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Clean-chemistry synthesis of biodegradable tertiary α-substituted carboxylic acids from the corresponding esters through enzymatic processes

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Abstract

A variety of lipases and esterases were tested for the hydrolysis of carboxylic acid ester **1** or similar tertiary α-substituted esters. Carboxylic acid ester **1** is not readily biodegradable under normal hydrolytic conditions. However, the resulting carboxylic acid **2** is biodegradable. In our study we have found that of the biocatalysts used, the most effective were *Candida antarctica* lipase A (CAL-A), *C. antarctica* lipase B (CAL-B), and pig liver esterase (PLE). In addition, different ratios of cosolvents have been studied. CAL-B and PLE catalyzed the hydrolysis of ester **1** in 4 h at 30 °C, whereas CAL-A hydrolyzed **1** in 22 h.

Keywords: Enzymatic hydrolysis; Biotransformations; Lipases; Esterases; Famoxate®

1. Introduction

Enzyme-catalyzed chemical transformations have been recognized as practical alternatives to conventional organic syntheses [1–4]. In general, these catalysts are inexpensive and in many cases able to accept a wide range of structurally different substrates [5–7]. Moreover, biocatalysts are ecologically beneficial natural catalyst. In recent years, new catalytic synthetic methods in organic chemistry, which satisfy increasingly stringent environmental constraints, are in great demand by the pharmaceutical and chemical industries. In our work related to an alternative synthesis of the fungicide Famoxate® [1] small amounts of carboxylic acid ester intermediates remained that needed to be hydrolyzed since they are not readily biodegradable. In contrast, the corresponding carboxylic acids are environmental friendly and easily degraded. At present, hydrolysis by conventional procedure involves high temperatures and long reaction times. The hydrolysis of esters by means of hydrolytic enzymes, such as proteases [3,8], lipases [9,10], and esterases [11,12] has become a well-established method for their resolution [13]. Here we have studied in detail the biodegradation

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[1] Famoxate® is a DuPont registered trademark for technical DPX-JE874.

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process, by means of an enzymatic reaction, of ethyl 2-hydroxy-2-(4-phenoxyphenyl)propanoate (**1**), the main byproduct among several tertiary α-substituted carboxylic acid esters that are present in the mother liquids of the alternate synthetic pathway to fungicide Famoxate®.

2. Experimental

2.1. Chemicals

Chromobacterium vicosum lipase (CVL, 3800 U/mg of solid) and *Candida antarctica* lipase B (CAL-B, Novozym 435, 7300 PLU/g) were a gift from Genzyme and Novo Nordisk Co., respectively. Immobilized *Pseudomonas cepacia* lipase (PSL-C, 783 U/g) was obtained from Amano Pharmaceutical Co. Porcine pancreas lipase (PPL, type II, 46 U/mg of protein using triacetin), *Candida rugosa* lipase (CRL, type VII, 950 U/mg of solid), and porcine liver esterase [PLE, 260 U/mg of protein, 3.2 M $(NH_4)_2SO_4$ solution pH 8] were purchased from Sigma. *C. antarctica* lipase A (CAL-A, chirazyme L-5, c-f, lyo., 1000 U/g using tributyrin) was obtained from Roche. Esterase from *Rhizomucor miehei* (RM, 25.4 kU/ml) was purchased from Jülich Fine Chemicals. Esterase E020 (5.5 U/mg) was a gift from ThermoGen.

2.2. Analytical HPLC

High performance liquid chromatography (HPLC) analyses were carried out in a Hewlett Packard 1100 chromatograph UV detector at 230 nm using a Zorbax C8 column (0.46 cm × 25 cm) and 43% MeCN/H_2O (pH 3) as gradient eluent. The column temperature was 40 °C. Flow 0.9 ml/min. Pressure column ≈300 bars. Substrate **1** appeared at 10.48 min and product **2** at 6.02 min.

2.3. Enzymatic hydrolysis of ethyl 2-hydroxy-2-(4-phenoxyphenyl)propanoate (1)

In a standard procedure, CVL (20 mg), CAL-B (60 mg), CRL (120 mg), PSL-C (60 mg), PLE (100 μl), PPL (120 mg), RM (134 ml), E020 (15 mg) or CAL-A (120 mg) were added to a solution of ester **1** (100 mg, 0.349 mmol) in 3.5 ml of 0.1 M KH_2PO_4 (pH 7) and the appropriate cosolvent (ratios are indicate in Tables 1–3). The suspension was shaken at 250 rpm and the progress of the reaction was followed by HPLC analysis. The mixture was filtered, organic solvents were evaporated and the solution was extracted with CH_2Cl_2 (3 ml × 5 ml). The organic phase was dried and concentrated in vacuum. The crude residue was sonicated in hexane (50 ml) and the solid was filtered and dried to give compound **2** as a white solid. Melting point: 126–128 °C; IR (KBr): ν 3421, 2978, 2935, 2650, 1726, 1590, and 1492 cm^{-1};

Table 1
Hydrolysis of ester **1** catalyzed by CAL-B using several cosolvents

Entry	Solvent	Ratio (buffer:cosolvent)	*t* (h)	**2** (%)[a]
1	None		4	99
2	1,4-Dioxane	10:0.1	4	98
3	THF	10:0.1	5	99
4	MeCN	10:0.1	6	99
5	Acetone	10:0.1	3	98
6	1,4-Dioxane	10:1	4	97
7	THF	10:1	22	94
8	MeCN	10:1	13	98
9	Acetone	10:1	13	97
10[b]	THF	10:1	16	97
11[b]	MeCN	10:1	4	97
12[b]	Acetone	10:1	4	96
13[b]	1,4-Dioxane	10:2	8	96
14[b]	THF	10:2	32	92
15[b]	MeCN	10:2	32	96
16[b]	Acetone	10:2	32	97

[a] Percentage of hydrolysis calculated by HPLC.
[b] These reactions were set with 120 mg of CAL-B, instead of 60 mg.

Table 2
Hydrolysis of ester **1** catalyzed by CAL-A using several cosolvents

Entry	Solvent	Ratio (buffer:cosolvent)	*t* (h)	**2** (%)[a]
1	None		22	94
2	1,4-Dioxane	10:1	46	58
3	THF	10:1	22 (46)	1 (24)
4	MeCN	10:1	46	23
5	Acetone	10:1	46	85
6	1,4-Dioxane	10:2	46	39
7	THF	10:2	22	17
8	MeCN	10:2	46	75
9	Acetone	10:2	46	85

[a] Percentage of hydrolysis calculated by HPLC.

Table 3
Hydrolysis of ester **1** catalyzed by PLE using several cosolvents

Entry	Solvent	Ratio (buffer:cosolvent)	*t* (h)	**2** (%)[a]
1	None		4	97
2	1,4-Dioxane	10:1	22	94
3	THF	10:1	70	90
4	MeCN	10:1	56	88
5	Acetone	10:1	70	80
6	1,4-Dioxane	10:2	94	82
7	THF	10:2	94	46
8	MeCN	10:2	80	35
9	Acetone	10:2	70	34

[a] Percentage of hydrolysis calculated by HPLC.

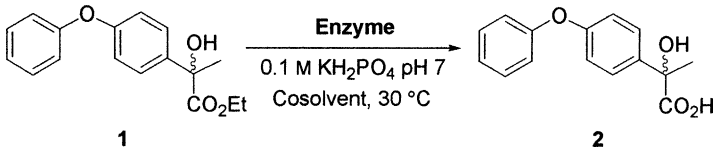
^{1}H NMR (MeOH-d_4, 200 MHz): δ 1.95 (s, 3H, Me) and 7.10–7.77 (m, 9H, ArH); ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ: 27.9, 77.2, 119.7, 120.4, 125.0, 128.6, 131.4, 140.4, 158.7, 159.1, and 179.1; MS (ESI$^-$, m/z): 258 (M^-, 18%), 257 [$(M–H)^-$, 100], and 213 [$(M–CO_2H)^-$, 51].

3. Results and discussion

For an initial screening of suitable lipases and esterases, enzymatic hydrolyses of **1** were carried out in 0.1 M phosphate buffer (pH 7) with CAL-B, CAL-A, PSL, PSL-C, CRL, PPL, CVL, E020, PLE, and RM as biocatalysts (Scheme 1). Among all lipases studied only CAL-A and CAL-B catalyzed the hydrolysis of **1** to **2** (Fig. 1). Shorter reaction times were achieved with CAL-B as compared to CAL-A. CAL-B completely hydrolyzes ester **1** in just 4 h at 30 °C. In contrast, 22 h are needed in the case of CAL-A. Of the esterases tested, PLE was the only active catalyst in the hydrolysis of **1**. Reaction times for this enzyme are similar to those for CAL-B.

The influence of added cosolvents on the catalytic activity of the enzymes was studied using different ratios of a variety of organic solvents. In CAL-B catalyzed hydrolyses, 1,4-dioxane, THF, acetonitrile, and acetone were employed as cosolvents in an initial ratio of buffer:solvent 10:0.1 (entries 2–5, Table 1). The progress of the enzymatic reaction in 1,4-dioxane was very similar to the process in absence of solvent (entry 1, Table 1). After 1 h, a 1:1 mixture of **1** and **2** was obtained, being isolated almost exclusively **2** after 4 h at 30 °C. Longer reaction times were observed when THF or acetonitrile were employed. If acetone is used a 98% conversion was achieved in 3 h. As evident from entries 6–9 in Table 1, an increase in the ratio buffer:cosolvent upto 10:1 leads to a decrease in the activity of CAL-B, except in the case of 1,4-dioxane. The order of reactivity was 1,4-dioxane > acetonitrile ≈ acetone > THF. When the same process was carried out with double amount of CAL-B, the hydrolyses of **1** were faster being maintained the above mentioned relative reactivity in the different solvents (entries 10–12, Table 1). As expected the enzymatic reactions using a ratio buffer:cosolvent of 10:2 were slower and longer reaction times were required to reach high conversions (entries 13–16, Table 1). This effect is particularly evident in Fig. 2, in which the formation percentage of compound **2** using CAL-B at different ratios buffer:THF is plotted. Thus, two first curves from the left, corresponding to no cosolvent (●) and ratio 10:0.1 (□), are very similar. In approximately 4 h the reactions were complete with 98–99% conversion. In contrast, 22 h was necessary to obtain 94% conversion when the ratio was 10:1 (◆). If the amount of biocatalyst is doubled, the hydrolysis is faster (curves (○) versus (◆)). It is possible to see clearly that if the process takes place in a ratio 10:2, even with double amount of the enzyme, the reaction is slower (■).

In the hydrolyses catalyzed by CAL-A two ratios of buffer:cosolvent (10:1 and 10:2) were tested. In a 10:1 ratio the order of reactivity as a function of cosolvent was acetone > 1,4-dioxane > acetonitrile ≈



1 2

Scheme 1.

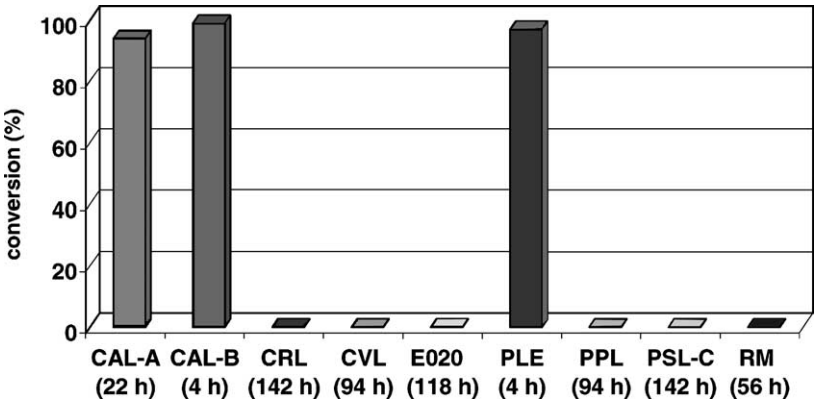


Fig. 1. Screening of lipases and esterases in the hydrolysis of ester **1**.

THF (since at 46 h, 24% of compound **2** was formed) (entries 2–5, Table 2). Similar to CAL-B, the hydrolysis of **1** is faster in absence of solvent (entry 1, Table 2). When the reaction takes place with a 10:2 ratio, CAL-A has shown similar catalytic activity in acetone but lower in 1,4-dioxane in comparison with the process at 10:1 (entries 2 and 5 versus 6 and 9, Table 2). However, if THF or acetonitrile are used as cosolvents, an increase in the activity of this lipase as a result of a larger ratio buffer:cosolvent were observed (entries 3 and 4 versus 7 and 8, Table 2).

Enzymatic hydrolysis experiments of **1** with PLE in a 10:1 ratio (buffer:cosolvent) revealed that, as CAL-B and CAL-A, shorter reaction times are achieved without organic solvent (entry 1, Table 3). PLE activity decrease in the order 1,4-dioxane > acetonitrile > THF > acetone (entries 2–5, Table 3). In the case of larger ratio of organic solvent (i.e. 10:2) this effect is higher being necessary several days to reach conversions closest to 50% (entries 6–9, Table 3).

Similar results, as above mentioned to compound **1**, were achieved when tertiary α-substituted methyl ester was used (Fig. 3).

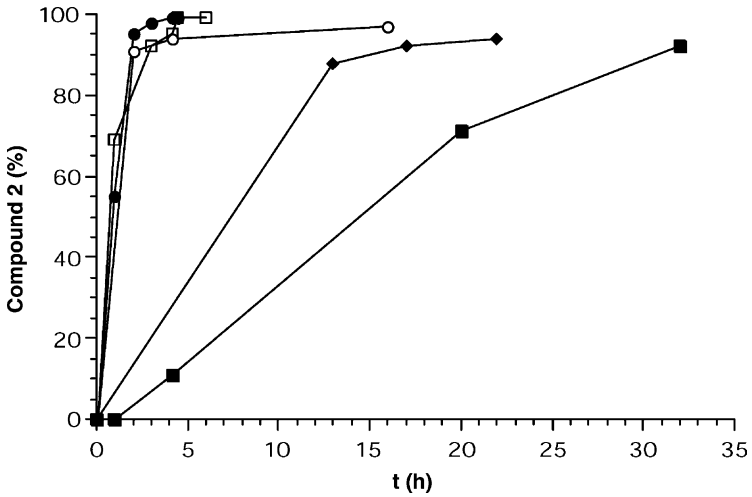


Fig. 2. Hydrolysis of 1 with CAL-B at different ratios buffer:THF; (●) none, 60 mg CAL-B; (□) 10:0.1, 60 mg CAL-B; (◆) 10:1, 60 mg CAL-B; (○) 10:1, 120 mg CAL-B; (■) 10:2, 120 mg CAL-B.

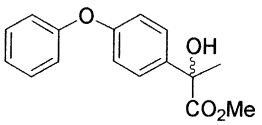


Fig. 3.

4. Conclusions

Lipases CAL-A and CAL-B, and esterase PLE catalyzed the hydrolysis of ester **1** or similar intermediates to the corresponding carboxylic acid, which is a biodegradable product. A study regarding the influence of several cosolvents at different ratios has been carried out. For the three active enzymes, shorter reaction times were achieved in absence of organic solvents. CAL-B and PLE hydrolyzed ester **1** in 4 h at 30 °C whereas 22 h was necessary with CAL-A. The enzyme of choice for this industrial biodegradation is CAL-B since this lipase gives shorter reaction times and it is immobilized on a polymer-supported being easily recovered by conventional filtration techniques and reused in successive runs.

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