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Ultrasound in enzymatic resolution of ethyl 3-hydroxy-3-phenylpropanoate

Carlos Magno R. Ribeiro,* Elisa N. Passaroto and Eugênia C. S. Brenelli

Universidade Federal Fluminense, Instituto de Química, Departamento de Química Orgânica, Campus Valonguinho, Niterói, 24020-150 Rio de Janeiro, Brazil

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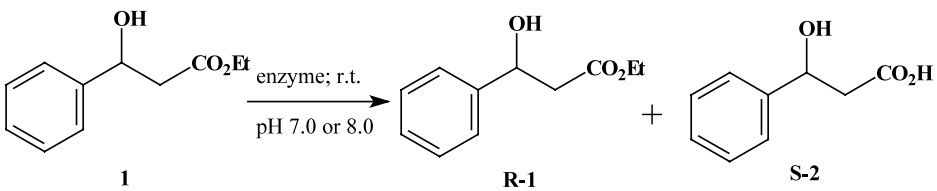
Abstract—The enzymatic hydrolysis of ethyl-3-hydroxy-3-phenylpropanoate using ultrasound bath and PCL, PLE and CRL enzymes was studied. The application of ultrasound bath led to an appreciative decrease in the reaction time of enzymatic hydrolysis without a significant change in the yield or enantiomeric excess of reaction products, when compared with the use of magnet stirring.

Enzyme catalysis field has achieved the interest of organic chemists due to their synthetic application.[1] The production of enantiomerically pure natural products and synthons for use in asymmetric synthesis, as well as resolution of alcohol and esters, are some useful examples of these methodologies. During the development of this approach, many kinds of enzymes have been used, for example pig liver esterase (PLE), *Pseudomonas cepacia* lipase (PCL), *Candida rugosa* lipase (CRL), *Aspergillus oryzae* protease (AOP).[1]

The enzymatic hydrolysis of ethyl 3-hydroxy-3-phenylpropanoate (**1**) and of other ester derivatives has attracted the interest of research groups because of the use of 3-hydroxy-3-phenylpropanoic acid (**2**) as an important intermediary in antidepressants synthesis.[2] Such reactions have been reported with *Pseudomonas* sp.,[2] PLE[3] (Eq. (1)), and in the hydrolysis of 1-*O*-acetyl derivative using pennicillin G amidohydrolase[4a] (PGA) and lipase A (Amano).[4b]



1 **R-1** **S-2** (1)

Due to our interest in this reaction, we decided to reinvestigate the enzymatic hydrolysis of ethyl 3-hydroxy-3-phenylpropanoate (**1**).[5] Table 1 lists some of our results using magnetic stirring (method A, entries 1–4).

The use of ultrasound is an interesting tool in organic chemistry: numerous reviews and papers have demonstrated its importance.[6] However, the applications of ultrasound in enzymatic reactions have not been extensively investigated.[7]

In this paper, we evaluated the influence of ultrasound[8] in the enzymatic hydrolysis of ethyl 3-hydroxy-3-phenyl propanoate (**1**). The ester **1** was obtained by a classic Aldol reaction,[9] and the enzymes used here were PCL, PLE and CRL (purchased from Sigma), as also used in our first study.[5] The results using an ultrasound bath[10] are presented in Table 1 (method B, entries 5–8).

As shown in Table 1 (entries 5–8), as expected, the best results were obtained when PCL was used. The enzymatic hydrolysis of racemic **1** at 50% conversion gave the remaining *R*-**1** in >98% e.e. and the acid *S*-**2** in 76% e.e. (entry 6). When PLE was used, the ester *R*-**1** was recovered in 60% yield (29% e.e.) and the acid *S*-**2** was prepared in 38% yield and 91% e.e. (entry 7). When CRL was used, the recovered ester **1** was obtained as a racemate and the acid **2** was obtained in 11% yield in

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* Corresponding author. Fax: 55-21-7193349; e-mail: gqocmrr@vm.uff.br

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Table 1. Enzyme-catalysed hydrolysis of **1** at rt, with magnetic stirring (A) and ultrasound bath (B)

Entry	Method	Enzyme (units)	Reaction time	pH	Conversion (%)	% *R*-**1**[a] (% e.e)[b]	% *S*-**2**[a] (% e.e.)[c]	*E*[e]
1	A	PCL (0.45)	6 h	7.0	22	60 (25)	22 (79)	21
2	A	PCL (0.45)	43 h	7.0	50	49 (>98)	47 (85)	458
3	A	PLE (50)	40 min	8.0	40	60 (36)	23 (58)	5
4	A	CRL (150)[d]	7 days	7.0	40	60 (15)	32 (37)	2
5	B	PCL (0.45)	3 h	7.0	22	71 (26)	22 (81)	32
6	B	PCL (0.45)	24 h	7.0	50	35 (>98)	50 (76)	458
7	B	PLE (50)	0.5 h	8.0	40	60 (29)	38 (91)	3
8	B	CRL (150)[d]	27 h	7.0	40	56 (04)	11 (46)	1

[a] Determined based on the sign of the specific rotation previously described.
[b] e.e. determined by ^{1}H NMR with Eu(tfc)$_3$.
[c] Determined by $[\alpha]_D$.
[d] Value in mg.
[e] E = enantiomeric ratio = $\ln[(1-c)(1-\text{e.e.}(S))]/\ln[(1-c)(1+\text{e.e.}(S))]$.[12]

46% e.e. (entry 8). A simple separation of the ester *R*-**1** and the acid *S*-**2** was achieved by aqueous acid–base extraction. The same extraction sequence has been applied in enzymatic resolutions and racemization of *S*-**2** was not observed under these conditions.[2,5] The pH of the enzymatic hydrolysis was maintained constant with the continuous addition of 0.25 M aqueous NaOH until the desired conversion.

Comparison of the results with magnetic stirring and ultrasound bath (methods A and B, respectively), we observed in all experiments an appreciative decrease in the reaction time of the enzymatic hydrolysis without a significant change in the yield or enantiomeric excess of the products (Fig. 1 and Table 1). When PCL was used, the application of the ultrasound bath did not alter the reaction enantioselectivity. On the other hand, some different results were observed when PLE and CRL were tested under these conditions. These results can be observed through the *E* values on Table 1. When PLE was used the e.e. decreased in the recovered ester *R*-**1**, from 36 to 29%, but the e.e. of the *S*-**2** acid increased from 58 to 91% (entries 3 and 7). The reverse result was observed when CRL was used (entries 4 and 8).

Ethyl 3-cyclohexyl-3-hydroxy-3-phenylpropanoate[11] hydrolysis using PLE was also tested and the results led to the same conclusion. The enzymatic hydrolysis of racemic ester at 40% conversion gave the remaining *R*-ester in 50% yield (5% e.e.), and the racemic corresponding acid in 32% yield (method A).[5] When an ultrasound bath was used at the same conversion, the *R*-ester was recovered in 45% yield (3% e.e.), and the racemic acid was prepared in 48% yield.[11] In this case, there was also observed an appreciative decrease in the reaction time of the enzymatic hydrolysis without a significant change in the yield or enantiomeric excess of the products, despite the poor enantioselectivity reaction, 5 days to hydrolysis without ultrasound and 27 h when ultrasound was used. From these results (method A or B) a decrease in the enantiomeric excess and an increase in the reaction time may be observed when a hydrogen on C-3 is replaced by a cyclohexyl group, showing that for larger groups at C3 the enzyme was not selective.

The results described in this paper complement studies which were carried out in different reaction conditions. They have demonstrated that the use of ultrasound bath in enzymatic resolutions can reduce the reaction time in the enzymatic hydrolysis, without altering the reaction enantioselectivity and the enzyme activity. Therefore, this methodology is of interest in synthetic application. Others substrates are under current investigation in order to extend the generality of and to improve the methodology.

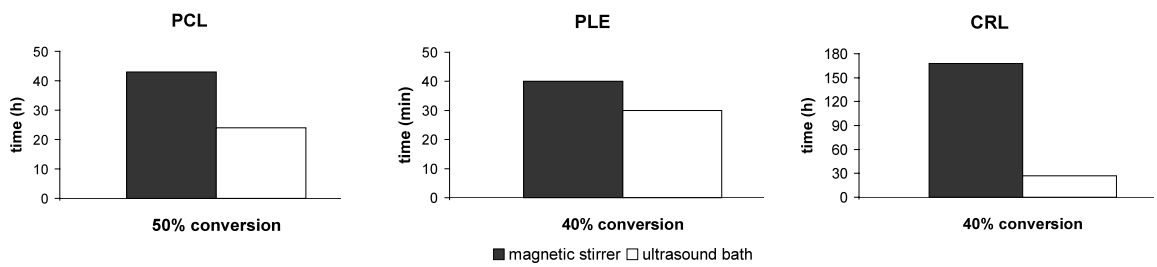

Figure 1. Magnetic stirrer versus ultrasound bath in reaction time of ester **1** hydrolysis.

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CAPES and PROPP/UFF.

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10. Typical procedure: Racemic ester **1** (1 mmol) was combined with pH 7 or 8 (Table 1) phosphate buffer (10 ml) and the enzyme (units; Table 1) at room temperature. The reaction was maintained in an ultrasound bath, during the time specified in Table 1. The pH was maintained constant by continuous addition of 0.25 M aqueous NaOH. After the conversion (Table 1) the reaction mixture was diluted with aqueous $NaHCO_3$ and extracted three times with ether, the combined organic solution was dried ($MgSO_4$) and concentrated to afford (*R*)-**1** (the % yield and e.e. are presented in Table 1). The combined aqueous solution was acidified to pH 1 with 3N HCl and extracted three times with ether. The extracts were dried ($MgSO_4$) and concentrated to afford acid (*S*)-**2** (the % yield and e.e. are presented in Table 1). Ethyl 3-hydroxy-3-phenylpropanoate **1**:[2] Colorless oil. **^{1}H NMR** (300 MHz, $CDCl_3$), δ (ppm): 1.27 (3H, t, $J=7.2$ Hz); 2.70 (1H, dd, $J=16.4$ Hz and 4.8 Hz); 2.77 (1H, dd, $J=16.4$ Hz and 8.4 Hz); 3.27 (sl; -OH); 4.18 (2H, q, $J=7.2$ Hz); 5.13 (1H, dd, $J=8.4$ and 4.8 Hz); 7.26–7.40 (5H, m). **^{13}C NMR** (75 MHz, $CDCl_3$), δ (ppm): 14.0; 43.2; 60.8; 70.2; 125.6; 127.7; 128.4; 142.4; 172.3. 3-Hydroxy-3-phenylpropanoic acid **2**:[2] mp = 115°C. **^{1}H NMR** (300 MHz, $CDCl_3$), δ (ppm): 2.78 (1H, dd, $J=16.5$ Hz and 3.9 Hz); 2.86 (1H, dd, $J=16.5$ and 9.0 Hz); 3.80 (sl; -OH); 5.17 (1H, dd, $J=9.0$ and 3.9 Hz); 7.28–7.41 (5H, m). **^{13}C NMR** (75 MHz, $CDCl_3$), δ (ppm): 44.0; 70.3; 125.9; 127.4; 128.4; 144.8; 172.2.
11. Ethyl 3-cyclohexyl-3-hydroxy-3-phenylpropanoate: Colorless oil; **^{1}H NMR** (300 MHz, $CDCl_3$), δ (ppm): 0.97–1.77 (12H, m), 1.03 (3H, t, $J=7.2$ Hz), 2.85 (1H, d, $J=15.6$ Hz), 3.01 (1H, d, $J=15.6$ Hz), 3.96 (2H, q, $J=7.2$ Hz), 7.18–7.49 (5H, m); **^{13}C NMR** (75 MHz, $CDCl_3$), δ (ppm): 13.7, 26.2, 26.9, 42.2, 42.2, 48.6, 60.4, 77.1, 125.7, 126.5, 127.6, 144.9, 173.4. 3-Cyclohexyl-3-hydroxy-3-phenylpropanoic acid: mp 174°C; **^{1}H NMR** (300 MHz, $CDCl_3$), δ (ppm): 0.88–1.74 (11H, m), 2.90 (1H, d, $J=15.9$ Hz), 3.06 (1H, d, $J=15.9$ Hz), 7.20–7.36 (5H, m); **^{13}C NMR** (75 MHz, $CDCl_3$), δ (ppm): 26.1, 26.3, 26.4, 26.7, 26.9, 41.5, 48.7, 77.0, 125.5, 126.8, 127.8, 144.3, 177.3.
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