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Tetrahedron: *Asymmetry* 19 (2008) 549–553

# Screening of liver acetone powders in the resolution of 1-phenylethanols and 1-phenylpropanols derivatives

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Received 5 November 2007; accepted 31 January 2008

**Abstract**—Hydrolases from the liver acetone powders (LAPs) of bovine, cat, chicken, turkey, lamb, pig, rabbit, and rat were assessed for the enantioselective hydrolysis of acetates of 1-(4-chlorophenyl)ethanol, 1-(3-bromophenyl)ethanol, 1-(4-chlorophenyl)propanol, 1-(4-bromophenyl)propanol, and 1-(3-bromophenyl)propanol. The enantioselectivity of the hydrolytic reaction was dependent upon the liver hydrolase, substrate, pH of the reaction media, and the cosolvent. The most ester selective LAP was from chicken, and the resulting alcohols had the highest ee (80% to >99%). All of the LAPs tested catalyzed the hydrolysis of 1-(4-chlorophenyl)ethanol, except for lamb LAP.


## 1. Introduction

Optically active secondary alcohols are useful intermediates in the synthesis of biologically active compounds, such as drugs and agrochemicals. For example, chlorophenylpropanols have antifungal properties against *Botrytis cinerea*, which damages economically important crops,[1] while other phenylethanol derivatives also have antifungal properties.[2,3] Optically active *sec*-alcohols can be prepared by asymmetric reduction of the corresponding ketone,[4,5] and by resolution of the racemic *sec*-alcohols via enantioselective oxidation.[6–8] Asymmetric hydrogen transfer is based on alcohol dehydrogenases that require nicotinamide cofactors, which have the disadvantage of requiring cofactor recycling.[9] The resolution of racemic *sec*-alcohols via enantioselective hydrolysis of their corresponding esters can be carried out using hydrolases that include lipases from microbial origin[10] and esterases from animal origin, specifically from the liver.[11] Liver acetone powders (LAPs) from different animals were used as crude sources of esterases,[11] which have the advantage of not going through the tedious and expensive process of purification. These LAPs constitute inexpensive and accessible sources of this type of enzymes.

Herein, LAPs from bovine (BLAP), cat (CALAP), chicken (CLAP), turkey (TLAP), lamb (LLAP), pig (PLAP), rabbit (RLAP) and rat (RALAP) were used in the resolution of 1-phenylethanols and 1-phenylpropanols by the hydrolysis of their corresponding acetates. The influence of the reaction conditions was evaluated to obtain high conversion and enantiomeric excess.

## 2. Results and discussion

### 2.1. Effect of pH

Esterases from bovine, cat, chicken, turkey, lamb, pig, rabbit and rat LAPs were tested to perform the hydrolysis of **1a** (Fig. 1). As pH is an important factor that can affect the rate and enantioselectivity of enzymatic reactions, the hydrolysis of **1a** was investigated at pH values of 6.0, 7.0 and 8.0 using the LAPs previously mentioned. The results are shown in Table 1.

The rate and enantioselectivity of the reaction varied markedly depending on the source of the enzyme employed, although the stereochemical preference remained unchanged. The preferred absolute configuration of the resulting alcohol was *R*, irrespective of the LAP used. Of the esterases tested, LAPs from turkey, lamb, rat and

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doi:10.1016/j.tetasy.2008.01.041

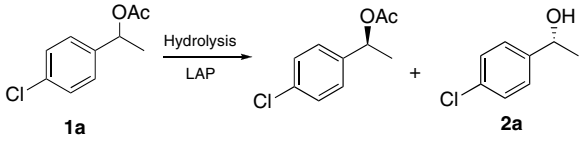


**Figure 1.**

**Table 1.** Effect of pH and LAP source on the biocatalyzed hydrolysis of **1a**

| Entry | LAP | pH | t (h) | % Conv. **2a**[a] | % ee **2a**[b] |
|---|---|---|---|---|---|
| 1 | BLAP | 6.0 | 24 | 10 | 46 |
| 2 | BLAP | 7.0 | 24 | 10 | 76 |
| 3 | BLAP | 8.0 | 24 | 13 | 72 |
| 4 | CALAP | 6.0 | 24 | 12 | 89 |
| 5 | CALAP | 7.0 | 24 | 22 | 82 |
| 6 | CALAP | 8.0 | 24 | 23 | 82 |
| 7 | CLAP | 6.0 | 24 | 25 | 80 |
| 8 | CLAP | 7.0 | 24 | 43 | 74 |
| 9 | CLAP | 8.0 | 6 | 35 | 60 |
| 10 | PLAP | 6.0 | 6 | 31 | 85 |
| 11 | PLAP | 7.0 | 4 | 53 | 78 |
| 12 | PLAP | 8.0 | 2 | 41 | 75 |
| 13 | RLAP | 6.0 | 24 | 44 | 45 |
| 14 | RLAP | 7.0 | 24 | 69 | 3 |
| 15 | RLAP | 8.0 | 24 | 55 | 0 |
| 16 | RALAP | 6.0 | 24 | 12 | 28 |
| 17 | RALAP | 7.0 | 24 | 63 | 18 |
| 18 | RALAP | 8.0 | 24 | 40 | 22 |
| 19 | TLAP | 7.0 | 24 | 57 | 11 |
| 20 | LLAP | 7.0 | 48 | 8 | nd |

Cosolvent: acetonitrile; *T* 25 °C; nd: not determined due to low conversion.
[a] Determined by GC.
[b] Determined by chiral HPLC.

rabbit biocatalyzed the hydrolysis of **1a**, but the stereoselectivity of the reaction was very low (Table 1, entries 14–20). With regard to the other LAPs tested, the conversion and enantioselectivity were dependent on the pH of the reaction media. In the case of the LAPs from cat, chicken and pig, the highest ee was reached at pH 6.0 (entries 4, 7, and 10, which correspond to 89%, 80% and 85% ee for cat, chicken and pig LAPs, respectively, Table 1); however, the conversion was better at pH 7.0 for CLAP and PLAP and at pH 8.0 for CALAP. With BLAP, the best enantioselectivity was obtained at pH 7.0 (Table 1, 76% ee, entry 2); however, the conversion remained low at all the pHs tested.

## 2.2. Effect of the cosolvent

As **1a** is water insoluble and the reaction was carried out in aqueous media, it was necessary to use an organic solvent to help the dissolution of **1a** in the reaction media. Water miscible solvents THF, 1,4-dioxane, DMSO, DMF and acetonitrile were used to determine their influence on the enantioselectivity of the hydrolysis of **1a**. The pH of the reaction media, for each LAP, was selected from the best results shown in Table 1. The results shown in Table 2 enabled us to establish that the cosolvents tested had little impact on the ee of **2a** when TLAP, LLAP, RLAP and RALAP were used as the biocatalysts. These facts confirm that these sources of esterases were not stereoselective

**Table 2.** Effect of the cosolvent on the biocatalyzed hydrolysis of **1a**

| Entry | LAP | Cosolvent | pH | % Conv. **2a**[a] | % ee **2a**[b] |
|---|---|---|---|---|---|
| 1 | BLAP | THF | 7.0 | 8 | nd |
| 2 | BLAP | Dioxane | 7.0 | 34 | 88 |
| 3 | BLAP | DMSO | 7.0 | 48 | 69 |
| 4 | BLAP | DMF | 7.0 | 23 | 89 |
| 5 | BLAP | Acetonitrile | 7.0 | 10 | 76 |
| 6 | CALAP | THF | 8.0 | 9 | 70 |
| 7 | CALAP | Dioxane | 8.0 | 32 | 81 |
| 8 | CALAP | DMSO | 8.0 | 36 | 78 |
| 9 | CALAP | DMF | 8.0 | 16 | 74 |
| 10 | CALAP | Acetonitrile | 8.0 | 23 | 82 |
| 11 | CLAP | THF | 6.0 | 25 | 75 |
| 12 | CLAP | Dioxane | 6.0 | 28 | 71 |
| 13 | CLAP | DMSO | 6.0 | 29 | 78 |
| 14 | CLAP | DMF | 6.0 | 74 | 40 |
| 15 | CLAP | Acetonitrile | 6.0 | 25 | 80 |
| 16 | TLAP | THF | 7.0 | 5 | nd |
| 17 | TLAP | Dioxane | 7.0 | nd | nd |
| 18 | TLAP | DMSO | 7.0 | 86 | 11 |
| 19 | TLAP | DMF | 7.0 | 63 | 22 |
| 20 | TLAP | Acetonitrile | 7.0 | 57 | 11 |
| 21 | LLAP | THF | 7.0 | 1 | nd |
| 22 | LLAP | Dioxane | 7.0 | 2 | nd |
| 23 | LLAP | DMSO | 7.0 | 5 | nd |
| 24 | LLAP | DMF | 7.0 | 2 | nd |
| 25 | LLAP | Acetonitrile | 7.0 | 8 | nd |
| 26 | PLAP | THF | 6.0 | 23 | 90 |
| 27 | PLAP | Dioxane | 6.0 | 38 | 81 |
| 28 | PLAP | DMSO | 6.0 | 35 | 79 |
| 29 | PLAP | DMF | 6.0 | 37 | 82 |
| 30 | PLAP | Acetonitrile | 7.0 | 31 | 85 |
| 31 | RLAP | THF | 7.0 | — | nd |
| 32 | RLAP | Dioxane | 7.0 | 10 | nd |
| 33 | RLAP | DMSO | 7.0 | 98 | 5 |
| 34 | RLAP | DMF | 7.0 | 66 | 18 |
| 35 | RLAP | Acetonitrile | 7.0 | 69 | 3 |
| 36 | RALAP | THF | 7.0 | — | nd |
| 37 | RALAP | Dioxane | 7.0 | 25 | 28 |
| 38 | RALAP | DMSO | 7.0 | 75 | 17 |
| 39 | RALAP | DMF | 7.0 | — | nd |
| 40 | RALAP | Acetonitrile | 7.0 | 63 | 18 |

*T* 25 °C, 24 h; nd = not determined due to low conversion.
[a] Determined by GC.
[b] Determined by chiral HPLC.

toward the hydrolysis of **1a** under the reaction conditions used.

The biocatalytic behavior of CALAP, BLAP, CLAP and PLAP depended on the cosolvent used. The enantioselectivity of the reaction biocatalyzed by CALAP was favored in the presence of dioxane and acetonitrile (81% and 82% ee of **2a**, entries 7 and 10, respectively, Table 2), by BLAP in the presence of dioxane and DMF (88% and 89% ee of **2a**, entries 2 and 4, respectively, Table 2), by CLAP in the presence of DMSO and acetonitrile (78% and 80% ee of **2a**, entries 13 and 15, respectively, Table 2), and by PLAP in the presence of THF and acetonitrile (90% and 85% ee of **2a**, entries 26 and 30, respectively, Table 2). Among the LAPs tested, BLAP, CLAP, CALAP and PLAP proved to be the most enantioselective toward the biocatalyzed hydrolysis of **1a** and hence the next experiments were conducted with these LAPs only.

### 2.3. Effect of the structure of acetates 1a–1e

Acetates **1a**–**1e** (Fig. 2) were selected to determine the effect of the alcohol structure on the enantioselectivity of the hydrolytic reaction biocatalyzed by BLAP, CLAP, CALAP and PLAP. The reactions were carried out under the best conditions determined in the previous experiments for each LAP (Tables 1 and 2), and the results are shown in Table 3.

Acetate **1a** was well accepted by BLAP, CALAP, CLAP and PLAP, the enantiomeric excess of alcohol **2a** was higher than 80% (Table 3, entries 1–4), but the conversion extent depended on the LAP used; the highest enantioselectivity and conversion were observed using BLAP as biocatalyst (88% and 34%, respectively, $E = 34$, entry 1, Table 3). It was interesting to note that the biocatalyzed hydrolytic reaction of 3-bromophenylethanol acetate **1b** proved to be highly enantioselective with three of the four LAPs tested, BLAP, CALAP and CLAP (>99% ee **2b**, $E > 100$, entries 5–7, Table 3); furthermore, when CALAP was used as a biocatalyst, the conversion was the highest possible in this kind of resolution (51%, entry 6, Table 3).

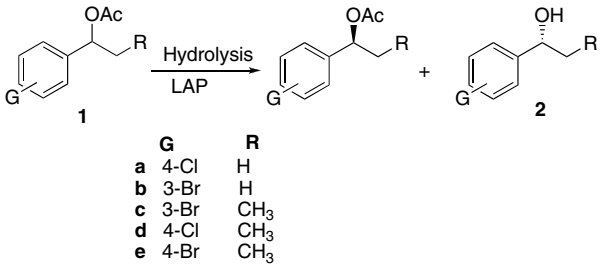


**Figure 2.**

With the other *meta*-substituted, 3-bromophenylpropanol acetate **1c**, the results were irregular, the conversion was high in the case of BLAP, but the enantioselectivity was moderate (48% and 63% of **2c**, respectively, entry 9, Table 3); in contrast to this, the hydrolysis biocatalyzed by CLAP was highly enantioselective, but the conversion was lower (>99% and 20% of **2c**, respectively, entry 11, Table 3). For the hydrolysis of acetate **1d** using, as the source of hydrolase, BLAP and CLAP, the enantioselectivity was similarly high, but the conversion was lower with BLAP and moderate with CLAP (ee >99%, $E > 100$%, 17% and 30% of conversion of **2d**, respectively, entries 13 and 15, Table 3). In the case of acetate **1e**, only CLAP gave a high enantiomeric excess of alcohol **2e**, but a low conversion (91% and 12%, respectively, entry 19, Table 3). Although PLAP is the most widely used source of esterase in synthetic procedures, it proved to be the least active LAP in this work.

In all cases, the configuration of the main enantiomer of the resulting alcohols **2a**–**2e** was (*R*), which was determined by HPLC comparing the retention times against authentic samples.[7,8] The configuration of **2a** was determined by comparing the specific rotation values with data from the literature.[6]

## 3. Conclusion

Most of the LAPs tested biocatalyzed the hydrolysis of acetate **1a**. RALAP and TLAP hydrolyzed the acetate to a high extent but without enantioselectivity. LAP from lamb

**Table 3.** Effect of the structure of acetates **1a**–**1e**

| Entry | Substrate | LAP | pH | Cosolvent | *t* (h) | % Conv. **2**[a] | % ee **2**[b] | *E*[c] |
|---|---|---|---|---|---|---|---|---|
| 1 | **1a** | BLAP | 7.0 | Dioxane | 24 | 34 | 88 | 34 |
| 2 | **1a** | CALAP | 8.0 | Dioxane | 24 | 32 | 81 | 13 |
| 3 | **1a** | CLAP | 6.0 | Acetonitrile | 24 | 25 | 80 | 11 |
| 4 | **1a** | PLAP | 6.0 | THF | 24 | 23 | 90 | 24 |
| 5 | **1b** | BLAP | 7.0 | Dioxane | 73 | 29 | >99 | >100 |
| 6 | **1b** | CALAP | 8.0 | Dioxane | 50 | 51 | >99 | >100 |
| 7 | **1b** | CLAP | 6.0 | Acetonitrile | 49 | 12 | >99 | >100 |
| 8 | **1b** | PLAP | 6.0 | THF | 50 | 12 | 40 | 2.5 |
| 9 | **1c** | BLAP | 7.0 | Dioxane | 24 | 48 | 63 | 7.8 |
| 10 | **1c** | CALAP | 7.0 | Dioxane | 48 | 22 | 35 | 2.3 |
| 11 | **1c** | CLAP | 6.0 | Acetonitrile | 49 | 20 | >99 | >100 |
| 12 | **1c** | PLAP | 6.0 | THF | 73 | 6 | nd | — |
| 13 | **1d** | BLAP | 7.0 | Dioxane | 73 | 17 | >99 | >100 |
| 14 | **1d** | CALAP | 8.0 | Dioxane | 49 | 40 | 60 | 5.9 |
| 15 | **1d** | CLAP | 6.0 | Acetonitrile | 73 | 30 | >99 | >100 |
| 16 | **1d** | PLAP | 6.0 | THF | 73 | 7 | nd | — |
| 17 | **1e** | BLAP | 7.0 | Dioxane | 24 | 57 | 76 | 16 |
| 18 | **1e** | CALAP | 7.0 | Dioxane | 48 | 5 | nd | — |
| 19 | **1e** | CLAP | 6.0 | Acetonitrile | 49 | 12 | 91 | 23 |
| 20 | **1e** | PLAP | 6.0 | THF | 73 | 3 | nd | — |

*T* 25 °C; nd = not determined due to low conversion.
[a] Determined by GC.
[b] Determined by chiral HPLC.
[c] Ref. 12.

did not catalyze the hydrolytic reactions studied. Among all the LAPs used, only BLAP, CALAP, CLAP and PLAP proved to be enantioselective, but to different extents, toward the hydrolysis of acetates **1a**–**1e**. Their biocatalytic activity was strongly influenced by the reaction conditions such as pH and cosolvent. With regard to the alcohol structure, the most active LAP was CLAP because the resulting alcohols **2a**–**2e** had the highest enantiomeric excesses (81% to >99%).

## 4. Experimental

### 4.1. Materials and instruments

Infrared spectra were recorded on a Perkin–Elmer Paragon 1600 FT as liquid films, $^1$H NMR spectra on a Varian 400 MHz instrument in $CDCl_3$ using tetramethylsilane as internal reference and TLC on Silica Gel 60 $GF_{254}$ Merck. HPLC analysis was performed on an Agilent 1100 liquid chromatograph, equipped with a diode array detector, and using Chiracel OB-H and OJ-H columns. GC analysis was performed on a Hewlett–Packard HP 6890 gas chromatograph, equipped with a flame ionization detector and HP-5 column (30 m × 0.33 mm) and nitrogen as carrier.

All racemic alcohols were prepared from the corresponding aldehyde and Grignard reagent, while the alcohols were converted to their *O*-acetyl esters following the standard procedure using acetic anhydride and triethylamine. All compounds were purified by silica gel column chromatography using hexanes–ethyl acetate as eluent and were characterized by IR and $^1$H NMR.

### 4.2. Liver acetone powders (LAPs)

The corresponding livers were purchased in local stores or obtained as gifts from the University animal facilities. First, the excess fat was removed from the liver, and then washed with water. It was then ground three times with acetone in a blender, and the powder was filtered, dried, and stored at 5 °C.

### 4.3. General procedure for enzyme-mediated hydrolysis

About 0.4 mL of a buffer phosphate solution (0.1 M, pH 6.0, 7.0 and 8.0) and 10 mg of LAP were added to 5 mg of **1a**–**1e** in 0.1 mL of a cosolvent. The mixture was stirred at 25 °C (for reaction times, refer to Tables 1–3), then extracted twice with methylene chloride. The organic phase was dried over $Na_2SO_4$ and the solvent evaporated under reduced pressure to dryness. Conversion % was determined by GC ([peak area of alcohol/peak area of alcohol + peak area of acetate] × 100) and % ee by HPLC using a chiral column.

### 4.4. (±)-1-(4-Chlorophenyl)ethanol, (±)-2a

GC, column HP5, 140 °C, 1 mL/min, $t_{\mathbf{2a}} = 1.69$ min, $t_{\mathbf{1a}} = 2.39$ min; HPLC, OB-H column, hexanes–isopropanol 98:2, 0.7 mL/min, $t_R = 18.57$ min, $t_S = 21.31$ min; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.44 (3H, d, $J = 6.4$ Hz), 4.83 (1H, q, $J = 6.4$ Hz), 7.2–7.45 (4H, m).

### 4.5. 1-(3-Bromophenyl)ethanol, (±)-2b

GC, column HP5, 120 °C, 1 mL/min, $t_{\mathbf{2b}} = 2.88$ min, $t_{\mathbf{1b}} = 4.51$ min; HPLC, OB-H column, hexanes–isopropanol 98:2, 0.7 mL/min, $t_R = 19.93$ min, $t_S = 23.39$ min; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.45 (3H, d, $J = 6.4$ Hz), 4.84 (1H, q, $J = 6.4$ Hz), 7.19–7.48 (4H, m).

### 4.6. 1-(3-Bromophenyl)propanol, (±)-2c

GC, column HP5, 120 °C, 0.8 mL/min, $t_{\mathbf{2c}} = 4.66$ min, $t_{\mathbf{1c}} = 8.34$ min; HPLC, OJ-H column, hexanes–isopropanol 97:3, 0.7 mL/min, $t_R = 38.19$ min, $t_S = 41.63$ min; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.83 (3H, t, $J = 7.2$ Hz), 1.58–1.77 (2H, m), 4.45 (1H, t, $J = 6.7$ Hz), 7.12–7.40 (4H, m).

### 4.7. 1-(4-Chlorophenyl)propanol, (±)-2d

GC, column HP5, 120 °C, 0.8 mL/min, $t_{\mathbf{2d}} = 3.03$ min, $t_{\mathbf{1d}} = 4.99$ min; HPLC, OB-H column, hexanes–isopropanol 99:1, 0.6 mL/min, $t_R = 15.99$ min, $t_S = 17.79$ min; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.88 (3H, t, $J = 7.5$ Hz), 1.62–1.84 (2H, m), 4.55 (1H, t, $J = 6.5$ Hz), 7.12–7.40 (4H, m).

### 4.8. 1-(4-Bromophenyl)propanol, (±)-2e

GC, column HP5, 120 °C, 0.8 mL/min, $t_{\mathbf{2e}} = 5.0$ min, $t_{\mathbf{1e}} = 8.133$ min; HPLC, OJ-H column, hexanes–isopropanol 99:1, 0.6 mL/min, $t_R = 33.69$ min, $t_S = 35.34$ min. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.83 (3H, t, $J = 7.2$ Hz), 1.59–1.74 (2H, m), 4.44 (1H, t, $J = 6.5$ Hz), 7.11–7.41 (4H, m).

## Acknowledgements

The authors wish to thank Dr. Julia Cassani for the $^1$H NMR spectra. They also thank the partial financial support of Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico, Grant 37272N.

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