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Size selectivity in lipase catalysed tetrol acylation

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ABSTRACT

Size selectivity of *Candida antarctica* lipase B (CAL-B) was examined in the acylation of pentaerythritol with oleic acid. Biolubricant mixtures consisting of mono-, di-, tri-, and tetraoleates were expected in variable excess. Enzymatic tetraoleate formation was suppressed under solvent conditions; however, this size selectivity was lost without solvent and tetra-acylated pentaerythritol accumulated in up to 93%. The lipase caused size selectivity persisted over a broad temperature range from 35 to 95 °C. A Fischer–Speier esterification showed that substrate bulkiness was only a minor contributor to observed size selectivity. All in all, switch on/off size selectivity using CAL-B allowed to vary pentaerythritol biolubricant compositions in an unprecedented manner.

1. Introduction

Lipase catalysed acylation of symmetric pentaerythritol with fatty acids potentially yields mono-, di-, and triacylated esters, and eventually the less obvious tetraester. Native lipases catalyse mono-, di-, and triglycerolester hydrolysis and by inverse hydrolysis its formation; a fourth acylation would lead to an unusual large fat molecule. Such tetraester formation is possible with achiral pentaerythritol, which is a tetrol and acylated once more than the triol glycerol. The lipase mediated synthesis of pentaerythritol tetraesters is therefore a good platform to test lipase controlled size selectivity. Pentaerythritol tetraester hydrolysis was examined before with rat pancreatic lipase and no hydrolysis occurred [1]. Every lipase is different and in this work, *Candida antarctica* lipase B is used for its potential in size selective catalysis. The entry channel with native CAL-B is rather narrow, 10 Å × 4 Å wide and 12 Å deep [2]. In view of this small entry to the catalytic site it is well possible that larger pentaerythritol tetraolates cannot be synthesised. This assumption is in line with observations that short chain fatty acids are esterified faster than longer ones [3]. The hydrolysis rate of common triglycerides was even further reduced by engineering a more restricted entry channel which enhanced size selectivity [4].

Size selectivity is a phenomenon with consequences for stereo and regio selectivity. For example the asymmetrisation of cyclic meso-1,2-dicarboxylates using porcine liver esterase showed exceptional size selectivity effects [5]. The size of the meso ring influenced R/S meso-trick hydrolysis selectivity as it was reversed from S to R with increasing ring size. Another example is lactone hydrolysis by CAL-B where increasing lactone ring sizes reversed R and S selectivity in ring opening polymerisation [6]. Also other polycondensations showed size selectivity using lipases [7]. Size selectivity is also reported for proteases. Serine prolyl oligopeptidase hydrolysed peptide chains after proline in terminal protein sections with up 30 amino acids [8].

Lipase catalysed esterifications in organic solvents is widely described [9]. Hydrophobic organic solvents with a log *P* around 2 support well water free (0.01% H_2O content) lipase catalysis [10,11]. Solvent-free lipase catalysis is another possibility and an attractive option for industrial application. CAL-B showed before size selectivity with trimethylolpropane, which is in this work further explored [12].

To examine size selectivity effects in pentaerythritol oleate synthesis a fast and reliable analytical method was required. Gas chromatography (GC) and high pressure liquid chromatography (HPLC) are not well suited as oleic acid mixtures contain also other fatty acids (>10%). This multiplies product peaks with each additional acylation on a tetrol. Any chromatographic method is therefore of limited use. Pentaerythritol tetraesters are widely described but it is not excluded that in many instances they

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were less pure than thought due to the absence of a simple analytical method to assay its esterification degree using bio and chemical catalysis. Recently, an alternative convenient ^{1}H NMR method was developed and proven successful in the analysis of trimethylolpropan oleate biolubricant mixtures [12]. A 400 MHz NMR spectrometer in combination with THF-d_8 separated the three possible methylene singlets H-C(2) of the three acylation degrees and allowed product distribution analyses. Such chemical shift separation was not observed with other deuterated solvents such as chloroform ($CDCl_3$) [13] or methanol (CD_3OD). It was hypothesised that the four possible pentaerythritol oleates can equally be identified using this novel and convenient ^{1}H NMR method.

Pentaerythritol esters are high performance lubricants in aircraft engines [14] and various industrial machinery, but are also used as consistency wax in cosmetics [15]. It is also used, for example, as lubricant in new green refrigeration systems because it dissolves carbon dioxide that is nowadays used instead of halogen alkanes [16].

In the following pentaerythritol is acylated with oleic acid by CAL-B catalysis (Fig. 1). Size selectivity and product distribution will be influenced by various process parameters, such as presence and absence of solvent, stoichiometry, and temperature. A Fischer–Speier esterification shall distinguish substrate bulkiness from size selectivity by *C. antarctica* lipase B.

2. Experimental

2.1. Material

C. antarctica Lipase B, Novozym® 435 (10,000 PLU/g) was obtained from Novozymes A/S [Bagsværd, Denmark]. 3 Å Molecular sieve from Merck KGaA, [Darmstadt, Germany] was activated before use. Oleic acid (90%) and pentaerythritol were purchased at Sigma-Aldrich Chemie [Taufkirchen, Germany]. Tert-butanol (99%) was from Fluka Analytical [Buchs, Switzerland], and THF-d_8 from Armar AG [Döttingen, Switzerland].

2.2. General procedure for lipase catalysis in tert-butanol

0.27 g (2 mmol) pentaerythritol and 0.58 g (2 mmol) oleic acid were added (1:1 stoichiometry) to a 25 mL vial. Also other oleic acid quantities were used: 1.15 g (4 mmol), 1.70 g (6 mmol), and 2.26 g (8 mmol). 0.14 g Novozym® 435 (CAL-B) (50% weight of pentaerythritol) was employed with all substrate ratios. 0.8 g molecular sieve 3 Å was added to adsorb condensing water; and finally, 2 mL tert-butanol was filled in. The sealed vial was fixed vertically on a rotating cylinder (8 rpm) in a hybridisation oven and processed at 65 °C for up to 15 days. For reaction control an aliquot of reaction mixture was taken, tert-butanol evaporated, and a ^{1}H NMR/THF-d_8 recorded to determine conversion, yield and product (**1**–**4**) distribution.

2.3. Solvent screening for optimal lipase catalysis

A 1:4 (pentaerythritol/oleic acid) substrate mixture was screened using various organic solvents (2 mL) according to the general method. After processing the immobilised lipase and molecular sieves were filtered off and the solvent evaporated with a Rotavap at 25 mbar or on a high vacuum stand. An aliquot of the raw reaction mixture was analysed by ^{1}H NMR.

2.4. Solvent-free lipase catalysis

The same preparation and process conditions, without solvent, according to the general procedure.

2.5. Sulphuric acid catalysis

A series of 25 mL vials were filled with substrates as in the general procedure but without solvent. 5% concentrated sulphuric acid (96%) was added (based on pentaerythritol weight). The vials were sealed and fixed on a rotating (8 rpm) cylinder in a 100 °C preheated hybridisation oven. At defined times vials were removed and aliquots of reaction mixture were analysed by ^{1}H NMR/THF-d_8.

2.6. Conversion, yield and product distribution by ^{1}H NMR

A single ^{1}H NMR experiment provided complete reaction progress information. The solvent of any reaction mixture was evaporated and aliquots thereof dissolved in 0.5 mL THF-d_8 and a ^{1}H NMR recorded on a 400 MHz NMR spectrometer (Bruker Avance). This NMR-solvent separated the chemical shifts for all four possible products **1**–**4** and also oleic acid conversion was assayed at the same time. The pentaerythritol-oleates **1**–**4** distribution was determined by integrating H-C(2) singlets found at: 4.05 ppm (**1**), 4.06 (**2**) 4.08 ppm (**3**), and 4.11 ppm (**4**). Their integrams were divided by related acylation degrees (1, 2, 3, 4) and the results were normalized to 100%. The oleic acid conversion was quantified by the H-C(2′) triplet that shifted from 2.19 to 2.29 ppm upon esterification.

2.7. Analytical and preparative chromatography

High pressure liquid chromatography (HPLC) was realised with an Agilent PN 993967-906 and Eclipse XDB-C8 column, 4.6 × 150 mm, 5 μm; and a Charged Aerosol Detector, CAD-Ultra ESA Dionex. Fractions from selected peaks were collected and eluted a second time. Isolated compounds were then analysed by ^{1}H NMR for purity. Also preparative product isolation using silica gel normal pressure chromatography with EtOAc–heptane (1:2) was realised [12]. The separation quality was controlled by thin layer chromatography (TLC) and two separation runs were required.

2.8. Analytical data

2.8.1. Pentaerythritol monooleate (*1*)

$_\delta$H (400 MHz, THF-d_8): 5.33 (m, 2H, *HC*=*CH*, H-9′, H-10′); 4.05 (s, 2H, OCH_2, H_{b1}-2); 3.61 (t, 3H, O*H*, H-2); 3.51 (d, 6H, OCH_2, H_a-2); 2.27 (t, 2H, COCH_2, H-2′); 2.04 (m, 4H, CH_2. H-8′, H-11′); 1.57 (t, 2H, CH_2, H-3′); 1.32–1.29 (bm, 20H,CH_2, H-4′-7′, H-12′-17′), 0.89 (t, 3H, CH_3, H-18′).

$_\delta$C (100 MHz, THF-d_8): 173.23 (*C*=O, C-1′); 130.38 (*C*=*C*, C-9′, C-10′); 63.34 (C(O)OCH_2, C-2); 61.82 (HOCH_2, C-2); 45.15 (*C*(CH_2)$_4$, C-1); 34.49 (C(O)CH_2, C-2′); 32.75, 30.60, 30.57, 30.37, 30.16, 30.13, 30.09, 29.99, 29.96, CH_2, C-4′-7′, C-12′-16′; 27.9 (CH_2, C-8′,C-11′); 23.44 (CH_2, C-3′,C-17′); 14.32 (CH_3, C-18′).

2.8.2. Pentaerythritol dioleate (*2*)

$_\delta$H (400 MHz, THF-d_8): 5.32 (m, 4H, *HC*=*CH*, H-9′, H-10′); 4.06 (s, 2H, OCH_2, H_{b2}-2); 3.53 (m, 4H, OCH_2, H_a-2); 2.28 (t, 4H, COCH_2, H-2′); 2.03 (m, 8H, CH_2, H-8′, H-11′); 1.57 (t, 4H, CH_2, H-3′); 1.33–1.30 (bm, 40H, CH_2, H-4′-7′, H-12′-17′); 0.89 (t, 6H, CH_3, H-18′).

$_\delta$C (100 MHz, THF-d_8): 173.23 (*C*=O, C-1′); 130.38 (*C*=*C*, C-9′, C-10′); 63.34 (C(O)OCH_2, C-2); 61.82 (HOCH_2, C-2); 45.15 (*C*(CH_2)$_4$, C-1); 34.49 (C(O)CH_2, C-2′); 32.75, 30.6, 30.57, 30.37, 30.16, 30.13, 30.09, 29.99, 29.96 (CH_2, C-4′-7′, C-12′-16′); 27.9 (CH_2, C-8′,C-11′); 23.44 (CH_2, C-3′,C-17′); 14.32 (CH_3, C-18′).