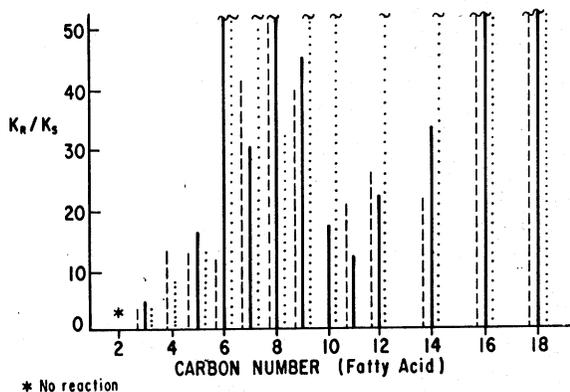


### Kinetic Resolutions of Aliphatic Alcohols with a Fungal Lipase from *Mucor miehei*<sup>†</sup>

A lipase preparation derived from the fungus *Mucor miehei* was employed recently to obtain (*R*)- and (*S*)-2-octanol by kinetic resolution through hydrolysis of the racemic octanoate ester and by esterification of the racemic alcohol with octanoic acid.<sup>1</sup> Of several commercially available lipases evaluated, only the lipase from this fungus exhibited a strong stereobias, thus making it useful for obtaining methyl-*n*-alkylcarbinols in high configurational purity. Moreover, of several *M. miehei* lipases, the preparations of NOVO Company (Lipozyme and NOVO-225) were of greatest value for obtaining these alcohols. a number of resolutions of alcohols (esters) catalyzed by esterases and lipases have been reported,<sup>2</sup> and the utility of enzymatic reactions conducted in organic solvents has



**Figure 1.** Esterification of 2-hexanol (---), 2-octanol (—), and 2-decanol (...) with *n*-alkanoic acids catalyzed by Lipozyme. Reactions were conducted at 30 °C in hexane (see Experimental Section).

<sup>†</sup>This manuscript is dedicated to Professor George Buchi, Camille Dreyfus Professor of Chemistry, Massachusetts Institute of Technology, on the occasion of his 65th birthday.

**Table I.** Esterification with Hexanoic Acid<sup>a</sup>

alcohol	$E_R$	alcohol	$E_R$
3-methyl-2-butanol	2.1	2-octanol	>50
2-pentanol	2.1	2-decanol	7.7
2-hexanol	9.5	2-dodecanol	14.6

<sup>a</sup> See Experimental Section.

been demonstrated.<sup>3</sup> However, esterases are not as readily available as are lipases, and the latter (excepting possibly that of *Candida rugosa*<sup>2</sup> have not been proved useful for the apparently difficult task of resolving straight chain methylcarbinols. Chiral secondary aliphatic alcohols have been the target of asymmetric reduction studies as well, employing chiral reagents<sup>4</sup> and oxidoreductases.<sup>5</sup> Recent research with chiral organoboranes and borohydrides emphasizes the interest in developing methods for such alcohols and the difficulty in achieving the goal.<sup>6,7</sup> The scope of the lipase-catalyzed resolution by esterification using NOVO's lipase from *M. miehei* is reported here.

Esterifications were conducted in hexane employing Lipozyme, a formulation of this lipase on an ion exchange resin.<sup>8</sup> The preparation is granular; reaction mixtures may be filtered, and the recovered resin may be used repeatedly. Analyses of reactions were performed by (1) free fatty acid determination to obtain mole fraction conversion (*C*) and (2) conversion of recovered alcohol to diastereomeric carbamates using (*S*)- $\alpha$ -methylbenzyl isocyanate followed by GLC analysis of obtain a value for enantiomeric excess of residual starting material expressed as a fraction (*ee*). These values may be employed to obtain the enantiomeric ratio (*E*) that is the ratio of the specificity constants (*V*/*K*) and is useful in gauging the relative reaction velocities of reacting enantiomers as long as (1) steady-state kinetics apply, (2) the reaction is irreversible, and (3) product inhibition of enzyme is absent.<sup>9</sup> The reaction conditions

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**Table II. Esterification of Selected Alcohols with Octanoic Acid<sup>2</sup>**

alcohol	$E_r$
1. cyclohexylmethylcarbinol	>50
2. phenylmethylcarbinol	42
3. 3-dodecyn-2-ol	>50
4. 3-octanol	1.2 <sup>b</sup>
5. 2-methyl-1-decanol	1.2
6. 2-methyl-1-dodecanol	1.3
7. 2-phenyl-1-propanol	0.29 <sup>c</sup>
8. 1,2-isopropylidenglycerol	1
9. citronellol	1.1 <sup>d</sup>
10. 4-methyl-2-pentanol	23.5
11. 3-methyl-2-hexanol	<i>e</i>

<sup>a</sup>Reactions were conducted at 30 °C in hexane. See Experimental Section. <sup>b</sup>The enantiomeric ratio was 1 with propanoic acid and 4.6 with lauric acid. <sup>c</sup> $k_S/k_R = 3.4$ . <sup>d</sup>Diastereomer elution order not determined. <sup>e</sup>Two stereoisomers react significantly faster.

and degree of conversion for analyses (20–50%) minimized error due to deviation from the first two criteria, and inhibition to lipase-catalyzed esterification byproduct has not been reported to our knowledge. Several methyl-*n*-alkylcarbinols were subjected to the esterification conditions with hexanoic acid. The results indicated a very strong bias (for the *R* enantiomer) only in the case of 2-octanol (Table I). However, when the acid employed was octanoic acid the strong bias extended to 2-hexanol and 2-decanol. It seemed that the (presumed) enzyme-acid complex could be unique for each acid. In other words, the chain length of the acid evidently constitutes structural information that can be transmitted to the vicinity of the enzyme-bound carbonyl in a manner that alters the energies for approach to that carbonyl of the two enantiomeric alcohols. The esterification of 2-octanol with several fatty acids was conducted and the results shown in Figure 1 indicate that (1) acetic acid is unreactive, (2) enantiomeric ratio increases to >50 for C<sub>6</sub> and C<sub>8</sub> fatty acids, and (3) the ratio declines beyond C<sub>8</sub> to rise again >50 for C<sub>16</sub>. Similar observations were made for 2-hexanol and 2-decanol. Because of the heterogeneity of these reactions, rates of conversion may depend on stirring (particle size is altered due to mechanical breaking). However, a rough evaluation of overall reactivity is that rates increased from propanoic to heptanoic acid and then remained fairly constant. Interestingly, although acetic acid itself was unreactive, an experiment in which racemic 2-octanol was exposed to 1:1 acetic and octanoic acids resulted in a reduction of *E* from >50 (without acetic acid) to ca. 9.

Esterifications of several other alcohols with octanoic acid using Lipozyme were conducted (Table II). Cyclohexyl- and phenylmethylcarbinols showed strong stereobias, *R* alcohols reacting preferentially though the effect of the increased bulk adjacent to the carbinol carbon reduced reactivity. Reaction of 3-dodecyn-2-ol also showed a strong preference for the *R* enantiomer. By contrast phenyl(trifluoromethyl)carbinol reacted extremely sluggishly (6% conversion in 96 h). Racemic 2-octanol, in the presence of phenyl(trifluoromethyl)carbinol, reacted at a markedly reduced rate (37% to 13% in 96 h); however, the stereobias was unaffected.

The relative rate of reaction, i.e., the stereodiscrimination, of enantiomers was dramatically reduced for 3-octanol. Evidently discrimination between methyl and *n*-alkyl is much easier than between ethyl and *n*-alkyl. Reduced

stereobias were also observed for reactions of primary alcohols with aliphatic asymmetric centers on the  $\beta$ - or  $\gamma$ -carbon (Table II, entries 5–8). Data for the ee of such alcohols were obtained by oxidation of the recovered alcohols to the acid followed by conversion via acid halides to the diastereomeric amides with (*S*)- $\alpha$ -methylbenzylamine for analysis by GLC. The reduced enantioselectivity exhibited by *M. miehei* lipase for 3-octanol and citronellol as well as for  $\alpha$ -branched 2-carbinols such as 3-methyl-2-butanols indicates that strong stereoselection requires that the carbinol carbon be the asymmetric center and that the larger alkyl group preferably be unbranched in the immediate vicinity of that carbon. Finally, esterifications conducted at 40 °C proceeded much faster, but stereobias was virtually lost. At 20 °C reactions were impractically slow.

In conclusion, the lipase preparation Lipozyme has the ability to usefully resolve methyl-*n*-alkylcarbinols. Because of the nature of commercial enzyme preparations, the enzymatically active principle may not be the same as the lipolytically active protein present. If such is the case a more enzymatically active preparation for this type of resolution would be possible. Optimum reaction temperatures are 30–35 °C, and stereoselectivity can be adjusted to some extent for a given alcohol by altering the fatty acid. Stereoselection is greatest for methylcarbinols. The enzyme's stereoselectivity was reduced in the presence of acetic acid without much loss in reactivity, and its reactivity was altered without loss in stereoselection when phenyl(trifluoromethyl)carbinol was present in the reaction mixture. The availability of this enzyme and the simplicity of the procedures employed should promote its use for the kinetic resolution of simple chiral aliphatic alcohols.

### Experimental Section<sup>17</sup>

Gas-liquid chromatography was performed with a Shimadzu GC-mini-2 instrument using an SPB-1 column (2.05 mm  $\times$  30 m) with a 50:1 split ratio and He carrier gas.

Infrared data were recorded with a Perkin-Elmer 1310 spectrophotometer. Free fatty acid titrations were performed with a Radiometer pH instrument comprised of an ABU-80 Autoburette module. The lipase preparations from *Mucor miehei* were used as obtained from NOVO Company, Wilton, CT; Lipozyme is the lipase formulated on a proprietary ion exchange resin and is intended for esterifications and transesterifications, while NOVO-225 is an aqueous formulation intended for hydrolytic application. Most of the alcohols were commercial samples and were employed without purification. The 3-dodecyn-2-ol was synthesized from lithio decynylide and acetaldehyde in the usual way,<sup>10</sup> bp 74–79 °C (0.05 mm); 2-methyl-1-decanol and 2-methyl-1-dodecanol had been prepared from decanoic and lauric acids, respectively, for a previous study.<sup>11</sup> Methoxy(trifluoromethyl)phenylacetic acid (MTPA) was obtained from Aldrich Chemical Co. and converted to the acid chloride. (*S*)- $\alpha$ -Methylbenzylamine was purchased from Hexcel Corp. and determined to be 99.4% *S* by GLC as its MTPA derivative.<sup>12</sup> The amine was converted to (*S*)- $\alpha$ -methylbenzyl isocyanate by using the procedure of Pirkle and Hauske.<sup>13</sup>

**Analysis of Reaction Mixtures.** The reactions were conducted with 2.5 mmol each of alcohol and acid, 1.0 g of Lipozyme (specific activity on olive oil: 0.095  $\mu$ mol fatty acid generated/min, mg), and 10 mL of Nanograde hexane. The mixtures were stirred magnetically at 30 °C for 70–96 h. They were then diluted with 100 mL of ethanol and titrated to pH 9.5 with 0.1 N NaOH. Samples of unreacted alcohols were obtained by dilution of the

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titrated mixtures with water and extraction with pentane, etc. In order to recover 2-pentanol and 2-hexanol, the organic extract was carefully boiled down under a Vigreux column. Reaction products from long chain fatty acids were freed of the ester products prior to GLC analysis by passage through small columns of silica gel in hexane. The alcohol was obtained by elution with moist ether. Derivatization of the recovered alcohols with the isocyanate (using excess isocyanate to avoid fractionating enantiomers) was as previously described.<sup>1</sup> Analytical GLC data for the carbamates follows ( $T$  (°C),  $k'$  for  $R,S$ ,  $k'$  for  $S,S$ ): 3-methyl-2-butanol (185, 3.08, 3.15), 2-pentanol (175, 4.50, 4.65), 2-hexanol (185, 4.85, 5.10), 2-octanol (220, 3.58, 3.77), 2-decanol (240, 3.40, 3.20), 2-dodecanol (260, 3.23, 3.42), cyclohexylmethylcarbinol (220, 4.40, 4.60), phenylmethylcarbinol (220, 4.30, 4.50), 3-dodecyn-2-ol (260, 3.18, 3.32), 3-octanol (220, 2.42, 2.50), 4-methyl-2-pentanol (185, 3.48, 3.65), and 3-methyl-2-hexanol (185, 5.78, 5.91, 6.09, 6.22). Primary alcohols were oxidized to acids by using Jones Reagent,<sup>14a</sup> the acids were then converted to acid halides with  $\text{SOCl}_2/\text{DMF}$  in ether<sup>14b</sup> and then to amides with excess ( $S$ )- $\alpha$ -methylbenzylamine. Control experiments with chiral 1,2-isopropylidenglycerol indicated that no racemization occurred when using this sequence. Similarly  $\alpha$ -branched primary carbinols suffer no loss of configurational integrity during Jones oxidation.<sup>15</sup> Analytical GLC data for the amides follows ( $T$  (°C),  $k'$  for  $R,R$ ,  $k'$  for  $R,S$ ): 2-methyl-1-decanoic acid (240, 3.36, 3.59), 2-methyl-1-dodecanoic acid (260, 3.32, 3.55), 2-phenylpropanoic acid (220, 3.14, 3.45), isopropylidenglyceric acid (210, 2.23, 2.04), and citronellic acid (225, [4.04, 4.12]). Order of elution for diastereomers for citronellic acid was not determined.

**Examples of Resolutions of Several Methylcarbinols.** The procedure employed was similar to that previously described for obtaining the enantiomers of 2-octanol.<sup>1</sup> The racemic alcohol (2.5 mmol), octanoic acid (8.0 mL, 50 mmol), and 3.0 g of Lipozyme were swirled in 20 mL of either pentane or hexane at 30 °C for a period of 3–5 weeks. The progress of the resolution was monitored by derivatizing samples of the mixture directly with ( $S$ )- $\alpha$ -methylbenzyl isocyanate followed by GLC analysis. The mixture was worked up by suction filtration; the resin was washed thoroughly with solvent and stored at 0–5 °C for future use. The

combined organic phase was washed with 1.25 N NaOH and then with  $\text{H}_2\text{O}$ . After drying ( $\text{MgSO}_4$ ), the solvent was removed by careful distillation, and the concentrate was fractionally distilled to obtain the  $S$  alcohol and ( $R$ )-octanoate ester. The ester was saponified by heating with 25 mL of 6 N KOH and 20 mL of methanol under reflux for 16 h. The resulting  $R$  alcohol was recovered from the saponification step in the usual manner and then distilled. In this way we obtained ( $S$ )-2-hexanol [0.90 g (34.6%), bp 55–60 °C (30 mm), 87.2% ee,  $[\alpha]_D^{25} +8.69^\circ$  (c 5.35, EtOH) [lit.<sup>16</sup>  $[\alpha]_D^{20} +11.6^\circ$ ]], ( $R$ )-2-hexanol [1.11 g (47.7%), 83.2% ee,  $[\alpha]_D^{25} -7.74^\circ$  (c 5.55, EtOH)], ( $S$ )-2-decanol [2.10 g (52.5%), bp 112–115 °C (30 mm), 83.0% ee,  $[\alpha]_D^{25} +4.22^\circ$  (c 6.28, EtOH)], ( $R$ )-2-decanol [1.49 g (40.3%), 87.0% ee,  $[\alpha]_D^{25} -5.37^\circ$  (c 5.31, EtOH)]; ( $S$ )-4-methyl-2-pentanol [1.03 g (39.6%), bp 54–57 °C (30 mm), 47.2% ee,  $[\alpha]_D^{25} +7.93^\circ$  (c 5.49, EtOH) [lit.<sup>16</sup>  $[\alpha]_D^{20} +20.54^\circ$ ]], ( $R$ )-4-methyl-2-pentanol [0.63 g (29.5%), 87.4% ee,  $[\alpha]_D^{25} -16.47^\circ$  (c 5.24, EtOH)], ( $S$ )-cyclohexylmethylcarbinol [1.35 g (42%), bp 100 °C (30 mm), 67% ee,  $[\alpha]_D^{25} +3.59^\circ$  (c 5.10, EtOH)], ( $R$ )-cyclohexylmethylcarbinol [1.18 g (37%), 86% ee,  $[\alpha]_D^{25} -2.39^\circ$  (c 5.53, EtOH)], ( $S$ )-phenylmethylcarbinol [0.31 g (10%), bp 99–100 °C (30 mm), 71% ee,  $[\alpha]_D^{25} -16.6^\circ$  (c 4.72, EtOH) [lit.<sup>17</sup> for  $R$   $[\alpha]_D^{20} +42.86^\circ$ ]], and ( $R$ )-phenylmethylcarbinol [0.34 g (11%), 87% ee,  $[\alpha]_D^{25} +33.2^\circ$  (c 4.67, EtOH)].<sup>18</sup>

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