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Letter

Abstract

A series of ketones and aldehydes were reduced using plant cell preparations from fruits' barks of *Passiflora edulis* in water as solvent. The reduced products were obtained in very good yields, and low to moderate enantiomeric excesses were reached with aromatic ketones and a β-ketoester. This is the first time that the biotransformation of carbonyl compounds have been successfully achieved using *Passiflora* species.

Keywords: *Passiflora edulis*; Bioreduction; Biocatalysis; Aromatic aldehydes and ketones; β-Ketoester

Bioreduction of aromatic aldehydes and ketones by fruits' barks of *Passiflora edulis*

1. Introduction

Passiflora edulis is a wide spread plant cultivated around all tropical countries of the world. In Brazil, the fruits are commonly known as "maracuja" and the fruit pulp yields a delicious juice which is exported to several countries [1,2]. During industrial production of "maracuja juice", several thousands of fruits' barks are discharged, being considered an organic waste (Fig. 1).

Plant cell cultures, whole plant cell, microorganisms and enzymes have been studied as potential agents for biotransformation reactions specially for obtention of chiral alcohols as intermediates of pharmaceutical, and other potential compounds to be used in industrial scale [3–8a].

In order to determine the potential source of enzymes from Brazilian northeastern plants to be used as biocatalysts, an investigation of different tropical fruits and vegetables as bioreduction agents was carried out. Recently, we described successful regio- and enantioselective bioreductions of aromatic and aliphatic ketones and aldehydes, and also a β-ketoester using Manihot species as biocatalysts [8].

Following a program to find source of reductases from Brazilian plants to be used as biocatalysts [8,9], fruits' barks of *P. edulis* were investigated. Since fruits' barks are in general considered industrial organic waste and a potential source of reductase, they could be considered an alternative reagent in organic synthesis for production of important optically enriched products. To the best of our knowledge, this is the first report of the use of *P. edulis* as a biocatalyst agent.

2. Experimental

2.1. General

Optical rotations were measured in a PerkinElmer 341 digital polarimeter using chloroform as solvent. The reaction products and the pure starting materials were analyzed by GC–MS on a Hewlett-Packard (Model 5971) using a (5%-phenyl)-methylpolysiloxane DB-1 capillary column (30 m × 0.25 mm) with film thickness 0.1 μm; using helium as carrier gas and a flow rate 1 mL/min with split mode. The injector temperature and detector temperature were 250 and 200 °C, respectively. The column temperature was programmed at 4 °C/min from 35 to 180 °C, and then at 10 °C/min from 180 to 250 °C. NMR spectra were recorded on a Bruker Advance DRX-500 (500 MHz) using $CDCl_3$ as solvent. Chemical shifts, are given on the δ scale and referenced to the residual, undeuterated portion of the deuterated $CHCl_3$ solvent (δ_H 7.27). Column chromatography was run using silica gel 60 (70–230 mesh, Vetec). All aldehydes and ketones as well as racemic compounds **1a**, **2a** and **11a** were purchased from Aldrich Chemical Co. Chiral HPLC analyses were performed using a Hewlett-Packard 1100 chromatograph UV detector at 210 nm equipped with a Daicel Chiralcel OD column (25 cm × 4.6 mm i.d.). Chiral GC analyses were carried out using a Hewlett-Packard 6890 Series II apparatus equipped with a Restek RtβDEXse column (30 m × 0.25 mm × 0.25 μm, 1.0 bar N_2). For the determination, the injector temperature was 225 °C and the FID temperature was 250 °C.

2.2. Plant material

Fresh fruits of *P. edulis* were obtained from a specimen located at Campus do Pici-UFC, Fortaleza-Ceara-Brazil and

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Fig. 1. Photography of fruit and fruits' barks of *P. edulis* ("Maracuja" fruit).

identified by the botanist Prof. Edson P. Nunes. Voucher specimen (#12.811) is deposited at the Herbarium Prisco Bezerra (EAC) from Departamento de Biologia, Universidade Federal do Ceará, Brazil.

2.3. General bioreduction procedure for ketones *1* and *2*, aldehydes *3–10* and β-keto-ester *11* using *P. edulis*

Fruits' barks of *P. edulis* were rinsed with 5% sodium hypochlorite solution and sterile distilled water, and cut into small pieces (approx. 1 cm long slice).

In separate experiments, the corresponding substrate **1–11** (200 mg) was added to slices of fruits' barks of *P. edulis* (20 g) in 100 mL of water, and the mixture was incubated in a shaker (160 rpm) at room temperature for 72 h, according to previously described procedures [8–10].

The aqueous solution was then extracted with EtOAc (3 mL × 100 mL), and the solvent evaporated under reduced pressure. The resulting residue was filtered on a short silica gel column, using $CHCl_3$ as eluent (30 mL), to afford the reduced product (except **6**): **1a** (128 mg), **2a** (135 mg), **3a** (152 mg), **4a** (162 mg), **5a** (170 mg), **7a** (159 mg), **8a** (147 mg), **9a** (134 mg), **10a** (158 mg) or **11a** (165 mg). Conversions were quantified by GC–MS, and results are shown in Table 1.

Table 1
Conversion of **1–11** to the corresponding products **1a–11a** using fruits' barks of *Passiflora edulis*

Product	Conversion (%)[a]	*ee* (%)
1a	74	23[b]
2a	30	64[b]
3a	96	–
4a	84	–
5a	67	–
6	NR	–
7a	41	–
8a	35	–
9a	95	–
10a	97	–
11a	81	43[c]

NR: no reaction.
[a] Determined by GC–MS.
[b] Determined by chiral HPLC.
[c] Determined by chiral GC.

2.3.1. For experiments of bioconversion versus time

Compounds **1**, **3**, **7** and **9** were selected and the same procedure previously described in Section 2.3 was used. Aliquots were analyzed by GC–MS at reaction times varied from 12, 24, 36, 48, 60–72 h. Duplicated experiments were performed and the results are presented in Fig. 2.

2.4. Spectroscopical data

^{1}H NMR spectra of the isolated products **1a–11a** are presented below, and are in agreement of literature data [13].

2.4.1. (−)-(S)-1-Phenylethanol *1a*

$[\alpha]_D^{20} = -37,5$ (c 1.6, MeOH) [10a], $[\alpha]_D^{20} = -39,1$ (c 3.0, MeOH), for (*S*)-configuration. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.39 (d; 3H, $J = 5.48$ Hz), 2.18 (brs; 1H), 4.77 (q; 1H, $J = 6.45$ Hz), 7.16–7.28 (m; 5H).

2.4.2. (−)-(S)-1-(3-Methoxylphenyl)ethanol *2a*

$[\alpha]_D^{20} = -28.2$ (c 0.76, $CHCl_3$) [11] $[\alpha]_D^{20} = -30$ (c 1.75, $CHCl_3$), for (*S*)-configuration. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.18 (s; 1H); 2.59 (d; 3H, $J = 5.88$ Hz); 3.84 (s; 3H); 4.87 (q; 1H, $J = 6.40$ Hz); 7.16–7.28 (m; 5H).

2.4.3. Phenylmethanol *3a*

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.52 (s; 1H); 4.60 (s; 1H); 7.33–7.73 (m; 5H).

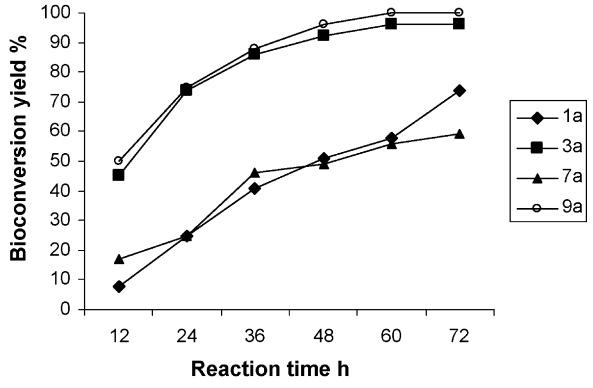


Fig. 2. Bioconversion vs. time: acetophenone (**1**), benzaldehyde (**3**), cinnamaldehyde (**7**) and furfuryl (**9**) to the corresponding alcohols 1-phenyl-ethanol (**1a**), benzyl alcohol (**3a**), cinnamyl alcohol (**7a**), and furfuryl alcohol (**9a**) using fruits' barks of *P. edulis*.

*2.4.4. 4-Methoxy-benzyl-alcohol **4a***

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.61 (s; 1H); 3.70 (s; 3H); 4.60 (s; 1H); 6.93 (d; 1H, J = 5.31 Hz); 6.93 (d; 1H, J = 5.31 Hz); 7.33 (d; 1H, J = 6.24 Hz); 7.33 (d; 1H, J = 6.24 Hz).

*2.4.5. 3-Methoxy-benzyl-alcohol **5a***

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.61 (s; 1H); 3.82 (s; 3H); 4.6 (s; 1H); 6.96 (m; 1H); 6.96 (m; 1H); 6.80 (dt; 1H, J = 4.64 Hz); 7.26 (t; 1H, J = 3.74 Hz).

*2.4.6. Cinnamyl alcohol **7a***

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.87 (s; 1H); 4.34 (d; 2H); 6.71 (m; 1H); 6.50 (d; 1H, J = 7.77 Hz); 7.30–7.50 (m; 2H).

*2.4.7. 1-Methyl-phenylallyl-alcohol **8a***

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.24 (s; 1H); 2.42 (s; 3H); 4.18 (s; 2H); 6.50 (s; 1H); 7.20–7.50 (m; 5H).

*2.4.8. Furfuryl alcohol **9a***

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.65 (s; 1H); 4.60 (s; 2H); 6.34 (m; 1H); 7.36 (d; 1H, J = 7.54 Hz); 7.40 (d; 1H, J = 2.73 Hz).

*2.4.9. Cyclohexan-1ol **10a***

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.51 (s; 1H); 3.62 (m; 1H); 1.70 (m; 2H); 1.51 (m; 2H).

*2.4.10. (+)-(S)-Hydroxyethyl butyrate **11a***

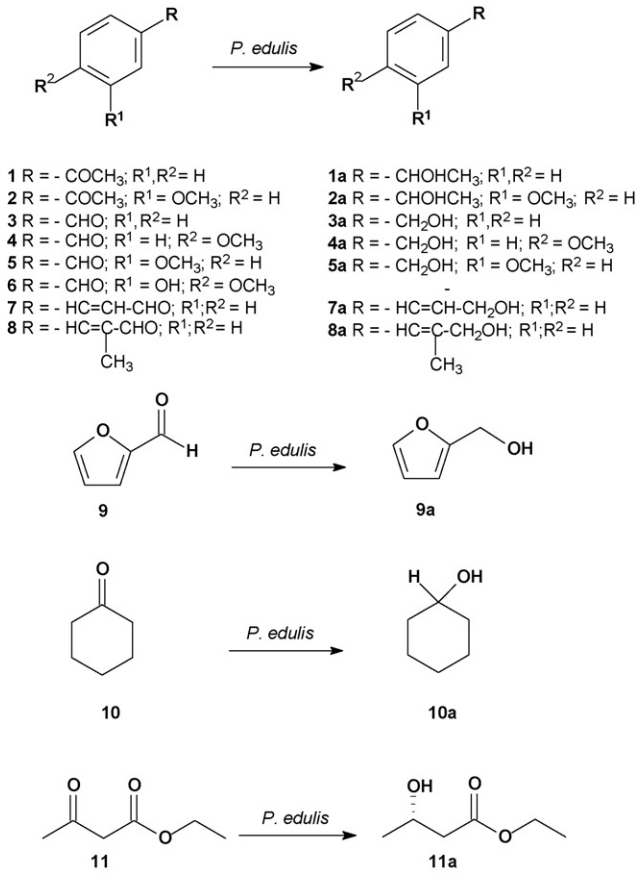
$[\alpha]_D^{20} = +35.7$ (c 0.5, $CHCl_3$) [14] $[\alpha]_D^{20} = +39.5$ (c 1.3, $CHCl_3$), for (*S*)-configuration. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21 (s; 3H); 1.29 (s; 3H); 2.18 (d; 1H, J = 3.06 Hz); 2.46 (dd; 1H, J = 1.26 Hz; J = 4.14 Hz); 4.20 (m; 1H).

*2.5. Acylation of compound **11a** and determination of enantiomeric excess*

The enantiomeric excess of **11a** was determined through the corresponding acylated derivative. Commercially available racemate **11a** and the reaction product obtained by reduction of **11** with *P. edulis* were separately acylated with Ac_2O in the presence of pyridine at room temperature [12]. Both acylated products were analyzed by Chiral GC and the *ee* of the bioreduction process was determined as 43%. GC conditions: 80 °C (10 min), 2 °C/min, 160 °C (10 min); t_R (*S*) 25.2 min, t_R (*R*) 27.1 min.

*2.6. Determination of enantiomeric excesses of compounds **1a** and **2a***

Commercially available racemic **1a** and **2a**, and reaction products obtained by reduction of **1** and **2** with *P. edulis* (water as solvent) were separately analyzed by HPLC and the enantiomeric excesses were determined as 23% and 64%, respectively. For both compounds, HPLC conditions: *n*-hexane/*i*-propanol 95:5, 0.8 mL/min; 20 °C; t_R (*S*)-**1a** 10.6 min, t_R (*R*)-**1a** 12.5 min; t_R (*S*)-**2a** 15.9 min, t_R (*R*)-**2a** 18.8 min.



Scheme 1.

3. Results and discussion

Experiments of biotransformations were carried out using the aromatic carbonyl compounds **1**–**11** following previously described procedures [8,10], treating aldehydes and ketones with freshly slices of fruits' barks in aqueous solution. The products were analyzed by GC–MS and the results are presented in Table 1. Except for aldehyde **6**, all tested carbonylic compounds were bioreduced yielding the corresponding alcohols with conversions from 30 to 97% (Scheme 1).

Both cinnamic aldehydes **7** and **8** yielded **7a** and **8a** in 41 and 35%, respectively, being considered lower conversions than those found for the others aromatic aldehydes. Reducing process for **7** and **8** were chemoselective, with the transformation occurring only in the carbonyl group, keeping the double bond located in the side chain intact. The aromatic ketones **1** and **2** yielded chiral alcohols **1a** and **2a** in different percentage, 74 and 30%, respectively. The *ee* of these compounds were determined by chiral HPLC as 23 and 64%, respectively.

The *Passiflora* reductase enzyme system was also extending to aliphatic carbonyl compounds. Cyclohexanone (**10**) and β-keto-ethyl-butyrate (**11**), presenting two different carbonyl groups) were selected as starting materials. The reduction of cyclohexanone yielded the corresponding alcohol (**10a**) in excellent yield (97%). The β-ketoester (**11**) was reduced with complete chemoselectivity to the corresponding β-hydroxyester

in good yield (81%). In this case, the *ee* was determined as 43% by chiral GC analysis of its acetylated derivative.

Configurations of the major enantiomers **1a**, **2a** and **11a** were established as "*S*" after comparison of their specific rotation with those described in the literature [10,11], what is in agreement with Prelog model for bioreduction [12].

The bioconversion process versus time was studied using compounds **1**, **3**, **7** and **9**, representing one ketone and three aldehydes, and the results are shown in Fig. 2. Maximum conversion for all selected compounds was observed after 72 h. As expected, aldehydes were more reactive than acetophenone (**1**), and benzaldehyde (**3**) and furfuraldehyde (**9**) were more reactive than cinnamaldehyde (**7**).

4. Conclusion

These results demonstrate that fruits' barks of *P. edulis* have enzyme system with ability to reduce carbonyl compounds to the corresponding alcohols with usually high conversions and good isolated yields. Low to moderate *ee* were found for the chiral alcohols **1a**, **2a** and **11a** and the major enantiomers showed the "*S*" configuration.

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