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Full Length Research Paper

Plant dried powders as biocatalysts: Hydrolysis of 1-phenylpropanol acetate

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The hydrolytic ability of plant dried powders, lyophilized or acetone dried, was tested on the hydrolysis of racemic 1-phenylpropanol acetate. Most of the twenty powders tested showed hydrolytic activity, however the best values of conversion and enantioselectivity were reached with the lyophilized powder of nopal (27% conversion, 72% enantiomeric excess of 1-phenylpropanol) and the acetonic powders of cherry (50% conversion, 74% enantiomeric excess of 1-phenylpropanol) and red plum (27% conversion, 75% enantiomeric excess of 1-phenylpropanol).

Key words: Acetonic powder, lyophilized powder, 1-phenylpropanol, plant hydrolases.

INTRODUCTION

The biological activity of chiral compounds is different for each enantiomer. One of the enantiomers is responsible for the activity of interest while the other enantiomer could be inactive, an antagonist of the active enantiomer or have other activity that might be desirable or undesirable. Considering these possibilities, there are major advantages in using stereochemically pure drugs, such as a reduction of the total administered dose and side effects, enhanced therapeutic window and a more precise estimation of dose–response relationships (Agranat et al., 2002). These factors have led to an increasing preference for the use of single enantiomers in both industry and sanitary legislation (Patel, 2006; Hava et al., 2004).

Biocatalysts have been increasingly used to assist in synthetic routes to enantiopure molecules of industrial interest (Pollard and Woodley, 2006; Collado et al., 2006). Advantages of biocatalyst over chemical synthesis include the fact that enzyme catalyzed reactions are often highly enantioselective and regioselective. They can be carried out at room temperature and atmospheric pressure, thus avoiding the use of more extreme conditions that can cause problems like isomerization, racemization, epimerization and rearrangement.

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Hydrolases are biocatalysts widely used for the preparation of optically active alcohols and carboxylic acids, which are useful in the preparation of drugs or agrochemicals. Most of the studies on biocatalyzed hydrolysis have been performed using microbial (Hasan et al., 2006) and animal hydrolases (Basavaiah, 2001; Solís et al., 2008a, b). On the other hand, there are some reports about the presence of hydrolases in plants, for example in the Caricaceae family, mainly in *Carica papaya* (Alcántara et al., 2006), *Euphorbiaceae* (Palocci et al., 2003) and *Asclepiadaceae* (Palocci et al., 2005). In spite of these reports, plant enzymes have rarely been employed for synthetic purposes. However, these enzymes are very attractive due to their low cost, availability, ease of handling and purification (Giri et al., 2001; Cordell et al., 2007).

In this study, different plants extracts were tested to determine their hydrolytic capability towards the hydrolysis of 1-phenylpropanol acetate (Figure 1). One of the disadvantages of plants is that they are not available all through the year and they cannot be conserved for long periods of time even at low temperatures. To overcome these disadvantages, the biological material can be dehydrated and stored at low temperature. Dehydration of biological materials can be done in two ways. This could either be by lyophilization or with acetone. The plants tested in this study were seeds from capulín (*Prunus capuli*), cherry (*Prunus avium),* red plum

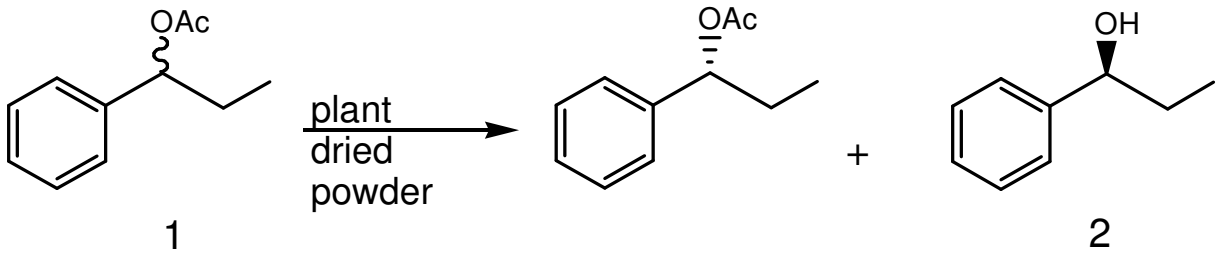

Figure 1. Biocatalyzed hydrolysis of 1-phenylpropanol acetate (1).

(Prunus domestica, cv *moscatel),* almond (*Prunus dulcis*), peach (*Prunus persica*), apricot (*Prunus armeniaca*) mamey (*Pouteria sapota*), guanabana (*Anona muricata*); fruits of tomatillo (*Physalis philadelphica*), zucchini (*Cucurbita pepo*), green pea (*Pisum sativum*), chayote (*Sechium edule*) and pineapple (*Ananas comosus*); leaves of guanabana (*Anona muricata*), nopal (*Opuntia ficus-indica*) and pitahaya (*Hylocereus undatus*) and carrot root (*Daucus carota*).

MATERIALS AND METHODS

Analytical methods

Infrared spectra were recorded on a Perkin-Elmer Paragon 1600 FT spectrophotometer; ^{1}H NMR spectra were recorded on a Mercury 400 MHz instrument, in $CDCl_3$ using tetramethylsilane as internal reference. High performance liquid chromatography (HPLC) analysis was performed on an Agilent 1100 liquid chromatograph, equipped with a diode array detector and a Chiralcel OD column. The mobile phase was *n*-hexane–isopropanol (97:3, v/v) and the flow rate was 0.6 mL/min, retention time of (*R*)-1-phenylpropanol was 15.67 min and for (*S*)- (*R*)-1-phenylpropanol was 17.78 min. 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), oven at 140°C, N_2 as carrier with a flow rate of 0.8 mL/min, retention time of 1-phenylpropanol acetate was 2.19 min, retention time of (*R*)-1-phenylpropanol was 1.62 min.

Substrates, solvents and biocatalysts

(±)-1-Phenylpropanol was purchased from Aldrich (USA), acetic anhydride and triethylamine were purchased from Baker (México). (±)-1-Phenylpropanol acetate was prepared following the standard procedure of acetic anhydride and triethylamine. Solvents were HPLC grade and were purchased from Tecsiquim (México). Guanabana and pitahaya leaves were from a farm located in Veracruz, México, whereas nopal leaves and the fruits were purchased in markets from Xochimilco, México.

Preparation of the plant dry powders

Lyophilized powder

The biological material was washed with soap and water and then cut into small pieces and frozen. After this treatment, the material was lyophilized. Lyophilized dried powders were used without any further purification as enzyme source. The powders were stored at 4°C.

Acetone dried powders

The biological material was washed with soap and water and then grinded with cold acetone. Since the seeds were fatty and were grinded three times with acetone, the mixture was filtered, the acetone discharged and the solid was left to dry. Acetone dried powders were used without any further purification as enzyme source. The powders were stored at 4°C.

General procedure for the enzyme-mediated hydrolysis

5 mg (0.03 mol) of 1-phenylpropanol acetate in 0.05 mL of acetonitrile were added to a suspension of 0.45 mL of distilled water and 10 mg of the plant dry powder. The mixture was stirred at 25°C for 48 h. After this, extraction was carried out twice with methylene chloride. The organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure to dryness. Conversion percentage was determined by GC and enantiomeric excess (ee) by chiral HPLC.

RESULTS AND DISCUSSION

The hydrolytic ability of dehydrated plants was studied on the hydrolysis of 1-phenylpropanol acetate. The plants selected were dried in two different ways; by lyophilization or with acetone. The lyophilized powders were leaves of nopal and fruits of chayote, green pea, tomatillo, zucchini and pineapple and the acetone dried powders were carrot root, leaves of guanabana, nopal and pitahaya, fruits of green pea, zucchini and tomatillo, seeds of capulín, guanabana, cherry, almond, red plum, peach, apricot and mamey. It is worth mentioning that due to the oily character of the seeds, they were not lyophilized. The reaction was carried out in distilled water and acetonitrile was selected as cosolvent. From results in Table 1, it was observed that all the lyophilized powders catalyzed the hydrolysis of 1-phenylpropanol acetate, except the pineapple and tomatillo powders. The hydrolytic activity was similar for nopal, green pea and zucchini. Conversions to (*R*)-1-phenylpropanol were between 25 and 33%. The lowest was with chayote (12%). On the other hand, the best enantiomeric excess of the resulting alcohol (*R*)-1-phenylpropanol was reached using the nopal powder (72%).

With regard to the biocatalytic activity of the acetone dehydrated powders, the results of the hydrolysis of 1-phenylpropanol acetate are summarized in Table 2. It

Table 1. Hydrolysis of 1-phenylpropanol acetate using lyophilized powders of plants.

Plant	% Conversion[a]	% Enantiomeric excess of (*S*)-2[b]
Zucchini [d]	33	54
Nopal [c]	27	72
Green pea [d]	25	0
Chayote [d]	12	46
Pineapple [d]	< 5	nd
Tomatillo [d]	<5	nd

aConversion to alcohol; bDetermined by chiral HPLC; cleaves; dfruit; nd = not determined due to low conversion; 2 = determined by GC.

was noticed that most of the acetonic powders catalyzed the hydrolysis of 1-phenylpropanol acetate, except that from peach and apricot seeds, carrot root and green pea fruit. The most active powders were from cherry seeds and guanabana leaves and seeds, with conversions between 60 and 50%. A little less active were nopal and tomatillo powders (40 and 36% conversion, respectively). The activity of the rest of the powders was between 17 and 30% of conversion to (*R*)-1-phenylpropanol. The enantioselectivity of the reaction catalyzed by the acetonic powders was variable with the majority of the active powders. The enantiomeric excess of the resulting alcohol (*R*)-1-phenylpropanol was between 53 and 75% and the best enantiomeric excess was obtained with the powders of cherry and red plum seeds, 74 and 75%, respectively (Table 2).

It is interesting to notice the different behavior of the hydrolases present in nopal, green pea, tomatillo and zucchini according to the way they were dehydrated, with acetone or lyophilized, as can be stated from results in Tables 1 and 2. Nopal acetone powder was more active than the corresponding lyophilized, which showed 40 and 27% of conversion, respectively. However, the enantioselectivity was better, using the lyophilized powder than with the acetonic powder (72 and 43% enantiomeric excess of (*R*)-1-phenylpropanol, respectively). In the case of zucchini, the biocatalytic activity of the lyophilized and acetonic powders was similar (33 and 28% conversion of (*R*)-1-phenylpropanol, respectively), but the enantioselectivity was higher with the acetonic powder (63% enantiomeric excess) than with the lyophilized powder (54%). In the case of the powders from green pea, the acetone dried was inactive, whereas the lyophilized catalyzed the hydrolysis of 1-phenylpropanol acetate in 25%, but without any enantioselectivity. Something similar occurred with tomatillo, where the acetonic was inactive, but with the lyophilized the conversion was 36 and the enantiomeric excess of (*R*)-1-phenylpropanol was one of the best (65%).

Some of the powders tested have been stored for almost a year at 4°C and we have not found an appreciable loss in their biocatalytic activity. In all the cases, the preferred enantiomer was (*S*)- (*R*)-1-phenylpropanol. The configuration was assigned comparing the results of the HPLC analysis using a Chiralcel OD column with the results reported by Uzura (2001).

Table 2. Hydrolysis of 1-phenylpropanol acetate using acetone dried powders of plants.

Plant	% Conversion[a]	% Enantiomeric excess of (*S*)-2[b]
Nopal [c]	40	43
Guanabana [c]	52	54
Pitahaya [c]	17	46
Tomatillo [d]	36	65
Zucchini [d]	28	63
Green pea [d]	< 5	nd
Carrot [e]	< 5	nd
Guanabana [f]	60	53
Cherry [f]	50	74
Mamey [f]	30	28
Red plum [f]	27	75
Capulín [f]	26	66
Almond [f]	23	35
Peach [f]	<5	nd
Apricot [f]	<5	nd

aConversion to alcohol; bDetermined by chiral HPLC; cleaves; dfruit; eroot; fseed; 2 = determined by GC; nd = not determined due to low conversion.

Conclusion

Most of the plants tested showed hydrolytic activity, though the enantioselectivity was not very high with the majority of these plants. They are good candidates to be more extensively studied in order to find the conditions to improve conversion and enantiomeric excess. One of the most important contributions of this work is the fact that the lyophilized or the acetone dried powders did not loss their biocatalytic activity and that they can be stored for longer periods of time at low temperature, unlike fresh material that only can be stored for a few days. A lot of plants are not accessible all through the year, but since the dry powders can be stored, then they are always available like any other reagent.

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