rationalize why **1**–TMEDA and **1**-bisquinuclidine should have vastly different exchange rates. The condition that a change in solvation drastically could change the preferred lithium arrangement has been demonstrated recently in dilithium salts of benzocyclobutadienes.[11]

The results above are in accord with the proposal that the energy surface is rather shallow for systems of this kind and that other factors, such as crystal packing forces, may determine the actual crystal structure.[7,11,12]

It is also evident from this study that solid-phase ^{13}C CP/MAS NMR studies can provide a valuable source of structural information of organoalkali compounds. Variable-temperature studies are possible without changing the ion-pair structure as in solution. The method has a time scale that, in contrast to X-ray crystallography, can give more direct information about various dynamic processes.[13] Moreover, dipolar line broadening due to the coupling to the quadrupolar alkali nuclei can give detailed information about the nature of the carbon–alkali interaction.

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Enzyme-Facilitated Transport and Separation of Organic Acids through Liquid Membranes

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The selective separation of organic acids is important for the production of numerous biologially functional molecules, including amino acids, peptides, fats, and pharmaceuticals.[1] Enzymes, specifically lipases and proteases, have been used to selectively separate a variety of organic acids; however, multiple steps are usually required.[2] In addition, carbonic anhydrase has been used in a variety of liquid membranes for transport of CO_2.[3] In the present study, we have coupled the selectivity of enzymes with liquid membranes to provide a single-step method to selectively separate and purify organic acids.

Our experimental strategy was to use facilitative transport of a desired organic acid through a liquid membrane. This was carried out by the lipase-catalyzed esterification of the desired organic acid with a hydrophobic alcohol contained in an organic liquid membrane (see Figure 1A). The resulting ester partitions into the organic phase or is hydrolyzed to the parent acid. Once in the organic phase, the ester diffuses across the membrane where a second lipase catalyzes ester hydrolysis into the alcohol and the parent acid. If the enzyme-facilitated pathway is significantly faster than transport of the organic acids through the membrane, this will result in selective purification of the desired acid. The system employed for these studies is shown in Figure 1B.

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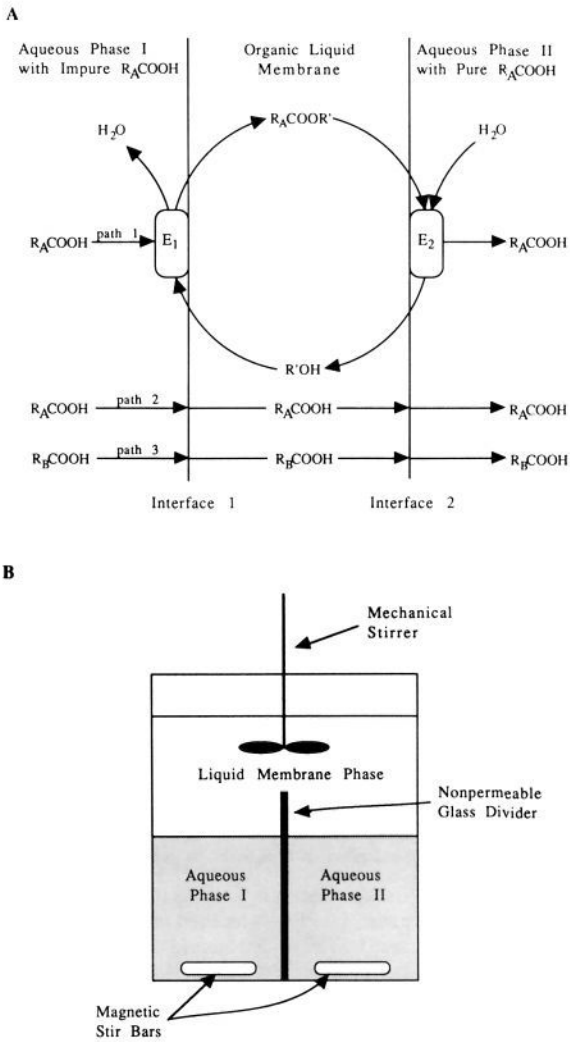

Figure 1. (A) Schematic of enzyme-facilitated liquid membrane transport for organic acid separations. R_ACOOH, R_BCOOH, R'OH, R_ACOOR', E_1, and E_2 represent the organic acids to be transported (where A is the desired acid), carrier alcohol, resulting ester, enzyme in aqueous phase I, and enzyme in aqueous phase II, respectively. Path 1 represents the enzyme-facilitated transport of the desired organic acid. Paths 2 and 3 represent nonfacilitated transport of both organic acids. (B) Enzyme-assisted membrane apparatus. The separation scheme was put into practice with a 1-L beaker with a diameter of 11 cm containing a 6-cm high impermeable glass septum separating the aqueous phases. Each aqueous phase contained 125 mL of solution and had a liquid height of 4 cm. The isooctane liquid membrane (450 mL) resided above each phase, yet the top of the isooctane phase was 3 cm above the top of the glass septum. Hence the organic solvent acted as a membrane between the two aqueous phases. Both aqueous phases were magnetically stirred at 150 rpm, and the isooctane phase was mechanically stirred at 75 rpm.

The results for the transport of 2-phenoxypropionic acid (PPA) are shown in Figure 2.[4] Lipase from *Candida cylindracea*[6] (CCL)

(4) 2-Phenoxypropionic acid was chosen as a model organic acid because it belongs to a known class of substrates of commercial lipase-catalyzed esterifications,[5] is easily analyzed by reversed-phase HPLC, and has the correct degree of hydrophobicity such that in its anionic form it resides almost solely in the aqueous phase while in its esterified form it partitions favorably into the organic liquid membrane.
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(6) Lipase from Candida cylindracea has been shown to be an effective esterification catalyst.[5] Lipase from porcine pancreas is a well-known hydrolytic catalyst[7] as well as transesterification catalyst yet is unable to catalyze esterification reactions.[5a] Hence reverse esterification and transport from aqueous phase II to phase I is eliminated.
(7) *Lipases*; Borgstrom, B., Brockman, H. L., Eds.; Elsevier: Amsterdam, 1984.

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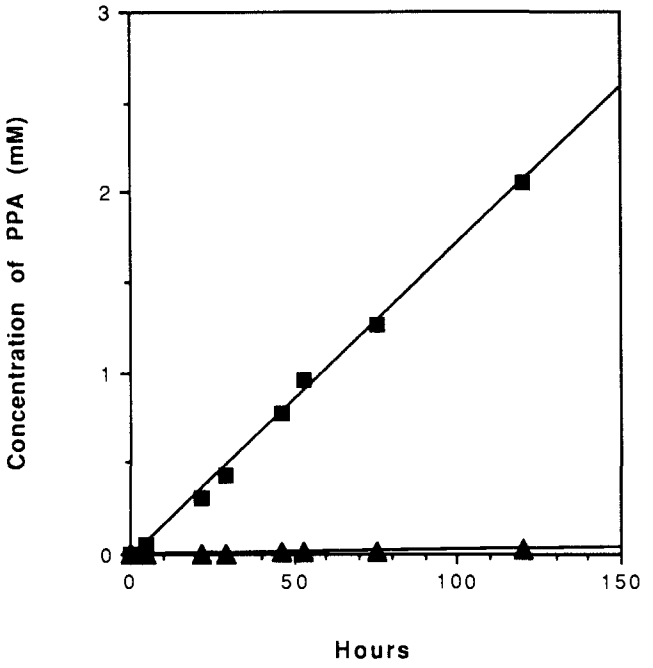

Figure 2. Enzyme-facilitated transport of 2-phenoxypropionic acid (PPA) across an organic liquid membrane: (■), enzyme-facilitated; (▲), control, no enzyme. Aqueous phase I initially consisted of 50 mM NaCl, pH 6.3,[10] 10 mM PPA, and 30 mg/mL CCL, while aqueous phase II initially consisted of 50 mM NaCl, pH 6.3, and 20 mg/mL PPL. The rate of PPA transport across the liquid membrane was dependent on the concentration of both enzymes and reached a maximum at the enzyme concentrations given above. PPA was quantitatively measured by using reverse phase HPLC and detection at 280 nm. This experiment represented the average of five independent runs with a standard error of ±5%.

was used as the esterification catalyst in aqueous phase I, while lipase from porcine pancreas (PPL) was used as the hydrolysis catalyst in aqueous phase II. The organic liquid membrane phase consisted of isooctane[8] supplemented with 1 M *n*-butyl alcohol. A control was performed by omitting the enzymes. The addition of enzymes resulted in a 70-fold increase in the amount of organic acid transported through the liquid membrane. Specifically, after 120 h, 2.05 (±0.10) mM PPA was measured in aqueous phase II in the enzyme-assisted case, while less than 0.03 mM PPA was transported in the control. Furthermore, the ester, butyl 2-phenoxypropionate, was observed in the isooctane phase only in the presence of the enzymes, while no PPA was observed in the organic phase, indicating that the species transporting through the liquid membrane is the butyl ester of the 2-phenoxypropionic acid. An increase in the PPA concentration to 25 and 100 mM resulted in 40 and 32% transport, respectively, after 10 days of operation.

In addition to PPA, we have studied the transport of other organic acids (Table I). Phenylacetic acid and 2-(4-chlorophenoxy)propionic acid were transported across the liquid membrane with efficiencies similar to those obtained for PPA. Furthermore, *n*-octyl alcohol was capable of replacing *n*-butyl alcohol and lipase from *Penicillium* sp. (lipase G) could replace CCL, although in the latter substitution, the rate of transport was significantly reduced. No transport was observed with PPL employed as the esterification catalyst.

The enzyme-assisted liquid membrane was also shown to be selective. Mandelic acid could not be transported across the liquid membrane. This selectivity was induced by the great selectivity afforded by CCL.[9] To demonstrate selective separation, a solution

(8) Isooctane was selected as the liquid membrane phase because it is immiscible with and less dense than water, provides for a favorable partitioning of the resulting ester, and has a low vapor pressure that limits evaporation during the separation.

(9) In independent experiments, it was found that CCL could not catalyze the esterification of mandelic acid with butanol in either toluene or hexane under conditions analogous to organic acid esterifications in nearly anhydrous media.[5a]

Table I. Enzyme-Facilitated Transport Rates of Organic Acids through an Organic Liquid Membrane[a]

enzyme[b] (30 mg/mL)	organic acid (10 mM)	alcohol (1 M)	initial rate of transport (μmol/L-h)
CCl	PPA	*n*-butyl alcohol	60
CCl	PPA	*n*-octyl alcohol	28
CCl	4-Cl-PPA[c]	*n*-butyl alcohol	67
CCl	phenylacetic	*n*-butyl alcohol	123
CCl	mandelic	*n*-butyl alcohol	0
PPL	PPA	*n*-butyl alcohol	0
lipase G	PPA	*n*-butyl alcohol	5

[a] For experimental conditions, see text. [b] Enzymes were obtained from commmercial suppliers and were used witout prior pretreatment. Lipase G is from a *Penicillium* sp. [c] 2-(4-Chlorophenoxy)propionic acid.

of 10 mM 2-phenoxypropionic acid and 10 mM mandelic acid was prepared in aqueous phase I (all other conditions as described above). After 170 h, 3.3 (±0.17) mM PPA was transported across the liquid membrane with the total exclusion of mandelic acid (<0.01 mM detected in aqueous phase II). Hence, ca. 100% selectivity was achieved, and this selectivity was imparted by the enzyme.

Our findings demonstrate that coupling enzymes with liquid membranes provides a simple, single-step method to selectively separate and purify organic acids through a facilitative transport mechanism. We are presently expanding this methodology to include optical resolutions and purifications as well as alternate membrane geometries. It can ben envisioned that by varying the enzyme used, it may be possible to control membrane selectivity for specific separations.

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(10) It may be expected that the CCL-catalyzed esterification of organic acids occurs through the acid form of the substrate. Hence, it is desirable to operate at the lowest pH possible without significant loss of enzyme activity. In this case, pH 6.3 fulfilled both requirements.

Stereochemistry of the Thermal Homodienyl Hydrogen Shift Reverse Ene Reaction. Stereoelectronic Control of Stereogenicity Transfer through the Anisotropic Influence of a Cyclopropane Ring

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Powerful stereoelectronic effects control the rate[1,2] and stereochemistry[2] when a C–C bond of a cyclopropane or cyclobutane ring breaks in concert with other participating bonds in homo-Diels–Alder cycloreversions. If similar influences operate generally, the stereochemical outcome of homodienyl 1,5-hydrogen shifts should be predictable from the necessity for good overlap of the reacting C–H σ- and C–C π-bond orbitals with the breaking ring bond (C_2–C_3).[3,4] From the tendency of orbital phase

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