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Selectivity in the transesterification of esters by supported enzymes

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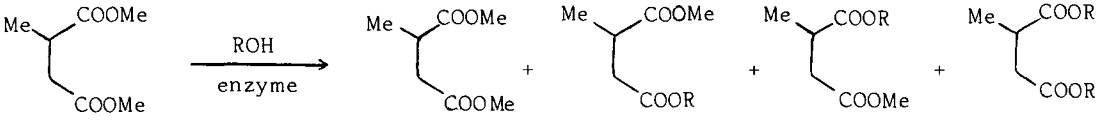
Abstract : The regio and enantioselectivities of the transesterification of racemic dimethyl esters by supported enzymes in absence of solvent are studied : preparations of chiral succinates and N-acetyl aminoesters are reported.

Enzymatic transesterification had been used for the enantioselective preparation of alcohols and esters (1). The enzyme was usually suspended in an organic solvent. To avoid enzyme-organic medium interface problems and to favor enzyme recovery, its immobilization on different supports had been reported for ester synthesis (2a), transesterification (2b) and recently ester hydrolysis (2c) : for these reactions a biphasic aqueous-organic mixture was often used.

We now report transesterifications by supported enzymes without added solvent. After transesterification of racemic dimethyl methylsuccinate 1, four compounds could be obtained : the remaining dimethyl ester 2 (racemic or not), the β and α monotransesterified compounds 3, 4 and the ditransesterified product 5. We tested three supports : alumina, silicagel and Florisil (3) and four different enzymes (4) : Pig pancreatic (PPL) and Candida cylindracea (CCL) lipases, α-chymotrypsin (α-CT) and Horse liver esterase (HLE). The supports were dried by heating two hours at 250°C under high vacuum then the enzyme-powders were added (5) and the mixture was stirred for one hour with a magnetic bar. The supported enzymes could be stored at room temperature for several months without loss of enzymatic activity. Transesterifications were made by a standard procedure : to one gram of the supported enzyme mixture 0.3g (1.875 mmol) of dimethyl methylsuccinate 1 and 0.4 g (6.66 mmol) of 1-propanol were added. After stirring (30 minutes) the mixture was left several days at 35°C then ether (5 ml) was added. After filtration, the filtrate was concentrated and analyzed by V.P.C. (overall yields 90-100% ; capillary quartz column 25 m CPSIL 5). The supported enzymes could be repeatedly used (with PPL the same reaction was made five times without rate change). If the supports were dried at 20°C (6) the reaction was faster (with PPL for example, we observed 50% reaction in four days instead of ten days), however the chemical yield was lower (70% instead 90% for the PPL reaction), probably due to a competitive hydrolysis (1).



(R,S) 1 2 3 4 5

To compare the selectivity of different enzyme-support couples, the transesterification of dimethyl methylsuccinate 1 with 1-propanol was stopped after seven days and compared with hydrolysis. The results are reported in the table. We found that on Florisil (or alumina) after 45 or 55% of transformation (7 or 10 days) (R)-dimethyl methylsuccinate 2 (95% ee (11)) and (S)-methylpropyl methylsuccinate 3 (95% ee) were respectively obtained.

Table : Transesterification and hydrolysis of dimethyl methylsuccinate 1

Entry	Enzyme	Transesterification (R = Propyl)						Hydrolysis (R = H)	
		Support	time (days)	Product ratio				Product ratio	
				2	3	4	5	3	4
a	PPL	Alumina	7	56	38[b]	4	2	90[b](7)	10
b		Silicagel	7	64	33[b]	2	1		
c		Florisil	7	52[a]	45[b]	1	2		
d	CCL	Alumina	7	73[c]	4	22[c]	1	10	90[c](8)
e		Florisil	7	86[c]	1	13[c]	0		
f	α-CT	Alumina	7	92[c]	4[c]	4[c]	0	10	90(9)
g		Alumina	21	80[c]	9[c]	9[c]	2		
h	HLE	Alumina	1	12[c]	15[c]	34[c]	39[c]	70[c]	30[c](8)

a) ee ⩾ 95%, R enantiomer ; b) ee⩾ 95%, S enantiomer ; c) Racemate (or low ee), (11).

While all enzyme catalyzed hydrolysis rates were similar, 50% of transesterification by CCL and α-CT occurred in one and two months respectively. Besides with α-CT we observed a modification of the regioselectivity (10)

Other primary alcohols gave similar results. For example with PPL-Florisil, allylic alcohol led from racemate 1 mainly to R-dimethyl methylsuccinate 2 (95% ee) (11) and S-mixed ester 3 (90% ee) (11) (overall yield 90%). Treatment of ester 3 with tributyltin hydride in the presence of palladium catalyst (12) gave the (S) -monomethyl ester (cleavage of the allyl ester) which was easily separable of the unchanged (R)-ester 2.

The transesterification of solid compounds was also possible even if they are poorly soluble in organic solvents (hexane, ether, ...) : racemic dimethyl N-acetylaspartate and dimethyl N-acetylglutamate in presence of PPL-alumina reacted with 1-propanol to give (in 7 hours and 24 hours respectively) quantitatively the D-dimethyl esters and the L- α-propyl— methyl esters (11) (overall yield : 90%).

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3) We used Florisil 100-200 mesh (Fluka), pH 9.2 ; neutral aluminum oxide, 100-125 mesh (Fluka Type 507C) pH 8.5 and silicagel 70-230 mesh (SDS), pH 7.

4) All enzymes purchased from Sigma were used as received. PPL was the crude enzyme and HLE a liver acetone powder.

5) 3 g of crude enzyme for 10 g of support (PPL, α-CT and CCL) or 6 g of acetone powder for 10 g of support (HLE).

6) At 20°C under high vacuum Florisil loss 1.2% of water (in weight) instead of 6% when heat at 250°C.

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11) ee determined by ^{1}H NMR (250 MHz) in the presence of Eu-(hfc)$_3$ (20-30% mol/mol).

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