Resolution of Racemic Mixtures via Lipase Catalysis in Organic Solvents

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Abstract: Yeast lipase and porcine pancreatic lipase have been found to vigorously function in nearly anhydrous organic solvents and catalyze the rections of esterification and transesterification, respectively, in a highly stereoselective manner. With use of these enzymatic processes, a number of optically active alcohols, carboxylic acids, and their esters have been prepared on a gram scale.

Lipases have been widely used for the resolution of racemic alcohols and carboxylic acids through asymmetric hydrolyses of the corresponding esters.[1] In addition to hydrolysis, lipases should also be able to catalyze different reactions where compounds other than water serve as nucleophiles (e.g., alcohols, amines, thiols, oximes, etc.). Although these lipase-catalyzed processes would undoubtedly offer new synthetic opportunities, they have not been exploited because in aqueous solutions they are suppressed by hydrolysis; likewise, the reversal of the hydrolysis reaction is not feasible due to an unfavorable equilibrium position.

In principle, these limitations can be overcome if biphasic aqueous–organic mixtures are employed as reaction media instead of water.[2] For example, we have been able to prepare a number of optically active alcohols on a gram scale using transesterification reactions catalyzed by yeast lipase or pig liver carboxyl esterase dissolved in an aqueous buffered solution confined to the pores of a porous support (Sepharose or Chromosorb), which was in turn suspended in a water-immiscible organic medium.[3] The main drawbacks of this approach were tediousness and poor reproducibility of entrapment of enzymes in porous supports[4] and relatively low operational stability of the esterases in the aqueous milieu.

The overall fraction of water in the aforementioned biphasic systems was a small percent. It was, therefore, intriguing to find out what would happen to the lipase activity if even that water is removed. Following this train of thought, we have recently discovered[5] that porcine pancreatic and yeast lipases can vigorously function and catalyze numerous reactions[6] even in nearly anhydrous organic solvents. In the present paper, we report that in such media the lipases can be used for facile, preparative, and efficient resolution of racemates of organic compounds. These resolutions via asymmetric, unusual reactions (esterifications and transesterifications) in organic solvents have some important advantages over conventional asymmetric hydrolyses in water.

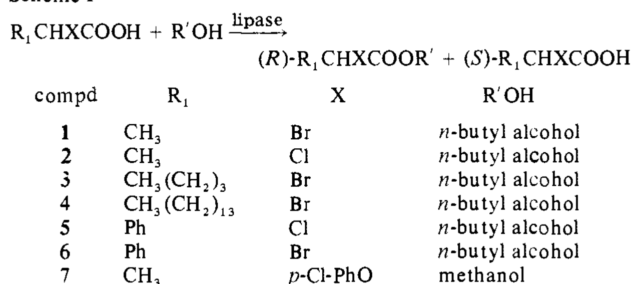
Results and Discussion

Yeast (*Candida cylindracea*) and porcine pancreatic lipases (E.C. 3.1.1.3) are ideal candidates for practical transformations because they are commercially available and relatively inexpensive,[7] require no cofactors for their action, and have a broad substrate specificity.[8]

When the concentration (activity) of water is decreased, the position of the thermodynamic equilibrium in (lipase-catalyzed) hydrolysis shifts in favor of the esters.[9] In nearly anhydrous organic media, the hydrolysis reactions (e.g., catalyzed by porcine pancreatic or yeast lipases) are nearly completely reversed.[6] In this work, it was found that not only did *Candida cylindracea* lipase almost quantitatively convert a carboxylic acid and an alcohol to the ester in organic solvents but, when a chiral acid was used, it did so in a highly stereoselective manner.

The general reaction catalyzed by yeast lipase in this work is shown in Scheme I. A typical experimental procedure is illustrated below by using racemic **1** as an example. A mixture of

Scheme I



$R_1CHXCOOH + R'OH \xrightarrow{\text{lipase}} (R)\text{-}R_1CHXCOOR' + (S)\text{-}R_1CHXCOOH$

compd	R_1	X	R′OH
1	CH_3	Br	*n*-butyl alcohol
2	CH_3	Cl	*n*-butyl alcohol
3	$CH_3(CH_2)_3$	Br	*n*-butyl alcohol
4	$CH_3(CH_2)_{13}$	Br	*n*-butyl alcohol
5	Ph	Cl	*n*-butyl alcohol
6	Ph	Br	*n*-butyl alcohol
7	CH_3	*p*-Cl-PhO	methanol

(±)-**1** and a 3-fold molar excess of *n*-butyl alcohol was dissolved in hexane, and then powdered lipase[10] from *Candida cylindracea* was added. The suspension (the lipases are insoluble in this and

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(3) Cambou, B.; Klibanov, A. M. *J. Am. Chem. Soc.* **1984**, *106*, 2687–2692.

(4) We have found that even minor variations in the water content of the system may have a dramatic effect on the rate and stereospecificity of enzymatic transesterifications.

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(6) Zaks, A.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3192–3196.

(7) Crude yeast and porcine pancreatic lipases obtained from Sigma Chemical Co. cost $51/100 g and $24/500 g, respectively.

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(10) It may seem that here and hereafter relatively large quantities (grams) of the lipases have been employed, suggesting low catalytic efficiency. However, it should be stressed that the lipase preparations used were very crude (less than 1% purity) which was done deliberately to keep the cost down. Therefore the lipase amounts quoted in the text are somewhat misleading as to the actual amount of the *active* enzyme present (the great majority of the weight is that of catalytically inert proteins).

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Table I. Production of Optically Active Esters from Racemic Acids via Scheme I with Yeast Lipase in Organic Solvents[a]

acid	solvent	reaction time, h	degree of conversion, %	yield, g (%)	bp, °C (mmHg)	purity,[b] %	$[\alpha]^{25}_D$, deg	isomer	ee, %
1	hexane	6	45	3.3 (88)	84–86 (14)	97	+18.4 (*c* 1, $CHCl_3$)	*R*	96[c]
2	hexane	6	42	2.4 (87)	71–73 (10)	99	+7.5 (*c* 1, $CHCl_3$)	*R*	95[c]
3	hexane[d]	19	30	1.4 (91)	78–80 (2)	97	+12.4 (*c* 1, $CHCl_3$)	*R*	99[c]
4	hexane[d]	22	47	1.4 (76)	194–196 (1)	97	–9.7 (*c* 1, $CHCl_3$)	*R*[e]	*c*
5	hexane + butyl ether (1:1)[d]	168	8	0.23 (82)	118–120 (2)	95	+40.1 (*c* 1, ether)	*R*	99[c]
6	hexane + butyl ether (1:1)[d]	216	20	0.44 (81)	127–129 (2)	95	+61.4 (*c* 1, ether)	*R*	99[c]
7	hexane + butyl ether (1:1)[d]	40	45	1.5 (89)	85–87 (2)	96	+32.6 (*c* 1, EtOH)	*R*	79[f]

[a] Conditions: the amounts of the racemic acids were 0.04 mol (6.1 and 4.34 g, respectively) in the case of **1** and **2**, 0.02 mol (3.9 g) of **3**, 0.01 mol (3.35 g) of **4**, 0.015 mol (2.56 g) of **5**, 0.01 mol (2.15 g) of **6**, and 0.01 mol (2.0 g) of **7**; a threefold molar excess of the alcohol (R'OH) was used in all instances (11 mL of butanol for **1** and **2**, 5.5 mL of butanol for **3**, 2.75 mL of butanol for **4**, 4.12 mL of butanol for **5**, 2.75 mL of butanol for **6**, and 0.4 mL of methanol for **7**); the volumes of the solvents used were 400 mL for **1** and **2**, 200 mL for **3**, 150 mL for **5**, and 100 mL for **4**, **6**, and **7**; the amounts of yeast lipase used[10] were 2.0 g for **1** and **2**, 3.0 g for **3** and **5**, 1.0 g for **4**, **6**, and **7**; *n*-butyl alcohol was used as a nucleophile (R'OH) in all instances except for **7** where methanol was used instead (because it affords a higher ee for the ester formed: 79% vs. 65%; the esterification rates were the same with both alcohols); 30 °C and shaking at 250 rpm. [b] Determined by gas chromatography. The purity was also tested by TLC and no spots except for the one corresponding to the ester were detected. [c] Attempts to determine the enantiomeric excess using $Eu(dcm)_3$ as a chiral shift reagent[15] were unsuccessful as no satisfactory separation of the peaks of diastereotopic hydrogens was observed. Therefore, where possible (**1–3**, **5**, and **6**), the ee values were determined on the basis of the comparison of the observed $[\alpha]^{25}_D$ with that of a chemically synthesized, optically pure authentic compound (see Experimental Section). [d] Distilled water (0.1%) was added. [e] To our knowledge, optically active **4** or its esters have never been prepared. By analogy with the other acids, one can assume that the reactive isomer has the *R* configuration. [f] Lit.[19] $[\alpha]^{25}_D$ +41.1° (*c* 50, EtOH).

Table II. Production of Optically Active Acids from Racemates via Scheme I with Yeast Lipase in Organic Solvents[a]

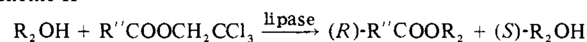
acid[b]	solvent	reaction time, h	degree of conversion, %	yield, g (%)	bp, °C (mmHg)	purity,[c] %	$[\alpha]^{25}_D$, deg	isomer	ee, %
1	hexane	14.5	78	1.3 (96)	97–98 (11)	99	–25.2 (*c* 15, $CHCl_3$)	*S*	99.6[d]
2	hexane	14.5	68	1.3 (96)	81–83 (11)	97	–15.1 (*c* 1, $CHCl_3$)	*S*	95[e]
3	hexane[f]	48	67	1.1 (89)	101–103 (2)	97	–16.6 (*c* 5, EtOH)	*S*	62[g] or 73[h]
4	hexane[f]	32	57	0.96 (67)	mp 51–52	96	+13.0 (*c* 1, $CHCl_3$)	*S*[i]	*j*
7	hexane + butyl ether (1:1)[f]	168	65	0.67 (96)	92–95 (2)	96	–26.2 (*c* 1, EtOH)	*S*	65[k]

[a] Conditions were the same as in footnote *a* to Table I. [b] In the case of **5** and **6**, the reactions were too slow to surpass a 50% degree of conversion which is necessary to produce the acids of a reasonable optical purity. [c] Determined by gas chromatography. [d] Lit.[20] $[\alpha]^{25}_D$ –25.3° (*c* 15, $CHCl_3$). [e] The authentic acid was synthesized from L-alanine[21] and had $[\alpha]^{25}_D$ +15.9° (*c* 1, $CHCl_3$); this acid had $[\alpha]^{25}_D$ +14.6° (neat) which exactly coincided with the literature[22] value. [f] Distilled water (0.1%) was added. [g] Lit.[22] $[\alpha]^{25}_D$ –27.0° (*c* 5, EtOH). [h] Obtained from comparison with $[\alpha]^{25}_D$ of the (*S*)-**3** chemically synthesized by us. [i] To our knowledge, optically active **4** has never been prepared. By analogy with the other acids, one can assume that the unreactive isomer has the *S* configuration. [j] Attempts to determine the ee value by NMR[15] were unsuccessful for the reason mentioned in footnote *c* to Table I. [k] Lit.[24] $[\alpha]^{25}_D$ –40.1° (*c* 1, EtOH).

other organic media used) was vigorously shaken at 30 °C, and periodically the liquid phase was analyzed by gas chromatography. The time course of the enzymatic esterification in hexane revealed that the reaction considerably slowed down near 40% conversion. Since only minor (see below) inactivation of lipase during the reaction was observed,[11] this phenomenon implied stereoselectivity of the lipase-catalyzed esterification. The general theory of enzyme-catalyzed kinetic resolutions[12] suggests that the optical purity of the ester formed decreases and that of the remaining acid increases upon an increase in the degree of conversion. Accordingly, in order to optimize the production of the optically active *ester* the reaction was stopped (by filtering out the enzyme) at a 45% conversion, and to optimize the production of the optically active *acid*, the reaction was terminated at a 78% conversion. As a result, optically active butyl (*R*)-(+)-2-bromopropionate (line 1, Table I) and (*S*)-(–)-**1** (line 1, Table II) were obtained.

Thus yeast lipase catalyzed the esterification of the *R* isomer of **1** in hexane with the overwhelming preference over its *S* counterpart.[13] Whichever the desirable isomer,[14] the other one could be readily racemized (and then resolved again): butyl (*R*)-(+)-2-bromopropionate was dissolved in concentrated HBr and incubated at 90 °C overnight. As a result, optically inactive **1** was obtained, indicating that this treatment afforded both hydrolysis and racemization of the ester. The (*S*)-(–)-acid was

Scheme II



compd	R_2OH	R''
8	2-octanol	$CH_3(CH_2)_2$
9	3-octanol	$CH_3(CH_2)_2$
10	2-dodecanol	$CH_3(CH_2)_2$
11	2-hexadecanol	$CH_3(CH_2)_2$
12	2-methyl-2,4-pentanediol	$CH_3(CH_2)_2$
13	2-methylcyclohexanol	$CH_3(CH_2)_5$
14	*sec*-phenethyl alcohol	$CH_3(CH_2)_2$

(11) As established by two independent methods: the enzyme was recovered by filtration and assayed both in water (hydrolysis of tributyrin) and in hexane (repeated esterification).

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(13) Both enzymatic esterification and hydrolysis occur via a common acylenzyme intermediate.[8] We have found that, as expected, yeast lipase is also stereoselective in the *hydrolysis* of esters of **1**, hydrolyzing butyl (*R*)-(+)-2-bromopropionate much faster than the (*S*)-(–) isomer.

(14) Both **1** and **2** are used for the synthesis of a number of derivatives of 2-phenoxypropionic acid having wide applications as potent herbicides (Cremlyn. R. "Pesticides, Preparation and Mode of Action"; Wiley: Chichester, 1978; p 143). Only the *R* isomer of 2-phenoxypropionic acid derivatives is biologically active (Jager, G. In "Chemistry of Pesticides"; Buchel, K. H., Ed.; Wiley: New York, 1983; p 361), thereby requiring (*S*)-**1** or (*S*)-**2** as starting materials (a Walden inversion takes place during subsequent coupling).

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Table III. Production of Optically Active Esters from Racemic Alcohols via Scheme II with Porcine Pancreatic Lipase in Organic Solvents[a]

alcohol	solvent	reaction time, h	degree of conversion, %	yield, g (%)	bp, °C (mmHg)	purity,[b] %	$[\alpha]^{25}_D$, deg	isomer	ee, %[c]
8	ether	130	47	6.0 (70)	48–50 (4)	95	−7.0 (*c* 10, ether)	*R*	95[d]
9	ether	133	45	6.4 (75)	45–48 (4)	95	+2.5 (neat)	*R*	70[e]
10	ether	46	46	6.0 (87)	311–314 (760)	95	−4.8 (*c* 5, EtOH)	*R*[f]	100[g]
11	ether	50	46	4.3 (83)	342–344 (760)	95	−4.0 (*c* 5, EtOH)	*R*[f]	98[g]
12	heptane	95	48	9.8 (61)	108–110 (6)	97	−14.7 (neat)	*R*[f]	87[g]
13	heptane	96	48	14.3 (72)	65–67 (1)	80[h]	−28.7 (neat)	*R*[i]	88[g,i]
14	ether	133	45	8.9 (75)	63–65 (4)	93	+97.9 (*c* 10, ether)	*R*	95[j]

[a] Conditions: the amounts of the alcohols were 0.080 mol (10.4 g) of **8** and **9**, 0.053 mol (10.0 g) of **10**, 0.032 mol (8.0 g) of **11**, 0.169 mol (20.0 g) of **12**, 0.175 mol (20.0 g) of **13**, and 0.122 mol (15.0 g) of **14**; a 20% molar excess of the ester (**15**) was used in all instances (17.7 g for **8** and **9**, 12.0 g for **10**, 7.3 g for **11**, 37.6 g for **12**, and 27.3 g **14**) except for **13** where 2,2,2-trichloroethyl heptanoate was used instead of **15**, and its molar ratio to the alcohol was 0.66:1 (30.2 g of the ester); the volumes of the solvents used were 55 mL for **8** and **9**, 90 mL for **10**, 60 mL for **11**, 290 mL for **12**, 126 mL for **13**, and 90 mL for **14**; the amounts of porcine pancreatic lipase used[10] were 8.0 g for **8** and **9**, 15.0 g for **10**, 14.0 g for **11**, 100.0 g for **12**, 26.2 g for **13**, and 12.3 g for **14**; all reaction mixtures were dehydrated prior to the addition of the enzyme by extensive shaking with molecular sieves; the reaction temperature was 25 °C for **8**, **9**, **10**, **11**, and **14** and 60 °C for **12** and **13**; in the case of **8**, **9**, **10**, **11**, and **14**, the mixture was shaken at 250 rpm, and in the case of **12** and **13** they were stirred mechanically at 300 rpm. [b] Determined by gas chromatography. [c] Determined on the basis of the optical purity of the alcohol produced from the ester by alkaline hydrolysis (see Experimental Section). [d] $[\alpha]^{25}_D$ for the **8** produced was −12.7° (*c* 10, ether) as compared to $[\alpha]^{25}_D$ −13.4° (*c* 10, ether) for the authentic (*R*)-(−)-**8** (obtained from Aldrich). [e] For the **9** produced $[\alpha]^{25}_D$ −9.0° (*c* 6, ether) as compared to lit.[26] $[\alpha]^{25}_D$ −12.9° (*c* 6, ether). [f] To our knowledge, optically active **10**, **11**, **12**, and their esters have not been prepared before. By analogy with the other alcohols, one can assume that the reactive isomer has the *R* configuration. [g] Determined by ^{19}F NMR with the MTPA esters.[27] [h] The remainder was 2,2,2-trichloroethyl heptanoate. [i] Analysis[28] with the Mosher esters[27] revealed that two major peaks (94% of total) correspond to 1(*R*),2(*R*)- and 1(*R*),2(*S*)-**13**. Hence the configuration and optical purities presented in the table refer to C-1. [j] For the **14** produced $[\alpha]^{25}_D$ +37.6° (neat) as compared to lit.[29] $[\alpha]^{25}_D$ +39.5° (neat).

Table IV. Production of Optically Active Alcohols from Racemates via Scheme II with Porcine Pancreatic Lipase in Organic Solvents[a]

alcohol	solvent	reaction time, h	degree of conversion, %	yield, g (%)	bp, °C (mmHg)	purity,[b] %	$[\alpha]^{25}_D$, deg	isomer	ee, %
8	ether	130	47	3.1 (60)	84–86 (20)	95	+12.0 (*c* 10, ether)	*S*	90[c]
9	ether	133	45	2.9 (56)	85–86 (20)	93	+7.3 (*c* 6, ether)	*S*	57[d]
10	ether	46	46	4.4 (88)	104–106 (13)	95	+6.9 (*c* 5, EtOH)	*S*[e]	95[f]
11	ether	50	46	3.4 (85)	*g*	96	+5.6 (*c* 5, EtOH)	*S*[e]	100[f]
12	heptane	95	48	5.2 (52)	87–90 (6)	96	+15.0 (*c* 1, EtOH)	*S*[e]	92[f]
13	heptane	96	48	5.9 (59)	67–69 (10)	93	+38.5 (*c* 2.7, EtOH)	*S*[h]	86[f]
14	ether	133	45	4.2 (56)	43–45 (4)	93	−37.1 (neat)	*S*	90[i]

[a] For experimental conditions see footnote *a* to Table III. [b] Determined by gas chromatography. [c] Determined from comparison with $[\alpha]^{25}_D$ +13.4° (*c* 10, ether) of the authentic (*S*)-(+)-**8** (purchased from Aldrich). [d] Lit.[26] $[\alpha]^{25}_D$ +12.9° (*c* 6, ether). [e] To our knowledge, optically active **10**, **11**, and **12** have not been prepared before. By analogy with the other alcohols, one can assume that the unreactive isomer has the *S* configuration. [f] Determined by ^{19}F NMR with the MTPA esters.[27] [g] This compound is a solid with mp 45–47 °C. [h] Analysis[28] with the Mosher esters[27] revealed that two major peaks (93% of the total) correspond to 1(*S*),2(*R*)- and 1(*S*),2(*S*)-**13**. Hence the configuration and optical purity presented in the table refer to C-1. [i] Lit.[29] $[\alpha]^{25}_D$ −41.3° (neat).

racemized by using the same procedure.

The enzymic catalyst recovered after the esterification was washed with hexane and repeatedly employed for preparative resolutions. After each use, no more than 10% of the enzymatic activity was lost. The yeast lipase-catalyzed esterification of **1** with butanol was examined in solvents other than hexane. Toluene was equally effective; the reaction in chloroform was significantly slower and in ether slower still. No reaction was observed in butanol and the water-miscible solvents dioxane, tetrahydrofuran, and methanol. When *n*-butyl alcohol was replaced with methanol as a nucleophile, no reaction took place in hexane.

Half a dozen other acids were successfully esterified in organic media in a stereoselective manner with yeast lipase as a catalyst and the procedure described above. As one can see from Tables I and II, high enantiomeric excesses were obtained in most instances. In the case of **5** through **7**, a 1:1 (v/v) mixture of hexane and butyl ether was used as the reaction medium because the acids were poorly soluble in pure hexane. In the case of **3** through **7**, the enzymatic esterification was extremely slow unless 0.1% water was added to the system. Yeast lipase was quite tolerant with respect to the nature of R_1 (Scheme I): it accepted both aliphatic and aromatic R_1, both small and large. On the other hand, the enzyme required an electron-withdrawing X (a halogen or a halophenoxy group). Acids in which X was an alkyl or a hydroxyl were unreactive; amino acids were insoluble in water-immiscible organic solvents.

In contrast to yeast lipase, porcine pancreatic lipase displayed a very low catalytic activity in the esterification reactions in organic media (Scheme I). For example, in the case of **1**, even 16 g of lipase in 400 mL of the hexane solution resulted in only a 15% degree of conversion after 5 days (the enantiomeric excess of the ester formed was 45%); in other instances the reactions were even slower. However, porcine pancreatic lipase was found to be both quite active and very stereoselective in another reaction in anhydrous organic solvents—transesterification. This process was used for preparative resolution of a number of racemic alcohols (Scheme II) as illustrated below with **8** as an example. A mixture of (±)-**8** and a 20% molar excess of 2,2,2-trichloroethyl butyrate (**15**)[25] was dissolved in ether, and then the solution was dehydrated with molecular sieve absorbents. Powdered porcine pancreatic lipase[10] was added, and the suspension was vigorously shaken at 25 °C. The liquid phase was periodically assayed by gas chromatography. The degree of conversion of the lipase-catalyzed

(25) A variety of esters of different structures and degrees of activation have been tested: ethyl acetate, methyl butyrate, 2-chloroethyl acetate, ethyl cyanoacetate, methyl bromoacetate, 2-chloroethyl trichloroacetate, 2,2,2-trichloroethyl trichloroacetate, tributyrin (glyceryl tributyrate), **15**, and 2,2,2-trichloroethyl heptanoate. The initial rates obtained with these esters in ether (1 M **8** and 1.2 M ester) were (μmol/min·g enzyme): 0.0056, 0.087, 0.017, 0.10, 0.26, 0.10, 0.13, 0.61, 1.05, and 1.8, respectively. Hence, the last two afforded the highest rates of the lipase-catalyzed transesterification in ether; **15** was used in most instances as the smallest of the two. In addition to being activated esters and, hence, resulting in high reaction rates, 2,2,2-trichloroethyl esters are also advantageous because of the position of the thermodynamic equilibrium in Scheme II (the equilibrium is shifted more to the right as the nucleophilicity of the ester's alcohol moiety decreases: Koskikallio, J. In "The Chemistry of Carboxylic Acids and Esters"; Patai, S., Ed.; Interscience: London, 1969; Chapter 3).

(26) Pappo, R.; Collins, P.; Jung, C. *Ann. N.Y. Acad. Sci.* **1971**, *180*, 64–75.

(27) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543–2549.

(28) Jones, J. B.; Takemura, T. *Can. J. Chem.* **1982**, *60*, 2950–2956.

(29) "Aldrich Catalog/Handbook of Fine Chemicals"; Aldrich Chemical Co.: Milwaukee, WI, 1984; p 864.

transesterification reached almost 50%, and then the reaction nearly stopped. The latter was not due to enzyme inactivation because, as in the case of yeast lipase, the porcine pancreatic enzyme recovered was catalytically active. Following removal of the enzyme by filtration, the liquid phase was separated and, as a result, optically active (*R*)-(−)-2-octyl butyrate and (*S*)-(+)-**8** were obtained.

On the basis of a high optical purity of the ester (line 1, Table III) and the alcohol (line 1, Table IV) prepared, one could conclude that porcine pancreatic lipase was very stereoselective in the transesterification in ether. This conclusion was confirmed when the reaction was carried out with authentic *R* and *S* isomers of **8**: the difference in the initial reaction rates was 93-fold (the same conditions as in Table III).

Numerous other racemic alcohols were tested instead of **8** in the porcine pancreatic lipase-catalyzed asymmetric transesterifications (Scheme II). In the case of **9** through **14**, the reactions virtually stopped between 45 and 50% conversion. The products were separated and in most instances displayed a high degree of optical purity. One can see that the stereoselectivity decreased when the difference in size between the substituents at the asymmetric carbon diminished (transition from **8** to **9** in Scheme II). In agreement with that conclusion 1-methoxy-2-propanol, 1-chloro-2-propanol, *sec*-butyl alcohol, and 3-butyn-2-ol exhibited only slight stereoselectivity.[30] Sterically hindered secondary alcohols[32] as well as bulky tertiary alcohols[33] were unreactive presumably due to steric reasons. Primary alcohols[34] readily reacted in the lipase-catalyzed transesterification in ether but exhibited a low, if any, stereoselectivity.[35]

Porcine pancreatic lipase was quite flexible with respect to experimental conditions of the transesterification reactions depicted in Scheme II. In addition to ether and heptane (Tables III and IV), the enzymatic process could also be carried out in many other organic solvents, both water-immiscible (different paraffins, toluene, carbon tetrachloride) and water-miscible (acetone, acetonitrile, dioxane). In all instances water had a detrimental effect: in the presence of even a small percent of water the enzymatic transesterification was severely suppressed by hydrolysis. Therefore, all reaction mixtures were dehydrated prior to the addition of the enzyme.

Lipase can be used as a catalyst in organic solvents over a wide temperature range: upon a temperature increase from 25 to 60 °C the enzymatic transesterification accelerates by more than sevenfold. The enzyme loses virtually no catalytic activity after each transesterification run at 25 °C (and therefore can be used repeatedly) and less than a quarter of the initial activity if the reaction is carried out at 60 °C.

In conclusion, it is reported in this study that two different lipases can act as highly stereoselective, practical catalysts in nearly anhydrous organic solvents. Under such "unnatural" conditions the enzymes can asymmetrically catalyze[37] reactions of esterification and transesterification which are not feasible in aqueous solutions because of the domination of hydrolysis. As a result, a number of optically active alcohols, carboxylic acids, and esters have been prepared on a gram scale. Advantages of lipase-catalyzed resolutions in organic media as compared to water (via asymmetric hydrolysis) are the following: (i) there is no need to convert an alcohol or an acid to an ester prior to the enzymatic resolution, thereby eliminating one synthetic step; (ii) stability of enzymes is much greater in organic solvents than in water;[5] (iii) there is no need to immobilize enzymes to make them reusable because they are insoluble in organic solvents and, therefore, can be easily recovered by filtration and used repeatedly; and (iv) some substrates or products are unstable in aqueous solutions, e.g., toward racemization or other degradation reactions, but stable in organic solvents.[38]

Experimental Section

Materials. Porcine pancreatic and *Candida cylindracea* lipases were purchased from Sigma Chemical Co. and had a specific activity of 54 and 2415 olive oil units per mg of solid, respectively. The lipases were in the form of a powder which contained 3.6 and 6.1% (w/w) water,[5] respectively. In all experiments the lipases "straight from the bottle" were employed, except for porcine pancreatic lipase in the resolutions of **12** and **13** where the enzyme was dried under vacuum for 2 days (which brings its water content to 0.5% (w/w))[5] to enhance its stability at 60 °C.

All racemic acids and alcohols (as well as individual optical isomers when available) used in this work were obtained commercially. Butyl esters of racemic **1** through **6** and methyl ester of **7** were synthesized by using a standard procedure.[39] Their boiling points coincided with those of the esters produced enzymatically listed in Table I. Optically active *S* isomers of **1**, **2**, and **3** were synthesized from L-alanine (the first two) and L-norleucine with the method of Fu et al.[21] The boiling points of the acids coincided with those of the racemic authentic samples. The specific optical rotations obtained were −25.3° (*c* 15, $CHCl_3$), −15.9° (*c* 1, $CHCl_3$), and −22.8° (*c* 1, EtOH), respectively. Butyl esters of *S* isomers of **1**, **2**, and **3** were prepared by using a standard procedure[39] from the corresponding acids. The boiling points coincided with those listed in Table I. The specific optical rotations were −19.2° (*c* 1, $CHCl_3$), −7.9° (*c* 1, $CHCl_3$), and −12.5° (*c* 1, $CHCl_3$), respectively. Butyl esters of *S* isomers of **5** and **6** were synthesized from (*R*)-(+)-mandelic acid first by esterifying it with butanol[16] and then by nucleophilic displacement of OH with Cl[17] and Br,[18] respectively. The specific optical rotations obtained were +43.5° (*c* 1, $CHCl_3$) and +62.0° (*c* 1, ether), respectively. Esters of all racemic alcohols and all activated esters were synthesized from the corresponding acyl chlorides.[40] The boiling points of the esters of **8** through **14** coincided with those listed in Table III. The boiling points of other esters were 29–30 °C (1 mmHg) for **15**, 51–55 °C (1 mmHg) for 2,2,2-trichloroethyl heptanoate, 65–67 °C (4 mmHg) for 2-chloroethyl trichloroacetate, 55–57 °C (1 mmHg) for 2,2,2-trichloroethyl trichloracetate, 63–65 °C (15 mmHg) for 3-butyn-2-yl butyrate, and 67–70 °C (4 mmHg) for 2-phenyl-1-propyl butyrate.

All organic solvents used in this work were of analytical grade. Prior to use they were dehydrated by shaking with 4-Å molecular sieves (Linde) (1 g of the sieves per 10 mL of the solvent) for 24 h at room temperature. The water content of the dried solvents in all instances did not exceed 0.02% (w/w), as determined by the optimized Fischer method.[41]

Assays. All alcohols, acids, and esters in this work were determined by gas chromatography using a 5-meter capillary column with 530 μm-fused silica gel (Hewlett-Packard) (N_2 carrier gas, 30 mL/min, detector and injector port temperature 250 °C). (i) In the case of **1**, **2**, and their butyl esters, the temperature of the column was 60 °C for 3 min, and then it was increased to 120 °C at 35 °C/min. The retention times observed were 1.35 min for **1**, 0.75 min for **2**, 2.85 min for butyl 2-bromopropionate, and 1.44 min for butyl 2-chloropropionate. (ii) In the case of **3** through **7** and their esters, the temperature of the column was increased from 60 to 250 °C at 25 °C/min. The retention times observed were 2.28 min for **3**, 6.46 min for **4**, 3.13 min for **7**, 2.72 min for butyl 2-bromohexanoate, 6.99 min for butyl 2-bromohexadecanoate, 3.57 min for butyl 2-chlorophenylacetate, 3.95 min for butyl 2-bromophenyl-

(30) E.g., the transesterification reaction with 3-butyn-2-ol was stopped at about 40% conversion, and the ester obtained had ee = 15% (for the alcohol obtained from the ester by alkaline hydrolysis $[\alpha]_D^{25}$ + 52.0° (*c* 3, dioxane)).

(31) Vigneron, J. P.; Bloy, V. *Tetrahedron Lett.* **1979**, *29*, 2683–2686.

(32) The following were tested: (±)-mandelonitrile, (±)-mandelic acid and its methyl ester, (±)-2-bromo-1-indanol, (±)-menthol, (±)-pantoyllactone, and (±)-propranolol.

(33) The following were tested: (±)-3-methyl-3-hexanol, (±)-terpineol, and (±)-linalool.

(34) The following alcohols were tested: (±)-3-methoxy-1-butanol, (±)-2-methyl-1-butanol, (±)-3-methyl-1-pentanol, (±)-3,7-dimethyl-1-octanol, (±)-citronellol, (±)-2,3-dichloro-1-propanol, (±)-2-phenyl-1-propanol, and (±)-2,2-dimethyl-1,3-dioxolane-4-methanol.

(35) As evidenced by the following two examples: The initial rates of the porcine pancreatic lipase-catalyzed reaction between **15** and individual (commercially purchased) isomers of 2,2-dimethyl-1,3-dioxolane-4-methanol in ether were the same. When the transesterification reaction between **15** and 2-phenyl-1-propanol was stopped at 25% conversion, the ester obtained had ee of 50% (for the alcohol obtained from the ester by alkaline hydrolysis $[\alpha]_D^{25}$ −8.7° (neat) as compared to lit.[36] $[\alpha]_D^{25}$ −17.4° (neat)).

(36) Bakshi, S. P.; Turner, E. E. *J. Chem. Soc.* **1961**, 171–173.

(37) It should be stressed that the catalytic activities observed are not merely due to a general nucleophilicity of the protein because when the lipases were inactivated by the active center-specific reagent *p*-nitrophenyl diethyl phosphate, no reactions were observed.[6]

(38) Brandstrom, A. *J. Mol. Catal.* **1983**, *20*, 93–103.

(39) Allen, C. F. H.; Spangler, F. W. "Organic Syntheses"; Horning, E. C., Ed.; Wiley: New York, 1955; Collect. Vol. III, pp 203–204.

(40) Sonntag, N. O. V. *Chem. Rev.* **1953**, *52*, 237–416.

(41) Laitinen, H. A.; Harris, W. E. "Chemical Analysis", 2nd ed.; McGraw-Hill: New York, 1975; p 361.

acetate, and 3.76 min for methyl 2-(4-chlorophenoxy)propionate. (iii) For all other compounds, the temperature of the column was increased from 60 to 250 °C at 35 °C/min. The retention times observed were 0.74 min for **8**, 0.72 min for **9**, 2.25 min for **10**, 3.63 min for **11**, 0.45 min for **12**, 0.54 min for **13**, 0.88 min for **14**, 1.89 min for 2-octyl butyrate, 1.88 min for 3-octyl butyrate, 3.33 min for 2-dodecyl butyrate, 4.49 min for 2-hexadecyl butyrate, 1.45 min for 2-methyl-2-hydroxypent-4-yl butyrate, 2.87 min for 2-methylcyclohexyl heptanoate, 2.08 min for *sec*-phenethyl butyrate, 1.29 min for 2-phenyl-1-propanol, 2.20 min for 2-phenyl-1-propyl butyrate, 1.31 min for **15**, and 2.47 min for 2,2,2-trichloroethyl heptanoate.

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

GC–MS was used to verify that upon both chemical and enzymatic acylation of **12** only the secondary hydroxyl was modified.

The purity of the esters of **1** through **7** was confirmed (in addition to GC) by TLC with precoated silica gel 60 F-254 plates (EM Reagents) and toluene as a solvent. The spots were developed by spraying with 5% vanillin in concentrated H_2SO_4, followed by heating at 140 °C for 2 min.

Distillations. The fractional distillation apparatus employed by us consisted of a Vigreux distilling column (12.5 cm height), water-cooled condenser, and a three-necked 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 1 mL.

Chromatographic Separations. In some instances enzymatically prepared esters were separated from acids or alcohols by using liquid chromatography on a silica gel column.[42] In the case of the esters of **3**, **4**, **7**, and **12**, toluene was used as a solvent; the R_f values determined were 0.15 for **3**, 0.56 for the butyl ester of **3**, 0.17 for **4**, 0.64 for the butyl ester of **4**, 0.14 for **7**, 0.52 for the methyl ester of **7**, 0 for **12**, and 0.21 for the butyric ester of **12**. After the esters exited the column, **3**, **4**, **7**, and **12** were eluted with ethyl acetate. In the case of **8**, **9**, **10**, **11**, and **14**, a 2:1 (v/v) mixture of toluene and ethyl acetate was used as a solvent; the R_f values measured were 0.23 for **8**, 0.19 for **9**, 0.65 and 0.64 for butyric esters of **8** and **9**, respectively, 0.37 for **10**, 0.40 for **11**, 0.58 and 0.69 for butyric esters of **10** and **11**, respectively, 0.30 for **14**, and 0.44 for the butyric ester of **14**.

Yeast Lipase-Catalyzed Production of Optically Active 1–7 and Their Esters. A solution of a racemic acid and an alcohol in a given solvent was supplemented with powdered lipase from *Candida cylindracea* (for details, see footnote *a* to Table I). The suspension was placed in an Erlenmeyer flask and shaken on an orbit shaker at 250 rpm and 30 °C to reach a certain degree of conversion (Tables I and II). Then the enzyme was removed by filtration, and the liquid phase was washed with three 80-mL portions of 0.5 M aqueous $NaHCO_3$. The organic phase obtained was dried with $MgSO_4$, and the solvent was evaporated in a rotary evaporator. To recover the ester, the remainder was distilled in the case of **1**, **2**, **5**, and **6** and chromatographed in the case of **3**, **4**, and **7**. The aqueous phase was acidified with 6 N HCl to pH 1, and then the acids were extracted with three 80-mL portions of CH_2Cl_2. The combined methylene chloride fractions were dried with $MgSO_4$, followed by evaporation of the solvent. The acids were isolated from the residue either by distillation or by liquid column chromatography as mentioned above. The properties of the enzymatically prepared **1** through **7** and their esters are presented in Tables I and II.

Porcine Pancreatic Lipase-Capalyzed Production of Optically Active 8–14 and Their Esters. A solution of a racemic alcohol and **15** (or 2,2,2-trichloroethyl heptanoate) in ether or heptane was dehydrated and then supplemented with powdered lipase from porcine pancreas (for details, see footnote *a* to Table III). The suspension was placed in a round-bottomed flask and either shaken on an orbit shaker at 250 rpm or mechanically stirred at 300 rpm. When the degree of conversion reached 45–50% and the reaction virtually stopped, the enzyme was removed by filtration. The liquid phase was dried with $MgSO_4$, followed by evaporation of the solvent in a rotary evaporator. In the case of **8**, **9**, and **14**, the remainder was subjected to liquid column chromatography; then the esters were separated from **15** by distillation and the alcohols from 2,2,2-trichloroethanol by aqueous extraction of the latter. In the case of **10**, **11**, and **12**, first **15** and trichloroethanol were removed by distillation, and then the alcohols were separated from their butyric esters by liquid chromatography. In the case of **13**, the remainder was separated by distillation. The properties of the enzymatically prepared **8** through **14** and their esters are presented in Tables III and IV.

Alkaline Hydrolysis of Esters of 8–14. Enzymatically prepared optically active esters (Table III) have been converted to the corresponding optically active alcohols by alkaline hydrolysis. In a typical experiment, 4 g of the ester (butyric or heptanoic) were dissolved in 30 mL of a 1 M solution of KOH in absolute ethanol. The solution was stirred for 2 h at 25 °C, after which time the ester was completely hydrolyzed (determined by GC). Then ethanol was evaporated in a rotary evaporator, the residue was extracted with ether, the extract was washed and dried with $MgSO_4$, and the solvent was evaporated.

Racemization of 1, 2, and Their Esters. One gram of optically active **1** or **2** was dissolved in 5 mL of 48% aqueous HBr or 36% HCl, respectively, and the solution was stirred at 90 °C for 14 h. After cooling to the ambient temperature, the reaction mixture was extracted with CH_2Cl_2. The organic phase was dried with $MgSO_4$, and the solvent was evaporated. The remainder was optically inactive. The same procedure applied to butyl esters of **1** and **2** resulted in simultaneous racemization and hydrolysis (as determined by GC) of the esters. The yields in all instances were 95–97%.

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(42) Loibner, H.; Seidl, G. *Chromatographia* **1979**, *12*, 600–604.