Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 51 (2008) 49–55
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Comparative study in the asymmetric bioreduction of ketones by plant organs and undifferentiated cells

Alejandro A. Orden, Fabricio R. Bisogno, Oscar S. Giordano, Marcela Kurina Sanz *

INTEQUI-CONICET, Área de Química Orgánica, Facultad de Química, Bioquímica y Farmacia, UNSL, Chacabuco y Pedernera, 5700 San Luis, Argentina

Received 2 July 2007; received in revised form 26 September 2007; accepted 10 October 2007
Available online 18 October 2007

Abstract

Stereoselective reduction of prochiral ketones was studied by using both cell cultures and wild tissues of various endemic plant species. Dedifferentiated cell cultures of *Baccharis crispa* reduced regioselectively, with excellent stereocontrol, and in an anti-Prelog manner the methyl ketone moiety of a diketone, meanwhile *Tessaria absinthioides* cell cultures yielded the other antipode of the secondary alcohol with an optical purity higher than 98% *ee*. Therefore, it is possible to prepare the *S* and *R* isomers of a secondary alcohol by reduction of the corresponding prochiral ketone with these two biocatalysts. Moreover, significant differences in the reductive capacity of cell cultures and differentiated tissues of the same plant species were found. This fact allows improving the yields and optical purities and, even more interesting, obtaining the antipode of a secondary alcohol with the same plant species by using alternatively both biocatalytic systems.

Keywords: Enantioselective; Regioselective; Bioreduction; Prochiral ketones; Plants

1. Introduction

There is a considerable interest in efficient routes to obtain enantiopure chiral alcohols since they are useful building blocks in the synthesis of more complex compounds. They can be obtained with high optical purity from the reduction of prochiral ketones either by chemical or biological methods. The use of ruthenium(II) and iridium(I) complexes, as well as the CBS-oxaborolidine developed by Corey et al., are good examples of the former methodology [1 and references therein]. The use of locally available vegetables may offer an alternative opportunity for countries to investigate their local resources for the effective conduct of key synthetic transformations with significant economic and ecological implications [2]. One of the main advantages in using whole plant cells instead of isolated dehydrogenases is a relatively simple handling that does not require complex protein separation procedures or the addition and/or recycling of exogenous cofactors. However, the use of whole cell systems to metabolize xenobiotic substrates does not afford highly productive processes due to the toxicity of substrates and products towards living organisms. This fact leads to the necessity of employing large amounts of biomass to obtain reasonable yields. Biotransformations based on wild plant tissues and organs are very simple owing to the availability of biomass and the use of water with or without carbon sources as reaction media that makes the work up procedure easy and reduces costs. Despite that, these systems have some drawbacks regarding the possible use of various cultivars for the same species and the diverse activity of cells in different harvest seasons and geographical regions.

Following with our studies in asymmetric bioreductions of diketones with plants, we have tested the biocatalytic abilities of some South American endemic species. Their regio- and enantioselectivities were compared with *Daucus carota* and *Gardenia augusta* (ex. *jasminoides*). The former is well known for its selective reductive capacity; meanwhile *G. augusta* transformed acetophenone in a two-step pathway leading to the *R*-1-phenylethanol [3]. *D. carota* is by far the most widely used plant for the reduction of prochiral ketones yielding the corresponding chiral alcohols with excellent enantioselectivity and conversion rates. This biocatalyst has been employed as undifferentiated suspension cells [4–6], hairy roots [7] and fresh roots

* Corresponding author. Tel.: +54 2652 439909; fax: +54 2652 426711.
E-mail address: mkurina@unsl.edu.ar (M. Kurina Sanz).

1381-1177/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2007.10.003

[8–15]. For most cases, the reduction of the carbonyl group by carrots proceeds stereoselectively following the Prelog's rule to furnish the *S*-alcohol. Consequently, it is very important to continue the screening in order to identify species that yield the *R* alcohol as the main biotransformation product.

This work is focused on the screening of plants capable of reducing substrates bearing two different prochiral ketones, a methyl aryl ketone moiety and a chroman-4-one ring. Thereby, two commercial reagents, acetophenone (**1a**) and chroman-4-one (**2a**) were used as substrates with both dedifferentiated cell cultures and organs of endemic plants. Also, the natural product 6-acetyl-2,2-dimethyl-2,3-dihydro-4*H*-chromen-4-one (**3a**), was chosen as a substrate to study the regio- and stereoselectivity of the bioreduction process since it bears both ketone moieties in the same molecule. This product was reduced with stereo- and regiocontrol by *Brassica napus* hairy roots in a previous work [16].

Although many papers, as the ones previously cited, have focused on the reduction of ketones by plants, the use of diketones is not a frequent approach. Among the reports dealing with this topic, it is worth mentioning the reduction of camphorquinone and 1,2-cyclohexanedione by various vegetables [13] and the biotransformation of 3,6-dialkylcyclohexane-1,2-dione by plant cells of *Marchantia polymorpha* [17].

Most of the endemic plant species used in this work such as *Baccharis crispa*, *B. flabellata*, *Tessaria absinthioides*, *Grindelia pulchella* and *Buddleja cordobensis* are used in folk medicine and/or their crude extracts or isolated compounds have exhibited some in vitro bioactivity [18–23].

2. Experimental

2.1. General methods

The NMR spectra were obtained using a Bruker ARX 300 or AMX-400 instruments. For ^{1}H NMR, instruments were operated at 300 MHz or 400 MHz (Mosher's esters) and for ^{13}C NMR at 75.5 MHz. Chemical shifts are expressed in ppm values using TMS as an internal standard. Electron Impact MS were collected at 70 eV on a GCQ Plus Finnigan Mat. Optical rotations were obtained on a Perkin-Elmer 341 polarimeter. Biotransformation reactions were monitored by GC (FID) analysis in a Perkin-Elmer Clarus 500 instrument equipped with an Elite 5-column (30 m, 0.25 mm ID and 0.25 μm d.f.) or a β-DEX-column (60 m, 0.25 mm ID and 0.25 μm d.f.). CC procedures were performed on silica gel G 70–230 mesh Merck. TLC was carried out on Si gel 60 F_{254} Merck (0.2 mm-thick plates).

2.2. Substrates and standards

The substrates acetophenone (1a) and chroman-4-one (**2a**) were purchased to Sigma-Aldrich Argentina SA.

2.2.1. Substrates *1a* and *2a*

Acetophenone **(1a)** and chroman-4-one **(2a)** were purchased to Sigma-Aldrich Argentina S.A.

2.2.2. Isolation of *3a*

Compound **3a** was isolated from dried aerial parts of *Ophryosporus axilliflorus* (Griseb.) Hieron as it was described in [24] or prepared from the secondary metabolite 1-(5-acetyl-2-hydroxyphenyl)-3-methylbut-2-en-1-one by non enzymatic cyclation as it was described in [16]. Comparison of its spectral data with those reported confirmed their structures.

6-Acetyl-2,2-dimethyl-2,3-dihydro-4H-chromen-4-one (**3a**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50 (6H, s, H-3′, H-4′), 2.60 (3H, s, H-2′), 3.28 (2H, s, H-3), 7.00 (1H, d, J = 8.2 Hz, H-7), 8.15 (1H, dd, J = 8.2, 2.0 Hz, H-8), 8.45 (1H, d, J = 2.0, H-5). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 26.2 (q, C-3′, C-4′), 26.3 (q, C-2′), 48.1 (t, C-3), 80.0 (s, C-2), 118.6 (d, C-8), 118.8 (s, C-10), 127.0 (d, C-5), 129.2 (s, C-6), 134.3 (d, C-7), 162.8 (s, C-9), 192.1 (s, C-1′), 195.3 (s, C-4). EI MS *m/z* (relative intensity %): 218 (5), 203 (100), 165 (30), 163 (22), 161 (36), 147 (35), 119 (10).

2.2.3. Preparation of the racemic and diastereomeric mixtures for analysis correlations by gas chromatography

2.2.3.1. Synthesis and structural determination of the racemic alcohols. Compounds **1a**, **2a** and **3a** were reduced to the corresponding racemic alcohols with $NaBH_4$ in MeOH. The reduction products were purified by CC on Si gel, eluting with *n*-hexane-EtOAc. Their chemical structures were confirmed by ^{1}H NMR.

4-Chromanol (**2b**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.00 (2H, m, H-3), 2.72 (1H, bs, -OH), 4.20 (2H, m, H-2), 4.69 (1H, t, H-4), aromatic ring: 6.88 (2H, m) and 7.20 (2H, m).

6-(1-Hydroxyethyl)-2,2-dimethylchroman-4-one (**3b**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (6H, s, H-3′, H-4′), 1.49 (3H, d, J = 7.0 Hz, H-2′), 2.71 (2H, s, H-3), 4.87 (1H, q, H-1′), 6.93 (1H, d, J = 8.9 Hz, H-8), 7.56 (1H, dd, J = 8.9, 2.0 Hz, H-7), 7.83 (1H, d, J = 2.0 Hz, H-5). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 25.2 (q, C-2′), 27.0 (q, C-3′, C-4′), 48.5 (t, C-3), 69.5 (d, C-1′), 79.0 (s, C-2), 118.5 (d, C-8), 120.3 (s, C-10), 122.8 (d, C-5), 133.0 (d, C-7), 138.5 (s, C-6), 159.1 (s, C-9), 192.5 (s, C-4). EI MS *m/z* (relative intensity %): 220 (23), 205 (100), 165 (44), 149 (87), 121 (47), 91 (26), 65 (42).

1-(4-Hydroxy-2,2-dimethylchroman-6-yl)ethanone (**3c**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (3H, s, H-3′), 1.40 (3H, s, H-4′), 1.83 (1H, dd, J = 12.0, 8.5 Hz, H-3a), 2.15 (1H, dd, J = 12.0, 6.2 Hz, H-3b), 2.47 (3H, s, H-2′), 4.82 (1H, dd, J = 8.5, 6.2 Hz, H-4), 6.75 (1H, d, J = 8.6 Hz, H-8), 7.73 (1H, dd, J = 8.6, 2.3 Hz, H-7), 8.05 (1H, d, J = 2.3 Hz, H-5). ^{13}C NMR (75.5 MHz, $CDCl_3$): Δ 25.1 (q, C-4′), 25.3 (q, C-3′), 27.9 (q, C-2′), 41.3 (t, C-3), 62.2 (d, C-4), 74.4 (s, C-2), 116.3 (d, C-8), 122.7 (s, C-10), 128.1 (d, C-5), 128.3 (s, C-6), 128.8 (d, C-7), 156.1 (s, C-9), 195.3 (s, C-1′). EI MS *m/z* (relative intensity %): 220 (7), 187 (73), 165 (21), 164 (23), 149 (100).

1-(4-Acetoxy-2,2-dimethylchroman-6-yl)ethyl (**3d**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (3H, s, H-3′), 1.35 (3H, s, H-4′), 1.40 (3H, d, J = 6.5 Hz, H-2′), 1.76 (1H, dd, J = 13.5, 8.8 Hz, H-3a), 2.77 (1H, dd, J = 13.5, 6.1, H-3b), 4.74 (2H, m, H-4, H-1′), 6.68 (1H, d, J = 8.4 Hz, H-8), 7.38 (1H, dd, J = 8.4, 2.2 Hz, H-7), 7.95 (1H, m, H-5). ^{13}C NMR (75.5 MHz, $CDCl_3$): Δ 23.8 (q, C-2′), 24.9 (q, C-3′), 27.9 (q, C-4′), 41.8 (t, C-3), 62.6 (d, C-4), 69.0 (d, C-1′), 74.3 (s, C-2), 116.2 (d, C-8), 123.1

(s, C-10), 123.7 (d, C-5), 125.5 (d, C-7), 136.5 (s, C-6), 151.6 (s, C-9). EI MS *m/z* (relative intensity %): 222 (17), 207 (35), 189 (82), 151 (100), 123 (23), 91 (24), 77 (67).

1-(4-Acetoxy-2,2-dimethylchroman-6-yl)ethyl acetate (*acetyl* **3d**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38 (3H, s, H-3′), 1.44 (3H, s, H-4′), 1.52 (3H, d, $J = 4.5$, H-2′), 2.00 (1H, m, H-2a), 2.05 (3H, s, acetate moiety), 2.14 (3H, s, acetate moiety), 2.20 (1H, m, H-2b), 5.83 (1H, q, $J = 4.5$ Hz, H-1′), 5.99 (1H, m, H-4), 6.80 (1H, d, $J = 6.0$ Hz, H-8), 7.20 (1H, d, $J = 1.8$ Hz, H-5), 7.24 (1H, dd, $J = 6.0$, 1.8 Hz, H-7).

2.2.3.2. Synthesis of acetate derivatives. The extracts from the biotransformations carried out with substrate **3a** and its racemic alcohols obtained by chemical reduction were treated with acetic anhydride and pyridine. The reaction mixtures were incubated in darkness at room temperature for 24 h. After this period, they were washed with $CuSO_4$ 10%, extracted with EtOAc and dried over anhydrous Na_2SO_4.

2.2.3.3. Assignment of the absolute configurations for the alcohols. Optical rotations of the purified alcohols **1b** and **2b**, obtained by biotransformations were measured and compared with literature. ***S*-1b**: $[\alpha]_D^{25} = -46°$ (methanol; *c* 5) and ***S*-2b**: $[\alpha]_D^{25} = -67°$ (chloroform; *c* 0.5). The absolute configuration of **3b** was determined by Mosher's method as it was described by Orden et al. [16]. ***S*-3b**: $[\alpha]_D^{25} = +36.4°$ (acetone; *c* 1.1).

(S)-(MTPA) Ester of **3b**: ^{1}H NMR (400 MHz, $CDCl_3$) Δ 1.56 (3H, d, $J = 7.0$ Hz, H-2′), 2.72 (2H, s, H-3), 6.07 (1H, q, $J = 7.0$ Hz, H-1′), 6.91 (1H, d, $J = 8.9$ Hz, H-8), 7.38 (1H, dd, $J = 8.9$, 2.0 Hz, H-7), 7.86 (1H, d, $J = 2.0$ Hz, H-5).

(R)-(MTPA) Ester of **3b**: ^{1}H NMR (400 MHz, $CDCl_3$) Δ 1.62 (3H, d, $J = 7.0$ Hz, H-2′), 2.71 (2H, s, H-3), 6.04 (1H, q, $J = 7.0$ Hz, H-1′), 6.85 (1H, d, $J = 8.9$ Hz, H-8), 7.35 (1H, dd, $J = 8.9$, 2.0 Hz, H-7), 7.77 (1H, d, $J = 2.0$ Hz, H-5).

2.3. Biotransformation procedures

2.3.1. Biotransformations with undifferentiated cells (calli)

The plant cell calli used in this research were initiated from leaves and maintained on Murashige Skoog agar [25] supplemented with sucrose (30 g l^{-1}) and auxins and cytokinins at different ratios. The calli were mechanically disrupted and placed in flasks containing 20 mL of liquid MS media without phytoregulators (5 g per flask) or in 30 mM phosphate buffer (pH 6). 0.026 mmoles of substrates **1a** (3 μl), and **2a** (3.8 mg) and 0.013 mmoles of **3a** (2.8 mg) dissolved in 30 μl of *N*,*N*-DMF were added to the each flask and incubated in an orbital shaker at 120 rpm for 3 and 6 days. The suspension was filtered and the media was extracted with EtOAc (×3), dried over anhydrous Na_2SO_4 and evaporated in vacuo.

2.3.2. Biotransformations with wild plant tissues and organs

Fresh plants of *B. crispa*, *B. cordobensis*, *T. absinthioides* and *G. pulchella* were collected in Departamento Capital, San Luis, in summer (December 2006–March 2007). A voucher specimen of each species is deposited at the Herbarium of the UNSL (376, 4868, 7922 and 3616, respectively). *G. augusta* and *B. flabellata* (voucher number UNSL-434) were collected from the UNSL Botanic Garden.

The leaves of each plant were excised and surface disinfected with EtOH 70% for 3 min and NaClO 20% for 10 min and rinsed several times with sterile distilled water. Leaves were cut and put in flasks containing 20 ml of distilled water (5 g per flask).

Fresh *D. carota* roots purchased at the local market were peeled off, submerged in NaClO 20% for 10 min, chopped into small pieces and inoculated in sterile water (5 g per flask of 20 mL).

The substrates were added as it was described in Section 2.3.1 and the flasks incubated under vigorous shaking (170 rpm).

Blank assays without substrates and without biomass were carried out in parallel. The results are the media of the repetition of three experiments.

2.4. Conversion rates analysis

After 3 and 6 days of incubation, biomass was separated from the culture media by either filtration or centrifugation. Culture media was extracted with EtOAc (×3), the solvent dried over anh. Na_2SO_4 and removed in vacuo. For biotransformations with **3a**, the organic extracts were acetylated, prior to analysis, as it was described in Section 2.2.3.2. Aliquots of the residues (1 μl) were analyzed by GC-FID equipped with Elite-5 column (30 m, 0.25 mm ID and 0.25 μm d.f.).

1a-b, **2a-b**: Oven $T_1 = 100\,°C$ (4 min), $T_2 = 200\,°C$ ($\Delta t = 30\,°C$/min), injector $T = 200\,°C$, carrier gas N_2 46 cm/seg, FID $T = 350\,°C$. Compound **1a** ($R_t = 3.06$ min) and (***R*/*S*)-1b** ($R_t = 2.95$ min). Compound **2a** ($R_t = 6.22$ min) and (***R*/*S*)-2b** ($R_t = 6.26$ min).

3a–d: Oven $T_1 = 100\,°C$, $T_2 = 320\,°C$ ($\Delta t = 25\,°C$/min), injector $T = 240\,°C$, carrier gas N_2 35 cm/seg, FID $T = 350\,°C$. Compound **3a** ($R_t = 5.13$ min), acetyl derivatives of (***R*/*S*)-3b** ($R_t = 5.48$ min), (***R*/*S*)-3c** ($R_t = 5.66$ min) and (***R*,*R*/*R*,*S*/*S*,*R*/*S*,*S*)-3d** ($R_t = 5.98$ and 6.04 min).

2.5. GC analysis for determination of the enantiomeric excess (ee)

The reaction progress was monitored by collecting a flask every 3 and 6 days. These samples were extracted with EtOAc (×3). The organic phase of the bioconversion with **3a** was derivatized as it was described in Section 2.2.3.2. Samples (1 μl) were analyzed by chiral GC on a β-DEX-column (60 m, 0.25 mm ID and 0.25 μm d.f.). The products of the biocatalyzed reactions were compared with racemic mixtures and standards of ***S*-1b**, ***S*-2b** and ***S*-3b** obtained by bioreduction with *D. carota* and *B. napus* hairy roots.

GC conditions:

1a–b: Oven $T_1 = 120\,°C$, $T_2 = 150\,°C$ ($\Delta t = 3\,°C$/min), $T_3 = 160\,°C$ ($\Delta t = 5\,°C$/min); injector $T = 200\,°C$, carrier gas N_2 25 cm/seg; FID $T = 300\,°C$. Compound **1a** ($R_t = 13.55$ min), ***R*-1b** ($R_t = 16.55$ min), ***S*-1b** ($R_t = 16.91$).

2a–b: Oven $T = 155\,^{\circ}C$; injector $T = 220\,^{\circ}C$, carrier gas N_2 28 cm/seg; FID $T = 300\,^{\circ}C$. Compound **2a** ($R_t = 28.32$ min), ***R*-2b** ($R_t = 39.36$ min), ***S*-2b** ($R_t = 39.92$).
3a–b: Oven $T = 185\,^{\circ}C$; injector $T = 240\,^{\circ}C$, carrier gas N_2 27 cm/seg; FID $T = 350\,^{\circ}C$. Compound **3a** ($R_t = 64.61$), acetyl-***S*-3b** ($R_t = 82.87$ min), acetyl-***R*-3b** ($R_t = 83.91$ min).

3. Results and discussion

Among the plant cell suspension tested, *G. augusta* and *G. pulchella* reduced **1a** in an anti-Prelog manner, but with different conversion rates and, particularly with different stereoselection, since the former reached an *ee* higher than 80% and *G. pulchella* only gave 22% *ee* (Table 1). Conversely, leaves of these species yielded mainly the ***S*-1b** isomer. When chroman-4-one **2a** was used as substrate, the conversion rates and optical purities increased considerably by using both cells and differentiated tissues of *G. pulchella* as biocatalysts (Table 2). It is noteworthy that the optical purity of the alcohol ***S*-2b** decreased during the incubation time with leaves, suggesting that it would exist a competition between reduction and oxidation reactions with different stereoselection. A similar behavior was observed with *B. flabellata*.

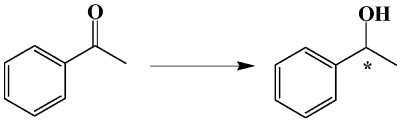
Another species that showed significant differences in yields and *ee* in the reduction of **1a** along the incubation time was *T. absinthioides* (cell cultures). It was observed a remarkable increase in the conversion rates and also an improvement in stereoselectivity. However, these results are not in agreement with the ones observed in the process catalyzed by *T. absinthioides* wild tissues, in which the *ee* diminished as the conversion rates increased during the incubation. On the other hand, *T. absinthioides* cell cultures reduced with very good yield and optical purity the pirane ring ketone of **2a**. The selective bioreduction of the methyl ketone of **3a** furnished the alcohol ***S*-3b** as the main product; in this case, the optical purity of this enantiomer was excellent (*ee* > 98%). For this reason, it results in a valuable system to obtain an alcohol following the Prelog's rule (Table 3).

No bioreduction products for **1a** and **2a** were detected with undifferentiated cells of *B. cordobensis*. Nevertheless, its leaves showed excellent conversion rates and *ee* (>98% at day 3) of **1b** and **2b**. Conversely, only calli were capable of reducing the methyl ketone of **3a** with good optical purity but with low yields (Tables 3 and 4).

Most of the biocatalysts employed reduced **3a** with a strong regio-preference for the methyl aryl ketone. In fact, undifferentiated cells of *G. augusta* were the sole biocatalyst that furnished the enantio- and diastereomeric mixtures of the diol **3d** as a main product. During the bioconversion of **3a** with *G. augusta* calli, the alcohol **3b** was consumed as the amount of **3d** increased.

Cell cultures of *B. crispa* reduced the ketone **2a** and the diketone **3a** yielding the alcohols ***R*-2b** and ***R*-3b** respectively, showing quite good conversion rate (66%) and excellent optical purity for the isomer ***R*-3b** (*ee* > 98%). Since there is no variation in the *ee* of the *R* isomer during the biotransformation process, it is possible to assume that the reduction is highly stereoselective. Remarkably, **1a** was reduced to ***S*-1b** with low conversion

Table 1
Bioreduction of **1a**

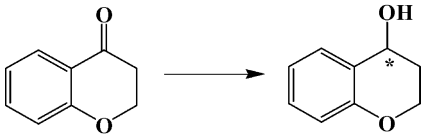


1a **1b**

Plant species	*t* (days)	1-Phenylethanol **(1b)**					
		Undifferentiated cells (calli)			Differentiated tissues (organs)		
		Conv.	*ee*	Config.	Conv.	*ee*	Config.
B. crispa	3	21	45	S	14	67	S
	6	25	42	S	30	82	S
B. flabellata	3	40	70	S	34	42	S
	6	nb	–	–	19	72	S
B. cordobensis	3	nb	–	–	95	98	S
	6	nb	–	–	98	93	S
D. carota	3	45	>98	S	95	>98	S
	6	54	>98	S	>98	>98	S
G. augusta	3	41	82	R	77	22	S
	6	52	87	R	92	16	S
G. pulchella	3	37	20	R	33	77	S
	6	31	22	R	21	70	S
T. absinthioides	3	18	80	S	8	52	S
	6	96	89	S	26	31	S

Conversion rates (Conv.) and *ee* determined by GC. Config.: absolute configuration. nb: no bioreduction products detected.

Table 2
Bioreduction of **2a**

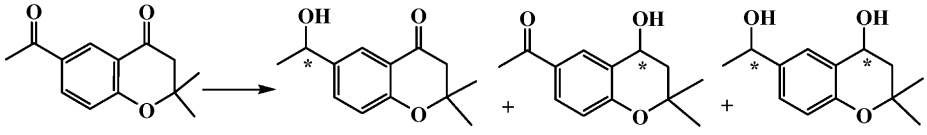


2a **2b**

Plant species	*t* (days)	1-Chromanol (**2b**)					
		Undifferentiated cells (calli)			Differentiated tissues (organs)		
		Conv.	*ee*	Config.	Conv.	*ee*	Config.
B. crispa	3	34	30	R	28	79	S
	6	9	24	R	42	80	S
B. flabellata	3	29	61	S	53	88	S
	6	24	45	S	13	72	S
B. cordobensis	3	nb	–	–	98	98	S
	6	nb	–	–	98	80	S
D. carota	3	98	>98	S	98	>98	S
	6	97	>98	S	98	>98	S
G. augusta	3	11	9	S	95	83	S
	6	7	47	S	94	90	S
G. pulchella	3	55	79	S	41	83	S
	6	47	72	S	41	36	S
T. absinthioides	3	41	96	S	42	89	S
	6	87	95	S	51	82	S

Conversion rates (Conv.) and *ee* determined by GC. Config.: absolute configuration. nb: no bioreduction products detected.

Table 3
Bioreduction of **3a** by undifferentiated cell cultures



3a **3b** **3c** **3d**

Plant species	*t* (days)	**3a** (%)	**3b**			**3c**	**3d**	
			Conv.	*ee*	Config.	Conv.	Conv.	
							a	b
B. crispa	3	59	34	>98	R	6	<1	
	6	26	66	>98	R	6	1	1
B. flabellata	3	81	9	nd	–	10	<1	
	6	70	15	90	S	15	<1	
B. cordobensis	3	84	14	95	–	2	<1	
	6	82	16	95	–	2	<1	
D. carota	3	8	81	>98	S	10	<1	<1
	6	4	74	>98	S	14	1	6.5
G. augusta	3	2	79	69	S	4	9.0	6.0
	6	<1	18	13	R	6	46.0	29.0
G. pulchella	3	59	39	63	R	2	<1	
	6	65	28	–	–	6	<1	<1
T. absinthioides	3	66	42	>98	S	2	<1	
	6	25	68	>98	S	5	2.0	

Conversion rates (Conv.) and *ee* determined by GC. Config.: absolute configuration. a and b correspond to the peaks for each diastereomer. <1 means traces of the products.

Table 4
Bioreduction of **3a** by differentiated tissues and organs

Plant species	*t* (days)	**3a** (%)	**3b**			**3c**	**3d**
			Conv.	*ee*	Config.	Conv.	Conv.
B. crispa (l)	3	87	9	–	–	4	–
	6	73	16	37	S	11	–
B. flabellata (l)	3	79	15	12	S	6	–
	6	59	27	15	S	14	–
B. cordobensis (l)	3	100	nb	–	–	–	–
	6	100	nb	–	–	–	–
D. carota (r)	3	6	91	>98	S	2	1
	6	–	95	>98	S	4	1
G. augusta (l)	3	76	20	48	S	4	–
	6	62	29	57	S	9	–
G. pulchella (l)	3	98	1	–	–	1	–
	6	95	2	–	–	3	–
T. absinthioides (l)	3	77	22	41	S	1	–
	6	60	36	43	S	4	–

Conversion rates (Conv.) and *ee* determined by GC. Config.: absolute configuration. (l): leaves. (r): roots

rate and *ee*. These results indicate that the biocatalytic system is more stereoselective when the methyl aryl ketone moiety is positioned in a more complex molecule. On the contrary, differentiated tissues of *B. crispa* furnished the *S*-enantiomers as the main products. Except for the diketone **3a**, both conversion rates and optical purities of the chiral alcohols increased when leaves were used as biocatalysts (Table 4). *B. crispa* calli reduced **3a** with good conversion rates and excellent *ee* leading to ***R*-3b**; meanwhile, very low yield and optical purity of the isomer ***S*-3b** was observed with leaves. According to these results, it could be assumed a further oxidation of the secondary alcohols, with a slight preference to the *R* isomer in the differentiated tissues but not in calli. Another explanation could be the lack or low expression of the callus deshydrogenase in leaves.

Unfortunately, none of the biocatalysts assayed, reduced the carbonyl group on C-4 of the diketone substrate **3a** with regioselectivity as they did with the methylketone. The low amounts of **3c** obtained by bioconversion were not sufficient to determine the absolute configuration or the *ee*.

A quite different behavior was observed in the bioreduction of ketones carried out with organs from wild plants and with undifferentiated tissues cultured in vitro. Other authors have reported that differentiated tissues were more efficient in the reduction than calli [12]. This assertion was not always in agreement with our results. As this regards, the reduction of substrates **1a** and **2a** with *T. absinthioides* is particularly noticeable. Except for the well-known case of *D. carota* roots which stores large amounts of sugar, the differentiated tissues employed in our experiments were parenchyma of the leaves. The lack of sugars, as reduction equivalents, necessary to recycle NAD(P)H, could explain the above-mentioned discrepancy. However, when the biocatalyst was *B. cordobensis*, there was not bioreduction of substrates **1a** and **2a** with cell cultures, although excellent yields and optical purities were afforded with leaves.

For these reasons, several assays using different tissues and organs should be performed before ruling out a particular species in bioreduction processes. On the other hand, the use of calli in biocatalysis allows working under reproducible conditions, avoiding seasonal, geographical and cultivar variations.

4. Conclusion

In this work we compare the ability of differentiated and undifferentiated tissues from some South American endemic plants to reduce ketones to their corresponding alcohols. We could detect two cell lines capable of reducing the methyl aryl ketone moiety of the diketone **3a** with opposite stereochemistry and in both cases with excellent regio- and stereocontrol. This fact gives us the possibility of preparing both ***S*-3b** and ***R*-3b** enantiomers.

Based on the results here reported, we can conclude that there are profound differences in the reductive capacity of undifferentiated cells and organs of the same plant species. Consequently, it is possible, not only to improve the yields and optical purities, but also to obtain both antipodes of a *sec*-alcohol by using alternatively differentiated and undifferentiated plant tissues as biocatalysts.

Acknowledgements

The authors want to thank M. Ferrari and H. Lancelle for the technical assistance and C. Ardanaz for MS spectra. Financial support from CONICET (PIP 6228 and A.A.O. doctoral fellowship) and UNSL (Project 22Q-505) is gratefully acknowledged. This work is part of A.A.O. Doctoral Thesis.

References

[1] A. Hage, D. Petra, J. Field, D. Schipper, J. Wijnberg, P. Kamer, J. Reek, P. van Leeuwen, R. Wever, H. Schoemaker, Tetrahedron: Asymmetry 12 (2001) 1025–1034.
[2] G. Cordell, T. Lemos, F. Monte, M. de Mattos, J. Nat. Prod. 70 (2007) 478–492.

[3] Y. Akakabe, Y. Naoshima, Phytochemistry 32 (1993) 1189–1191.
[4] Y. Naoshima, Y. Akakabe, Phytochemistry 30 (1991) 3595–3597.
[5] R. Villa, F. Molinari, M. Levati, F. Aragozzini, Biotechnol. Lett. 20 (1998) 1105–1108.
[6] B. Baskar, S. Ganesh, T. Lokeswari, A. Chadha, J. Mol. Catal. B Enzyme 27 (2004) 13–17.
[7] D. Caron, A. Coughlan, M. Simard, J. Bernier, Y. Piché, R. Chênevert, Biotechnol. Lett. 27 (2005) 713–716.
[8] J. Comasseto, A. Omori, A. Porto, L. Andrade, Tetrahedron Lett. 45 (2004) 473–476.
[9] J. Yadav, S. Nanda, P. Thirupathi Reddy, A. Bhaskar Rao, J. Org. Chem. 67 (2002) 3900–3903.
[10] J. Yadav, G. Reddy, G. Sabitha, A. Krishna, A. Prasad, Hafeez-U-R-Rahaman, K. Vishwaswar Rao, A. Bhaskar Rao, Tetrahedron: Asymmetry 18 (2007) 717–723.
[11] W. Mączka, A. Mironowicz, Tetrahedron: Asymmetry 15 (2004) 1965–1967.
[12] R. Bruni, G. Fantin, S. Maietti, A. Medici, P. Pedrini, G. Sacchetti, Tetrahedron: Asymmetry 17 (2006) 2287–2291.
[13] T. Utsukihara, S. Watanabe, A. Tomiyama, W. Chai, C. Akira Horiuchi, J. Mol. Catal. B Enzyme 41 (2006) 103–109.
[14] D. Scarpi, E. Occhiato, A. Guarna, Tetrahedron: Asymmetry 16 (2005) 1479–1483.
[15] F. Baldassarre, G. Bertoni, C. Ciappe, F. Marioni, J. Mol. Catal. B Enzyme 11 (2000) 55–58.
[16] A. Orden, F. Bisogno, D. Cifuente, O. Giordano, M. Kurina Sanz, J. Mol. Catal. B Enzyme 42 (2006) 71–77.
[17] Y. Matsuura, W. Chai, E. Endoh, J. Suhara, H. Hamada, C. Horiuchi, Phytochemistry 61 (2002) 669–673.
[18] J. Ceñal, O. Giordano, P. Rossomando, C. Tonn, J. Nat. Prod. 60 (1997) 490–492.
[19] V. Juan Hikawczuk, M. López Verrilli, E. Borkowsky, M. Sosa, O. Giordano, R. Saad, C. Tonn, Nat. Prod. Res. 20 (2006) 813–819.
[20] M. Kurina Sanz, X. Hernandez, C. Tonn, E. Guerreiro, Plant Cell Rep. 19 (2000) 821–824.
[21] X. Hernandez, A. Orden, O. Giordano, M. Kurina Sanz, Electron. J. Biotechnol. 8 (2005) 276–283, doi:10.2225/vol8-issue3-fulltext-6 (online).
[22] C. Pungitore, M. Juri Ayub, M. García, E. Borkowski, M. Sosa, G. Ciuffo, O. Giordano, C. Tonn, J. Nat. Prod. 67 (2004) 357–361.
[23] C. Duschatzky, M. Possetto, L. Talarico, C. García, F. Michis, N. Almeida, M. De Lampasona, C. Schuff, E. Damonte, Antiviral Chem. Chemother. 16 (2005) 247–251.
[24] L. Favier, C. Tonn, E. Guerreiro, A. Rotelli, L. Pelzer, Planta Med. 64 (1998) 657–659.
[25] T. Murashige, F. Skoog, Plant Physiol. 15 (1962) 473–497.