Enzymatic Hydrolysis of N-protected 2-Hydroxymethylpiperidine Acetates

Aida Solís,* Artemio Nava, Herminia Inés Pérez, Norberto Manjarrez, Héctor Luna, Julia Cassani

Departamento de Sistemas Biológicos, Universidad Autónoma Metropolitana-Xochimilco, Calzada del Hueso 1100, Col. Villa Quietud, Coyoacán, México, D.F. CP 04960, Phone: (55) 5483-7255, Fax: (55) 5483-7237.
Email: asolis@correo.xoc.uam.mx

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Abstract. Liver acetone powders (LAPs) were used as hydrolase sources to biocatalyze the hydrolysis of N-protected-2-hydroxymethylpiperidine acetates (N-Ts and N-Boc). Different reaction conditions were evaluated, like source of LAP (cat, guinea pig, chicken, dog, sheep, bovine, rat, mouse, pig and rabbit), proportion of cosolvent, type of cosolvent and pH.
Key words: Liver acetone powder, 2-hydroxymethylpiperidine, biocatalysis, hydrolysis.

Introduction

The impact of biological methods in organic synthesis has been extremely significant in recent times, particularly for the many contributions made to asymmetric synthesis; an attractive feature of this methodology is that the biological catalysts can be used like any other standard laboratory reagent, often needing no special handling or experience. Some advantages of biocatalysis over chemical synthesis are that enzyme-catalyzed reactions are often highly enantioselective and regioselective; they can be carried out at ambient temperature and atmospheric pressure, thus avoiding the use of more extreme conditions that can cause problems like isomerization, racemization, epimerization and rearrangement. Then biocatalysis can be applied in the production of fine chemicals and optically active compounds of industrial interest, in processes that represent an effective and environmentally-friendly alternative to chemical synthesis. As a result of these attributes biocatalyzed process are widely used in the industrial production of bulk chemicals, pharmaceutical and agrochemical intermediates, active pharmaceuticals and food ingredients [1].

The piperidine ring is an ubiquitous structural feature in numerous secondary metabolites and biologically active compounds, for example (S)-pipelic acid a non proteinogenic aminoacid [2], the anesthetic (S)-bupivacaine [3], k-receptor agonists [4], immunosuppressants as FK506 and rapamycin [5]. (+)-2-Hydroxymethylpiperidine is a precursor of compounds having the piperidine ring, and it can be resolved by the biocatalyzed hydrolysis of their N-protected acetates. The resolution of racemic alcohols via enantioselective hydrolysis of their corresponding esters can be carried out using hydrolases, that include lipases from microbial origin [6] and esterases from animal origin, specifically from the liver [7,8]. Liver acetone powders (LAPs) from different animals have been used as crude sources of esterases [7,8], without the tedious and expensive process of purification, then LAPs constitute cheap and accessible sources of this kind of enzymes.

In this work we studied the effect of the hydrolase source on the hydrolysis of the racemic acetates of N-tosyl-2-hydroxymethylpiperidine (1a) and of N-boc-2-hydroxymethylpiperidine (1b), as hydrolase sources we used liver acetone powders from cat, guinea pig, chicken, dog, sheep, bovine, rat, mouse, pig and rabbit.

Results and Discussion

Effect of the source of liver and the proportion of cosolvent on the biocatalyzed hydrolysis of 1a.

The hydrolysis of 1a was carried out in phosphates buffer (0.1M, pH 7.0), at 25ºC for 24 h, since 1a is water insoluble, acetonitrile was used as cosolvent. From Fig. 1 it can be observed that the source of enzyme is very important on the hydrolysis of acetate 1a. LAPs from mouse, rat, pig and chicken gave conversions higher than 50% of the corresponding alcohol 2a; with rabbit, bovine and guinea pig LAPs, the conversions were moderate (30-40%), with cat and dog LAPs the conversions were very low (12 and 5%, respectively), but sheep LAP practically did not show any biocatalytic activity towards 1a.

Very interesting was the effect of the cosolvent proportion on the reaction media, the conversion was reduced dramati-
cally upon an increase in the cosolvent content (Fig. 1); most of the LAPs catalyzed the hydrolysis of 1a using 10% (v/v) of acetonitrile as cosolvent, but a rise to 20% (v/v) of acetonitrile practically suppressed the biocatalytic activity of pig, chicken, bovine, guinea pig and cat LAP, in the case of mouse and rat LAPs the activity was decreased more than 70% with respect to the experiments using 10% of cosolvent.

With regard to the enantiomeric excess of 2a, it also was dependent on the LAP source; we analyzed the enantioselectivity of the reaction with 10% (v/v) of acetonitrile. Using the LAPs from mouse, rat and pig the conversions were very good (68, 64 and 60%, respectively, Fig. 1), but the reaction was not enantioselective (less than 7% ee, Fig. 2). The LAPs that gave the highest enantiomeric excess of 2a were from bovine, cat and dog (41, 38, 33%, respectively, Fig. 2).

**Effect of cosolvent and pH**

From the fact that the cosolvent had a strong influence on the biocatalyzed hydrolysis of 1a, we tested DMSO, dioxane, DMF, acetonitrile and diethyl ether to determine if they affect the conversion extent or the enantioselectivity of the hydrolytic reaction, using as biocatalyst only bovine LAP, since with this LAP the % ee of 2a was the highest (Fig. 2); the results are summarized in Table 1. It can be observed that in presence of DMSO and dioxane the conversion was improved significantly but the % ee was lower (entries 1 and 2, Table 1). The hydrolytic activity of the enzyme was similar in presence of DMF, diethyl ether and acetonitrile (39% conversion, entries 3-5 Table 1), but the % ee was still low (30 – 36%, entries 3-5 Table 1).

From results in Table 1, acetonitrile was chosen to determine the effect of the pH on the biocatalyzed hydrolysis of 1a, using bovine LAP as biocatalyst.

It can be observed that pH had an important effect on conversion and enantioselectivity, (Table 2) at pH 8.0 the conversion was high but the enantioselectivity was diminished significantly (54 and 19% respectively), at pH 7.0 the conversion was lower but enantiomeric excess is higher than at pH 8.0, at pH 6.0 the conversion was the lowest of the three pHs tested, but the enantioselectivity was the highest. The configuration of the main enantiomer of 2 was assigned as S, by comparison of the optical rotation of the isolated compound with the reported optical rotation literature, and according to the reported retention times of (R)-2 and (S)-2 using a Chiralcel OJ-H column [8].

**Effect of N-protecting group on the bovine LAP biocatalyzed hydrolysis of acetate 1.**

The nitrogen of piperidine nucleus was protected with tosylate or boc (1a or 1b); bovine LAP was chosen to carry out

### Table 1. Effect of cosolvent on the biocatalyzed hydrolysis of 1a, using bovine LAP.

<table>
<thead>
<tr>
<th>entry</th>
<th>Cosolvent</th>
<th>% conv 2a</th>
<th>% ee 2a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMSO</td>
<td>72</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>dioxane</td>
<td>71</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>DMF</td>
<td>39</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>diethyl ether</td>
<td>39</td>
<td>34</td>
</tr>
<tr>
<td>5</td>
<td>acetonitrile</td>
<td>39</td>
<td>36</td>
</tr>
</tbody>
</table>

*aDetermined by GC; b determined by chiral HPLC. 10% (v/v) of cosolvent, pH 7.0, 25º C, 24 h.*

### Table 2. Effect of pH on the % of conversion and % of ee of 2a using bovine LAP.

<table>
<thead>
<tr>
<th>pH</th>
<th>% conv 2a</th>
<th>% ee 2a</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>23</td>
<td>37</td>
</tr>
<tr>
<td>7</td>
<td>38</td>
<td>34</td>
</tr>
<tr>
<td>8</td>
<td>54</td>
<td>19</td>
</tr>
</tbody>
</table>

*aDetermined by GC; b determined by chiral HPLC. 10% (v/v) of acetonitrile, 25º C, 24 h.*
the hydrolytic reaction of 1a and 1b, at pH 7.0 using acetonitrile as cosolvent (10% v/v), at 25°C. Under these reaction conditions the hydrolysis of tosylate 1a yield 2a in 38% of conversion and 34% of ee (Table 2). But the change from tosylate to Boc had a negative effect on the enantioselectivity of the biocatalyzed reaction; the conversion of 1b was the same as that from 1a (38%), however the ee diminished to only 8%.

Conclusion

All the LAPs tested catalyzed the hydrolysis of 1a, except sheep LAP that did not catalyze the reaction, however the enantiomeric excess was moderate to low in all the cases, the highest value was obtained using bovine LAP. The proportion of cosolvent in the reaction media had a strong impact on the biocatalyzed reaction; an increase to 20% practically suppressed the reaction with acetonitrile. The type of cosolvent also has an important influence on the reaction, acetonitrile gave the best results. Besides, the nature of the N-protecting group changed the biocatalytic behavior of bovine LAP, using the N-Ts (1a) as substrate the enantioselectivity of the reaction is moderate, however using the N-Boc (1b) as substrate the enantioselectivity was practically lost.

Experimental

Infrared spectra were recorded on a Perkin-Elmer Paragon 1600 FT spectrophotometer; 1H NMR spectra were recorded on a Varian 400 MHz instrument, in CDCl₃ using tetramethylsilane as internal reference; TLC on silica gel 60 GF plate, Merck. HPLC analysis was performed on an Agilent 1100 liquid chromatograph, equipped with a diode array detector, using a Chiracel OJ-H column. GC analysis was performed on a Varian 400 MHz instrument, in CDCl₃, detection at 215 nm, tf = 15.40 min, ts = 16.83 min. GC analysis on a HP-5 column, of length 30 m and 0.25 mm ID, 0.25 mm film, N₂ as carrier gas, isocratic at 220°C, with a flow rate of 1.2 mL min⁻¹, retention time 7.3 min.

(R,S)-N-Ts-2-hydroxymethylpiperidine acetate (1a). A solution of 2a (500 mg, 1.85 mmol) and triethylamine (0.3 mL) in acetic anhydride (2.5 mL) was stirred at room temperature for 24 h, then CH₂Cl₂ (5 mL) was added, this solution was washed twice with saturated sodium carbonate solution, then with water, the organic layer was dried with anhydrous sodium sulfate and evaporated under vaccuo, was obtained a yellow solid, pF 45°C. The compound was identified and characterized by IR, 1H NMR, GC and HPLC, data are in agreement with literature. IR (cm⁻¹): 2867,1940, 1740, 1649, 1330, 1281, 1185; 1H NMR (CDCl₃) δ: 7.75-7.76 (m, 2H), 7.27-7.29 (m, 2H), 4.26-4.30 (m, 2H), 4.07-4.09 (m, 1H), 3.75-3.80 (m, 1H), 2.98-3.08 (m, 1H), 2.42 (s, 3H), 2.03 (s, 3H), 1.2-1.85 (m, 6H). HPLC analysis on a Daicel Chiralcel OJ-H column, eluent, hexane/2-propanol: 80:20, flow rate of 0.6 mL min⁻¹, detection at 215 nm, tf = 22.30 min, ts = 32.10 min. GC analysis on a HP-5 column, of length 30 m and 0.25 mm ID, 0.25 mm film, N₂ as carrier gas, isocratic at 220°C, with a flow rate of 1.2 mL min⁻¹, retention time 9.2 min.

(R,S)-N-Boc-2-hydroxymethylpiperidine (2b). The 2-hydroxymethylpiperidine (3 g, 26 mmol) was dissolved in 35 mL of a mixture of dioxane/water (10% v/v), then di-tert-butylcarbonate (8.52 g, 39 mmol) and NaOH (1.5 g, 39 mmol) were added, the mixture was stirred overnight at room temperature. Dioxane was evaporated under vaccuo, and 20 mL of water were added to the resulting solution, it was extracted with ethyl acetate (3x10 mL). The organic layer was separated with water, the organic layer was dried with anhydrous sodium carbonate, then the solvent evaporated to dryness. A yellow solid was obtained, pF 66°C. The compound was identified by IR, 1H NMR, GC and HPLC. Data are in agreement with literature. IR (cm⁻¹): 3422, 2930, 1661, 1413, 1363, 1161; 1H NMR (CDCl₃) δ: 4.27-4.31 (m, 1H), 3.92–3.96 (m, 1H), 3.79–3.84 (m, 1H), 3.59–3.63 (m, 1H), 2.86 (m, 1H), 1.4-1.71 (m, 5H), 1.34-1.55 (s, 1OH). HPLC analysis on a Daicel Chiralcel OJ-H column, eluent, hexane/2-propanol: 80:20, flow rate of 0.65 mL min⁻¹, detection 220 nm, tf = 15.30 min, ts = 16.10 min. GC analysis, HP-5 column, length 30 m, ID 0.25 mm, film 0.25 mm, carrier N₂, isocratic at 220°C, flow rate 0.8 mL min⁻¹, retention time 2.2 min.
(R,S)-N-Boc-2-hydroxymethylpiperidine acetate (1b). The reaction was carried under the same conditions as stated for 1a. A yellow liquid was obtained, the compound was identified and characterized by IR, NMR $^1$H, GC and HPLC. Data are in agreement with literature [10]. IR (cm$^{-1}$) n: 2931, 1739, 1684, 1412, 1362, 1223, 1167; $^1$H NMR (CDCl$_3$) $\delta$: 4.46-4.54 (m, 1H), 4.24–4.27 (m, 1H), 4.07–4.12 (m, 1H), 2.77-2.83 (m, 1H), 2.05 (s, 3H), 1.58–1.65 (m, 5H), 1.38-1.52 (s, 10H).

HPLC analysis on a Daicel Chiralcel OJ-H column, eluent, hexane/2-propanol, 99:1, flow rate 0.65 mL min$^{-1}$, detection 220 nm, $t_R$ 8.80 min, $t_S$ 9.50 min. GC analysis, HP-5 column, length 30 m, ID 0.25 mm, film 0.25 mm, carrier N$_2$, isocratic at 200°C, flow rate 0.8 mL min$^{-1}$, retention time 2.7 min.

General procedure for enzyme-mediated hydrolysis. To 5 mg of 1a or 1b in 0.05 mL of a cosolvent, were added 0.45 mL of a phosphate buffer solution (0.1M, pH 6.0, 7.0 or 8.0) and 10 mg of LAP, the mixture was stirred at 25°C, for 24h then it was extracted twice with methylene chloride, the organic phase was dried over Na$_2$SO$_4$, and the solvent was then evaporated under reduced pressure to dryness. Conversion % was determined by GC and % ee was determined by HPLC using a Chiracel OJ-H column, eluent hexane/2-propanol=80/20, flow rate 0.6 ml/min, detection at 215 or 220 nm.

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References