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Bioreduction of aldehydes and ketones using *Manihot* species

Luciana L. Machado [a], João Sammy N. Souza [a], Marcos Carlos de Mattos [a],
Solange K. Sakata [a], Geoffrey A. Cordell [b], Telma L.G. Lemos [a,*]

[a] *Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, 60451-970 Fortaleza-Ceara, Caixa Postal 60021, Brazil*
[b] *Department of Oral Medicine and Diagnostic Sciences, College of Dentistry, Department of Medicinal Chemistry and Pharmacognosy,*
College of Pharmacy, University of Illinois at Chicago, Chicago, Il 60612, USA

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Dedicated to Professor Rod Croteau on the occasion of his 60th birthday.

Abstract

Biocatalysis constitutes an important tool in organic synthesis, especially for the preparation of chiral molecules of biological interest. A series of aliphatic and aromatic aldehydes and two ketones were reduced using plant cell preparations from *Manihot esculenta* and *Manihot dulcis* roots. The reduced products were typically obtained in excellent yields (80–96%), and with excellent enantiomeric excess (94–98%), except for vanillin. Esters, a nitrile, and an amide were also examined, but were not reduced. Preliminary conversion rate studies are reported. This is the first attempt to perform the biotransformation of carbonyl compounds using *Manihot* species.

Keywords: *Manihot esculenta*; *Manihot dulcis*; Euphorbiaceae; Biocatalytic reduction; Aliphatic and aromatic aldehydes and ketones; Ester hydrolysis

1. Introduction

In the past decades, biocatalysis has undergone significant development, and a number of reactions have been introduced and optimized, especially for the synthesis of chiral molecules of industrial and biological interest, such as drugs (Luna, 2004; Sharma et al., 2005), cosmetics (Veit, 2004), and other industrial chemicals (Liu et al., 2004). Several studies have demonstrated the high versatility, efficiency, selectivity, and the important chemical aspects, such as regioselectivity, chemoselectivity, and enantioselectivity of reactions involving biocatalysis (Castro and Knubovets, 2003; Shaw et al., 2003). One of the most important reactions in organic synthesis, and a key step in the manufacture of numerous pharmaceutical and specialty chemicals is the reduction of an aldehyde or a ketone to an alcohol, frequently in the presence of other reducible groups. Additionally, in certain instances, it may be critically important that the reduction product be chiral. Biocatalysis has been studied for the conduct of this reaction (Kroutil et al., 2004), and there are several reports where such reductions with yeasts (Mahima et al., 1993; Anderson et al., 1995; Toneva et al., 2002) and *Geotrichum candidum* (Matsuda et al., 2003) have led to high yields of product and excellent enantioselectivity.

In recent years, plant cell cultures and whole plant cells, in addition to microorganisms and enzymes, have been studied as potential agents for biotransformation reactions. Yadav et al. have investigated the function of *Daucus carota* roots for the asymmetric reduction of aliphatic and aromatic ketones, cyclic ketones, β-ketoesters, and azidoketones in aqueous medium (Yadav et al., 2002a,b). More recently, Comasseto et al. have explored the potential of *Daucus carota* roots for the preparation of chiral organochalcogeno-α-methylbenzyl alcohols (Comasseto et al., 2004). Enantioselective hydrolysis of racemic acetates in appreciable yield has also been conducted using comminuted tissues

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[*] Corresponding author. Tel.: +55 85 40089438; fax: +55 85 40089782.
E-mail address: tlemos@dqoi.ufc.br (T.L.G. Lemos).

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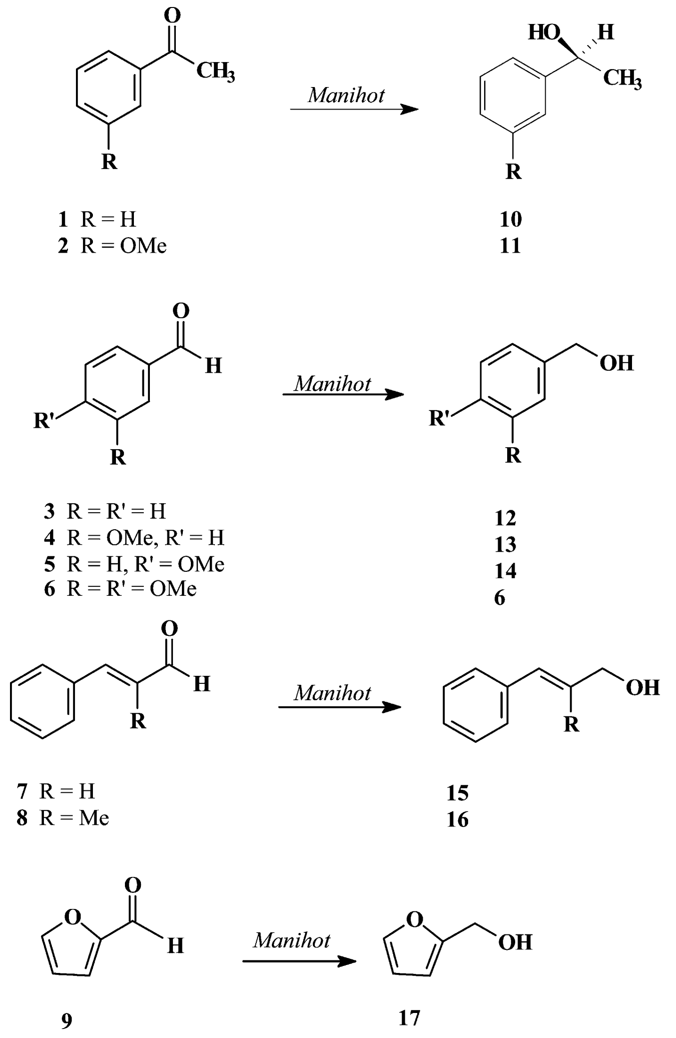
from ripe vegetable roots (Maczka and Mironowicz, 2002). Thus, it is possible that a wide range of plant materials, roots, tubers, seeds, and fruits from the Brazilian northeast used for nutritional and medicinal purposes could be a valuable source of preparations for a variety of biocatalytic reactions.

As part of an evaluation process for the discovery of chemical reactions catalyzed by the roots of local plants, the efficacy of two regional tubers were investigated as potential enzyme sources for the bioreduction of several aromatic aldehydes and ketones to their corresponding alcohols. In particular, this report describes a preliminary exploration of the potential of the roots of two local tubers *Manihot esculenta* Crantz and *Manihot dulcis* (J.F. Gemel) Pax (Euphorbiaceae) to serve as biocatalysts. The roots of *M. esculenta* and *M. dulcis* are valuable for their contemporary economic importance; both are tropical roots used widely in a large variety of traditional food preparations, especially in Brazilian northeast, Latin America, Africa, and Asia (Braga, 1976). In the process of the manufacture of the flour of *M. dulcis,* popularly known as mandioca, several thousand liters of water called "manipueira" are discarded. The potential use as a surfactant has previously been identified (Nitschke et al., 2004). Hydroxynitrile lyases catalyze the stereoselective addition of hydrocyanic acid to aldehydes and ketones (Schmidt and Griengl, 1999), and one of these, from *Manihot esculenta*, which provides an *S*-configured product, has been cloned and over-expressed for potential industrial application (Johnson and Griengl, 1999). The encouraging results obtained here using aqueous extracts for biocatalysis may offer new possibilities for the reduction of selected carbonyl compounds as a critical step in a synthetic organic pathway; and specifically avoiding the use of non-sustainable, hydride reducing agents.

2. Results and discussion

As a part of an evaluation process to find reductases from common vegetables, a series of plants used as food in the Brazilian northeast were evaluated, using acetophenone **1** (Scheme 1) as the substrate and a known methodology (Yadav et al., 2002a; Souza, 2003) using selected vegetable species (Table 1). The crude reaction mixtures were initially analyzed by TLC observing the formation of alcohol **10**, which was visualized practically in all of the tested materials. The samples were then analyzed by IR to observe the presence of OH stretching. Finally, the samples were analyzed by ^{1}H NMR spectroscopy to obtain a semi-quantitative measure of the respective yields using the integration area of the methyl ketone singlet at δ_H 2.61, compared to the quartet in the secondary alcohol product **2** δ_H 4.80 (Table 2). The results of this analysis for the selected species are reported in Table 1. The experiments, as well as the ^{1}H NMR spectroscopic analysis were performed in duplicate and the results reflect an arithmetic average.



Scheme 1.

Table 1
Relative percentage yield using the ^{1}H NMR integration of the methyl group of acetophenone **1** compared with integration of the carbinolic hydrogen of alcohol **10**

Plant material	Yield of **1** (%)	Yield of **10** (%)
Solanum melongena L.	57.8	42.1
Manihot dulcis Crantz	16.2	85.5
Manihot esculenta Crantz	10.7	89.3
Daucus carota L.	53.7	46.3
Colocasia esculenta (L.) Schott	44.2	55.8
Ipomoea batatas (L.) Lam	57.4	42.6

According to the data, the two *Manihot* species yielded the product alcohol in highest yields. Consequently, these *Manihot* species were selected for further development. The initial experiment was to determine the total protein in the enzymatic systems using the Hartree method (Hartree, 1972). For both species, the total protein was about 0.7%, in accordance with literature values of about 1% (Nassar, 1986). A series of simple aromatic aldehydes and ketones (**1**–**9**, Scheme 1) was treated with the freshly cut roots of *M. esculenta* and *M. dulcis* in aqueous solution