860, 830, 740, 680, 635 cm^{-1}; exact mass calcd, m/e 485.2419; found, m/e 485.2441; mass spectrum, m/e 485 (parent), 470 (P - Me), 397, 276 (P - (2(OSi=)-(OMe))), 179 (Ph-CH-OSi=, 148 ((MeO)$_2$—C=CPhH), 131 (Ph—CH=CHC=O), 28 (CO).

Synthesis of 31. To a solution of complex **1** (375 mg, 1.5 mmol) in petroleum ether (50 mL) in a Pyrex Erlenmeyer flask under argon at 25 °C was added 328 mg (1.5 mmol) of the *N*-(L-valine methyl ester)-benzylideneimine. The flask was irradiated with either direct sunlight or the 20-W Vitalites. After 30 h, TLC showed unreacted imine still present. An additional 100 mg of the carbene was added, and the solution was irradiated for 48 h. Oxidation, filtration, and concentration gave 285 mg (62%) of a yellow oil. High-field NMR spectra indicated that the sample contained both the *S,R,S* and the *R,S,S* isomers and was free of detectable amounts of side products. The diastereomeric excess was 60%. Further characterization was not attempted. IR (neat) 1740-1730 cm^{-1} (strong, broad).

Minor isomer: NMR (360 MHz) 0.94 (d, J = 6.8 Hz, 3 H, CH_3), 1.13 (d, J = 6.8 Hz, 3 H, CH_3), 1.64 (s, 3 H, CH_3), 2.57 (m, 1 H, CH), 3.04 (s, 3 H, OCH_3), 3.46 (s, 3 H, CO_2CH_3), 3.72 (d, J = 8.6 Hz, 1 H, CH), 4.39 (s, 1 H, PhCH), 7.34-7.45 (m, 5 H, Ar).

Major isomer: NMR 0.78 (d, J = 6.8 Hz, 3 H, CH_3), 0.91 (d, J = 6.8 Hz, 3 H, CH_3), 1.63 (s, 3 H, CH_3), 2.03 (m, 1 H, CH), 3.05 (s, 3 H, OCH_3), 3.73 (s, 3 H, CO_2CH_3), 4.04 (d, J = 7.2 Hz, 1 H, CH), 4.65 (s, 1 H, PhCH), 7.34-7.45 (m, 5 H, Ar).

X-ray Structure Determinations.[38] For compound **27** ($C_{13}H_{15}NO_2S$) at -130 °C, a = 6.887 (2) Å, b = 13.042 (6) Å, c = 13.257 (8) Å; $P2_12_12_1$, ρ_c = 1.39 g cm^{-3} (Z = 4, formula weight = 249.32). The intensities of 1257 reflections ($h, k, l \geq 0$) were measured by $\theta - 2\theta$ scans on the Nicolet $R3m/E$ diffractometer (Mo K_α radiation, graphite monochromator). Intensities of 974 unique, observed reflections ($I > 2_\sigma(I)$) were used in refinement of the structure. The structure was solved (by Patterson interpretation and successive Fourier map analyses) and refined by using the SHELXTL programs supplied with the $R3m/E$ computing system. Anomalous scattering of Mo K_α radiation was not of sufficient magnitude to unambiguously fix the absolute configuration of the enantiomer contained in the data collection crystal. The final structural model included anisotropic thermal parameters for non-hydrogen atoms, together with placement of hydrogen atoms in idealized positions. This model refined to convergence with R = 0.039 R_w = 0.039, and GOF = 1.60.

For compound **28** ($C_{11}H_{17}NO_4S$) at 20 (1) °C, a = 6.357 (2) Å, b = 9.815 (4) Å, c = 20.987 (6) Å; $P2_12_12_1$, ρ_c = 1.32 g cm^{-3} (Z = 4, formula weight = 259.32). The intensities of 1417 reflections ($h, k, l \geq 0$) were measured by θ-2θ scans on the Nicolet $R3m/E$ diffractometer (Mo K_α radiation, graphite monochromator). Intensities of 1297 unique, observed ($I > 2\sigma(I)$) reflections were used in refinement of the structure. The structure was solved (by direct methods) and refined using the SHELXTL programs supplied with the $R3m/E$ computing system. The final structural model, which was consistent with the known absolute configuration at C3, included anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms were placed in idealized positions. This model refined to convergence with R = 0.042, R_w = 0.047, and GOF = 1.38.

(38) Structural work performed by Professor Oren Anderson, Colorado State University. Full details of these structural studies will be published elsewhere.

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Preparative Production of Optically Active Esters and Alcohols Using Esterase-Catalyzed Stereospecific Transesterification in Organic Media

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Abstract: A novel enzymatic approach to the production of optically active alcohols and esters from racemates is developed. It involves the use of esterase catalyzed transesterifications carried out in biphasic aqueous-organic mixtures. Water-insoluble substrates constitute the organic phase, while the enzyme is located in the aqueous phase. Since the fraction of the latter phase can be made very low, such an arrangement solves the problem of both the competition of an alcohol (the nucleophile) with water in the enzymatic reaction and poor solubility of most organic esters and alcohols in water. By use of porous supports (Sepharose or Chromosorb) filled with aqueous solutions of hog liver carboxyl esterase as a stereoselective catalyst and methyl propionate as a matrix ester, the following optically active alcohols and their propionic esters were produced on a preparative scale: 3-methoxy-1-butanol, 3-methyl-1-pentanol, 3,7-dimethyl-1-octanol, and β-citronellol. To overcome a rather narrow substrate specificity of hog liver carboxyl esterase, a nonspecific lipase from yeast (*Candida cylindracea*) also was employed as a stereoselective transesterification catalyst. Using an aqueous solution of this enzyme confined to the pores of Chromosorb and tributyrin as a matrix ester, we have prepared gram amounts of the following optically active alcohols and their butyric esters: 2-butanol, *sec*-phenethyl alcohol, 2-octanol, 1-chloro-2-propanol and 2,3-dichloro-1-propanol (subsequently nonenzymatically converted to optically active propylene oxide and epichlorohydrin, respectively), 6-methyl-5-hepten-2-ol, and 1,2-butanediol.

Most hydrolases can catalyze not only their "natural" reaction of hydrolysis but also that of transesterification.[2] For example, carboxylesterase[3] is capable of accepting nucleophiles other than water, such as various alcohols.[4] The ability of hog liver carboxylesterase to asymmetrically hydrolyze chiral and prochiral esters has been widely used for the preparative syntheses of op-

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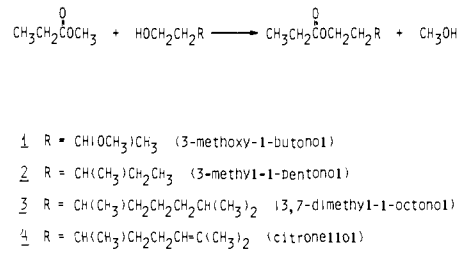

Figure 1. Hog liver carboxylesterase catalyzed transesterification.

tically active compounds.[5] And yet, despite their obvious potential, transesterifications catalyzed by the enzyme have never been utilized for synthetic purposes. This is caused by a problem common to all hydrolase catalyzed transesterifications: since the concentration of water in aqueous solutions is high and that of competing nucleophiles is relatively low, the yield of the transesterification products is usually low. In addition, the majority of esters of interest to organic chemists are poorly soluble in water, and therefore enzymatic reactions must be carried out in dilute aqueous solutions, which further reduces their preparative value. Attempts to solve both of the above problems by partial replacement of water with organic cosolvents have not been particularly successful because enzymes lose their catalytic activity when the concentration of the nonaqueous solvent becomes high.[6]

We are proposing a novel general approach to preparative enzymatic transesterifications. It involves the use of biphasic aqueous–organic mixtures where the substrates—the ester and the alcohol (the nucleophile)—constitute the organic phase and the enzyme is dissolved in the aqueous phase. Since proteins are insoluble in water-immiscible organic solvents,[7] the enzyme will remain in the aqueous phase. The substrates will freely diffuse into the latter and undergo the enzymatic conversion, and then the products will diffuse back into the organic phase. The fraction of water in such a biphasic system can be made extremely low, and hence transesterification will be greatly favored over hydrolysis.

Conceptually, the aforedescribed system represents an emulsion of an aqueous solution of an enzyme in a mixture of substrates. However, from the experimental standpoint it is advantageous instead to use porous supports whose pores are filled with an aqueous solution of an enzyme: first, such beads are more mechanically robust than water droplets; second, they can be readily separated from products at the end of the enzymatic reaction; third, they can be used repeatedly. The organic (substrate) phase should be presaturated with an aqueous buffer (of the pH optimal for the enzyme action) to avoid loss of water from the beads due to its partitioning.

Results and Discussion

We report herein the application of the above approach for the preparative production of optically active esters and alcohols. Hog liver carboxylesterase, entrapped in such porous supports as Sepharose and Chromosorb, was initially used to asymmetrically catalyze transesterifications shown in Figure 1; methyl propionate was employed as a matrix in which the methyl moiety was stereoselectively replaced with alcohols **1**–**4**.[8]

(±)-**1** was dissolved in a molar excess of methyl propionate, and then the mixture was saturated with phosphate buffer, followed by addition of carboxylesterase entrapped in Sepharose beads. The system was vigorously shaken at room temperature; from time to time, the organic phase was analyzed gas chromatographically. Figure 2 shows the time course of the enzymatic transesterification: One can see that the concentration of **1** gradually decreases, while the concentration of the transesterification product, 3-methoxy-1-butyl propionate, simultaneously increases (no propionic acid was formed, indicating that hydrolysis was completely suppressed). After 20 h no further reaction takes place. The enzyme-containing beads were then recovered by filtration; the organic phase obtained was separated by aqueous extractions and evaporation under vacuum. As a result, optically active (+)-**1** and (−)-3-methoxy-1-butyl propionate were collected. We have found that the former was totally unreactive in the esterase catalyzed reaction with methyl propionate. On the other hand, the (−)-**1** produced from the (−) ester by an alkaline hydrolysis was nearly fully acylated in the enzymatic transesterification. These results suggest that hog liver carboxylesterase has essentially absolute stereoselectivity in the reaction of **1** with methyl propionate and that the transesterification stops at a 50% conversion (Figure 2) because the reactive (−) isomer is exhausted.

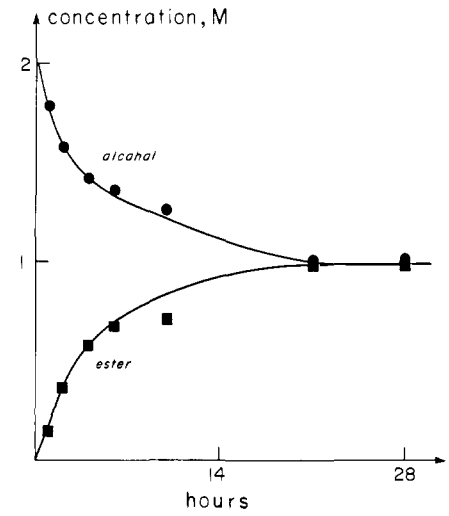

Figure 2. Time course of the reaction between methyl propionate and (±)-**1** catalyzed by hog liver carboxylesterase entrapped in Sepharose beads. Circles, disappearance of methyl propionate; squares, accumulation of (−)-3-methoxy-1-butyl propionate. The organic phase consisted of methyl propionate containing 2 M (±)-**1** (and was saturated with 0.1 M phosphate buffer, pH 8.0). The aqueous phase contained 9600 units of the enzyme entrapped in 6 g of Sepharose beads swollen with 0.1 M phosphate buffer (pH 8.0). The mixture was shaken at room temperature.

The enzymatic catalyst recovered after the preparative synthesis was rinsed with ether presaturated with phosphate buffer (to remove accumulated methanol) and repeatedly employed for the preparative transesterification. After each use, no more than a quarter of the enzymatic activity was lost (as judged on the basis of the half-life of the enzymatic reaction).

Sepharose is by no means a unique support for the enzyme entrapment; e.g., we have successfully used porcine liver carboxylesterase confined to Chromosorb beads for preparative production of three other couples of optically active esters and alcohols. Using the same approach as described above for **1**, we have found that only *S* (but not *R*) isomers of **2** and **3** can act as nucleophiles in the transesterification reaction shown in Figure 1 catalyzed by hog liver carboxylesterase. As a result, gram quantities of (*R*)-(+)-**2** and -**3**[9] (and their (*S*)-(−)-propionic esters) were prepared with a high enantiomeric excess.

Both *l*- and *d*-β-citronellols (**4**) are natural products which are widely used in perfumery.[10] Although methods for the synthesis

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(8) We have found that hog liver carboxylesterase has a rather narrow specificity with regard to the alcohols it accepts as nucleophiles in the transesterification reaction; only primary alcohols having no substituents in the first two methylene groups adjacent to the hydroxyl react at appreciable rates.

(9) Optically active **3** produced herein can be used as a convenient intermediate for the synthesis of *d*-α-tocopherol (vitamin E) and *l*-menthol: Valentine, D. H. U.S. Patent 4115417, 1978.

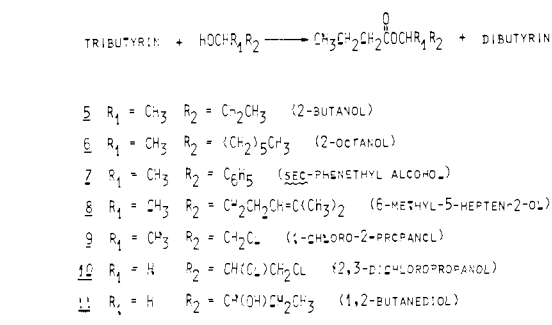

Figure 3. Yeast lipase catalyzed transesterification.

of the optical isomers of **4** have been developed, they are rather inefficient and usually afford low optical purity.[11] *d,l*-**4** can be easily made by reduction of synthetic citronellal or geraniol,[10] but its resolution, as well as that of most racemic alcohols,[12] is very cumbersome. We have succeeded in preparative resolution of *d,l*-**4** using our enzymatic approach. As in the case of the other alcohols tested, only (*S*)-(−)-**4** was reactive toward the enzyme. Hence hog liver carboxylesterase exhibits absolute *S* stereoselectivity in the transesterification reaction;[13] it is quite remarkable that the enzyme recognizes configuration of the chiral carbon which is two methylene groups away from the alcohol's reactive hydroxyl.

The major drawback of hog liver carboxylesterase as a practical catalyst is its narrow nucleophile specificity.[8] To overcome this obstacle, we have endeavored to search for another commercially available esterase which will be *both stereoselective and nonspecific* in the transesterification process. Such an esterase was found among lipases. Since the natural function of lipases is to hydrolyze triglycerides,[14] one of them, tributyrin (glyceryl tributyrate), was employed by us as a matrix ester.[15]

We have discovered that lipase from the yeast *Candida cylindracea*, when placed in a biphasic system where the aqueous phase is a solution of the enzyme in water confined to the pores of Chromosorb and the organic phase is a solution of an alcohol in tributyrin, (i) accepts a wide variety of alcohols (both secondary and primary) in the transesterification reaction (Figure 3) and (ii) if the alcohol is chiral, displays a marked stereoselectivity in accepting only one isomer as a nucleophile. In the case of all chiral alcohols tested, the time course of lipase catalyzed transesterifications resembled that shown in Figure 2. In all cases the process was carried out to apparent completion; no appreciable amount of butyric acid was detected (i.e., enzymatic transesterification totally suppressed hydrolysis). The general scheme of this process was conceptually the same as in hog liver carboxylesterase catalyzed resolutions and is illustrated in Figure 4.

To test the reusability of lipase entrapped in the Chromosorb beads, the enzymic catalyst was used for four consecutive tran-

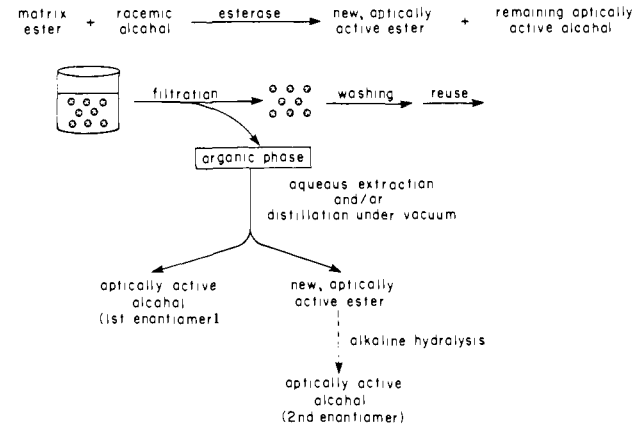

Figure 4. Schematic illustration of the resolution of racemic alcohols using esterase catalyzed transesterifications.

sesterifications with **5** as a nucleophile. After each resolution, about 75% of the initial enzymatic activity was retained.

Enzymatically prepared optically active alcohols and esters can then be chemically converted to other optically active compounds. We have illustrated this in the lipase catalyzed transesterification (Figure 3) using **9** and **10** as nucleophiles. In each case only the *R* isomer was reactive. Both the remaining *S* alcohols and butyric esters of the *R* alcohols were converted to epoxides by using KOH treatment.[17] All four optically active epoxides produced are useful as chiral building blocks,[18] for example: (*R*)-(+)-propylene oxide in the synthesis of (*R*)-reciferolide[19] or chiral α-methylene-γ-lactones;[20] (*S*)-(−)-propylene oxide for the production of (*S*)-sulcatol;[21] both (*R*)-(−)- and (*S*)-(+)-epichlorohydrin for the synthesis of a variety of pharmaceuticals.[18,22]

All the alcohols enzymatically resolved thus far were monohydric. It was of obvious interest to explore whether the lipase catalyzed transesterifications can be used for the preparation of optically active diols; 1,2-butanediol was chosen as a model. To avoid formation of both 1- and 2-esters, the enzyme, in addition to stereoselectivity, must also exhibit positional specificity. To examine this we studied the lipase catalyzed transesterification with 2 M 1-butanol (mimicking the primary hydroxyl in **11**) and with 2 M **5** (mimicking the secondary hydroxyl in **11**) as nucleophiles. It was determined that the half-life of the former reaction exceeds that of the latter by the factor of 12; that is, the enzyme is indeed regiospecific. This, along with the displayed stereoselectivity, led to the facile enzymatic resolution according to the scheme in Figure 4.

In closing, we have successfully tested our approach, enzymatic stereoselective transesterification in biphasic systems, with two different esterases—hog liver carboxylesterase and yeast lipase. As a result, a number of optically active alcohols and esters were produced with high yield and enantiomeric purity. It appears that our work represents a significant advance in the use of enzymes for preparative production of chiral compounds[23] as the methodology developed (i) is very simple, efficient, and readily amenable to a scale-up, (ii) is applicable to a wide variety of water-insoluble alcohols, (iii) employs commercially available enzymes, (iv) does not require expensive cofactors,[24] and (v) is carried out in the

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(13) There is a close parallelism between the stereospecificity of the enzyme in the transesterification reaction (Figure 1) and that in the reaction of hydrolysis. We have found that in water (pH 8.0) hog liver carboxylesterase catalyzes the hydrolysis of only the (−) isomer of propionic esters of **1**, **2**, **3**, or **4** (the reactions were carried out to completion in a pH-stat's cuvette; then the products were extracted with ether and characterized).

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(15) Tributyrin is a readily available and inexpensive matrix ester. Triacetin is too soluble in water and also is less reactive with most lipases.[14] Triglycerides of fatty acids are inferior because of their high molecular weight and cost per mole.

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presence of only a few percent of water which greatly simplifies product recovery.[24]

Experimental Section

Materials. Hog liver carboxylesterase (EC 3.1.1.1) was purchased from Sigma Chemical Co. in the form of a suspension in 3.2 M ammonium sulfate and had a specific activity of 160 units/mg of protein. Lipase from *Candida cylindracea* was also obtained from Sigma; it was a powder with a specific activity of 2415 units/mg of protein.

Porous supports used for "immobilization" of the enzymes, Sepharose 4B and Chromosorb 101, were purchased from Sigma. The former represented an aqueous suspension of 40–190-μm beads (agarose concentration 4%). Chromosorb 101 (commonly used as a carrier in gas–liquid chromatography) consisted of 125–150-μm particles with the pore diameter of 0.3–0.4 μm.

All alcohols used in this work, both in the racemic and in the optically pure form (when available), were obtained commercially. Propionic esters of **1–4** and butyric esters of **5–10** were synthesized from propionyl chloride and butyryl chloride, respectively, and the corresponding alcohol following the general procedure of Morris and Green.[25] The boiling points of the esters thus prepared were as follows (760 mmHg): propionic ester of **1** 180–181 °C, of **2** 173–175 °C, of **3** 242 °C, and of **4** 249–252 °C; butyric esters of **5** 151–152 °C, of **6** 209–211 °C, of **7** 249–250 °C, of **8** 223 °C, of **9** 169–170 °C, and of **10**, 219–220 °C. Methyl propionate and tributyrin employed in this study as matrix esters were used without further purification.

Assays. All alcohols and esters in this work were determined gas chromatographically by using a 6-ft glass column packed with Analab's "Super Pak 20 M" (N_2 carrier gas, 10 mL/min; detector and injector port temperature 250 °C). (i) In the case of **1** and **5** through **11**, the temperature of the column was increased from 51 to 240 °C at 16 °C/min. The retention times observed were 1.6 min for methyl propionate and methanol, 6.4 min for **1**, 7.1 min for 3-methoxy-1-butyl propionate, 5.2 min for propionic acid, 11–13 min for tributyrin and dibutyrin (a broad shapeless peak), 6.0 min for butyric acid, 1.8 min for **5**, 3.2 min for 2-butyl butyrate, 5.0 min for **6**, 6.6 min for 2-octyl butyrate, 6.6 min for **7**, 7.8 min for *sec*-phenethyl butyrate, 5.0 min for **8**, 6.7 min for 6-methyl-5-hepten-2-yl butyrate, 3.0 min for **9**, 4.9 min for 1-chloro-2-propyl butyrate, 6.1 min for **10**, 7.4 min for 2,3-dichloroprop-1-yl butyrate, 6.1 min for **11**, and 7.1 min for 2-hydroxybut-1-yl butyrate. (ii) In the case of **2**, the temperature of the column was 90 °C. The retention times observed were 1.1 min for methyl propionate and methanol, 2.0 min for propionic acid, 2.8 min for **2**, and 3.6 min for 3-methoxy-1-pentyl propionate. (iii) In the case of **3** and **4**, the temperature of the column was 135 °C. The retention times observed were 1.0 min for methyl propionate and methanol, 1.5 min for propionic acid, 3.0 min for **3**, 4.1 min for 3,7-dimethyl-1-octyl propionate, 3.5 min for **4**, and 4.5 min for citronellyl propionate.

All optical rotations were measured at 589 nm (sodium line) and 25 °C on a Perkin–Elmer 243 B polarimeter.

Distillations. The fractional distillation apparatus employed by us consisted of a Vigreux distilling column (12.5 cm height), water-cooled condenser, and a three-neck distilling receiver equipped with a vacuum adapter. The holdup of this apparatus with a typical amount of liquid we used in our work was about 0.7 mL. With the above apparatus, we succeeded in obtaining excellent separations of esters from alcohols (Table II) due to the very low rates of distillation used (2–5 mL/hr).

Preparation of Enzymatic Catalysts. Solutions of hog liver carboxylesterase were entrapped either in Sepharose or in Chromosorb as described below. (i) Swollen Sepharose 4B (2 mL) was washed with water and with 0.1 M phosphate buffer (pH 8.0). Then the resin was thoroughly dried on a glass filter, followed by cutting the resultant gel into small (approximately 2 mm × 2 mm) pieces with a razor blade. The beads produced were added to 4.6 mL of 10.9 mg/mL enzyme suspension and allowed to absorb it (there was no liquid left afterwards). (ii) Chromosorb 101 (1 mL) (100–120 mesh) was washed with water and 0.1 M phosphate buffer and then dried on a glass filter. After that, the beads were added to 1.1 mL of a 10.9 mg/mL enzyme suspension and allowed to completely absorb it. We have found that (for reasons not presently clear) Sepharose was a better support in the transesterification with **1** as a nucleophile, while Chromosorb was superior with **2**, **3**, and **4** (in terms of the half-life of the reaction at a given amount of the enzyme present in the system).

Lipase from *Candida cylindracea* was entrapped in Chromosorb beads only. One gram of the beads was impregnated with 0.1 M phosphate buffer (pH 8.0), followed by drying on a filter paper. Then 67 mg of the enzyme powder was added to the beads and mixed thoroughly, and the mixture was allowed to equilibrate at room temperature for 5 h.

Both enzymic preparations were stored at 4 °C for several days with no appreciable loss of the catalytic activity.

Enzymatic Production of Optically Active 1–11 and Their Esters. Racemic alcohols **1–4** were resolved through the transesterification reaction shown in Figure 1 catalyzed by hog liver carboxylesterase. In the case of **1**, the enzyme was entrapped in Sepharose beads; in the case of **2–4**, the enzyme was entrapped in Chromosorb beads (for both procedures see the previous section). Racemic alcohols **5–11** were resolved through the transesterification reaction shown in Figure 3 catalyzed by yeast lipase entrapped in Chromosorb beads as described above.

In all cases 2 M racemic alcohol (Table I) was dissolved in the matrix ester (methyl propionate or tributyrin). Then the mixture was saturated with 0.1 M phosphate buffer (pH 8.0), and the enzymic catalyst was added. The amount of the latter (including the weight of the support, of the buffer, and of the enzyme) was (i) 50 and 167 mg/mL of the reaction mixture for hog liver carboxylesterase entrapped in Sepharose and Chromosorb, respectively (which corresponds to 0.5 mg of the enzyme/mL of the reaction mixture), and (ii) 100 mg/mL of the reaction mixture for *Candida cylindracea* lipase (which corresponds to 3.34 mg of the enzyme/mL of the reaction mixture).

A mixture containing the matrix ester, the corresponding racemic alcohol, and the corresponding enzyme (prepared as described above) was placed in a 50–250-mL screw-cap bottle and shaken on an orbit shaker at 250 rpm and room temperature (21–25 °C) for a period of time required to complete the enzymatic transesterification. Experimental conditions for all the transesterifications examined are shown in Table I.

Following completion of the reaction, the enzymic catalyst was recovered by filtration. The liquid phase obtained was then separated by using (i) aqueous extractions with subsequent evaporation (in the case of **1** and **11**) or (ii) fractional distillation (in the case of **2–10**).

(i) The liquid phase was washed with 3 volumes of distilled water. The washings were combined, and water was evaporated at 50 °C and 15 mmHg; the remainder was washed with ether and dried with anhydrous $MgSO_4$. In the case of **1**, the organic phase (consisting of (−)-3-methoxy-1-butyl propionate and methyl propionate) left after aqueous extraction was evaporated at 40 °C and 15 mmHg to remove methyl propionate. In the case of **11**, the organic phase (consisting of (−)-2-hydroxy-1-butyl butyrate, dibutyrin, and tributyrin) left after aqueous extraction was distilled at 5 mmHg.

(ii) The liquid phase was subjected to fractional distillation by using the apparatus described above.

Table II summarizes the data concerning the properties of the enzymatically produced optically active alcohols and esters. (It is worth noting that the boiling points of the latter, propionic for **1–4** and butyric for **5–10**, were identical with those of the corresponding chemically synthesized esters.)

Alkaline Hydrolysis of Esters. Enzymatically prepared optically active esters (propionic or butyric) can be converted to the corresponding optically active alcohols by alkaline hydrolysis. (i) Propionic esters of **1**, **2**, **3**, and **4** and butyric esters of **5** and **11** were hydrolyzed at pH 12 and 37 °C in a pH-stat's cuvette: saturated solutions of the esters in 0.1 M aqueous solution of KCl with 10% of acetone were placed in a 100-mL water-jacketed cuvette and were hydrolyzed till completion (several hours). Then the solutions were partly evaporated under vacuum and the alcohols were extracted with ether, followed by its evaporation. (ii) Butyric esters of **6**, **7**, and **8** (about 10 g) were dissolved in 50 mL of dry methanol saturated with KOH. The esters were completely hydrolyzed within 1 h (as judged by gas chromatography). Then methanol was evaporated under vacuum and the precipitated KOH was removed by filtration; the resultant solution was washed with ether and dried with anhydrous $MgSO_4$. (iii) In the case of butyric esters of **9** and **10**, the alkaline treatment resulted both in alcoholysis and in formation of epoxides[17] of the alcohols produced. Butyric ester of **9** (11.9 g) and 10.5 g of butyric ester of **10** were dissolved in 11 mL of dry 1-butanol and 12 mL of dry methanol, respectively. Then the resultant solutions were added dropwise to 50 mL of dry 1-butanol or methanol, respectively, saturated with KOH, at 4 °C. The mixtures were stirred at room temperatures for 1 h, during which time the expoxide formation was completed as judged by gas chromatography. (Under the same conditions as in (i) of the Assays section, the retention times of both authentic, commerically obtained, and enzymatically prepared propylene oxide and epichlorohydrin were 1.3 and 2.5 min, respectively.) Then the mixtures

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Table I. Conditions for the Preparative Enzymatic Resolution of Racemic Alcohols via Transesterification in Biphasic Systems

alcohol		ester				reaction
compd	amount, g	name	amount, g	enzyme	support	time, h
1	25.0	methyl propionate	84.2	hog liver carboxylesterase	Sepharose	20
2	7.2	methyl propionate	20.4	hog liver carboxylesterase	Chromosorb	53
3	10.8	methyl propionate	16.5	hog liver carboxylesterase	Chromosorb	96
4	20.0	methyl propionate	36.3	hog liver carboxylesterase	Chromosorb	96
5	14.0	tributyrin	80.2	yeast lipase	Chromosorb	40
6	20.0	tributyrin	53.7	yeast lipase	Chromosorb	140
7	20.0	tributyrin	65.3	yeast lipase	Chromosorb	150
8	20.0	tributyrin	55.2	yeast lipase	Chromosorb	128
9	18.0	tributyrin	81.6	yeast lipase	Chromosorb	52
10	20.0	tributyrin	64.0	yeast lipase	Chromosorb	50
11	20.0	tributyrin	94.9	yeast lipase	Chromosorb	36

Table II. Preparative Enzymatic Resolution of Racemic Alcohols via Transesterification in Biphasic Systems[a]

		unreactive alcohol obtained					new ester produced				reactive alcohol derived from the new ester			
nucleophile	config of react isomer	bp, °C (mmHg)	yield, g (%)	purity,[b] %	$[\alpha]^{25}_D$, deg	ee, %	bp, °C (mmHg)	yield	purity,[b] %	$[\alpha]^{25}_D$, deg	yield, g (%)	purity,[b] %	$[\alpha]^{25}_D$, deg	ee, %
1	(−)[c]	158 (760)	11.7 (93)	97	+15.1 (neat)	*d*	180–181 (760)	16.7 (88)	98	−16.3 (neat)	6.1 (48)	97	−14.3 (*c* 9, MeOH)	*d*
2	*S*	53–55 (10)	2.6 (75)	96	+9.8 (*c* 10, MeOH)	94[e]	70–71 (10)	2.5 (44)	94	−8.6 (*c* 10, MeOH)				
3	*S*	103–105 (10)	3.3 (61)	96	+4.9 (*c* 5, ether)	97[f]	121–122 (10)	3.0 (40)	95	−5.1 (*c* 5, ether)				
4	*S*	108–110 (10)	6.9 (69)	96	+5.0 (neat)	96[g]	122–124 (10)	8.0 (58)	97	−3.8 (neat)	4.9 (48)	94	−4.4 (*c* 20, MeOH)	92[h]
5	*R*	26–28 (18)	4.8 (69)	97	+12.0 (neat)	89[i]	48–51 (18)	10.2 (75)	98	−11.3 (neat)	4.6 (65)	95	−12.8 (*c* 5, ether)	93[j]
6	*R*	32–35 (4)	7.7 (77)	95	+9.4 (neat)	95[k]	48–50 (4)	12.6 (82)	97	−11.8 (neat)	6.0 (60)	96	−9.1 (neat)	92[k]
7	*R*	43–45 (4)	7.9 (79)	94	−36.4 (neat)	88[l]	60–63 (4)	11.8 (82)	93	+42.3 (neat)	6.1 (61)	97	+33.6 (neat)	85[l]
8	*S*	29–32 (4)	8.3 (83)	97	−16.9 (neat)	91[m]	44–47 (4)	12.2 (79)	96	+14.8 (neat)	5.8 (58)	95	+16.5 (neat)	85[m]
9	*R*[31]	18–20 (4)	6.3 (70)	95	−5.7 (neat)	*n*	27–29 (4)	11.9 (76)	94	+6.3 (neat)	*o*	*o*	*o*	*n*
10	*R*[31]	28 (4)	8.2 (82)	94	+5.6 (neat)	*n*	51–54 (4)	10.5 (70)	95	−8.9 (neat)	*o*	*o*	*o*	*n*
11	*S*	191–192 (760)	7.2 (72)	96	+11.0 (*c* 16, EtOH)	89[p]	50–53 (5)	13.9 (79)	95	−14.6 (neat)	7.0 (70)	94	−11.6 (*c* 2.5, EtOH)	90[q]

[a] For conditions, see Experimental Section and Table I. The gist of the procedures involved is outlined in Figure 4. [b] Determined by gas chromatography. [c] The absolute configuration is not known. [d] To our knowledge, optically active 1 had not been synthesized before; therefore, we could not determine the enantiomeric excess of the enzymatically produced (+)- and (−)-1. [e] Lit.[26] $[\alpha]^{25}_D$ +10.4° (*c* 10, MeOH). [f] Lit.[27] $[\alpha]^{25}_D$ +5.1° (*c* 5.03, ether). [g] Lit.[10] $[\alpha]^{25}_D$ +5.2° (neat). [h] Determined from comparison with $[\alpha]^{25}_D$ −4.8° (*c* 20, MeOH) for the authentic sample of (*S*-(−)-**4** purchased from Pfaltz & Bauer. [i] Lit.[28] $[\alpha]^{25}_D$ + 13.5° (neat). [j] Determined from comparison with $[\alpha]^{25}_D$ −13.8° (*c* 5, ether) for the authentic sample of (*R*)-(−)-**5** purchased from Fluka. [k] Lit.[29] $[\alpha]^{25}_D$ +9.9° (neat) and −9.9° (neat). [l] Lit.[21] $[\alpha]^{25}_D$ −18.5° (neat) and +18.6° (neat). [n] The literature data are not available. [o] This optical isomer of the alcohol was not isolated as such because it was converted to the epoxide immediately following the alcoholysis of the "new ester produced". [p] Lit.[32] $[\alpha]^{25}_D$ +12.4° (*c* 16, EtOH). [q] Lit.[33] $[\alpha]^{25}_D$ −12.9° (*c* 2.5, EtOH).

were stored at 4 °C overnight to precipitate KCl and filtered, the solvents were evaporated under vacuum, and the solutions were filtered again, washed with ether, and dried with anhydrous $MgSO_4$.

The properties of the optically active alcohols prepared from the enzymatically produced esters by using the aforedescribed procedures are listed in Table II.

Formation of Epoxides from 9 and 10. The procedure used was a modification of the method of Blau et al.[17] and was identical with that described in (iii) of the preceding section except that 6.3 g of **9** and 8.2 g of **10** were used instead of their butyric esters.

In the case of **9**, (*S*)-(−)-**9**[31] (see Table II) was converted to 2.4 g of (*S*)-(−)-propylene oxide (43% overall yield, 93% purity by GC) with $[\alpha]^{25}_D$ −4.9° (*c* 1, chloroform) which corresponds to ee 68%[34] (lit.[35] $[\alpha]^{25}_D$ −7.2° (*c* 1, chloroform)). The (*R*)-(+)-1-chloro-2-propyl butyrate[31] enzymatically produced (see Table II) was converted to 2.7 g of (*R*)-(+)-propylene oxide (50% overall yield, 94% purity by GC) with $[\alpha]^{25}_D$ +4.8° (*c* 1, chloroform), which corresponds to ee 67%[34] (lit.[35] $[\alpha]_D^{25}$ +7.2° (*c* 1, chloroform)).

In the case of **10**, (*S*)-(+)-**10**[31] (see Table II) was converted to 3.6 g of (*S*)-(+)-epichlorohydrin (51% overall yield, 95% purity by GC) with $[\alpha]^{25}_D$ +23.5° (*c* 1.2, MeOH)). The (*R*)-(−)-2,3-dichloro-1-propyl butyrates[31] enzymatically produced (see Table II) was converted to 3.0 g of (*R*)-(−)-epichlorohydrin (43% overall yield, 95% by GC) with $[\alpha]^{25}_D$ −23.0° (*c* 1.5, MeOH), which corresponds to ee 67%[34] (lit.[22] $[\alpha]^{25}_D$ −34.3° (*c* 1.5, MeOH)).

(31) The absolute configurations of optically active **9** and **10** (and their butyric esters) are not available as such. Therefore we determined the absolute configurations of the enzymatically produced **9**, **10**, and their esters on the basis of those of the epoxides derived from the alcohols, using the Cahn–Prelog–Ingold rule.

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(34) The most likely reason for a relatively low ee obtained is temperature-induced racemization of optically active **9** and **10** (and their butyric esters). In agreement with this hypothesis, when the enzymatically produced **9** was distilled at a lower vacuum (20 mmHg) and higher temperature (38 °C) only 10% ee was obtained for the resultant propylene oxide.

(35) See ref 30, p 997.

Kinetic Studies on the Oxidation of Calf Liver Cytochrome b_5 with Inorganic Complexes

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Abstract: The complexes $Co(edta)^-$, $Co(NH_3)_6^{3+}$, $Pt(NH_3)_6^{4+}$, and $(NH_3)_5CoNH_2Co(NH_3)_5^{5+}$ have been used as oxidants for cytochrome b_5(II) at 25 °C, pH 7.4, I = 0.10 M (NaCl). With $Co(edta)^-$ a simple rate law first order in both reactants (k = 14.1 M^{-1} s^{-1}) is observed. The effects of pH (5.4–7.9) and temperature ($\Delta H^{\ddagger}$ = 8.6 kcal mol^{-1}, $\Delta S^{\ddagger}$ = −24.4 cal K^{-1} mol^{-1}) were studied. With the other oxidants a less than first-order dependence on oxidant (≥10-fold excess) is observed, consistent with association (K) prior to electron transfer (k_{et}). Values obtained are for $Co(NH_3)_6^{3+}$ (600 M^{-1}, 0.075 s^{-1}), $Pt(NH_3)_6^{4+}$ (14 800 M^{-1}, 0.080 s^{-1}), and $(NH_3)_5CoNH_2Co(NH_3)_5^{5+}$ (16 600 M^{-1}, 3.8 s^{-1}). Competitive inhibition is observed with the positively charged oxidants on addition of redox-inactive $Cr(en)_3^{3+}$ (K_{Cr} = 309 M^{-1}). With $Co(edta)^-$, however, an increase in rate constants is observed on addition of $Cr(en)_3^{3+}$. These results can be accounted for in terms of reaction by all four oxidants at a specific functional site on the protein, which includes the exposed heme edge, and are strongly influenced by acidic residues in this region. The magnitude of K values suggests an effective charge of 3−/4− at this binding site.

Cytochrome b_5 is a membrane-bound monoheme protein having a wide range of redox functions.[1] It can be detached from its membrane by detergent to give a protein of ca. 135 residues, which aggregates in solution. Much work has been carried out on the 93-residue (heme containing) soluble protein which is obtained by proteolysis.[2] This includes X-ray diffraction studies on the protein with Fe in the oxidized cytochrome b_5(III) (1.5-Å resolution) and reduced cytochrome b_5(II) (2.8 Å) states.[3] A preferred isolation procedure is that used in the present work involving trypsin digest, which gives an 84-residue protein. To obtain this the 50 C-terminal residues, which contain a high percentage of hydrophobic residues and attaches the protein to the membrane (it is also responsible for the aggregation), and 2 N-terminal residues are split off, leaving the 84-residue water-soluble heme peptide fragment, which is very resistant to further attack by trypsin. It has been demonstrated that trypsin digest of the 93-residue protein removes 7 C-terminal and 2 N-terminal residues to give the same 84-residue protein.[2] The heme group is situated in a hydrophobic pocket. There are no peptide to porphyrin linkages, but two histidines (39 and 63 in the 93-membered protein) are axial ligands to the Fe. There are no cysteines or methionines. The Fe is low spin in both the II (colored orange) and III (red) states. A redox potential of 20 mV has been reported for the protein in solution and when bound to microsomes.[4a] Recent studies indicate a value of 5 mV for the 84-residue protein at 25 °C, pH 7–8, I = 0.10 M (phosphate).[4b] From the amino acid composition[5] the charge on cytochrome b_5(II) and b_5(III) is estimated as 10− and 9−, respectively. A feature of the structure is the ring of acidic residues around the exposed heme edge.[1] The possible significance of two propionate groups attached to the porphyrin, both of which are exposed to solvent in hemoglobin but one of which is almost totally buried in cytochrome b_5, has been considered.[6]

A preliminary account of this work has appeared.[7] The reduction of cytochrome b_5(III) with $Fe^{II}(edta)^{2-}$ has been reported previously.[8] There have been no previous kinetic studies on the

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