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ENZYMATIC RESOLUTION OF ALCOHOLS VIA LIPASES IMMOBILIZED IN MICROEMULSION-BASED GELS

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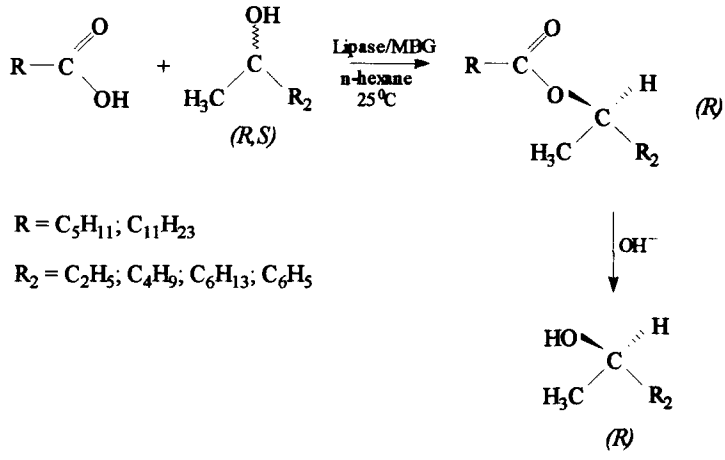
Abstract: Lipases immobilized in microemulsion-based gel (MBG) were used in the resolution of racemic alcohols, in a new, convenient method of catalysis in organic solvents which employs small amounts of the enzyme.

Enzymes have been increasingly utilized in organic synthesis in the resolution of racemic mixtures. Their use in organic media has considerably broadened the scope of reactions which are of interest to the synthetic chemist.[1, 2, 3] Lipases are among the most widely used enzymes, catalyzing esterifications of a large variety of substrates.[4, 5, 6]

The overwhelming majority of examples of enzymatic processes performed in organic solvents employ a large excess of the enzyme in a heterogeneous system.[7, 8] In addition, the biocatalyst is often degraded in the process so that its efficiency is lost after a few runs. In order to circumvent these limitations, we have developed a new method of enzyme immobilization which ensures stability and reusability of the catalyst. Small amounts of the enzyme are entrapped in the water-pool of a water-in-oil microemulsion of Aerosol-OT / n-hexane and the resulting system is congealed in a gelatin matrix. These microemulsion-based gels (MBG's) containing catalytic amounts of a lipase have been successfully used in the esterification of aliphatic and aromatic alcohols in organic media.[9, 10, 11]

As an extension of this line of work, we have investigated the use of our system for the resolution of secondary racemic alcohols (Scheme 1).

Four different lipases (*Chromobacterium viscosum lipase, lipase Microbial, Pseudomonas sp and Candida cylindracea*) were immobilized in MBG and used in the enantioselective esterification of (±)-butanol-2, (±)-hexanol-2, (±)-octanol-2, and (±)-1-phenyl-1-ethanol with hexanoic and dodecanoic acid.



Scheme 1

The preparation of the organic gel containing the immobilized lipases has been described previously.[9, 10] The esterifications were performed in n-hexane (30 mL) starting from equimolar amounts of the reactants (0.03 mol each) and 10g of the gel, containing 250 mg of the lipase. Firstly, the reaction mixture was shaken with the insoluble organic gel in a Dubnoff apparatus at 25°C for 5 days. Then, the course of the reaction was followed by nmr analysis of samples taken periodically from the reacting mixture. Isolation of the chiral ester was performed by column chromatography with silica gel Merck-60 (70 - 230 mesh), n-hexane:ethyl acetate 15:1 as eluent. Table 1 gives the yields of isolated chiral esters for each of the employed lipases. Basic hydrolysis of the obtained esters yielded the corresponding alcohols in high enantiomeric purity, as shown by comparison of their specific rotations with reported values from the literature. The observed $[\alpha]_D$ value and the estimated enantiomeric excess for each of these alcohols are given in Table 2. In this table, the values of $[\alpha]_D$ for 2- butanol are not shown due to the impossibility of isolating the optical isomers of the alcohol.

Inspection of Tables 1 and 2 shows that, with the exception of *Candida cylindracea*, all other lipases exhibited similar enantioselective efficiencies, differing however in the yields of obtained esters. Their overall efficiency thus decreased in the order *C.V.lipase* > *Microbial* >*Pseudomonas sp.* No reaction was observed with *Candida cylindracea* purchased from Sigma, presumably because of its low activity (71 units/mg). Regarding the employed acid, the more lipophilic dodecanoic acid reacted consistently faster and in higher yields than hexanoic acid. In order to confirm the catalytic activity of the *C.V.lipase* containing MBG, this system was repeatedly used in batchwise synthesis of (+)2-phenylethyl dodecanoate. The experiment was performed at 25°C as previously described. After 5 conversions, the catalytic activity is approx. 60% of the initial values. The ester was obtained in 14.4% yield (ee_s = 16%, ee_p = 95%, E = 47).

These results clearly demonstrate that very small amounts of enzymes can be conveniently entrapped in a microemulsion-based gel and used for the resolution of racemic alcohols.

TABLE 1 - Yields of isolated chiral esters obtained from the enantioselective enzymatic esterification of different alcohols and acids.

Obtained Ester	Enzyme	%Conversion[a]	Yield of isolated ester, %[b]
(−)-2-Butyl	*C.V.lipase*[c]	53	24
hexanoate	*Microbial*[d]	51	21
	Pseudomonas sp[e]	49	18
(−)-2-Hexyl	*C.V.lipase*[c]	26	31
hexanoate	*Microbial*[d]	20	14
	Pseudomonas sp[e]	16	11
(−)-2-Octyl	*C.V.lipase*[c]	18	32
hexanoate	*Microbial*[d]	16	26
	Pseudomonas sp[e]	15	20
(+)-2-Phenylethyl	*C.V.lipase*[c]	25	32
hexanoate	*Microbial*[d]	23	28
	Pseudomonas sp[e]	17	22
(−)-2-Butyl	*C.V.lipase*[c]	56	60
dodecanoate	*Microbial*[d]	40	31
	Pseudomonas sp[e]	53	16
(−)-2-Hexyl	*C.V.lipase*[c]	40	33
dodecanoate	*Microbial*[d]	27	23
	Pseudomonas sp[e]	22	15
(−)-2-Octyl	*C.V.lipase*[c]	43	46
dodecanoate	*Microbial*[d]	29	40
	Pseudomonas sp[e]	20	28
(+)-2-Phenylethyl	*C.V.lipase*[c]	40	35
dodecanoate	*Microbial*[d]	33	29
	Pseudomonas sp[e]	18	27

(a) Obtained from ^{1}H nmr spectra of the crude samples; (b) based on maximum 50% conversion; (c) from Genzyme, 3900 units/mg; (d) from Genzyme, 1600 units/mg; (e) from Genzyme, 1900 units/mg.

TABLE 2 - Enantiomeric excess of alcohols obtained from basic hydrolysis of chiral alkyl and aryl dodecanoates.

Alcohols	Enzyme	$[\alpha]^{20}/_{D}$, (c, $CHCl_3$)[a]	Enantiomeric excess, %		
			ee_s[b]	ee_p[c]	E[g]
(–)2-Hexanol[d]	*C.V.lipase*	–11.5 (1.1)	31	96	85
	Microbial	–12.1 (3.1)	20	100	>100
	Pseudomonas sp	–11.8 (9.1)	20	97	79
(–)2-Octanol[e]	*C.V.lipase*	–9.8 (6.4)	36	99	>100
	Microbial	–9.2 (7.4)	19	93	32
	Pseudomonas sp	–9.4 (5.1)	12	95	45
(+)1-Phenyl-	*C.V.lipase*	+41.9 (2.8)	46	98	>100
1-ethanol[f]	*Microbial*	+40.4 (3.1)	15	94	36
	Pseudomonas sp	+41.5 (3.9)	52	97	>100

(a) optical rotation of the reactive alcohol derived from the new ester; (b) ee_s is the enantiomeric excess of the unreactive alcohol obtained; (c) ee_p is the enantiomeric excess of the reactive alcohol derived from the new ester calculated by comparison with $[\alpha]_D$ values from the literature; (d) $[\alpha]^{20}/_{D}$= –12.04 (neat)[12]; (e) $[\alpha]^{20}/_{D}$= –9.9(neat) [12]; (f) $[\alpha]^{20}/_{D}$= +42.9 (neat) [12]; (g) **E** is the enantiomeric ratio , calculated according to reference 13.

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