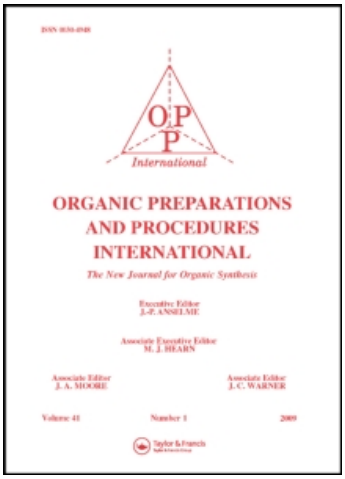

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LIPASE-CATALYZED TRANSACYLATION REACTIONS IN AZEOTROPIC DISTILLATION

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OPPI BRIEFS

LIPASE-CATALYZED TRANSACYLATION REACTIONS IN AZEOTROPIC DISTILLATION

Submitted by (12/03/92)

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The recent development of enzyme catalysis in organic solvents has attracted the attention of synthetic organic chemists because of their synthetic utility.[1-5] The lipase-catalyzed transacylation of secondary alcohols with acyl donors in organic solvents becomes a very popular method in asymmetric synthesis.[3] Many compounds, such as vinyl acetate, isopropenyl acetate, and 2,2,2-trifluoroethyl acetate, can be used as acyl donors in lipase-catalyzed transacylation due to their ability to shift the equilibrium toward the forward direction.[6,7] A major drawback of applying lipase-catalyzed transacylation reactions in organic synthesis is the low reaction rate. We described here a method based on azeotropic distillation to increase the lipase-catalyzed transacylation reaction rate. The basic idea for this modification is to remove continuously the lighter product from the reaction mixture by azeotropic distillation and shift the equilibrium to the forward direction.

Under azeotropic conditions, many enzymes such as porcine pancreatic lipase (PPL), *Candida cylindracea* lipase (CCL), *Pseudomonas species* lipase (PSL), crude cholesterol esterase from bovine pancreas acetone powder (PAP), phospholipase A2 from bee venom (PLA2) can enhance the transacylation catalysis rate (Table. 1). The reaction rate increases 2 to 10-fold from 25° to reflux. A further 2-7-fold increase in reaction rate is detected from reflux to azeotropic distillation. Benzene-diethyl ether (2/1, v/v) is the best solvent mixture for this reaction under azeotropic distillation. Under azeotropic conditions, hexanoic acid and palmitic acid still fail to react with 4-nitrophenol even under azeotropic conditions, and isopropenyl acetate (IPA) has a very high percentage (~30%) of nonenzymatic acylation.

For the lipase- and PLA2-catalyzed transacylations of (±)-α-tetralol ((±)-α-TL), (±)-indanol ((±)-ID) , and L-1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (L-PPC) with vinyl acetate, the stereospecificity and optical yield are hardly affected from 25° to azeotropic distillation (Table 1). But PAP-catalyzed transacylation of (±)-ID and lipase-catalyzed transacylation of *cis*-2-decalol exhibit poor stereoselectivities. This stereospecificity can fit into Burgess' model[8] for lipase from *Pseudomonas species*.

Table 1. Relative Rates of Transacylation Reactions in Different Conditions[a]

Reactants[b]	Solvents/ Condition[c]	Enzyme or Protein[d]	Relative Reaction Rate[e]	% ee of Unreacted Alcohol[f]	% ee of Reacted Alcohol[g]
PNP + VA	B-E(2/1)/25°	PPL + BSA	1.0[h]	-	-
PNP + VA	B-E (2/1)/reflux	PPL + BSA	10.7	-	-
PNP + VA	B-E (2/1)/D.-S./reflux	PPL + BSA	25	-	-
PNP + VA	B-E(2/1)/25°	CCL + BSA	0.006	-	-
PNP + VA	B-E (2/1)/reflux	CCL + BSA	0.13	-	-
PNP + VA	B-E (2/1)/D.-S./reflux	CCL + BSA	0.42	-	-
(±)-α-TL + VA	B-E(5/1)/25°	PPL + BSA	2.3	96; (+)-α-TL	96; (-)-α-TL
(±)-α-TL + VA	B-E (5/1)/reflux	PPL + BSA	3.8	-	-
(±)-α-TL + VA	B-E (5/1)/D.-S./reflux	PPL + BSA	26.2	95; (+)-α-TL	95; (-)-α-TL
(±)-ID + VA	B-E(5/1)/25°	PPL + BSA	2.4	96; (+)-ID	96; (-)-ID
(±)-ID + VA	B-E (5/1)/reflux	PPL + BSA	4.1	-	-
(±)-ID + VA	B-E (5/1)/D.-S./reflux	PPL + BSA	25.8	96; (+)-ID	96; (-)-ID
(±)-ID + VA	B-E(5/1)/25°	PAP + BSA	2.3	77; (+)-ID	76; (-)-ID
(±)-ID + VA	B-E (5/1)/reflux	PAP + BSA	4.0	-	-
(±)-ID + VA	B-E (5/1)/D.-S./reflux	PAP + BSA	25.2	76; (+)-ID	76; (-)-ID
(±)-ID + VA	B-E(5/1)/25°	PSL + BSA	23.4	97; (+)-ID	96; (-)-ID
(±)-ID + VA	B-E (5/1)/D.-S./reflux	PSL + BSA	250	96; (+)-ID	96; (-)-ID
(±)-DL + VA	B-E(5/1)/25°	PPL + BSA	2.1	32; (+)-DL	33; (-)-DL
(±)-DL + VA	B-E (5/1)/D.-S./reflux	PPL + BSA	24.4	32; (+)-DL	32; (-)-DL
L-PPC + VA	C-T (5/1)/25°	PLA2 + BSA	702	>99[i]	-
L-PPC + VA	C-T (5/1)/reflux	PLA2 + BSA	2,146	>99[i]	-

a) see Experimental Section b) PNP: 4-nitrophenol ; VA: vinyl acetate; (±)-α-TL: (±)-α-tetralol, (±)-1,2,3,4-tetrahydro-1-naphthol; (±)-ID: (±)-indanol; (±)-DL: (±)-*cis*-2-decalol (prepared according to reference 9); L-PPC: L-1-palmitoyl-2-hydroxy-*sn*-glcero-3-phosphocholine (Avanti Polar Lipids, Inc.) c) B: benzene; C: chloroform; E: diethyl ether; T: trichloroethylene; (2/1): ratio 2/1, volume by volume; D.-S. : Dean-Stark trap d) PPL: lipase from porcine pancreas (Sigma L0382); CCL: lipase from *Candida cylindracea* (Sigma L9767); PAP: bovine pancreas acetone powder (Sigma P3006); PSL: lipase from *Pseudomonas species* (Sigma L9518); PLA2: phospholipase A2 from bee venom (Sigma P1264); BSA (Bovine Albumin, fraction V, Sigma A-3350). e) NR: no reaction f) Optical purity of unreactive alcohol was calculated by OP = $[\alpha]_{D\ exp.}/[\alpha]_{D\ lit.}$. $[\alpha]_{D\ exp.}$ of (+)-α-TL is measured from a polarimeter at 25°, c2.5, $CHCl_3$. $[\alpha]_{D\ lit.}$ at 17° of (+)-α-TL is + 32° which is obtained from Aldrich Catalog 1992-1993. % ee was calculated by % ee = OP - (1- OP). g) The reactive alcohol ((-)-α-TL) is obtained from basic hydrolysis (0.1 N KOH, EtOH, 25°, 18h, 92%) of the ester and its % ee is determined as note f. h) As a standard (8.4×10^{-2} n mol/min mg enzyme or protein) at 25°. i) Calculated from the bee venom PLA2-catalyzed hydrolysis reaction from ^{31}P NMR.

In summary, reaction rates of lipases-catalyzed transacylation reactions with vinyl acetate are increased 4 to 70-fold by applying azeotropic distillation with this catalysis.

EXPERIMENTAL SECTION

(±)-*cis*-2-Decalol was prepared according to reference 9. L-PPC: L-1-palmitoyl-2-hydroxy-*sn*-glcero-3-phosphocholine was obtained from Avanti Polar Lipids, Inc. All chemicals were obtained from Aldrich and all biochemicals and enzymes were obtained from Sigma. Optical rotations were recorded from optical activity AA-5 polarimeter at 24°. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solution at 300 MHz on a Varian VXR-300S spectrometer operating at 299.95 and 75.42 MHz respectively, using TMS as an internal standard. ^{31}P NMR spectra were recorded in $CDCl_3$ solution at 300 MHz on a Varian VXR-300S spectrometer operating at 121.42 MHz, using H_3PO_4 as an external standard.

Typical Reaction.- To the alcohol substrate (PNP, (±)-ID, (±)-DL, (±)-α-TL, or L-PPC, 500 mg) in a solvent mixture (120 mL), enzyme (500 mg for all enzymes except PLA2, or 1 mg for bee venom PLA2), BSA (100 mg; Bovine Albumin, fraction V), and vinyl acetate (100 mole eq.) were added. The mixture was stirred under three different conditions (25°, reflux, and reflux with Dean-Stark trap) for 2-7 days. No reaction was detected for all control reactions (reactions without enzymes) under same conditions. Nonenzymatic acylation refers to the reaction with same mass equiv. of BSA without any enzyme under the same condition. In general, products and unreacted alcohols were purified by liquid chromatography on silica gel eluted with hexane-ethyl acetate solvent gradient. For PLA2-catalyzed acylation reaction, the product L-1-palmitoyl-2-acetyl-*sn*-glycero-3-phosphocholine were purified by liquid chromatography on silica gel eluted with chloroform-methanol-water (66:33:1, v/v/v). All products were characterized by ^{1}H NMR and ^{13}C NMR spectra which were identical to authentic samples. Relative rates of these reactions were estimated by TLC on silica gel (hexane-ethyl acetate, 5/1, v/v) and UV or ^{31}P NMR. In the TLC method, the reaction rate was determined from the slope of the number of moles of the product formed (<10 mol %) *vs* time plot. The molar yield of product was calculated by assuming that the area ratio of TLC spots is proportional to the weight ratio. In the UV method, the reaction rate was calculated from the negative slope of substrate absorbance *vs* time plot under standard conditions. In ^{31}P NMR method, the molar yield of product was calculated by assuming that the integration ratio is proportional to the weight ratio. The reaction rates from those experiments are about the same (within ±5%).

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THEXYL- AND ISOPINOCAMPHEYLHALOBORANES AS STEREOSELECTIVE REDUCING AGENTS

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Thexylchloroborane-dimethyl sulfide[1] (ThxBHCl•SMe_2) and thexylbromoborane-dimethyl sulfide[2] (ThxBHBr•SMe_2) have been shown to be attractive selective reducing agents, especially for the conversion of carboxylic acids to the corresponding aldehydes. This important conversion led us to investigate their reducing characteristics in greater detail.[3,4] In the course of the exploration of their selectivity, we found that these reagents reduce cyclic ketones in a much higher degree of stereoselectivity than that realized in reduction with $ThxBH_2$.[5] Furthermore, the stereoselectivity of ThxBBr•SMe_2 is much higher than that of ThxBHCl•SMe_2.[3,4] These results seem to suggest that the steric size of halogen substituent in the monoalkylboranes plays an important role in the stereoselective reduction of cyclic ketones. Accordingly, we have extended this investigation to the reaction of the iodo derivative, and finally to the halo derivatives of monoisopinocampheylborane ($IpcBH_2$), in the hope of providing a new class of stereoselective reducing agents.

As Table 1 shows, the halogen substituent in the monoalkylboranes plays an important role in the stereoselective reduction of typical cyclic ketones as expected. The stereoselectivity increased dramatically with increasing steric size of the substituent. For example, in the reduction of 4-*tert*-