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A CONVENIENT METHOD FOR ENZYMATIC BENZYL-ALKYL TRANSESTERIFICATION UNDER MILD NEUTRAL CONDITIONS

Arie L. Gutman,* Eleonora Shkolnik and Michal Shapira

Department of Chemistry, Technion - Israel Institute of Technology, Haifa 32000, Israel

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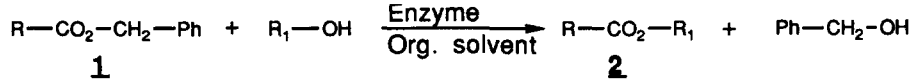
Abstract: Lipases from *Candida cylindracea* and from *Pseudomonas fluorescens* efficiently catalyse the benzyl to alkyl transesterification in organic solvents under mild conditions in nearly quantitative yields.

Benzyl esters are often chosen as carboxyl protecting groups since their transesterification is possible under mild conditions by a two step procedure: catalytic hydrogenation to the free carboxylic acid followed by esterification. The alternative one-step transesterification is usually carried out with acidic or basic catalysts under reflux.[1] Although several chemical[2] and enzymatic[3] methods have recently been reported on transesterification under mild conditions, in all cases the reactions involved the exchange of a light alkyl alcohol by a heavier or more bulky alcohol. One report describing a general method for mild transesterification specifically mentions its unsuitability for exchange of the benzyl ester group.[4] To our knowledge, only two groups reported on a benzyl to alkyl transesterification under mild conditions, however, the yields were relatively low and the procedures complicated.[5] As an extention of our studies on enzymatic reactions in organic solvents,[6] we explored the possibility of using lipases for transesterifications involving the exchange of a benzyl ester group by an alkyl group.

We now wish to report the facile and convenient enzyme-catalysed conversion of benzyl and dibenzyl esters into various alkyl esters (Scheme 1). Initial screening of five commercially available lipase preparations in organic solvents with benzyl benzoate (1a) and butanol revealed that the lipase from *Pseudomonas fluorescens* (PFL), and to a lesser extent the lipase from *Candida cylindracea* (CCL), catalysed the transesterification reaction.[8] PFL was shown to be active in transesterifying a wide range of other benzyl and dibenzyl esters with various aliphatic alcohols (Table I). CCL was also active in all cases, but catalysed the reactions at a lower rate, except for dibenzyl malonate (1e), where its efficiency was similar to that of PFL.



1 2

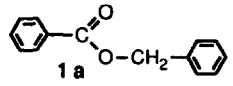
Scheme 1

Table I. Transesterification of benzyl esters with various alcohols catalysed by lipase from *Pseudomonas fluorescens* in organic solvents[a]

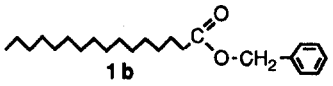
SUBSTRATE[7]	ALCOHOL (R_1OH)	% convers.[b] (24 hr)	%convers. (3 days)	%convers. (7 days)
Benzyl Benzoate (1a)	Methanol	59.0	73.7	82.0
	Ethanol	83.4	84.6	86.9
	n-Butanol	32.4	68.1	97.0
Benzyl Palmitate (1b)	Methanol	92.9	- -	- -
	Ethanol	87.2	- -	>99
	n-Butanol	96.8	97.7	>99
Benzyl Stearate (1c)	Methanol	92.5	--	>99
	Ethanol	93.9	--	>99
	n-Butanol	98.8	--	>99
Benzyl Mandelate (1d)	Methanol	21.7	- -	79.9
	Ethanol	6.6	- -	66.7
	n-Butanol	12.9	- -	89.2
Dibenzyl Malonate (1e)	Methanol	28.4	35.2	87.2
	Ethanol	51.2	65.3	67.8
	n-Butanol	68.6	88.2	94.7
Dibenzyl L-Tartrate (1f)	Methanol	66.9	- -	78.8
	Ethanol	73.4	- -	96.5
	n-Butanol	86.2	--	>99

a. The solvent was hexane, except for experiments with (1d) and (1f), which were carried out in tert-butyl methyl ether. No reaction took place in the absence of enzyme under the conditions used. The reactions were terminated by filtering off the enzyme and evaporating the solvent.

b. % conversion was monitored by comparing the relative intensities of the ^1H NMR signals corresponding to the esteric CH_2 benzyl group in the starting material and the esteric CH_2 or CH_3 alkyl groups of the product.

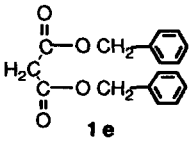


1a

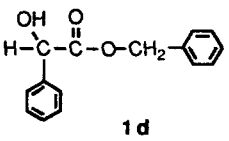


1b

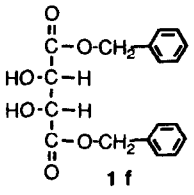
1c



1e



1d

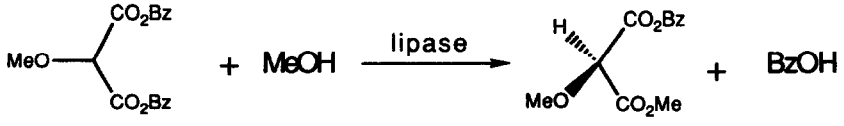


1f

As can be seen from Table I, the reaction rates were strongly dependent on the nature of the substrate and of the alcohol. Benzyl esters of simple, long-chain aliphatic acids (1b and 1c) were the most reactive and resulted in higher than 90% conversions with all three alcohols already after 24 hours. Transesterification rates of the less hydrophobic substrates were considerably lower and were dependent on the hydrophobicity and on the molar excess of the alcohol. Reactions with the least hydrophobic methanol were relatively slow, but reactivity improved with increasing hydrophobicity: MeOH < EtOH < n-BuOH. Examination of the influence of the molar ratio of alcohol to benzyl ester on the efficiency of transesterification revealed, that for methanol the optimal molar ratio was 2.5 and that any further increase in the methanol concentration resulted in the slowing down of the reaction, probably due to enzyme inactivation. On the other hand, with ethanol and n-butanol the reactions could be made considerably faster by increasing the molar ratio to 4.5 and 10 respectively. This observation is in line with the generally accepted rule, that enzymes are less stable in the presence of hydrophylic organic solvents, which are believed to inactivate the enzyme by stripping off its essential water layer.[9]

In a typical experiment 800 mg of powdered commercial lipase from *Pseudomonas fluorescens* were added to a solution of 500 mg benzyl palmitate (1b) and 0.52 mL ethanol (4.5 eq) in 15 mL of hexane, and the suspension was vigorously shaken at 39 °C and 200 rpm in a closed bottle. Aliquots were withdrawn periodically and their NMR spectra measured. Progress was monitored (to within ±5% accuracy) by comparing integration of the singlet at 5.09 ppm, corresponding to the CH_2 benzyl ester group of the starting material, and the triplet at 4.10 ppm, corresponding to the CH_2 ethyl ester group of the product. The reaction was allowed to proceed for 4 days until all of the starting material reacted. It was terminated by filtration to remove the insoluble enzyme, followed by evaporation of the solvent and excess ethanol. The product (2b) was separated from the resultant benzyl alcohol and from any traces of the starting material by bulb to bulb distillation to afford the pure ethyl palmitate in 82% yield.

Apart from proceeding under mild conditions, this enzyme-catalysed benzyl to alkyl transesterification may also have an advantage of catalysing the reaction in a stereospecific manner. Thus, we have shown, that when lipase from *Candida cylindracea* catalysed the transesterification of the prochiral methoxy-substituted dibenzyl malonate (3) with methanol in hexane, the reaction was highly stereospecific (Scheme 2).[6c] When the reaction was stopped at 50% conversion, examination of the isolated mixed diester (4) by polarimetry and HPLC on a chiral column revealed that the (+) enantiomer was formed with an ee value of 90-95%.[10] Higher conversion values were accompanied by significant formation of the byproduct, dimethyl ester, with a concomitant lowering in optical purity of (+)-4.



3 (+)-4

Scheme 2

In conclusion, it was shown that lipases from *Pseudomonas fluorescens* and from *Candida cylindracea* in organic solvents can act as practical catalysts in the benzyl to alkyl transesterification under mild conditions. This enzyme-catalysed benzyl to alkyl transesterification may also have an advantage of catalysing the reaction in a stereospecific manner.

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EXPERIMENTAL

General.

^1H NMR spectra were recorded on a bruker AM 200-MHz spectrometer in $CDCl_3$. All chemical shifts were reported in ppm with tetramethylsilane as internal standard. Enantiomeric excess (ee) was determined by Merck Hitachi HPLC on a chiral column (chiralcel OJ, Daicel). Optical rotations were determined on a JASCO digital polarimeter DIP-370. Distillations were performed on a glass tube oven Buchi GKR50. The shaker used for enzymatic experiments was a G24 environmental shaker incubator from New Bronswick Scientific Co.

The lipase from Candida cylindracea (CCL, EC 3.1.1.3) was purchased from Sigma Chemical Co. as a crude powder with a specific activity of 700 units/mg of solid. It has been recently redefined as lipase from *Candida cepacia*. Lipase from the Bacterium Pseudomonas fluorescens (PFL) was kindly provided by Amano Pharmaceutical Co. Unless otherwise stated, all solvents and other chemicals were obtained from commercial suppliers and were used without further purification. Substrates 1a and 1e were commercially available. Benzyl palmitate (1b) was synthesised from the commercially available ethyl palmitate and benzyl alcohol by the titanate-mediated transesterification according to the published method.[11] Methoxy dibenzyl malonate (3) was also prepared by the above method from the commercially available methoxy dimethyl malonate. Benzyl stearate (1c), benzyl mandelate (1d) and dibenzyl L-tartrate (1f) were prepared from the corresponding acids by esterification in the presence of the strongly acidic ion exchange resin Amberlyst 15, as described below for the preparation of 1c. All substrates were distilled prior to enzymatic reactions and their ^1H NMR spectra were consistent with structure.

Preparation of benzyl stearate (1c) (Representative procedure).

Stearic acid (14.2 g, 50 mmol) was dissolved in 30 ml benzyl alcohol and Amberlyst 15 (1 g) was added. The reaction mixture was kept under reflux overnight. After cooling, the Amberlyst was filtered off, the filtrate was diluted with 60 ml of ether, washed with 20 ml of saturated solution sodium bicarbonate and 20 ml of water and dried over sodium sulphate. The solvents were evaporated in vacuo and the residue was distilled by bulb to bulb distillation (at 70 °C and 0.05 mm Hg) to give 1c (12.1 g, 85% yield). 1d and 1f were prepared according to the same procedure in 83% and 84% yields respectively.

Kinetic Measurements (Representative procedure).

PFL-catalysed transesterification reactions were carried out as follows: a suspension of the enzyme (200 mg) in 3 ml of hexane containing the substrates (e.g. 29 μmole of benzyl palmitate and 72 μmol of methanol) was placed in a 4 ml screw-cap vial. The vial was shaken at 200 rpm and 40 °C. Periodically, different vials were withdrawn, the enzyme filtered off, the solvent evaporated and NMR spectra recorded. Reaction progress was determined to within ±5% accuracy by comparing the intensities of the singlet at 5.10 ppm, corresponding to the CH_2 benzyl ester group of the starting material, and the singlet at 3.64 ppm, corresponding to the CH_3 methyl ester group of the product. No reaction was observed in the absence of enzyme under the above mentioned reaction conditions.

Enzymatic benzyl-alkyl transesterification (Preparative representative procedure).

800 mg of powdered lipase from *Pseudomonas fluorescens* were added to a solution of 500 mg benzyl palmitate (1b) and 0.52 ml ethanol (4.5 eq) in 15 ml hexane and the suspension was vigorously shaken at 39 °C and 200 rpm in a closed bottle. Aliquots were withdrawn periodically and their NMR spectra measured. Progress was monitored in the same manner as during the kinetic measurements. The reaction was allowed to proceed for 4 days until all of the starting material reacted. It was terminated by filtration to remove the insoluble enzyme followed by evaporation of the solvent and excess ethanol. The product ethyl palmitate (2b) was separated from the resultant benzyl alcohol and from any traces of the starting material by bulb to bulb distillation (65 °C, 0.05 mm Hg) to afford 0.41 g of the pure ethyl palmitate (82% yield). ^1H NMR δ 0.84 (3H, t), 1.10-1.65 (29H, m), 2.25 (2H, t), 4.09 (2H, q).

Enzymatic transesterification of prochiral methoxy dibenzyl malonate with methanol.

Lipase from *Candida Cylindracea* (2.4 g) was added to a solution of methoxy dibenzyl malonate (3) (400 mg, 1.4 mmol) and methanol (0.32 g, 9.8 mmol) in 20 ml hexane. The suspension was shaken at 40 °C and 200 rpm. The reaction was terminated by filtering off the enzyme, followed by evaporation of the solvent. The components of this reaction were not separated and analysis was carried out on the crude residue, which also contained some of the remaining starting material (3). The sign of rotation was determined as (+) by polarimeter. The ee value of (+)-methoxy benzyl methyl malonate (4) was determined by HPLC analysis of the residue on a chiral column (Chiralcel OJ, Daicel) with a mixture of hexane and 2-propanol 95:5 as the mobile phase at the flow rate of 0.9 ml/min using detection at 258 nm. The R_t values were 85 min and 99 min (for the enriched (+) enantiomer). ^1H NMR of (4): δ 7.36 (5H, s), 5.26 (2H, s), 4.47 (1H, s), 3.77 (3H, s), 3.50 (3H, s).

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7. Substrates (1a) and (1e) were commercially available (Fluka and Aldrich respectively). All other substrates were prepared from the corresponding acids by esterification in the presence of the strongly acidic ion exchange resin Amberlyst 15 in benzyl alcohol, followed by conventional work-up procedure.

8. Only negligible transesterification was observed with porcine pancreatic lipase (Sigma), liver acetone powder horse (Sigma), liver acetone powder porcine (Sigma) and lipase SAM-II (Amano).

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10. The absolute configuration of (+)-(4), arbitrarily drawn as R, has yet to be determined.

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