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Enzyme catalysed synthesis of some adipic esters

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Abstract

Lipases from *Candida antarctica* (Novozym 435) and *Rhizomucor miehei* (Lipozyme IM) showed good reactivity in the alcoholysis of dimethyl adipate by racemic 2-ethylhexanol as well as neopentyl glycol. Novozym 435 was found to be the more reactive biocatalyst but less enantioselective compared with Lipozyme IM in the synthesis of methyl-2-ethylhexyl adipate. Di-2-ethylhexyl adipate obtained was optically inactive. Using dimethyl adipate and neopentyl glycol as starting material, oligomer esters with different range of molecular mass were synthesised (Novozym 435). An attempted of alcoholysis of dimethyl adipate by non-linear trihydric trimethylolpropane was unsuccessful.

Keywords: Lipase; Enantioselectivity; Alcoholysis; Adipic ester

1. Introduction

Some esters are used in lubrication because of their excellent properties, i.e. low volatility, high flash point, good thermal stability, low toxicity and good biodegradability [1]. One of the most important class of synthetic lubricants comprises esters of dicarboxylic acids, particularly adipic acid. Adipates are widely used due to their relatively low cost and good balance of properties. Simple dialkyl adipates (1) are made by reacting adipic acid or its dimethyl ester with monohydric alcohols. Oligomeric esters of adipic acid (2) can be formed in the reaction of dimethyl adipate with for example neopentyl glycol [2].

$$R_1\text{–OOC–}(CH_2)_4\text{–COO–}R_2$$
$$R_1, R_2 \rightarrow \text{alkyl group} \quad (1)$$

$$CH_3\text{–OOC–}(CH_2)_4\text{–CO[–OCH}_2\text{–C}(CH_3)_2\text{–CH}_2\text{–OOC–}(CH_2)_4\text{–CO]}_n\text{OCH}_3 \quad (2)$$

The method based on alcoholysis is especially suitable for synthesis of dicarboxylic esters and oligomeric esters. The alcoholysis reaction can be catalysed by both acids and bases or enzymatically [3]. The application of enzymes, including lipases, to organic synthesis has grown rapidly in recent years [4]. Generally, lipases are defined as glycerine hydrolases hydrolysing glycerides in oil–water systems [5]. However, lipases often show a wide activity spectrum, catalysing direct synthesis of some esters and their selective hydrolysis and alcoholysis. Analysing the catalytic activity of enzymes, one should pay close attention to their regio- and enantioselectivity [6]. Biocatalytic processes are used in the production of, e.g. pharmaceuticals [7,8], flavour materials [9,10], emulsifiers for food [11] and optically active surfactants [12].

This work presents an attempt to use lipases as biocatalysts for the synthesis of di-2-ethylhexyl adipate

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as well as oligomeric adipates of neopentyl glycol and trimethylolpropane.

2. Experimental

2.1. Materials

Immobilised lipases from *Candida antarctica* (Novozym 435) and from *Rhizomucor miehei* (Lipozyme IM) were kind gifts from Novo Nordisk. Dimethyl adipate, 2-ethylhexanol, neopentyl glycol and trimethylolpropane were purchased from Aldrich.

2.2. Synthesis of 2-ethylhexyl adipates

Methyl-2-ethylhexyl adipate was prepared by treating dimethyl adipate (0.1 mol) with 2-ethylhexanol (0.2 mol) in the presence of Novozym 435 or Lipozyme IM in the amount of 1 wt.%. The reaction was carried out in a glass open flask, which was shaken at 50°C on an incubator shaker. The progress of reaction was monitored by gas chromatography. GC analysis was performed using a HP 5890II chromatograph equipped with flame ionisation detector. The column used was a HP-Innowax (30 m × 0.32 mm × 0.25 μ). Upon completion of the reaction the reagents were filtered to remove the enzyme. The remaining substrates were separated by vacuum distillation.

The synthesis of di-2-ethylhexyl adipate was carried out analog to the above described conditions. However, the molar ratio of 2-ethylhexanol to dimethyl adipate was 4:1. The enantiomeric purity of 2-ethylhexanol was determined by GC technique using a HP-chiral-column (20% permethylated β-cyclodextrin, 30 m × 0.25 mm × 0.25 μ). The racemic 2-ethylhexanol was separated into two equal peaks of t_R 18.17 and 18.30 min. The enantiomeric excess of 2-ethylhexanol in synthesised adipates was determined after their alkaline hydrolysis (KOH in water–methanol solution).

The absolute configuration of chiral 2-ethylhexanol was determined by comparison of its optical rotation value, with that reported previously [12]. Optical rotations were measured by a Rudolf Research Autopol IV polarimeter.

2.3. Synthesis of oligomeric adipates

A mixture of neopentyl glycol (0.1 mol), dimethyl adipate (0.15, 0.2, 0.4, 0.6 mol) and Novozym 435 (1 wt.%) was shaken in an open glass flask at 50°C on an incubator shaker for the required period. The progress of the alcoholysis reaction was monitored by determining the amount of the methanol evolved. Typically the process was over after 10 h. The remaining dimethyl adipate was removed by vacuum distillation after separating lipase by filtration. The ^{1}H NMR spectra of synthesised compounds were taken on a Bruker Avauce DRX300 spectrometer for $CDCl_3$ solution with $(CH_3)_4Si$ as internal standard. Molecular weights of oligomeric esters were determined by Knauer pressure osmometer using chloroform as a solvent.

3. Results and discussion

3.1. Di-2-ethylhexyl adipate

Di-2-ethylhexyl adipate is formed in the following two-step reaction (3 and 4). Generally, these reactions are irreversible due to methanol removal from the reaction system.

$$CH_3OOC(CH_2)_4COOCH_3 + C_8H_{17}OH \rightarrow CH_3OOC(CH_2)_4COOC_8H_{17} + CH_3OH\uparrow \quad (3)$$

$$CH_3OOC(CH_2)_4COOC_8H_{17} + C_8H_{17}OH \rightarrow C_8H_{17}OOC(CH_2)_4COOC_8H_{17} + CH_3OH\uparrow \quad (4)$$

The test results were analysed with attention focused on the enantioselectivity of the used biocatalysts separately for the first and second step of the synthesis of di-2-ethylhexyl adipate (Table 1).

The synthesis of methyl-2-ethylhexyl adipate in the presence of Novozym 435 proceeded at a very high rate. Already after 45 min about 30% of the 2-ethylhexanol was reacted. Then the process was stopped to prevent the formation of di-2-ethylhexyl adipate. For Lipozyme IM a similar percentage of 2-ethylhexanol was reacted after 17 h. The syntheses of methyl-2-ethylhexyl adipate run, in general, with

Table 1
Two-step synthesis of di-2-ethylhexyl adipate using Lipozyme IM and Nowozym 435 as catalysts[a]

Substrates (mol)	Time (h)	The composition of post reaction mixture (mol)				α_D of remaining substrates	ee(*S*) (%)	ee(*P*) (%)	E[b]
		2EH	$AdMe_2$	AdMe2EH	$Ad(2EH)_2$				
$AdMe_2$ 0.1, 2EH 0.2; L	17	0.147	0.052	0.048	0.002	−0.07	10	20	2.0
$AdMe_2$ 0.1, 2EH 0.2; N	0.75	0.137	0.046	0.054	0.004	−0.16	4	8	1.3
$AdMe_2$ 0.1, 2EH 0.4; L	70	0.200	0.001	0.009	0.094	−0.04	2	2	1.1
$AdMe_2$ 0.1, 2EH 0.4; N	15	0.199	0.001	0.002	0.099	−0.01	0	0	1.0

[a] $AdMe_2$, dimethyl adipate; 2EH, 2-ethyl-hexanol; AdMe2EH, methyl-2-ethyl-hexyl adipate; $Ad(2EH)_2$, di-2-ethylhexyl adipate; L, Lipozyme IM; N, Nowozym 435.
[b] $E = \ln[(1 - c)(1 - \mathrm{ee}(S))]/\ln[(1 - c)(1 + \mathrm{ee}(S))]$.

low enantioselectivity. The values of the enantiomeric ratio (E) calculated according to Chen et al. [13] did not exceed 2.

The value of optical rotation of the remaining alcohol shows that the less reactive enantiomer was *R*-(−)-2-ethylhexanol ($\alpha_D^{20} = -3.3^0$, 55 g/l $CHCl_3$ [12]). This regularity was observed for both the reaction catalysed by Novozym 435 and the one catalysed by Lipozyme IM. The chromatographic data show that ee(*P*) of 2-ethylhexanol was 8 and 20% for the reaction catalysed by Novozym 435 and Lipozyme IM, respectively. In general, Novozym 435 in the synthesis of methyl-2-ethylhexyl adipate was characterised by higher catalytic activity but by lower enantioselectivity compared to Lipozyme IM.

For the synthesis of di-2-ethylhexyl adipate, twice as much 2-ethylhexanol was used as the amount given by the reaction stoichiometry. In the reaction proceeding in the presence of Novozym 435 all the dimethyl adipate was reacted to di-2-ethylhexyl adipate after 15 h. When Lipozyme IM was used to catalyse the alcoholysis reaction, this took 70 h.

The above results suggest that the substitution of the first methyl group by the 2-ethylhexyl group, catalysed by Novozym 435 or Lipozyme IM, was an enantioselective reaction and the preferred substrate was *S*-(+)-2-ethylhexanol. When the second group was substituted, it was found that the remaining 2-ethylhexanol did not show any significant optical activity. Also the chromatographically determined ratio of enantiomers in the remaining 2-ethylhexanol and in the 2-ethylhexanol obtained from the hydrolysis of di-2-ethylhexyl adipate showed that the ratio of the two enantiomers in them was close to 1.

To explain these phenomena, an additional experiment was performed. An attempt to synthesise di-2-ethylhexyl adipate using methyl-2-ethylhexyl adipate (separated by distillation) and racemic 2-ethylhexanol as starting material, was undertaken. Fig. 1 shows the dependence of extent of conversion (c) and excess of *R* enantiomer in remaining substrate ($\mathrm{ee_R}(S)$) on the reaction time. The extent of conversion of 2-ethylhexanol approached the value of 50% after 40 h of reaction. It appeared that this reaction also favoured *S*-(+)-2-ethylhexanol. However, on increasing the reaction time up to 70 h, a decrease in the enantiomeric purity of the remaining substrate, ee(*S*), from 16 to 4%, was observed.

Racemisation of di-2-ethylhexyl adipate probably occurred according to the following reversible reaction.

$$S\text{-}C_8H_{17}OOC(CH_2)_4COOC_8H_{17}\text{-}S + R\text{-}C_8H_{17}OH \leftrightarrow R\text{-}C_8H_{17}OOC(CH_2)_4COOC_8H_{17}\text{-}S + S\text{-}C_8H_{17}OH \quad (5)$$

The rate of above reaction was lower than that of synthesis of di-2-ethylhexyl adipate. Thus, the racemization was observed after prolonged reaction times.

3.2. Oligomeric adipates

The alcoholysis reaction of dimethyl adipate by neopentyl glycol is illustrated in Scheme 1. The product of this reaction is a mixture of compounds with average oligomerisation degree n. The oligomerisation degree theoretically should increase to infinity when the molar ratio of dimethyl adipate to neopentyl gly-

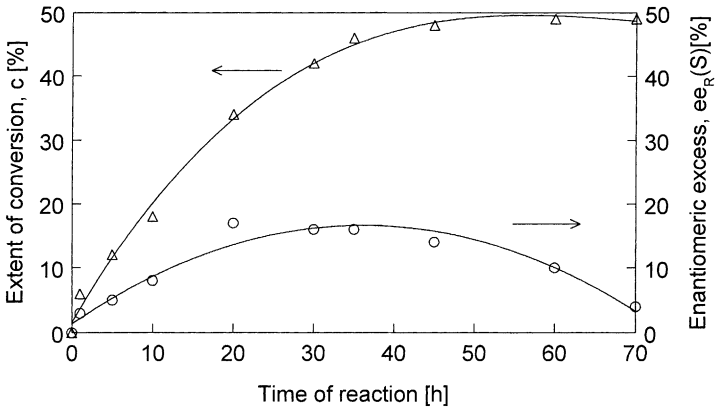


Fig. 1. Lipase (Lipozym 435) catalysed synthesis of di-2-ethylhexyl adipate using methyl-2-ethylhexyl adipate (0.1 mol) and (*R*, *S*)-2-ethylhexanol (0.2 mol) as starting material.

col approaches 1. In practice, the magnitude of n may be limited by many factors, e.g. by the enzyme's substrate selectivity.

To find out what the structure of a statistically average ester molecule is, one must determine, accurately enough, the ester's oligomerisation degree. The value of oligomerisation degree was estimated on the basis of the average molecular weight determined osmometrically and by a method based on ^{1}H NMR spectroscopy. The results of ^{1}H NMR spectroscopy of synthesised oligomeric esters are given below.

a, σ_1: 0.96 (18.6H, s); σ_2: 1.64–1.69 (16.2H, quintet, $J = 3.7$ Hz); σ_3: 2.32–2.37 (16.6H, t, $J = 7.5$ Hz); σ_4: 3.67 (6H, s); σ_5: 3.88 (12.3H, s); **b**, σ_1: 0.96 (11.6H, s); σ_2: 1.64–1.69 (12.2H, quintet, $J = 3.7$ Hz); σ_3: 2.32–2.37 (12.3H, t, $J = 7.5$ Hz); σ_4: 3.67 (6H, s); σ_5: 3.88 (7.7H, s); **c**, σ_1: 0.96 (7.4H, s); σ_2: 1.64–1.69 (9.5H, quintet, $J = 3.7$ Hz); σ_3: 2.32–2.37 (9.2H, t, $J = 7.5$ Hz); σ_4: 3.67 (6H, s); σ_5: 3.88 (5.2H, s); **d**, σ_1: 0.96 (7.2H, s); σ_2: 1.64–1.69 (9.0H, quintet, $J = 3.7$ Hz); σ_3: 2.32–2.37 (8.9H, t, $J = 7.5$ Hz); σ_4: 3.67 (6H, s); σ_5: 3.88 (4.7H, s).

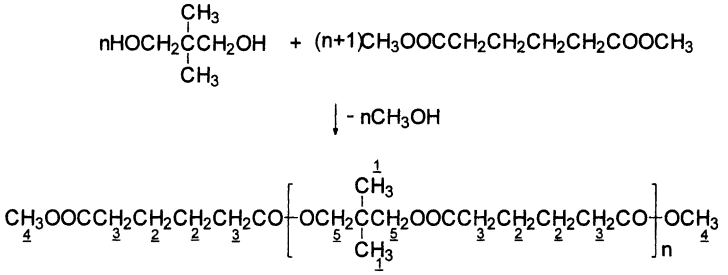
Proton signals from methyl group $-CH_3$ and methoxy group $-OCH_3$ are most easily identified. The ratio of the number of protons in $-CH_3$ and $-OCH_3$ groups is a good measure of oligomerisation degree. It follows from structural formula (Scheme 1) that

$$\sum H_{CH_3} = 6n \quad \text{and} \quad \sum H_{OCH_3} = 6$$

$$\frac{\sum H_{CH_3}}{\sum H_{OCH_3}} = n^{-1}$$

If n is known, then average molecular mass can be calculated from the equation

$$\langle M_{NMR} \rangle = 174 + 214n$$



Scheme 1. The synthesis reaction of oligomeric ester from neopentyl glicol and dimethyl adipate. The specified number refers to the same ^{1}H NMR chemical shift.

Table 2
The structural parameters and physicochemical properties of oligomeric esters synthesised from dimethyl adipate and neopentyl glycol using Novozym 435 as biocatalyst[a]

Substrates	n	M_{NMR}	M_O	Viscosity (cSt), 40°C	Melting point (°C)
Neopentyl glicol/dimethyl adipate					
a: 0.1/0.15	3.10	837	910	101.4	−49
b: 0.1/0.2	1.94	589	640	70.2	−50
c: 0.1/0.4	1.24	437	446	25.3	−53
d: 0.1/0.6	1.20	431	437	23.1	−55
Trimethylol propane/dimethyl adipate					
0.1/0.3	No reaction				

[a] n, Oligomerisation degree; M_{NMR}, molecular mass calculated from ^{1}H NMR; M_O, molecular mass determined osmometrically.

The average molecular mass for the synthesised esters and their basic physicochemical properties are presented in Table 2. The molecular mass values of synthesised oligomers calculated on the base of ^{1}H NMR were comparable with those determined osmometrically.

The obtained results show that Novozym 435 is an effective biocatalyst for the synthesis not only of simple alkyl esters of adipic acid but also of esters with the structure of linear oligomers. An attempt to transesterify enzymatically the trimethylolpropane, which possesses a non-linear structure was unsuccessful.

The degree of oligomerisation of enzymatically synthesised adipic esters depends strongly on the molar ratio of the substrates, dimethyl adipate to neopentyl glycol. For the ratio of 1.5:1, the oligomerisation degree is close to 3.1. When the dimethyl adipate to neopentyl glycol molar ratio increases to a level of 4:1 and 6:1, the average oligomerisation degree decreases quickly to a level of 1.23 and 1.20, respectively. As the oligomerisation degree decreases so does the viscosity of the synthesised esters and their melting points. It can be calculated that from the same parent substance one can obtain products varying widely in their physicochemical properties.

Oligomers of adipic acid and neopentyl glycol can be also made chemically [14]. The use of biocatalysts allows one to obtain compounds of similar molecular weight under very mild conditions. The proposed method makes it possible to produce oligomeric esters with a wide range of viscosity and good functional properties, which can be useful lubricants [1,14]. Conceivably, biocatalysis will be used to a great extent for the manufacture of lubricants in the future.

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