50 mmol of octanoic acid were combined. Catalyst

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Abbreviations: ee, enantiomeric excess; GLC, gas liquid chromatography; IR, infrared spectra; MS, mass spectra or spectrometry; NMR, nuclear magnetic resonance spectra.

(20 mg of powder or 0.20 g of immobilized enzyme) was added, the reaction mixture was stirred at 30°C and samples were analyzed by GLC (200°C) at 1.0 and 2.0 hr. The measurements were duplicated and averaged. Lipase (powder activity in  $\mu\text{mol}$  of ester formed;  $\text{hr}^{-1}$ , mg; immobilized enzyme activity): *C. rugosa* (1.1, 0.13); *A. niger* (0.33, 0.35). The pancreatic lipase was used as a powder; a useful preparation on Duolite was not obtained. Lipase 3A is immobilized on an ion-exchange resin, as purchased.

**Preparation of isopropenyl heptanoate.** Heptanoic acid (50 ml), isopropenyl acetate (200 ml) and  $\text{H}_2\text{SO}_4$  (0.3 ml) were heated under reflux for 5 hr. The mixture was cooled to ca. 25°C, stirred for 0.25 hr with 20 g of sodium acetate and 100 ml of anhydrous ether, and then filtered. The filtrate was concentrated on a rotary evaporator to remove ether and isopropenyl acetate. The concentrate was dissolved in 200 ml of ether and stirred for 2 hr with 25 g of  $\text{NaHCO}_3$  and 50 ml of water. The organic phase was recovered and dried ( $\text{Na}_2\text{SO}_4$ ). The solvent was removed, and the residue was distilled through a Vigreux column collecting the fraction bp 87–93°C (20 mm): 19.2 g, 32.2% yield, IR 1750, 865  $\text{cm}^{-1}$ , GC-MS  $m/e$  113 (heptanoyl ion), no molecular ion obtained;  $^{13}\text{C}$  NMR 14.09, 19.58, 22.60, 24.91, 29.01, 31.65, 34.37, 101.96, 153.01, 171.02. The product was contaminated with about 3–4% of the acetic heptanoic anhydride. Isopropenyl octanoate and oleate were similarly prepared; the latter was purified by column chromatography, as previously reported (11).

**Esterifications of 1-octanol.** Reaction mixtures were prepared that contained 0.50 mmol each of 1-octanol and the acylating reagent and 0.25 mmol of the hydrocarbon internal standard in 10 ml of wet solvent. Water-miscible solvents were fortified with 2  $\mu\text{l}$  of distilled water. The enzyme formulation was added, and the reaction mixture was stirred at 30°C. Aliquots were analyzed by GLC—acyl residue (hydrocarbon standard, GLC temperature): acetyl (undecane, 120°C); octanoyl (octadecane, 200°C); oleoyl (tetracosane, 280°C). The amount of catalyst used was adjusted downward, as necessary, to reduce percentage conversion to less than 20% during the first hour and thereby provide a calculated velocity close to an initial rate assay (the rate that would be calculated from the initial linear portion of the rate curve, with an excess of substrate to assure saturation of enzyme active sites). Most reactions proceeded to less than 5% conversion under the conditions employed.

**Esterifications of 2-octanol.** Reaction mixtures were prepared containing 0.50 mmol each of *rac*-2-octanol and octanoic acid (ester) and 0.25 mmol of octadecane in 10 ml of wet hexane. The enzyme (0.20 g of formulated material or 225 mg of pancreatic lipase powder) was added, and the mixture was shaken at 30°C for 4–6 days. Analysis by GLC provided percentage conversion. The configurational analysis for residual 2-octanol was obtained, as previously described (12), using (*S*)- $\alpha$ -methylbenzylisocyanate (13) to produce chromatographically separable diastereomers to determine the enantiomeric excess (ee) of the unesterified alcohol.

**Resolution of *rac*-2-hexadecanol.** 2-Hexadecanol (2.94 g, 10 mmol) was shaken in a solution of wet hexane (50 ml) containing isopropenyl oleate (3.23 g, 10 mmol) and *A. niger* on Duolite (5 g) at 30°C for 14 days. The mixture was suction filtered, and the resin was washed with small portions of hexane. The combined organic phase was

concentrated and chromatographed on silica gel, 60–200 mesh, 30 g (Grace Co., Baltimore, MD). The oleate ester was eluted with 2–5% ether-hexane, and the unreacted alcohol was eluted with 7.5–10% ether-hexane (1.36 g, 46.3% of 70.0 ee:S). The ester was saponified (20 ml of 50% 4 N KOH-methanol, reflux overnight) to produce alcohol (1.28 g, 43.5% of 86.6 ee:R). The ester, in other words, was 93.3% (R). The R-alcohol had  $\alpha_D^{25}$ -4.0 ( $C = 4.7$ , EtOH). A midfraction was recovered with 5% ether-hexane that was mostly alcohol. The chromatographic conditions for the diastereomeric carbamates used to assign configurational purity to the alcohols were: column temperature 280°C,  $k'$ s 4.167 (R,S-diastereomer), 4.375 (S,S-diastereomer) and a separation factor of 1.050.

## RESULTS AND DISCUSSION

**Esterification of 1-octanol.** Acetic, octanoic and Z-9-octadecenoic (oleic) acids were allowed to react with 1-octanol in wet hexane at  $30 \pm 0.2^\circ\text{C}$  with the following commercial lipase preparations: Enzeco *C. rugosa*, Amano Lipase K. (*A. niger*), NOVO Lipase 3A (*M. miehei*) and Sigma porcine pancreatic lipase. A hydrocarbon internal standard permitted an evaluation of conversion to 1-octanol ester, from which was calculated a value for reactivity in  $\mu\text{mol}$  ester formed,  $\text{min}^{-1}$ ,  $\text{g}^{-1}$ . Reactivities of ethyl, isopropenyl and 2,2,2-trichloroethyl esters were similarly calculated and the results tabulated as reactivity relative to the corresponding acid as unity (Table 1). A typical chromatogram is given in Figure 1. Under these conditions,

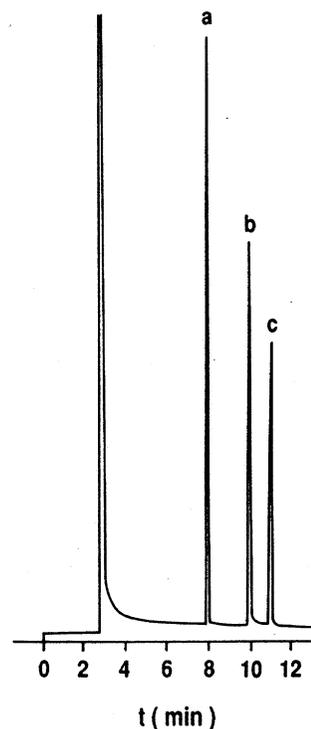


FIG. 1. Gas Chromatogram of a) 2-octanol octanoate, b) 1-octanol octanoate and c) octadecane, in a molar ratio of 1.765:1.797:1.000, using the SPB-1 column at 200°C. Similar chromatograms were used as standards to monitor lipase esterifications.

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TABLE 1

 Relative Rate of Esterification of 1-Octanol in Hexane<sup>a</sup>

Enzyme	Acid residue	Acid <sup>b</sup>	Ethyl <sup>c</sup>	Isopropenyl <sup>c</sup>	TCE <sup>d</sup>	SH <sup>e</sup>
<i>C. rugosa</i>	Acetyl	1.00	— <sup>f</sup>	19.8	23.4	15.3
	Octanoyl	1.00	—	1.7	8.5	ND
	Oleoyl	1.00	—	1.5	3.5	ND
<i>A. niger</i>	Acetyl	1.00	0.071	1.96	0.41	5.86
	Octanoyl	1.00	0.038	0.11	0.067	ND
	Oleoyl	1.00	<0.04	0.39	<0.04	ND
<i>M. miehei</i>	Acetyl	1.00	0.30	0.34	0.25	1.5
	Octanoyl	1.00	0.28	0.016	0.23	ND
	Oleoyl	1.00	0.27	0.29	0.27	ND
Pancreatic	Acetyl	1.00	0.32	0.62	2.07	2.57
	Octanoyl	1.00	<0.01	0.12	0.23	ND
	Oleoyl	1.00	— <sup>f</sup>	0.56	<0.15	ND

<sup>a</sup>See Materials and Methods. Reactions were duplicated and GLC analyses were reproducibly  $\pm 3\%$ . Activities presented are relative to the acid as 1.00; actual values obtained for free acids are: (acid, activity in  $\mu\text{mol}$  ester formed,  $\text{min}^{-1}, \text{g}^{-1}$ ) for *C. rugosa* (acetic, 0.058; octanoic, 1.25; oleic, 1.01), *A. niger* (acetic, 2.66; octanoic, 20.6; oleic, 10.3), *M. miehei* (acetic, 4.91; octanoic, 250; oleic, 310), and pancreatic (acetic, 0.32, octanoic, 8.4; oleic, 5.4).

<sup>b</sup>Free acid.

<sup>c</sup>Ester.

<sup>d</sup>2,2,2-Trichloroethyl ester.

<sup>e</sup>Thiolacetic acid.

<sup>f</sup>Very slow (less than 0.01).

ND, not determined.

TABLE 2

 Activity of Lipases in Various Organic Solvents (1-Octanol and Octanoic Acid)<sup>a</sup>

Enzyme	Hexane	Toluene	Ether	DME <sup>b</sup>	M <sup>c</sup>	Acetone
<i>M. miehei</i>	300	150	4.3 (260)	— <sup>d</sup> (100)	8.0 (220)	0.3 (250)
<i>A. niger</i>	20.6	2.5 (8.5)	— <sup>d</sup>	— (-)	— <sup>d</sup> (2.3)	— (0.77)
<i>C. rugosa</i>	1.25	— <sup>d</sup> (0.56)	— (0.77)	— (-)	— (0.083)	— (-)
Pancreatic	8.4	4.6	0.21 (3.4)	— (2.4)	0.55 (5.8)	0.20 (5.9)

<sup>a</sup>See Materials and Methods. Reactions were duplicated and GLC analyses were reproducibly  $\pm 3\%$ . Activities are in units of  $\mu\text{mol}$  of ester formed,  $\text{min}^{-1}, \text{g}^{-1}$ . Figures in parentheses are activities of recovered enzyme measured in hexane.

<sup>b</sup>1,2-Dimethoxyethane.

<sup>c</sup>Methylene chloride.

<sup>d</sup>Less than 0.01.

the ethyl esters were uniformly less reactive than the corresponding free acids. Because the carbonyl groups of the isopropenyl and 2,2,2-trichloroethyl esters render the ester carbonyls electron deficient, however, one might expect that the lipases would be more readily acylated. In fact, the "activated" esters were indeed more reactive with *C. rugosa* lipase, especially the acetates. But these esters were generally less reactive than the free acids using the other lipases. To the degree that an acylated enzyme has no memory of the source of the acyl residue, these relative reactivities suggest that carbonyl polarity in the substrate bears little relationship to the ease of enzyme acylation. It is interesting to note that thiolacetic acid was more reactive than acetic acid. The longer chain acids are favored by the enzymes over acetic acid, as

previously established. The counterunit (alkoxy group for an ester) is sometimes more effective in promoting reaction of the shorter acid, perhaps because acetic acid tends to self-associate in hexane, although its esters are monomeric and more hydrophobic.

The reactivity of enzymes in organic media has been reviewed by Klivanov (14), and it is clear that solvent can profoundly influence both stability and reactivity. We conducted esterifications of octanoic acid with 1-octanol, using the same lipase preparations in various organic solvents for 1 hr, and determined reactivities as before. The lipase from such reactions was recovered and used for the same esterification reaction in hexane, in order to determine residual lipase activity (Table 2). In each case, the esterification reaction was fastest in hexane, about

half as rapid in toluene for the lipases of *M. miehei* and porcine pancreas, and very slow otherwise. Evaluations of recovered activity often showed significant losses particularly for *A. niger* and *C. rugosa* lipases, especially in 1,2-dimethoxyethane. The loss in activity for the two aforementioned lipases in acetone is interesting, because that solvent is often used to precipitate crude protein preparations. An exact determination of the nature of these losses in each case is beyond the scope of this investigation, but it is apparent that reactivity in, and recovery of enzyme activity from, organic solvents would have to be uniquely determined and optimized for a particular reaction, enzyme and solvent.

**Esterification of 2-octanol.** The same lipase preparations were used to catalyze the esterification of 2-octanol with octanoic acid in hexane at  $30 \pm 0.2^\circ\text{C}$ . Acetic acid and its esters reacted too slowly, and octanoic acid was selected, because the results obtained for octanoic acid were expected to be similar to those that would be obtained for longer chain acids (12). The degree of conversion was obtained by GC (Fig. 1), and the stereobias (enantiomeric excess) in the unreacted 2-octanol was determined by conversion to diastereomeric carbamates with (S)- $\alpha$ -methylbenzylisocyanate that then could be analyzed by GC (Fig. 2). These data permitted the calculation of

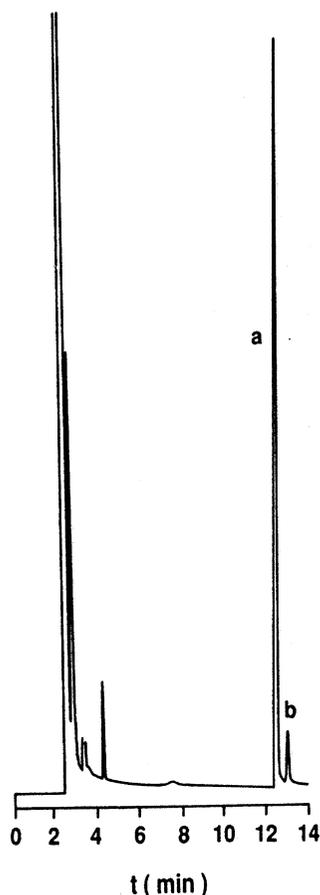


FIG. 2. GLC analysis of  $\alpha$ -methylbenzyl carbamates obtained by the reaction of 2-hexadecanol recovered from its oleate ester. The ester had been formed from alcohol and acid using *A. niger* lipase (Amano-Lipase K) on Duolite. Analysis uses an SPB-1 column at  $280^\circ\text{C}$ ; a) is the R,S-diastereomer (R-alcohol) and b) is the S,S-diastereomer.

enantiomeric ratio ( $E_R$ ), a number that expresses the relative reactivity of enantiomers with an enzyme (15) (Table 3). Although the derivation for  $E_R$  was based on pure enzyme, i.e., a single enzymatically active species, a mixture of enzymes such as an impure commercial preparation would, at most, cause the calculated  $E_R$  to drift, particularly at greater conversion. Conversions were less than 50% and are offered as a rough indicator of relative reactivity of the enantiomers.

As reported previously, the stereoselection of *M. miehei* lipase was high for octanoic acid; it is also high for the isopropenyl and 2,2,2-trichloroethyl esters. The Amano-Lipase K (*A. niger*) also was high enough to be useful and we partially resolved 2-hexadecanol to show the utility of such a procedure. Previous evaluation of this lipase (12) used the commercial powder that had low activity. Deposition of the *A. niger* lipase powder on Duolite, however, markedly increased its activity and allowed a more accurate determination of its stereobias in this reaction. The increased activity of the immobilized *A. niger* lipase is probably due to improved stability of the enzyme (16). The immobilization process frequently acts to reduce one or another of the denaturation processes, and the assay method that is conducted during a time interval leads to a higher calculated specific activity than did the original enzyme powder. The immobilized *A. niger* lipase was allowed to catalyze the esterification of 2-octanol with oleic acid. At 45% conversion, the residual alcohol was 85% S:15% R (70% ee:S), and the product oleate ester was 87% R (Fig. 2). The  $E_R$  would be ca. 29, and a conversion in the vicinity of 58% would assure a pure (S)-2-hexadecanol (17). The *C. rugosa* showed no bias with the octanoic acid and low, opposite biases with the two octanoyl esters. Enzyme recovered from reaction of the isopropenyl ester was used to esterify octanoic acid (no bias for octanoic acid), then recovered and used for the isopropenyl ester (same result for the ester). Thus, the alteration in selectivity is real and reproducible. More striking is the modest bias shown by pancreatic lipase with octanoic acid, and the very strong selectivity with the 2,2,2-trichloroethyl ester. Recently, Deleuze et al. (10) reported

TABLE 3

Stereoselectivities of Lipases in Octanoylation of 2-Octanol in Hexane<sup>a</sup>

Enzyme	Acid	Isopropenyl <sup>b</sup>	TCE <sup>c</sup>
<i>C. rugosa</i>	1.0	1.3 <sup>d</sup>	1.3
<i>A. niger</i>	>40 <sup>e</sup>	>40 <sup>e</sup>	>40 <sup>e</sup>
<i>M. miehei</i>	>40	>40	>40
Pancreatic	9.6	- <sup>f</sup>	>40

<sup>a</sup> See Results and Discussion for selectivity measurement. The (R)-2-octanol reacts fastest, except as noted.

<sup>b</sup> Ester.

<sup>c</sup> 2,2,2-Trichloroethyl ester.

<sup>d</sup> The (S)-2-octanol reacts slightly faster.

<sup>e</sup> Although calculation gives an exact figure, the analytical technique is insufficiently precise and the error in calculated value increases with higher relative rates. For practical purposes selectivities greater than 40:1 are likely to be useful in kinetic resolution.

<sup>f</sup> Reaction too slow.

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the results of competition experiments showing that the relative reactivity of pairs of esters with lipases was independent of the alcohol that was being esterified. Likewise, the relative reactivity of pairs of alcohols with acylated lipases was independent of the source (e.g., structure of the alcohol component of the ester) of acid residue. These observations are consistent with a generally accepted view that lipases first become acylated, and then undergo reaction with an available nucleophile in a discrete second phase of operation. Implicit is an assumption that the nonacyl component of the substrate is shed from the enzyme into the medium, where it becomes a minor component in the pool of available nucleophiles. Our observations do not confound this picture, but merely indicate that substrates and nucleophiles can, perhaps by virtue of alternate associations with the protein and their overwhelming presence (compared to enzyme concentration), alter somewhat the delicate balance of stereoselectivity of an acylated enzyme. Similar observations were reported earlier, for example, wherein stereoselectivity involving the esterification of *rac*-2-octanol with octanoic acid using *M. miehei* lipase was reduced significantly in the presence of the much less reactive acetic acid, though the same reaction proceeded at a slower rate with no change in stereoselectivity in the presence of unreactive phenyl(trifluoromethyl)carbinol (18).

**Lipase selectivity.** Much interest exists in exploring the selectivities of lipases for projected industrial reactions (3-6), and a number of patents have been issued that are based on preferential reactions of various lipases with fatty acids and their derivatives (19). Given the problem of selectively converting one reagent present in a mixture of potential reagents to a product, one is faced essentially with a kinetic problem. With sufficient reaction time, a

product mixture would be obtained that is at equilibrium thermodynamically. Therefore, how selective must a catalyst be to be useful?

Competitive reactions were conducted that were chosen based on the data of Table 1. Isopropenyl heptanoate and ethyl octanoate were allowed to transesterify 1-octanol competitively, using *C. rugosa* lipase on Duolite in hexane. Because isopropenyl esters are better substrates for this lipase than ethyl esters and will react irreversibly, one expects to see faster and more complete conversion to octyl heptanoate than to octyl octanoate. The data (Fig. 3) show the general predicament inherent in the use of enzymes as catalysts, when selectivity is modest. The heptanoate product contains only ~2% of the octanoate ester, if the reaction is terminated at ~19% conversion (5 hr). However, at 86% conversion (144 hr), the octanoate component is >17% of the product. In a related experiment that would yield the same two esters as products, but should lead to a mixture richer in octanoate ester (Fig. 4), isopropenyl heptanoate and 2,2,2-trichloroethyl octanoate were allowed to react with 1-octanol. In this case, it was expected that octyl octanoate ester would form more quickly. Indeed, this was so, and then the octyl heptanoate ester began to form. Eventually *trichloroethyl heptanoate* also was observed in the reacting mixture, indicating that trichloroethyl esters do react reversibly.

In summary, several acids and their esters have been evaluated in esterification reactions of 1- and 2-octanol as typical aliphatic alcohols, using several commercially available lipases. Reactions were fastest in hexane, as compared with several other common organic solvents,

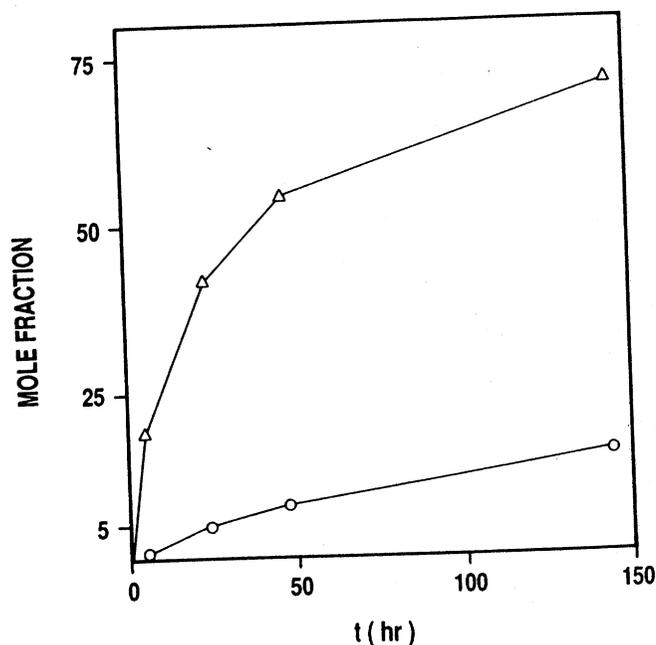


FIG. 3. Reaction of isopropenyl heptanoate (0.01 M), ethyl octanoate (0.10 M) and 1-octanol (0.12 M) in wet hexane with *C. rugosa* lipase (0.300 g/mmol of ester) at 30°C. Δ: 1-octanol heptanoate; ○: 1-octanol octanoate.

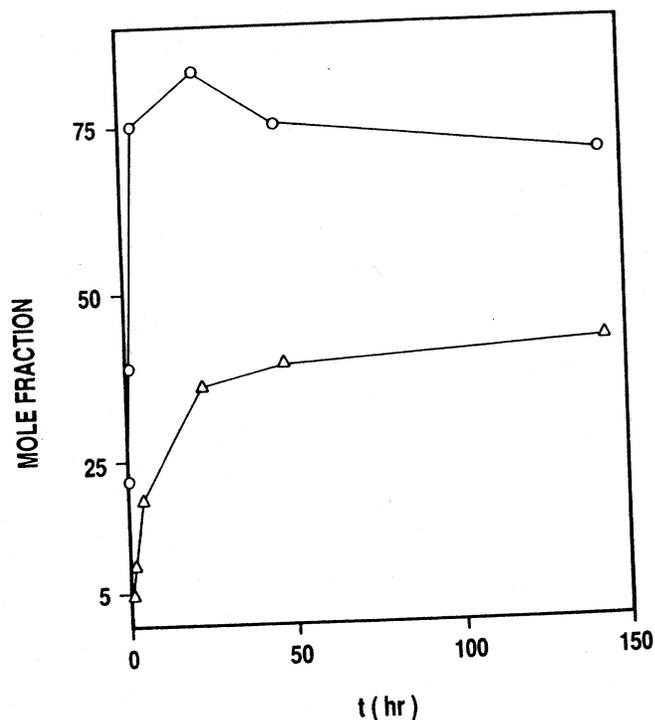


FIG. 4. Reaction of isopropenyl heptanoate (0.10 M), 2,2,2-trichloroethyl octanoate (0.10 M) 1-octanol (0.12 M) in wet hexane with *M. miehei* lipase (0.025 g/mmol of ester) at 30°C. Δ: 1-octanol heptanoate; ○: 1-octanol octanoate.

and the organic acid itself is usually the best substrate. An exception to this involves the reactions of *C. rugosa* lipase, which reacts more rapidly in hexane with isopropenyl and 2,2,2-trichloroethyl esters. Further evaluation of lipase selectivity using *rac*-2-octanol indicated a previously unobserved high stereoselectivity for the *A. niger* lipase and, unexpectedly, variation in stereobias with source of acyl residue using the lipases of *C. rugosa* and porcine pancreas. Exemplifications of lipase selectivity, based on relative reactivities of esters (Table 1), demonstrate the relationship between selectivity and product purity. Applications of lipase selectivities (as in kinetic resolution of enantiomers [16]) are best applied in the sense of purifying a starting material. Only if the selectivity of a lipase is such, to make the catalyst virtually specific, can it be used to yield a nearly pure product.

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