FAST MICROWAVE ASSISTED BIOREDUCTION OF AROMATIC ALDEHYDES USING *ALOE VERA*. A GREEN CHEMISTRY REACTION

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This paper is dedicated to Professor Pedro Joseph-Nathan in recognition of his 50 years of outstanding scientific trajectory.

ABSTRACT

A green chemistry methodology for fast and simple bioreduction of aromatic aldehydes to their corresponding aromatic alcohols was achieved using an extract of *Aloe vera* in aqueous suspension. Under microwave irradiation, this reaction was performed in a short reaction time. This methodology is an alternative preparation for aromatic alcohols that are extensively utilized as industrial and cosmetic reagents. *www.relaquim.com*

Keywords: aromatic aldehydes, aromatic alcohols, green chemistry, bioreduction, microwave, *Aloe vera.*

RESUMEN

Se desarrolló una metodología de química verde para la bioreducción rápida y sencilla de aldehídos aromáticos a alcoholes aromáticos utilizando un extracto de *Aloe vera* en suspensión acuosa. Por medio de radiación de microondas, esta reacción se llevo a cabo en un periodo de tiempo muy corto. El procedimiento desarrollado es una ruta alterna para preparar alcoholes aromáticos, los cuales son reactivos muy utilizados en la industria y en la preparación de cosméticos. *www.relaquim.com*

Palabras clave: aldehídos aromáticos, alcoholes aromáticos, química verde, bioreducción, microondas, *Aloe vera.*

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INTRODUCTION

The reduction of a carbonyl group present in aldehydes or ketones, is one of the most important reactions in organic chemistry (Wade, 2011). In general, this reaction is performed using hydrogen and a metal (Pd or Pt) as catalyst or a hydride of a metal (Na, Al or Li). However, few reduction methodologies have been developed taking into account the concepts of green chemistry in order to avoid the formation of toxic waste that may pollute the environment.

In the past decades, biocatalysis has undergone significant development, a large number of reactions have been introduced and optimized, specially those focused on the synthesis of organic molecules of industrial and biological interest such as pharmaceutical compounds, cosmetics and other industrial chemicals (Bruni *et al.*, 2002; Luna, 2004; Veit, 2004; Sharma *et al.*, 2005). Several studies have demonstrated the advantages of reactions involving biocatalysis such as regioselectivity, chemoselectivity and enantioselectivity (Castro and Knubovets, 2003). A relevant reaction in organic synthesis, and a key step in the manufacture of numerous pharmaceutical and fine chemicals, is the conversion of an aldehyde or ketone to alcohol, frequently in the presence of other reducible groups. There are some examples of the use of biocatalysis to perform this particular reaction (Koutril *et al.*, 2004; Machado *et al.*, 2006; Machado *et al.*, 2008; Suárez-Franco *et al.*, 2010).

In recent years, whole plant cells and plant cell cultures, in addition to microorganisms and purified enzymes, have been studied as potential agents for biotransformation reactions. The reduction of aliphatic and aromatic ketones in aqueous media by *Daucus carota* roots has been investigated (Yadav *et al.*, 2002). In another study, enantioselective hydrolysis of racemic acetates has also been conducted using comminuted tissues from ripe vegetable roots (Maczka and Mironowicz, 2002). Therefore it is quite possible that a wide range of plant materials (roots, tubers, seeds, fruits and *in vitro* cultures) could be a valuable medium for a variety of biocatalytic reactions.

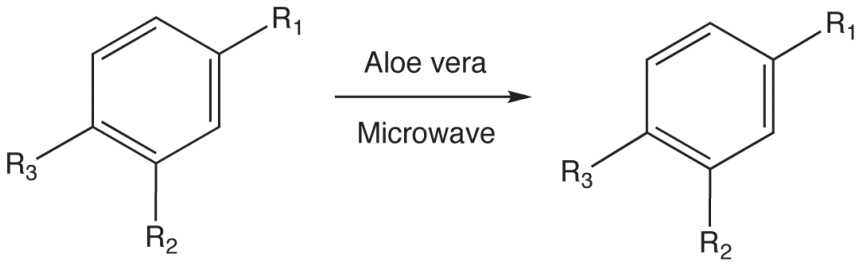
The application of microwave irradiation (mw) as a non-conventional energy source for the activation of reactions has become a very popular and useful technique in organic chemistry (Hayes, 2002; Tierney and Lidström, 2005; Nüchter, *et al.*, 2004). In general, the use of mw activation leads to enhanced conversion rates, higher yields, easier work-up and cleaner reactions demonstrating the real advantages of this methodology.

The synthesis of gold and silver nanoparticles, using an *Aloe vera* leaf extract as the reducing agent, has been recently reported as a simple procedure (Chandran *et al.*, 2006). This particular reaction was achieved reacting salts of gold or silver with the plant extract at 80 to 100 °C. Indeed, the use of heat-stable enzymes, called extremozymes, allows reactions to be completed faster due to rate enhancing by elevated temperatures (Solomons and Fryhle, 2004). We investigated the use of an extract of *A. vera* as a potential enzyme source for the bioreduction of several aromatic aldehydes to their corresponding alcohols. In addition, this bioreduction was studied under microwave irradiation (Scheme 1).

The encouraging results obtained using aqueous extracts of *A. vera* for biocatalysis may offer new possibilities for the reduction of selected carbonyl compounds which is a critical step in many synthetic organic pathways, avoiding the use of non-sustainable hydride reducing agents.

MATERIALS AND METHODS

All chemicals were obtained from Sigma-Aldrich, pre-coated TLC plates of silica gel 60GF254 and column chromatography silica gel grade 60 mesh were obtained from



1 **2**

1a R_1=CH=O, R_2=H, R_3=H; **2a** R_1=CH_2OH, R_2=H, R_3=H;
1b R_1=CH=O, R_2=NO_2, R_3=H; **2b** R_1=CH_2OH, R_2=NO_2, R_3=H;
1c R_1=CH=O, R_2=H, R_3=NO_2; **2c** R_1=CH_2OH, R_2=H, R_3=NO_2;
1d R_1=CH=O, R_2=OCH_3, R_3=H; **2d** R_1=CH_2OH, R_2=OCH_3, R_3=H;
1e R_1=CH=O, R_2=H, R_3=OCH_3; **2e** R_1=CH_2OH, R_2=H, R_3=OCH_3;
1f R_1=CH=O, R_2=CH_3, R_3=H; **2f** R_1=CH_2OH, R_2=CH_3, R_3=H;
1g R_1=CH=O, R_2=H, R_3=CH_3; **2g** R_1=CH_2OH, R_2=H, R_3=CH_3;
1h R_1=CH=O, R_2=Cl, R_3=H; **2h** R_1=CH_2OH, R_2=Cl, R_3=H;
1i R_1=CH=O, R_2=H, R_3=Cl, **2i** R_1=CH_2OH, R_2=H, R_3=Cl,
1j R_1=CH=O, R_2=OH,R_3=OCH_3 **2j** R_1=CH_2OH, R_2=OH,R_3=OCH_3
1k R_1=CH=CH-CH=O, R_2=H, R_3=H; **2k** R_1=CH=CH-CH_2OH, R_2=H, R_3=H;
1l R_1=CH=C(CH_3)-CH=O, R_2=H, R_3=H; **2l** R_1=CH=C(CH_3)-CH_2OH, R_2=H, R_3=H

Scheme 1. Bioreduction of aromatic aldehydes

Merck. IR spectra were recorded using a Nicolet i510 Thermo Scientific FT-IR spectrometer with ATR. The products obtained and the pure starting materials were analyzed by GC on a Shimadzu model GC-2014 using a (5%-phenyl)-methylpolysiloxane DB-5 capillary column (30 x 0.25 mm), carrier gas Helium, flow rate of 2 mL/min with split mode. The injector temperature and detector temperature were 220 and 200 °C, respectively. ^{1}NMR spectra were obtained in a 400 NMR Varian Mercury Spectrometer. Microwave reactions were performed in a CEM-Discover monomode microwave reactor (maximal power: 300 W and maximal frequency: 2455 MHz)

Bioreduction experiments were performed following the procedure previously reported by us with minor modifications (Hernández-González and Leyva-Ramos, 2011). *A. vera* plant was obtained from a commercial greenhouse and it was kept at -4 °C until used. The plant was washed with 5% sodium hypochlorite and rinsed with deionized water. An amount (63 g) of the pulp of *A. vera* was extracted, cut into small pieces (0.5 cm^3) and blended for 60 seconds. Then, aromatic aldehyde (0.6 g) was added and deionized water was also added to the reaction mixture for a total volume of 60 mL. The reaction mixture was placed in the microwave reactor at 100 watts potency (100 °C at 300 psi) for 70 minutes. The reaction was monitored by TLC. After reaction time, the hot mixture was allowed to cool to room temperature. The suspension was extracted with 50 mL of ethyl acetate three times. The organic phase was filtrated under vaccum to remove any plant solid, dried over anhydrous sodium sulfate and concentrated in a rotary evaporator. The residue was passed through a short silica gel column using $CHCl_3$ as eluent. The solvent was dried and removed to yield a clear liquid.

The product mixture was analyzed by IR and GC and each sample contained a small amount (less than 2 %) of starting aldehyde. All the products were also characterized by ^{1}NMR and were compared with previously reported spectra (Pouchert, 1981; Pouchert and Behnke, 1993).

The bioreduction experiments were also performed without microwave irradiation by stirring an aqueous suspension of an aromatic aldehyde with *Aloe vera* extract at room temperature for seven days. However, no reaction took place under these latter experimental conditions.

RESULTS AND DISCUSSION

Bioreduction of several aromatic aldehydes (Table 1) was performed by reacting them with aqueous extracts of *A. vera* under microwave irradiation. The product mixtures were analyzed by ^{1}NMR, IR and GC to confirm the reduction of aldehyde to alcohol. In general, aromatic aldehydes **1a-i** gave moderate yields of alcohols (55 to 65 %). A slightly better yield is observed when the aromatic benzaldehyde contains a substituent in *para*-position to the carbonyl functional group (**1c**, **1e**, **1g**, and **1i**). These results suggest that a better association between the reductive enzyme and the aldehyde is achieved in these cases. The interaction between the enzyme and the aldehyde most likely reflects a balance between steric repulsion, given by the relative size of the host (in this case the active site of enzyme) and the guest (the aldehyde), the hydrophobicity of the aromatic ring and the hydrophilicity of the aldehyde (-CH=O). It is expected that an aldehyde group (-CH=O) with certain polar and hydrophilic character would prefer to remain exposed to the bulk of the solution. When extra polar substituents (in position two or three in the aromatic ring) are present a weaker binding between the enzyme and the compound is expected. In contrast, a larger binding would be expected for *para*-substituted aldehydes. This may be due not only to a better fit between the enzyme and the aldehyde which allows for deeper penetration, but also to the more favorable hydrophobic interactions (Leyva *et al.*, 2001). In fact, we have reported a larger binding constant for *para*-substituted phenols with

Table 1. Structure and bioreduction of aromatic aldehydes

Aldehyde	R	R_1	R_2	Alcohol (% Yield)[a]
1a	CHO	H	H	65
1b	CHO	NO_2	H	60
1c	CHO	H	NO_2	65
1d	CHO	OCH_3	H	55
1e	CHO	H	OCH_3	62
1f	CHO	CH_3	H	55
1g	CHO	H	CH_3	61
1h	CHO	Cl	H	55
1i	CHO	H	Cl	65
1j	CHO	OH	OCH_3	NR[b]
1k	CH=CH-CHO	H	H	41
1l	CH=CH(CH_3)-CHO	H	H	31

[a]Yield was determined by weight of product and it is the average of three reactions.

[b]NR = no reaction observed.

cyclodextrins in an aqueous environment. Interactions of these latter substrates with organic compounds have been suggested to mimic enzyme-substrate interactions (Bender and Komiyama, 1997). Similar physicochemical interactions could explain the lack of reaction when a hydroxyl substituent is present in the aldehyde **1j**. In this case, the highly hydrophilic substituent will remain in the aqueous solution and a very weak binding would be expected .

A chemoselective bioreduction with both isomeric nitrobenzaldehydes, **1b** and **1c**, was observed. This reduction reaction proceeds almost quantitatively at the carbonyl group with no reduction of the nitro group. The presence of this nitro group in the aromatic alcohols, **2b** and **2c**, was verified by IR spectroscopy, since it is well known that an aromatic nitro group gives two strong bands due to asymmetric and symmetric -NO_2 stretching at 1520 and 1340 cm-^{1}. Conjugation of a nitro group with aromatic ring shifts both bands to lower frequencies (Pavia *et al.*, 2001).

Both cinnamic aldehydes, **1k** and **1l**, yielded the corresponding alcohols, **2k** and **2l**, in 41 and 31 % yield respectively. In these aldehydes, the reduction also took place on the carbonyl group keeping the double bond of the side chain intact. However, the low yields observed may indicate a rather weak interaction between the reductive enzyme and the reacting aldehyde due to steric grounds since these aldehydes contain a large carbon chain between the aromatic ring and the aldehyde group (–CH=O).

The biotransformation of an aromatic aldehyde to the corresponding aromatic alcohol could be easily determined analyzing starting material and reaction mixture by IR spectroscopy (Pavia *et al.*, 2001). The starting aromatic aldehyde **1** (Figure 1) gives two bands in the range between 2700 and 2900 cm^{-1} corresponding to the symmetric and asymmetric bond stretching

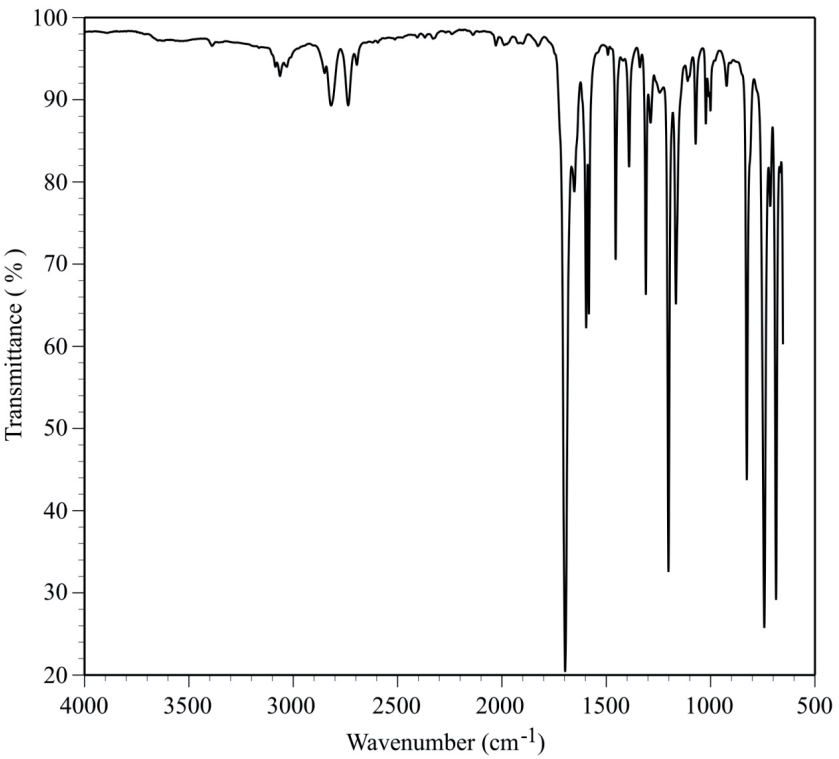


Figure 1. Infrared spectrum of starting aromatic aldehyde (Benzaldehyde **1a**).

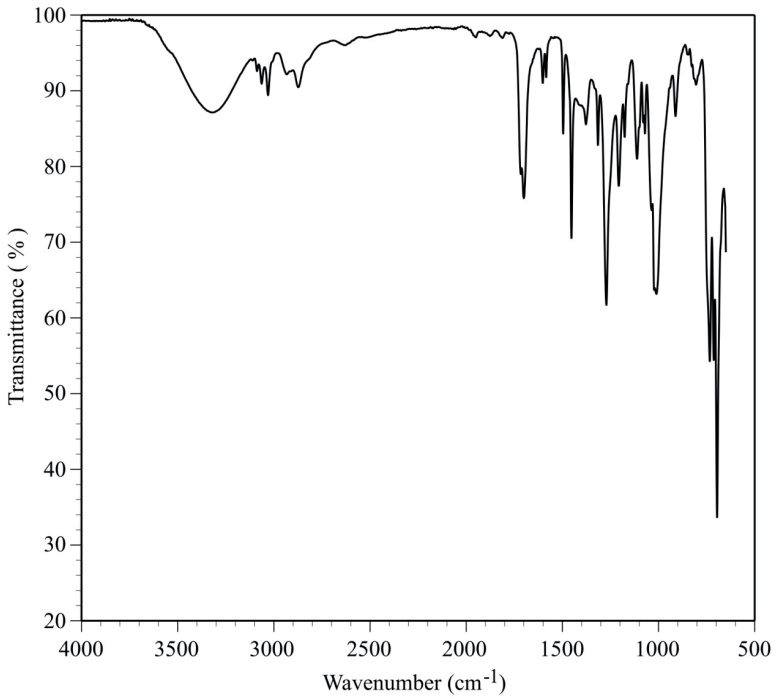


Figure 2. Infrared spectrum of reduction product aromatic alcohol (benzyl alcohol **2a**).

(H-C=O). The most intensive signal in the IR spectrum at 1687 cm^{-1} is due to stretching of a polar C=O aldehyde bond. An aromatic alcohol, the expected reduction product in the reaction mixture (Figure 2), gives a characteristic broad O-H stretching vibration band between 3500 and 3100 cm^{-1} due to hydrogen bonding. Furthermore, a strong band at 1262 cm^{-1} is due to C-O bond stretching. Aromatic alcohols usually present this band above 1200 cm^{-1} becouse of conjugation of oxygen with the ring. IR spectra of the reaction mixture shows a weak band at 1680 cm^{-1} indicating the presence of a small amount of starting aldehyde.

CONCLUSIONS

In the present paper, we describe a simple, fast and green biotechnological method for the preparation of a wide range of alcohols of industrial and pharmaceutical interest using *A. vera* extracts. To our knowledge, this procedure has not been previously reported in the literature. The results obtained suggest that a reductase system could be responsible for the biotransformation observed. The *A. vera* extract could be considered as a source of potential reagents for the reduction of simple aromatic aldehydes, providing an alternative to the most widely used metal or metal hydride mediated reductions. Furthermore, the use of microwave irradiation with heat-stable enzymes represents a fast and novel methodology.

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