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Deracemization of secondary alcohols by chemo-enzymatic sequence with plant cells

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ABSTRACT

A screening based on undifferentiated plant cells allowed identifying *Gardenia jasminoides* as the best biocatalyst to perform the kinetic resolution of 1-phenylethanol. This species was further tested for its ability to oxidize stereoselectively the (*S*)-isomers from racemic mixtures of secondary alcohols leaving their antipodes unaffected in Tris–HCl buffer. Those substrates which afforded the best results in the kinetic resolution were subjected to a chemo-enzymatic sequence of deracemization. *G. jasminoides* immobilized cells in calcium alginate were used for the oxidation of the (*S*)-enantiomers and, in a second step, $NaBH_4$ was added to the same vessel for the reduction of the corresponding ketone. The sequential repetition of these two steps allowed obtaining the *R*-alcohols in 82–90% yield in high optical purity (71–96% *ee*). Despite the viability of the cells is affected by the chemical reagent, their enzymes remain active due to the protective environment of the calcium alginate beads.

1. Introduction

Enantiopure alcohols are important building blocks for the synthesis of pharmaceuticals, flavors and fragrances, and they are required by industries as both (*R*) and (*S*) enantiopure isomers. They can be obtained by desymmetrization of prochiral ketones by chemical or biological methodologies, or by deracemization of secondary alcohols through kinetic resolution via acyltransferases or via oxidation (Voss et al., 2007). Hence, together with the search of new anti-Prelog ketoreductases to perform asymmetric reductions, the selective oxidation of the (*S*)-isomer from a racemic mixture is still a viable alternative to prepare pure (*R*)-alcohols due to the fact that the number of racemates will always be higher than that of prochiral compounds (Gadler et al., 2006).

Deracemization of secondary alcohols can be achieved by combining biocatalytic and chemical methods. Thus one of the enantiomers from a racemic mixture is selectively oxidized to the corresponding ketone by an ADH whereas its antipode (mirror image) remains untouched. In a further step, the ketone is reduced by a chemical reagent (e.g. $NaBH_4$, $NaCNBH_3$, amine–borane complex) to furnish the racemic alcohol. If this process is repeated sequentially, it is possible to obtain one of the enantiomers in a highly enriched manner. This methodology has been largely applied to produce optically pure unnatural aminoacids combining a D-amino acid oxidase and a chemical reductant (Hall and Bommarius, 2011).

Although microorganisms are the whole-cell biocatalysts of choice, plants have also been explored as a potentially important tool for organic synthesis. Among the first works on asymmetric biocatalysis with plant cell cultures, *Daucus carota*, *Nicotiana tabacum* and *Gardenia jasminoides* cells entrapped in calcium alginate beads were used for the desymmetrization of various ketones to yield secondary alcohols with very good yields and enantiomeric excess (*ee*) (Naoshima and Akakabe, 1989, 1991; Akakabe and Naoshima, 1994). Moreover, *G. jasminoides* was shown to carry out the biotransformation of acetophenone by a mechanism involving the low or non-stereoselective reduction of the ketone to give a mixture of (*R*)- and (*S*)-1-phenylethanol and, in a second step, a stereoselective oxidation of the (*S*)-alcohol to yield the (*R*)-1-phenylethanol reaching a maximum of 91% *ee* at 40% conversion (Akakabe and Naoshima, 1993). Despite these interesting results, the use of this biocatalyst for stereoselective oxidation of secondary alcohols has not yet been fully exploited. Recently, Itoh et al. (2008) reported the use of basil cultured cells for the kinetic resolution of 1-arylethanols by stereoselective oxidation affording the (*R*)-alcohols with 99% *ee*.

In this work we describe the use of *G. jasminoides* undifferentiated cells immobilized in calcium alginate beads in Tris–HCl buffer for the kinetic resolution of racemic secondary alcohols and the subsequent reduction of the ketone employing $NaBH_4$ in the same vessel where the biocatalytic process is carried out (Fig. 1).

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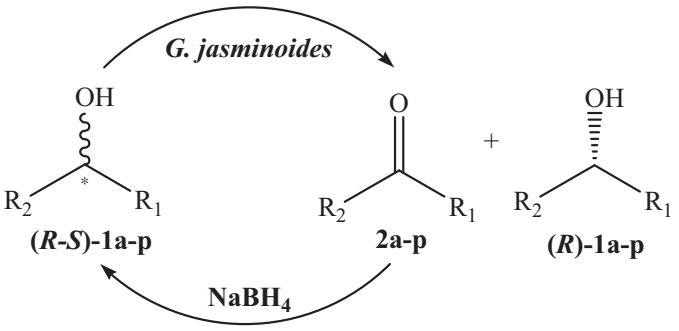

Fig. 1. Deracemization via an oxidation/reduction sequence.

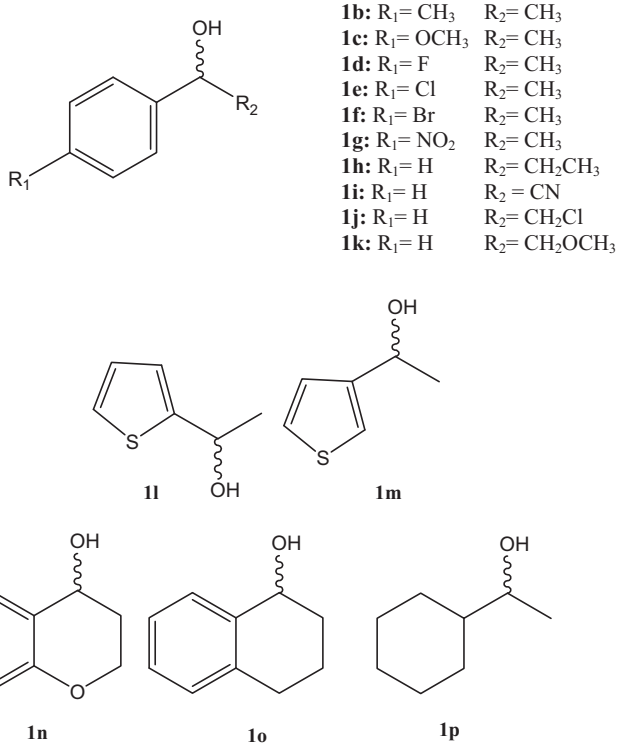

Fig. 2. Substrates for the kinetic resolution by *Gardenia jasminoides* plant cells.

2. Materials and methods

2.1. Biocatalysts

Plant calli from *Baccharis crispa*, *D. carota*, *Grindelia pulchella*, *Capsicum annum* and *Tessaria absinthioides* were initiated and maintained on Murashige Skoog (MS) agar (Murashige and Skoog, 1962) supplemented with sucrose (30 g l^{-1}) and auxins and cytokinins at different ratios as previously described (Orden et al., 2008).

Friable calli from *G. jasminoides* were initiated from leaves and stems. Calli were maintained on MS solid medium supplemented with sucrose (30 g l^{-1}) and α-naphthaleneacetic acid 10 μM and kinetin 0.46 μM, under a 16-h light/8-h dark cycle by fluorescent lamps at an irradiance of approximately 1.8 Wm^2. The 40-day-old calli were mechanically disrupted by gently pressing them with a spatula under sterile conditions. The mashed biomass was transferred to flasks containing 15 mL of 50 mM Tris–HCl or 30 mM phosphate buffer, pH 6.5 (2.5 g of fresh weight per flask).

2.2. Substrates and standards

Substrates **1a–p** (Fig. 2) were purchased from Sigma-Aldrich Argentina S.A. Solid substrates were dissolved in DMSO prior to addition in a final concentration of 2% (v/v). For standards preparation, compounds **1a–p** were treated with $NaBH_4$ in methanol to obtain the racemic mixtures of the corresponding alcohols. For the preparation of the (*S*)-*sec*-alcohols to be used as standards for chiral GC analysis, the corresponding ketones were biocatalytically reduced with *D. carota* roots in water as described by Yadav et al. (2002) and Aldabalde et al. (2007). The absolute configuration of the remaining alcohol after the kinetic resolution was determined by chiral GC-FID using a β-DEX-column by comparison with racemic mixtures and with the *S*-alcohols obtained by reduction with *D. carota*. The racemic alcohols, the (*S*)-enantiomers and the samples were analyzed as acetyl derivatives obtained as described in Section 2.3.

The $NaBH_4$ solution used for the racemization step was prepared a few minutes prior to use, dissolving the reagent in KOH (0.5 M) to reach a final concentration of 12% (w/v).

2.3. Chemical yields and enantiomeric excess (ee) determination

Chromatographic analysis was carried out by chiral GC-FID. For separation of the two enantiomers, the alcohols were derivatized by the addition of 200 μL of acetic anhydride and a catalytic amount of DMAP (4-dimethylaminopyridine, 2 mg) to the organic phase. The reaction mixture was shaken for 90 minutes at 130 rpm and 22 °C. Afterwards, 400 μL of water were added. The solution was centrifuged (4 min) and the organic phase dried over anhydrous Na_2SO_4 and analyzed by GC in a Perkin-Elmer Clarus 500 instrument. It was equipped with a chiral β-DEX-column Restek (30 m, 0.25 mm ID and 0.25 μm df), injector 200 °C, FID 300 °C and carrier N_2 (28 cm s^{-1}) for most compounds or chiral β-DEX-column Supelco (60 m, 0.25 mm ID and 0.25 μm df), injector 200 °C and FID 300 °C and carrier N_2 (25 cm s^{-1}) for **1e**, **1f** and **1g**.

Temperature program: (start temperature [°C]/holding time [min]/heating rate [°C/min]/plateau temperature [°C]/holding time [min]/heating rate [°C/min]/final temperature [°C]/holding time [min])

Compounds **1a**, **1h**. 120/2/1/126/1. Rt: **2a** 3.94; ***S*-1a** 5.33; ***R*-1a** 5.92; **2h** 5.70; ***S*-1h** 6.88; ***R*-1h** 7.32.
Compounds **1b**, **1d**. 120/2/1/131/1. Rt: **2b** 6.57; ***S*-1b** 8.32; ***R*-1b** 9.32; **2d** 4.09; ***S*-1d** 5.55; ***R*-1d** 6.64.
Compound **1c**. 110/5/2/130/0/5/170/1. Rt: **2c** 17.32; ***S*-1a** 19.53; ***R*-1a** 18.73.
Compound **1i**. 110/0/2/180/0/20/200/1. Rt: **2i** 28.91; ***S*-1i** 28.38; ***R*-1i** 28.76.
Compound **1j**. 70/4/20/110/0/10/130/15/20/200/10. Rt: **2j** 25.12; ***S*-1i** 26.01; ***R*-1i** 26.66.
Compound **1k**. 110/0/1/125/5/1/140/1. Rt: **2k** 22.50; ***S*-1k** 29.38; ***R*-1k** 29.79.
Compounds **1l**, **1m**. 120/2/1/126/1. Rt: **2l** 4.65; ***S*-1l** 5.37; ***R*-1l** 5.94; **2m** 4.98; ***S*-1m** 6.02; ***R*-1m** 7.54.
Compound **1n**. 105/0/1/120/7/1/125/7/1/130/1. Rt: **2n** 18.33; ***S*-1n** 25.40; ***R*-1n** 24.69.
Compound **1o**. 115/0/1/120/7/1/125/7/1/130/1. Rt: **2o** 20.87; ***S*-1o** 25.21; ***R*-1o** 24.10.
Compound **1p**. 106/4/1/110/0/5/130/1. Rt: **2p** 4.52; ***S*-1p** 7.28; ***R*-1p** 8.84.
Compounds **1e**, **1f**. 130/10/3/185/1. Rt: **2e** 16.21, ***R*-1e** 19.77, ***S*-1e** 20.10; **2f** 20.31, ***R*-1f** 24.81, ***S*-1f** 25.22.
Compound **1g**. 190/20/3/210/4. Rt: **2g** 15.83, ***R*-1g** 26.07, ***S*-1g** 26.52.

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2.4. General screening

Calli from 6 plant species (*B. crispa*, *D. carota*, *G. jasminoides*, *G. pulchella*, *C. annum* and *T. absinthioides*) were screened for their ability to oxidize 1-phenylethanol (**1a**) to its corresponding ketone. Disrupted calli (2.5 g fresh weight) were transferred to Erlenmeyer flask containing 15 mL of MS medium. Substrate **1a** (0.1 mmol) was added and incubated under orbital shaker at 120 rpm at room temperature, under illumination. Samples were taken at 2 and 6 days and analyzed by GC-FID.

2.5. Selection of the reaction media for G. jasminoides cells

G. jasminoides (2.5 g fresh weight) were suspended in 15 mL of MS medium, 50 mM Tris–HCl buffer (pH 6.5) or 30 mM phosphate buffer (pH 6.5). Substrate **1a** (0.1 mmol) was added and incubated under orbital shaker at 120 rpm at room temperature, under illumination. Samples were taken at different times.

2.6. General procedure for the study of the kinetic resolution of sec-alcohols by G. jasminoides suspension cells

The *G. jasminoides* disrupted calli (2.5 g fresh weight) were transferred to Erlenmeyer flasks containing 15 mL of 50 mM Tris–HCl buffer pH 6.5 and 0.1 mmol of racemic alcohols **1a–p** were added and incubated under orbital shaking at room temperature, under illumination. Blank assays without substrates and without cells were carried out. Samples were taken at different incubation times and extracted with ethyl acetate (EtOAc) prior to the addition of benzaldehyde (10 μmol) as internal standard. The solvent was dried over anhydrous Na_2SO_4 and centrifuged at 10,000 rpm. The progress of the bioconversion was followed by GC analysis. The results are the media of the repetition of three experiments.

2.7. Deracemization via an oxidation/reduction sequence

The deracemization was performed either with free cells or immobilized cells of *G. jasminoides*.

Free cells: After the kinetic resolution of **1c**, **1d**, **1l** (0.1 mmol) at 24 h carried out as described in Section 2.6, a $NaBH_4$ solution (30 mEq) was added. The pH of the reaction medium was adjusted to 6.5 an hour later by adding HCl (0.5 M). This procedure was named cycle and was repeated every 24 h, except the last cycle which lasted 48 h. The process was monitored by GC analysis at different times.

Immobilized cells: G. jasminoides cells (105 g) were suspended in 250 mL of 4% sterile sodium alginate and added dropwise to 1 L of 0.15 N calcium chloride solution. The beads with an average diameter of 0.45 cm, were stood in the solution for 1 h and then washed with distilled water. The entrapped cells, 2.5 g fresh weight approximately, were distributed in flasks containing 15 mL of sterile 50 mM Tris–HCl buffer pH 6.5. The substrates **1c**, **1d**, **1l** (0.1 mmol) were added to each flask and they were incubated under orbital shaking at room temperature. A $NaBH_4$ solution (30 mEq) was added and the cyclic deracemization process was continued as described for free cells.

2.8. Preparative scale deracemization

In a 2 L flask, containing 500 mL 50 mM Tris–HCl buffer (pH 6.5), 130 g of immobilized *G. jasminoides* cells were suspended and substrate **1a** (122 mg, 1 mmol) was added and incubated under orbital shaking at 150 rpm. After 24 h, a $NaBH_4$ solution (30 mEq) was added. The pH of the reaction medium was adjusted to 6.5 an hour later by adding HCl (0.5 M). This cycle was repeated 7 times during 4 days. The process was monitored by GC analysis at different times. The entrapped cells were further separated by filtration and the supernatant was extracted with EtOAc (3 times). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under vacuum. The extract was purified by chromatographic column eluting with hexane–EtOAc. The optical rotation of the main product was measured and compared to literature. The beads were washed several times with distilled water and utilized for a second time for a chemo-enzymatic sequence for deracemization of **1a**.

3. Results and discussion

3.1. Selection of the biocatalyst and reaction media

Out of the six cell lines tested for the oxidative ability towards **1a**, only *G. jasminoides* undifferentiated cells were able to resolve the racemic mixture in MS medium giving the (*R*)-isomer with good *ee* (90–92%) (Table 1). So far, biooxidation of racemic alcohols with plant cell cultures have been carried out in nutrient culture media and reversible processes usually take place. In our first experiments, we observed that the reaction in MS medium furnished the (*R*)-alcohol with an *ee* that only reached 92% even after 6 days of reaction which is in agreement with the data reported by Akakabe and Naoshima (1993). However, it was feasible to drive the equilibrium of the reaction towards the stereoselective oxidation of the alcohols to afford the (*R*)-enantiomer with a very good or excellent optical purity by using a biotransformation medium depleted of nutrients and reducing equivalents, achieving an efficient and faster transformation. Thus the (*R*)-**1b** was obtained with >99% *ee* in a shorter period of time (2 days).

Since the key bioreaction is a selective oxidation mediated by an oxidative enzyme which requires $NAD(P)^+$, we can assume that a medium depleted of reducing equivalents could be more suitable than a medium where the reduced form of the cofactors is more abundant. One possible explanation could be that the NAD(P)H generated by the selective oxidation might be rapidly consumed under these conditions for other more relevant processes for the cell to survive. So, the consumption of the NAD(P)H towards other processes would favor that the competitive reductive reaction does not take place.

Both phosphate and Tris–HCl buffers at pH 6.5 were assayed and no significant differences in the biotransformation were observed between the two conditions. Since the further cyclic deracemization process was intended to be carried out with immobilized cells in calcium alginate, we went on working with Tris–HCl buffer as it is more suitable for the stability of the beads.

3.2. Reduction of *2a* in MS and buffer

In a previous paper we reported that *G. jasminoides* cells reduced **2a** to the corresponding alcohol giving ***R*-1a** in 41% conversion rate and 82% *ee* in MS medium (Orden et al., 2008). In the reduction assays carried out in Tris–HCl buffer, negligible amounts of **1a** were detected even after 8 days of reaction. This fact helps explain why using this reaction medium is more effective for the kinetic resolution than the MS medium, since no reduction of the formed ketone is taking place simultaneously.

3.3. Kinetic resolution of sec-alcohols

We decided to explore the potential of *G. jasminoides* cells for the stereoselective oxidation of different racemic alcohols (Table 2). First, we studied the influence of the substituent at *para*-position on the aromatic ring of the phenylethanol derivatives (substrates **1a–g**). To a greater or lesser extent, *G. jasminoides* cell lines oxidized stereoselectively the (*S*)-enantiomers to the corresponding acetophenones, leaving the (*R*)-enantiomers with an optical purity

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Table 1
Kinetic resolution of ***rac*-1a** with plant growing cells in Murashige–Skoog medium.

Plant species	Time (days)	Ketone (%)	*R*-alcohol (%)	*S*-alcohol (%)	*ee* (%)	Config.
B. crispa	2	4	48	48	–	–
	6	6	48	46	–	–
D. carota	2	2	50	48	–	–
	6	5	55	40	16	*R*
G. jasminoides	2	48	49	3	90	*R*
	6	49	49	2	92	*R*
G. pulchella	2	4	49	47	–	–
	6	6	48	46	–	–
C. annuum	2	6	48	46	–	–
	6	13	46	41	6	*R*
T. absinthioides	2	3	49	48	–	–
	6	3	50	47	–	–

The data is expressed as percentage of conversion and *ee* (enantiomeric excess) and was determined by chiral GC analysis prior derivatization as acetates. Config.: absolute configuration.

ranging from 94 to 98% *ee* after 48 h of biotransformation. The only exception was the *para*-nitro derivative **1g** which was scarcely recognized by the alcohol dehydrogenases of *G. jasminoides* cells, and only a small amount of its corresponding ketone (3%) was detected after 4 days of bioreaction. On the other hand, excellent results were obtained in the kinetic resolution of substrates which bear electron-donor moieties in *para*-position, such as **1b** and **1c** with 92% and 98% *ee*, respectively. After 60 h of biotransformation the *ee* reached values higher than 99%, although incubation for longer periods of time led also to the oxidation of the (*R*)-enantiomer, therefore decreasing the overall yield of the process.

Significant differences were observed in the oxidation of racemic arylethanols with electron-withdrawing groups (EWGs) in *para*-position. The racemic substrate **1d** with fluorine as substituent afforded 47% of the *R*-alcohol (97% *ee*) after 74 h of incubation and there was not considerable conversion by extending the biotransformation to 100 h. However, for bulkier aryl halides, such as chloride and bromine, the reaction proceeds at a slower reaction rate and the (*R*)-enantiomer is also oxidized, affording a high proportion of the ketones and a small percentage of the alcohols (<25%), reaching only 90% *ee* of ***R*-1e** and 93% *ee* of ***R*-1f**, after a longer incubation time (100 h).

On the other hand, racemic substrates **1i**, **1j**, **1k** which are substituted in R_2 with EWGs, such as chloro, cyano and methoxy, were recovered unaffected (Table 3). In a screening with 60 commercial ADHs from different sources, other authors demonstrated that halodydrins are not oxidized at all, even with huge excess of cofactor (Lavandera et al., 2008). A thorough explanation of the complexity of oxidizing this type of alcohols bearing EWGs, considering both thermodynamic and kinetic effects, as well as substrate-enzyme interaction was provided by Bisogno et al. (2010).

Table 2
Kinetic resolution of phenylethanol derivatives by *G. jasminoides* suspension cells in Tris–HCl buffer.

Substrate	Time (h)	2 (%)	1 (%)		*ee* (%)
			R-1	*S*-1	
***rac*-1a**	24	45	49	6	81 (*R*)
	48	50	50	<1	98 (*R*)
	60	51	49	<1	98 (*R*)
	74	52	48	<1	98 (*R*)
	100	52	48	<1	98 (*R*)
***rac*-1b**	24	42	53	5	89 (*R*)
	48	52	45	3	92 (*R*)
	60	52	48	<1	>99 (*R*)
	74	63	37	<1	>99 (*R*)
	100	85	15	0	>99 (*R*)
***rac*-1c**	24	43	48	9	70 (*R*)
	48	53	46	1	98 (*R*)
	60	57	43	0	>99 (*R*)
	72	59	41	0	>99 (*R*)
	100	65	35	0	>99 (*R*)
***rac*-1d**	24	40	51	9	72 (*R*)
	48	46	52	2	94 (*R*)
	60	50	48	2	95 (*R*)
	74	52	47	1	97 (*R*)
	100	54	46	<1	98 (*R*)
***rac*-1e**	24	26	54	20	46 (*R*)
	48	39	53	8	73 (*R*)
	60	37	51	12	64 (*R*)
	74	52	43	5	80 (*R*)
	100	80	19	1	90 (*R*)
***rac*-1f**	24	18	55	27	35 (*R*)
	48	29	54	17	52 (*R*)
	60	33	53	14	58 (*R*)
	74	49	45	6	76 (*R*)
	100	75	24	1	93 (*R*)
***rac*-1g**	24	–	50	50	–
	48	1	50	49	–
	60	3	49	48	–

The data is expressed as percentage of conversion and *ee* (enantiomeric excess) and was determined by chiral GC analysis prior derivatization as acetates. Config.: absolute configuration.

Table 3
Kinetic resolution of racemic alcohols by *G. jasminoides* suspension cells in Tris–HCl buffer.

Substrate	Time (h)	2 (%)	1 (%)		*ee* (%)
			R-1	*S*-1	
***rac*-1h**	24	8	53	39	15 (*R*)
	48	13	56	31	28 (*R*)
***rac*-1i**	24	–	50	50	–
	48	–	50	50	–
***rac*-1j**	24	–	50	50	–
	48	–	50	50	–
***rac*-1k**	24	–	50	50	–
	48	–	50	50	–
***rac*-1l**	24	25	57	18	51 (*R*)
	48	42	56	2	93 (*R*)
***rac*-1m**	24	10	48	42	7 (*R*)
	48	12	47	41	6 (*R*)
***rac*-1n**	24	56	43	1	95 (*R*)
	48	72	28	–	>99 (*R*)
***rac*-1o**	24	39	47	14	54 (*R*)
	48	56	30	14	15 (*R*)
***rac*-1p**	24	6	52	42	11 (*R*)
	48	8	54	38	17 (*R*)

The data is expressed as percentage of conversion and *ee* (enantiomeric excess) and was determined by chiral GC analysis prior derivatization as acetates. Config.: absolute configuration.

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When R_2 was ethyl, a bulkier group than methyl (**1h**), the conversion rate was much slower and only a small amount of ***S*-1h** was oxidized.

On thiophene derivatives, the position of the ethanoyl group on the aromatic ring was significant for the recognition of the *G. jasminoides* ADHs. Thus, an excellent conversion of the derivative substituted in position 2 (**1l**) was reached with an important optical purity, but its isomer **1m** was scarcely transformed, detecting ***R*-1m** in a low amount and with poor *ee* after 48 h.

Another substrate that was well-accepted by *G. jasminoides* ADHs was **1n**. It is noteworthy that almost all the ***S*-1n** was oxidized even after 24 h as well as a small percentage of its antipode. At 48 h, the *R*-alcohol was obtained with excellent *ee* (>99%) but low yield (28%) and a high quantity of the ketone was detected (72%). It is evident that for this substrate the kinetic resolution is higher and shorter period of time should be necessary to optimize the process. Conversely, **1o** was oxidized with very low stereoselectivity and both stereoisomers were transformed simultaneously at similar rate reaching 30% of ***R*-1o** with poor *ee* (15%).

With the aim of study the recognition of cyclic aliphatic compounds by the ADHs from *G. jasminoides*, **1p** was subjected to kinetic resolution. In this case, we observed a very low enzymatic activity for this substrate and only 8% of the ketone was detected after 48 h.

3.4. Deracemization

With the information of the substrate scope in hands, we chose three alcohols with the best kinetic resolutions (**1c**, **1d** and **1l**) to carry out their deracemization by a chemoenzymatic way, employing *G. jasminoides* for the stereoselective oxidation to the corresponding ketones and $NaBH_4$ as a non-stereoselective reducing agent. In the first step of the process, the suspension cells were fed with the racemic alcohols and incubated for 24 h. After this first kinetic resolution, a solution of $NaBH_4$ was added and left for 1 h until the ketones were reduced to the racemic alcohols. The biotransformation medium was acidified to lower the pH at 6.5 and again incubated for 24 h. This procedure named cycle was repeated 6 times. In the first assays with free cells, we could see that the enzymatic activity was affected during the process, so we decided to prolong the last cycle to 48 h of incubation. Despite the *R*-alcohols could be obtained in an average of 80% for the three substrates and the ee_s were moderate (72–88%), the biocatalytic oxidation dropped after the fourth cycle demonstrating a loss of activity of the ADHs perhaps due to the hostile reaction conditions. Furthermore, as it was observed in the kinetic resolution of several substrates (entries ***rac*-1b**, ***rac*-1c**, ***rac*-1e** and ***rac*-1f** of Table 2) with free cells, the incubation for longer periods of time led also to the oxidation of the (*R*)-enantiomer. For these reasons, the ee_s could not be significantly improved within the next days.

In order to lessen the damage that could be caused to the *G. jasminoides* cells by $NaBH_4$ we decided to entrap them in calcium alginate. The deracemization sequence was followed in a similar manner than described before, using suspended beads instead. After 6 cycles of reduction/oxidation, there was not a significant improvement in the conversion or *ee* for substrate **1d** using immobilized cells. In this case, only 82% of ***R*-1d** (71% *ee*) was reached at the end of the process with entrapped cells similar to the first cycle of the assays carried out with free cells (72% of ***R*-1d** with 71% *ee*). However, it was possible to reach 90% and 85% of the *R*-alcohols with improved optical purities with ee_s of 96% and 86% for substrates **1c** and **1l**, respectively (Fig. 3). Thus, we optimized the deracemization process to obtain better yield, demonstrating that the cells entrapped in calcium alginate beads resulted to be more stable and therefore more compatible with the use of the chemical reductant.

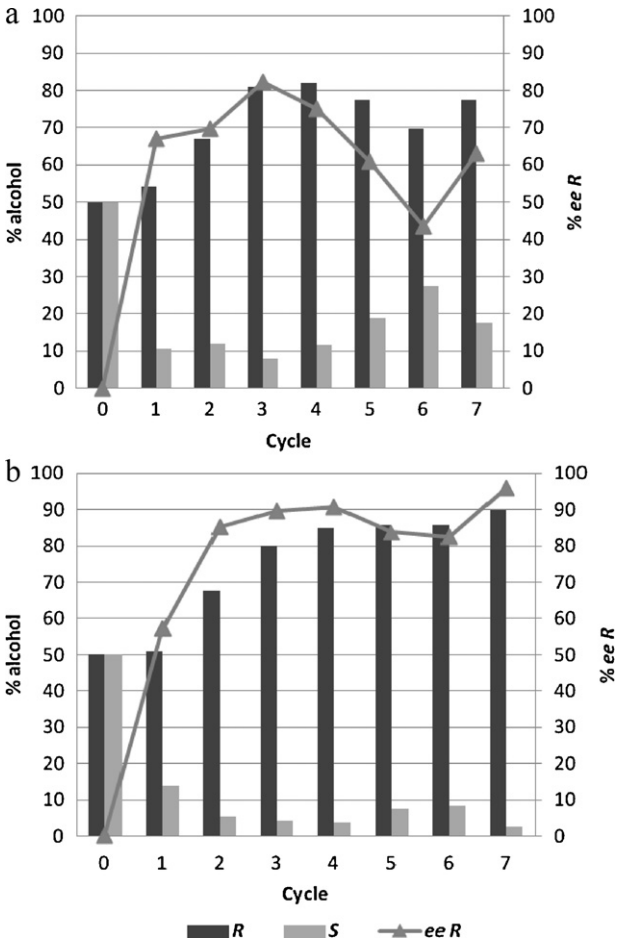

Fig. 3. Deracemization of **1c** by a chemoenzymatic process, employing *G. jasminoides* and $NaBH_4$ as a reducing agent. (a) Free cells, (b) immobilized cells.

3.5. Preparative scale

In order to prove whether the system was applicable for preparative scale, ***rac*-1a** was used as substrate for the cyclic deracemization in a 500 mL flask with 130 g of *G. jasminoides* cell immobilized in calcium alginate. Thus it was possible to enrich ***R*-1a** in 87% with an excellent optical purity (98% *ee*) after 4 days of repetition of the chemo-enzymatic cycles (7 cycles). Isolated yield of ***R*-1a** (30%, 37 mg, 98% *ee*). $[\alpha]_D^{20} = +35.9$ (*c* 0.53, MeOH), lit. (Okudomi et al., 2010) $[\alpha]_D^{20} = +34.5$ (*c* 1.77, MeOH).

The cells entrapped in calcium alginate were efficient for a second deracemization process through chemo-enzymatic sequence furnishing 84% of ***R*-1a** (97% *ee*) after 7 days (3 cycles).

4. Conclusion

We could find suitable biotransformation conditions to improve the enantiomeric excess in the kinetic resolution of arylethanols with *G. jasminoides* cells working in a non-conventional media for plant cells. Moreover, in the same reaction medium, it was possible to carry out the deracemization in a preparative scale of secondary alcohols in a chemoenzymatic manner employing $NaBH_4$ as a reductant in a two-step-sequence, combining the biological and chemical agents in the same pot. The use of cells immobilized in calcium alginate allowed protecting the activity of their ADHs from the unfriendly reaction conditions in the chemical reductive step.

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