Biocatalysis and Agricultural Biotechnology 4 (2015) 493–499

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Original Research Paper

Weeds as biocatalysts in the stereoselective synthesis of chiral phenylethanols used as key intermediates for pharmaceuticals

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ARTICLE INFO

Article history:
Received 16 June 2015
Received in revised form
30 July 2015
Accepted 1 August 2015
Available online 5 August 2015

Keywords:
Eryngium horridum
Biocatalysis
Bioreduction
Weeds
Substituted acetophenones
Chiral phenylethanols

ABSTRACT

This paper describes the search for novel vegetal biocatalysts for the stereoselective reduction of prochiral phenylketones. In this study, twenty native weeds were tested and *Eryngium horridum* Malme (Apiaceae) was proven to be an effective biocatalyst for the stereoselective reduction of acetophenone to (*S*)-1-phenylethanol (96% conversion, > 99.9 e.e.%). Using this biocatalyst, fourteen chiral (*S*)-phenylethanols with excellent enantiomeric excesses (> 98%) and variable conversions (30–100%) were obtained.

1. Introduction

The molecular complexity of currently used chemical compounds is rising and is characterized by a growing number of chiral centers. Moreover, because of safety and therapeutic and regulatory concerns, there has been an increasing interest in the development of processes capable of producing enantiomerically pure drugs (Hutt and Tan 1996; Rouhi 2003). Thus, it follow that the stereoselective production of enantiomerically pure molecules is the most critical step in the preparation of chiral building blocks for the pharmaceutical industry (Food and Drug Administration, 1992).

The asymmetric reduction of prochiral ketones represents a pivotal transformation in organic synthesis and can be performed using different catalytic processes (Singh, 1992). However, biocatalytic reduction provides an attractive means to reduce stereoselectively a broad range of ketones because of some comparative advantages (Wohlgemuth, 2010). Favorable characteristics of biocatalysts include their low cost, high versatility and efficiency, in addition to highly desirable chemical aspects such as chemoselectivity, regioselectivity and enantioselectivity. Moreover, there is the added advantage of using reagents for organic transformations that can be used on a sustainable basis, rather than depleting resources (Cordell et al., 2007). For these reasons, over the past decade the application of biocatalytic processes in the commercial synthesis of chiral alcohols has undergone a revolution. Biocatalysts are now often the preferred catalyst for the synthesis of chiral alcohols via ketone reduction (Huisman et al., 2010).

In recent years, chemical reactions using parts of plants as biocatalysts have received great attention because of their many advantages (Cordell et al., 2007). First of all, a large array of taxonomically different plants is available at a very low cost with these systems also having the advantage of being environmentally friendly due to the reaction being carried out in water as the solvent, and because the catalyst is biodegradable. For this reason, these processes generate less waste than conventional chemical reagents (Kumaraswamy and Ramesh, 2003).

In this sense, many transformations of different substrates, such as hydroxylation and oxidation reactions (*Gynostemma*

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http://dx.doi.org/10.1016/j.bcab.2015.08.001
1878-8181/

pentaphyllum, Sakamaki et al., 2005), hydrolysis of esters (*Solanum tuberosum, Helianthus tuberosus*, Mironowicz, 1998), bioreduction of ketones and aldehydes (*Daucus carota, Foeniculum vulgare, Cucurbita pepo, Phaseolus aureus, Cocos nucifera, Saccharum officinarum, Manihot dulcis, Manihot esculenta, Mespilus germanica, Citrus reticulata,* Cordell et al., 2007; Yadav et al., 2002; Bruni et al., 2006; Villa et al., 1998; Kumaraswamy and Ramesh, 2003; Fonseca et al., 2009; Baldassarre et al., 2000; Maczka and Mironowicz, 2004; Machado et al., 2006, 2008; Assunção et al., 2008; Blanchard and van de Weghe, 2006; Bennamane et al., 2014, 2015), enzymatic lactonization (*Malus sylvestris, Helianthus tuberosus*, Olejniczak et al., 2003), glycosylation (*Ipomoea batatas, Eucalyptus perriniana*, Shimoda et al., 2008), etc., have been performed using plants as biocatalysts, and have produced very good results. Moreover, the use of functionally intact cells ("whole plant cells") obtained directly from cut portions of plants have emerged, because the whole cells also ensure the recycling of the oxidized cofactors (Blanchard and van de Weghe, 2006). Additionally, these reaction systems do not need laborious cultivation or development operations to be performed, which are commonly employed in the management of microorganisms (Bohman et al., 2009).

This new methodology recently has been named as "*Botanochemistry*" and offers numerous advantages in terms of biodisponibility and economy of time, since fastidious steps of preparation, extraction, purification and multiplication of the biocatalyst are not necessary, thus promoting the preservation of a maximum catalytic activity of the enzymes (Vandenberghe et al., 2013). Additionally, botanochemistry is an interesting way to promote by-products of agriculture such as vegetable peelings that cannot be used for human consumption by food companies or for the use of those vegetable species which do not have any other reported practical utility and are simply considered to be weeds.

Recently, a project was commenced with the aim of identifying green procedures to obtain chemical intermediates using plants as biocatalyts. With this objective in mind, the screening of the some native weeds was initiated to search for plants that could be used as biocatalysts in the reduction of prochiral ketones in order to obtain chiral phenylethanols. In the particular case of these chiral alcohols, several were considered as key starting materials in the synthesis of scented substances for the pharmaceutical industries (Huisman et al., 2010). For this reason, this work is focused on the use of plants which are considered to be weeds for the stereoselective reduction of phenylketones as a sustainable alternative to traditional chemical methods.

2. Experimental

2.1. Generals

Ketones and $NaBH_4$ were purchased from Sigma-Aldrich S.A. (Argentina). Sterile deionizated water was used in all experiences. Ethyl acetate and hexane were purified by a simple distillation. GC analyses were made on a Shimadzu GC-14B instrument with a FID detector, and GC–MS analyses were carried out on a gas chromatograph Hewlett Packard HP 5890 Series II equipped with the Mass Detector HP 5970. The ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II 400 MHz using $CDCl_3$ as the solvent, and optical rotations were measured in a JASCO P-1010 polarimeter.

2.2. Biocatalysts

Healthy plants were collected in the Punilla Valley (Province of Córdoba, Argentina) and were identified by a botanist. Those selected had roots similar in form and texture to those a carrot (model vegetal biocatalyst) (Yadav et al., 2002). The aerial parts were discarded, and the roots were extensively washed with tap water to remove traces of soil.

2.3. General procedure for the bioreductions

Fresh plant roots were maintained in a 5% sodium hypochlorite aqueous solution for 10–20 min. Thus, they were washed with sterile deionized water again and the external layer was removed, with the remaining roots being cut into small thin slices (1 cm) with a sterile cutter. Treated and cut roots (10 g) were added to a sterile Erlenmeyer flask (250 mL) with sterile deionized water (75 mL), and ketone (50 mg) was added to this suspension. The reaction was carried out by stirring on an orbital shaker (120 rpm) at room temperature with the Erlenmeyer flask being closed. Then, the crude reaction was filtered through cotton, and the solution was extracted with ethyl acetate (3×40 mL). Finally, anhydrous calcium chloride was added to remove the dissolved water, and the organic solution was filtered and analyzed (1 μL) by chiral GC-FID and GC–MS. Thus, the organic solutions were evaporated, and the products were filtered on a short column with silica gel (70–230 mesh) using hexane-ethyl acetate in variable proportions as the eluent; the isolated yield was determined and the structure of the products was corroborated by ^1H NMR and ^{13}C NMR. Similarly, a control experiment was conducted without the addition of the corresponding phenylketone, and the crude reaction was analyzed by chiral GC-FID and GC–MS analyses.

2.4. Kinetic study of the bioreduction of acetophenone

In order to establish the optimal reaction time using *E. horridum* as biocatalyst, a kinetic study was made using acetophenone as the model substrat. The reaction progress was monitored by taking samples (2 mL) every 24 h, which were first extracted by shaking with ethyl acetate (2 mL) and the organic layer was collected. Then, anhydrous calcium chloride was added to remove the dissolved water, and the organic solution was filtered and analyzed (2 μL) by GC using the same general conditions described in GC-FID and GC–MS analyses. The reactions were made in triplicate.

2.5. GC-FID and GC–MS analyses

To establish the chromatographic conditions, all substrates used were previously reduced with $NaBH_4$ (Sigma-Aldrich S.A. Argentina) in ethanol to obtain the racemic mixture. The GC separations were performed on a fused silica capillary column Supelco β-Dex120 (phenyl-polysiloxane with 20% of permethylated β-Cyclodextrine, 30 m, 0.25 mm, 0.25 μm film thickness) with general GC conditions of: split mode 1/50; injector 220 °C; detector FID 220 °C; carrier gas N_2; head pressure 100 kPa. The conversion percentages of the reactions were determined by GC using the normalized peak areas without a correction factor, and the GC–MS (70 eV) analyses were performed using the same conditions as those in the GC analysis, but using a capillary column Hewlett Packard HP-5 (Crosslinked 5% PhMe Siloxane, 30 m, 0.3 mm, 0.25 μm film thickness).

2.6. GC and spectroscopic data

All products were identified by comparison of their GC retention times, MS, ^1H and ^{13}C NMR spectra with literature data. (Salvi and Chattopadhyay, 2001, 2008; Yu et al., 2011; Cheemala et al., 2007).

2.6.1. (−)-(S)-1-Phenylethanol

GC conditions: $T_1 = 80$ °C (1 min), $\Delta T = 2.5$ °C/min, $T_2 = 140$ °C. GC Rt acetophenone: 16.15 min, Rt (+)-(*R*)-1-phenylethanol: