Article

J. Mex. Chem. Soc. **2008**, *52(3),* 181-184

ISSN 1870-249X

Enzymatic Hydrolysis of *N*-protected 2-Hydroxymethylpiperidine Acetates

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Recibido el 18 de abril de 2008; aceptado el 24 de Mayo de 2008

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.

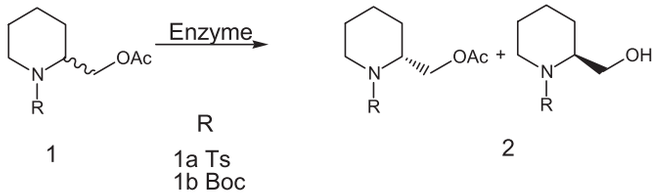
Resumen. Como fuente de hidrolasas se usaron polvos acetónicos de hígado (PAH), para biocatalizar la hidrólisis de los acetatos de la 2-hidroximetilpiperidina *N*-protegida (*N*-Ts y *N*-Boc). Se evaluaron diferentes condiciones de reacción, como la fuente del PAH (gato, cuyo, pollo, perro, borrego, res, rata, ratón, cerdo y conejo), la proporción de codisolvente, tipo de codisolvente y pH.
Palabras clave: Polvo acetónico de hígado, 2-hidroximetilpiperidina, biocatálisis, hidrólisis.

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*)-pipecolic 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.



Scheme 1

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 significatively 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 significatively (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].

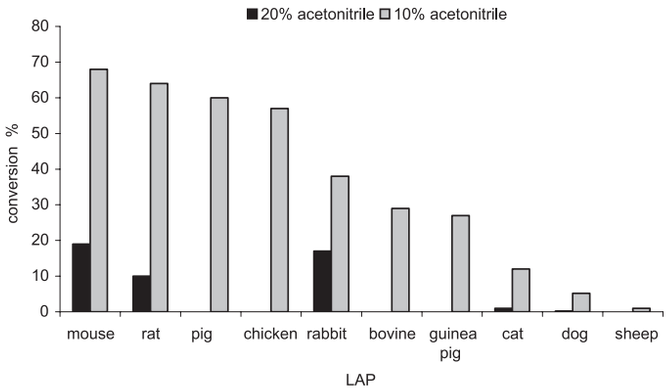


Fig. 1. Effect of LAP source and proportion of cosolvent:reaction media (% v/v) on the hydrolysis of **1a**, conversion % determined by GC.

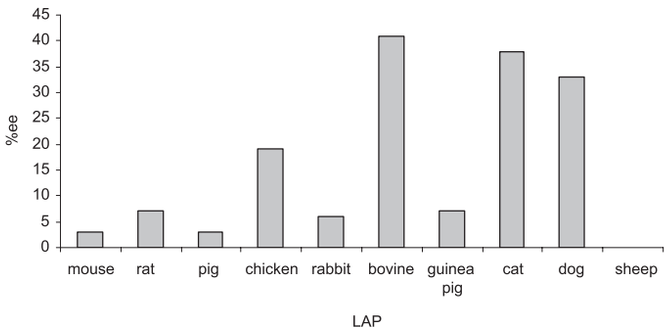


Fig. 2. Effect of LAP source on the enantioselectivity of the hydrolysis of **1a**, using 10% (v/v) acetonitrile, % ee determined by chiral HPLC.

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.

entry	Cosolvent	% conv **2a** [a]	% ee **2a** [b]
1	DMSO	72	11
2	dioxane	71	16
3	DMF	39	30
4	diethyl ether	39	34
5	acetonitrile	39	36

[a]Determined 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.

pH	% conv **2a** [a]	% ee **2a** [b]
6	23	37
7	38	34
8	54	19

[a]Determined 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 espectrofotometer; ^{1}H NMR spectra were recorded on a Varian 400 MHz instrument, in $CDCl_3$ using tetramethylsilane as internal reference; TLC on silica gel 60 GF_{254} 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 Hewlett-Packard HP 6890 gas chromatograph, equipped with a flame ionization detector and a HP-5 column (30 m x 0.33 mm).

Liver acetone powders (LAPs). The corresponding livers were purchased in local stores or were a gift from the University animal facilities. First the excess of fat was removed from the liver, then it was washed with water. Enough acetone was added to cover the liver in the blender vessel, and grinded at high speed for 2-5 min, after this time the mixture was filtered, the solid was grinded twice again in the same conditions, the resulting powder was dried in a hood and stored in a glass container at 5°C.

***(R,S)-N-Ts*-2-hydroxymethylpiperidine (2a)**. To an ice cooled solution of 2-hydroxymethylpiperidine (500 mg, 4.34 mmol) in CH_2Cl_2 (20 mL) and triethylamine (439 mg, 4.34 mmol), was added slowly a solution of 4-toluenesulfonyl chloride (993 mg, 1.2 eq.) in CH_2Cl_2 (20 mL). Then, it was warmed to room temperature and stirred 24 h, the solvent was evaporated under vaccuo, and the product was purified by column chromatography. A yellow solid was obtained, pf 62-65°C. The compound was identified and characterized by IR, ^{1}H NMR, GC and HPLC, data were in agreement with literature [9,10]. IR (cm^{-1}): 3411, 2932, 2868, 1710, 1661, 1597, 1447, 1326, 1154; ^{1}H NMR ($CDCl_3$) δ: 7.75-7.76 (m, 2H), 7.29-7.31 (m, 2H), 3.97-4.03 (m, 1H), 3.78-3.89 (m, 1H), 3.54-3.58 (m, 2H), 3.04-3.18 (m, 1H), 2.32 (s, 3H), 1.65 (m, 1H), 1.19-1.62 (m, 5H). 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, t_R= 15.40 min, t_S= 16.83 min. GC analysis on a HP-5 column, of length 30 m and 0.25 mm ID, 0.25 mm film, N_2 as carrier gas, isocratic at 220ºC, 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 CH_2Cl_2 (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, ^{1}H NMR, GC and HPLC, data are in agreement with literature.[10] IR (cm^{-1}): 2867,1940, 1740, 1649, 1330, 1231, 1155; ^{1}H NMR ($CDCl_3$) δ: 7.7-7.73 (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, t_R= 22.30 min, t_S= 32.10 min. GC analysis on a HP-5 column, of length 30 m and 0.25 mm ID, 0.25 mm film, N_2 as carrier gas, isocratic at 220ºC, 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 disolved in 35 mL of a mixture of dioxane/water (10% v/v), then di-*tert*-butyldicarbonate (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 and dried with anhydrous sodium sulphate, then the solvent evaporated to dryness. A yellow solid was obtained, pf 66°C. The compound was identified by IR, ^{1}H NMR, GC and HPLC. Data are in agreement with literature [11]. IR (cm^{-1}): 3422, 2930, 1661, 1413, 1363, 1161; ^{1}H NMR ($CDCl_3$) 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, 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 15.30 min, t_S 16.10 min. GC analysis, HP-5 column, length 30 m, ID 0.25 mm, film 0.25 mm, carrier N_2, isocratic at 200ºC, 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 ($CDCl_3$) δ: 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 200ºC, 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_2SO_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.

Acknowledgments

We thank the financial support of Consejo Nacional de Ciencia y Tecnología (CONACyT), México, Grant Num. 37272-N.

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