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Anti-Prelog reduction of ketones by hairy root cultures

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ABSTRACT

Raphanus sativus hairy roots were used in the anti-Prelog stereoselective reduction of a series of prochiral alkylaryl-ketones. Most of the bioreactions proceeded with high yields and excellent enantioselectivities. This novel biocatalyst is an easy handle system that allows the employment of the immense potential of plant enzymes in preparative asymmetric chemistry.

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1. Introduction

The desymmetrization of prochiral ketones is a wide spread methodology used to prepare *sec*-alcohols as pure enantiomers. Although the asymmetric reduction mediated by isolated enzymes has been extensively studied [1], the use of whole living cells is attractive since they do not require the addition of exogenous cofactors and cofactor regeneration systems [2]. Microorganisms, such as yeasts and filamentous fungi have been successfully used with this purpose [3]. However, most of these biocatalysts follow 'Prelog's rule' [4], thus the (*S*)-alcohol is usually obtained assuming that the smaller substituent of the ketone has the lower CIP priority. Only a few 'anti-Prelog'-(*R*)-specific whole cell biocatalysts have been described [5]. On the other hand, plants represent an alternative source of "new" enzymes to be used in organic synthesis. Many transformations of xenobiotics, such as hydroxylation and oxidation reactions [6], hydrolysis of esters [7], bioreduction of ketones [8], enzymatic lactonization [9], glycosylation [10], etc., can be performed by using plant tissues. Nevertheless, the use of "wild" plant organs and/or tissues presents the main drawback of the lack of reproducibility of the experiments, since some biochemical characteristics of these biocatalysts are easily changeable depending on several factors, such as the places they come from, the season of the year, the climatic conditions, etc. Recently, Matsuo et al. have reported the enantioselective reduction of α,α,α-trifluoroacetophenone and *o*-chloroacetophenone to the corresponding (*S*)-alcohols by *Raphanus sativus* L. sprouts obtained from seeds [11]. Plant cell cultures represent another valuable alternative in order to avoid the above-mentioned problems and they have been successfully used in many bioconversion procedures [12]. Although their enzymatic potential is great, this kind of cultures is not easy to manipulate by chemists. Moreover, they suffer, in many cases, of the lack of genetic and biochemical stability. Compared with other plant cell and tissue cultures, hairy roots (HR), obtained by the integration of a region (T-DNA) of the Ri plasmid from *Agrobacterium rhizogenes* to the plant genome, have important characteristics that give them potential applicability in fine chemistry and even in scaling up processes. Other authors have supported that they show genetic and biochemical stability, they generally present high growth rates without the addition of phytohormones and their cultures are mechanically stronger than cell suspensions [13]. There are some reports describing the use of HR cultures in biocatalysis. Among them, glycosylations are the most frequent reactions. Our research group is particularly interested in the stereoselective reductive capacity of this type of cultures. At the best of our knowledge, besides our previous work [14], there is only one report of the stereoselective reduction of ketones with HR [15]. In that paper, the authors describe the ability of *Daucus carota* HR in reducing ketones and ketoesters, according to Prelog's rule, to the corresponding chiral (*S*)-alcohols. The present work deals on the application of *R. sativus* HR in the stereoselective reduction of prochiral alkylaryl-ketones to afford the (*R*)-alcohols.

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2. Experimental

2.1. Biocatalyst

2.1.1. Establishment of R. sativus HR cultures

Leave and stem explants were aseptically taken from *in vitro* grown seedlings, wounded by transverse cuts with a sterile scalpel and then inoculated with a 48-h-culture of *A. rhizogenes* LBA 9402, grown in YMB liquid medium. Infected explants were transferred to a solid medium consisting of 0.9% (w/v) agar in water, and incubated in the dark for 2 days at 25 ± 2 °C. Then, these infected explants were transferred to solid MSRT medium [16], supplemented with ampicillin ($1\,g\,l^{-1}$), in order to eliminate *A. rhizogenes*. After 7–10 days, the first transformed roots appeared on the wounded surface of the explants. They were allowed to grow 2–5 cm before they were excised and cultured as individual lines on hormone-free MSRT medium, supplemented with ampicillin ($1\,g\,l^{-1}$). The results were expressed in percentage transformation frequency [17]. All the experiments were carried out in triplicate and the results were expressed as mean + SD. HR cultures were maintained at 22 ± 2 °C, in the dark on an orbital shaker at 100 rpm, and subcultured once a month to fresh liquid MSRT medium, using an inoculum of 100–200 mg of roots. Persisting *A. rhizogenes* contamination was eliminated by frequent subcultures on medium containing ampicillin.

2.1.2. Characterization of HR clones by PCR analysis

Total DNA was isolated according to a standard procedure [18]. The primers used were Rol 1 (5′-atggctgaagacgacctgtgt-3′) and Rol 2 (5′-gccgattgcaaacttgcactc-3′), for the amplification of a segment of 547 bp corresponding to the rol C gene of Ri plasmid. The reaction mixture contained 1 μg DNA sample, 200 μM dNTPs, 1.5 mM $MgCl_2$, reaction buffer 1×, 2 U Taq polymerase (Invitrogen) and 0.2 μM of each primer in 25 μl of total volume. The amplification was performed under the following conditions: initial denaturation at 94 °C for 5 min and 30 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min and extension at 72 °C for 1.5 min followed by one cycle of 72 °C for 5 min. PCR was carried out using an automatic thermocycler, PCRICYCLER from Bio-Rad. The amplified bands were observed under UV illumination after electrophoresis on 1.5% (w/v) agarose gels and staining with ethidium bromide.

2.2. Substrates and standards

Substrates **1**–**13** were purchased from Sigma–Aldrich, Argentina S.A. They were dissolved in dimethylformamide (DMF) in a 1:10 ratio to obtain stock solutions. For standards preparation compounds **1**–**13** were treated with $NaBH_4$ in methanol to obtain the racemic mixtures of the corresponding alcohols. The (*S*)-*sec*-alcohols were obtained by bioreduction with *D. carota* roots in water as described by Yadav et al. [8]. The retention times (Rt) of the biotransformation products and the ketones were compared with racemic mixtures by GC on a chiral β-DEX-column. The absolute configurations of the alcohols were determined by correlating the Rt with samples obtained by bioreduction of the ketones with *D. carota* fresh roots and comparing with literature [8,19].

2.3. Biotransformation procedures

Hairy roots (0.5 g) were transferred to fresh MSRT media (30 ml) and incubated in an orbital shaker at 120 rpm and 22 ± 2 °C under darkness. After 30 days, substrates dissolved in DMF (30 μl) were added to the cultures (3 mg or μl/flask) and incubated in dark at 22 °C with orbital shaking at 110 rpm. Blank assays without substrates and without hairy roots were carried out. The cultures were harvested and the media were filtered and extracted with EtOAc (3×), the solvent dried over Na_2SO_4 and removed in vacuo. The results are the media of the repetition of three experiments.

2.3.1. Isolation of compound *7b*

Biotransformation was performed as it was described above by adding 60 mg of **7a** distributed in 20 culture batches. After 7 days of incubation, the biomass was filtered and the media combined and extracted with EtOAc, the solvent dried over Na_2SO_4 and removed in vacuo. The residue was subjected to preparative TLC on Si gel, using *n*-hexane–EtOAc (7:3) to afford 41.5 mg (69% isolated yield) of compound **7b** which was confirmed by ^{1}H NMR and GC analysis.

2.4. Chemical yields and enantiomeric excess (ee) determination

The reaction progress was monitored at 2, 4 and 7 days of incubation by harvesting the cultures. These samples were extracted with EtOAc (3×) with the previous addition of *o*- or *p*-fluoroacetophenone as internal standards. Chromatographic analysis were carried out by chiral GC (FID) in a PerkinElmer Clarus 500 instrument equipped with β-DEX-column (60 m, 0.25 mm ID and 0.25 μm df), injector 200 °C and FID 300 °C. Compounds **1**–**13** and their derivatives were grouped in eight categories according to the GC analysis conditions in agreement with the following detail:

Compounds **1**, **3**, **8**, **9** and **10**: Oven: T_1 = 120 °C, T_2 = 160 °C (ΔT = 2.5 °C/min) for 5 min; carrier N_2 (28 cm/s). Rt: **1a** 11.88, ***R*-1b** 14.30, ***S*-1b** 14.57; **3a** 15.91, ***R*-3b** 17.22, ***S*-3b** 17.61; **8a** 11.45, ***R*-8b** 14.92, ***S*-8b** 15.30; **9a** 12.11, ***R*-9b** 17.56, ***S*-9b** 18.04; **10a** 9.71, ***R*-10b** 13.97, ***S*-10b** 14.30.

Compound **4**: Oven: T_1 = 130 °C for 45 min, T_2 = 150 °C (ΔT = 3 °C/min) for 3 min; carrier N_2 (32 cm/s). Rt: **4a** 41.16, ***R*-4b** 48.76, ***S*-4b** 50.29.

Compounds **6** and **7**: Oven: T_1 = 130 °C, T_2 = 185 °C (ΔT = 3 °C/min) for 10 min; carrier N_2 (25 cm/s). Rt: **6a** 20.31, ***R*-6b** 24.81, ***S*-6b** 25.22; **7a** 16.21, ***R*-7b** 19.77, ***S*-7b** 20.10.

Compound **2**: Oven: T_1 = 155 °C for 30 min, T_2 = 165 °C (ΔT = 10 °C/min) for 2 min; carrier N_2 (27 cm/s). Rt: **2a** 25.31, ***R*-2b** 25.89, ***S*-2b** 26.46.

Compound **5**: Oven: T_1 = 190 °C for 20 min, T_2 = 210 °C (ΔT = 3 °C) for 4 min; carrier N_2 (28 cm/s). Rt: **5a** 15.83, ***R*-5b** 26.07, ***S*-5b** 26.52.

Compound **11**: Oven: T_1 = 105 °C for 40 min, T_2 = 120 °C (ΔT = 1 °C/min) for 5 min, T_3 = 140 °C (ΔT = 3 °C/min) for 5 min, T_4 = 160 °C (ΔT = 5 °C/min) for 1 min; carrier N_2 (32 cm/s). Rt: **11a** 35.56, ***R*-11b** 66.16, ***S*-11b** 66.84.

Compound **12**: Oven: T_1 = 120 °C for 4 min, T_2 = 140 °C (ΔT = 1 °C/min) for 3 min; carrier N_2 (28 cm/s). Rt: **12a** 18.26, ***R*-12b** 22.12, ***S*-12b** 22.77.

Compound **13**: Oven: T_1 = 130 °C for 70 min, T_2 = 150 °C (ΔT = 3 °C/min); carrier N_2 (35 cm/s). Rt: **13a** 54.30, ***R*-13b** 72.20, ***S*-13b** 70.85.

α,α,α-Trifluoroacetophenone and α,α,α-trifluorophenylethanol: Oven: T_1 = 120 °C, T_2 = 155 °C (ΔT = 1 °C/min); carrier N_2 (28 cm/s). Rt: α,α,α-trifluoroacetophenone 6.36, (*S*)-α,α,α-trifluorophenylethanol 24.68 and (*R*)-α,α,α-trifluorophenylethanol 25.43.

3. Results and discussion

3.1. HR clones establishment and molecular characterization

For the establishment of HR cultures from radish through the infection with *A. rhizogenes* LBA 9402, aseptically growing plants from commercially available seeds, of approximately 48-day-old were used as explants, mainly leaves. After 10–50 days post-infection, the first transformed roots appeared on the wounded surface of the explants. Each main HR was excised and identified as an individual HR clone. The transformation frequency was 60%. These HR clones showed the typical characteristics of transformed

roots such as plagiotropic growth, large number of lateral roots, hormone independence and fast growing. To confirm the HR phenotype, amplification of rol C genes was carried out. The rol genes are essential for developing HR disease or phenotype, and they have other functions besides the production of many alterations in plant hormone concentrations [20]. Their presence has been used by several authors to demonstrate the HR condition [21,22]. Thus, genetic transformation was confirmed by PCR analysis, by amplifying a fragment of the rol C sequence from total DNA. We detected an expected band of 547 bp which corresponds to a rol C segment in all HR clones obtained, demonstrating the integration of the rol C gene into the plant genome. Only one HR clone was selected for further studies, based on the phenotypic stability and high growth index (data not shown).

3.2. Acetophenone bioreduction

The ability of the selected HR clone to perform stereoselective reductions was tested toward acetophenone as model substrate. The substrate was dissolved in DMF at a concentration that did not affect the HR viability. Conversion rates and *ee* were determined by chiral GC analysis using an internal standard added previously to the extraction, and no by-products were detected. Interestingly, the observed enantioselectivity was not in accordance with Prelog's rule (Fig. 1) affording the *sec*-alcohol with the (*R*) configuration. This fact represents one of the main advantages of this new system, since most of the biocatalysts follow Prelog's rule for this and other related substrates. As it has been mentioned, these results partially contrast with the ones recently reported by Matsuo et al. [11] for *R. sativus* sprouts, since these authors observed the opposite enantioselectivity in the reduction of *o*-chloroacetophenone. When comparing the bioreduction of acetophenone with HR, and α,α,α-trifluoroacetophenone with germinated radish, we could observe that the enantioselectivity was in accordance. However, when the reduction of this fluorinated substrate was tested with HR, the conversion rates were optimum (83%) but a scarce stereoselectivity was observed (15% *ee* of the *R*-alcohol). As it was pointed out by these authors to support their results, this fact could be explained by the presence of plural alcohol dehydrogenases (ADHs) with different substrate selectivity and stereopreference. These enzymes can even present different expression levels and/or activities in HR clones and untransformed plant tissues. In this regards, it is important to have into account the ability of wild freshly cut radish tissues to reduce acetophenone and *p*-chloroacetophenone according to Prelog's rule reported by Yang et al. [23]. On the contrary, but closer to our findings, Andrade et al. [24] reported that radish roots, in water, reduced acetophenone into (*R*)-1-phenylethanol with excellent optical purity (*ee* >98%), but with very low conversion rates (26%).

In our experiments, three different reaction times were tested (2, 4 and 7 days). At lower incubation times either yield or *ee* were moderate. However, when harvesting was carried out at the seventh day, both conversion rate and optical purity of (*R*)-1-phenylethanol were excellent. Due to the optical purity of the main alcohol increase along the incubation time, it is possible to assume that the enantioselective oxidation of the (*S*)-alcohol may be taking place.

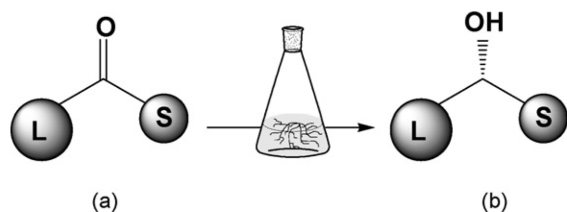

Fig. 1. Anti-Prelog reduction of ketones by *R. sativus* hairy roots.

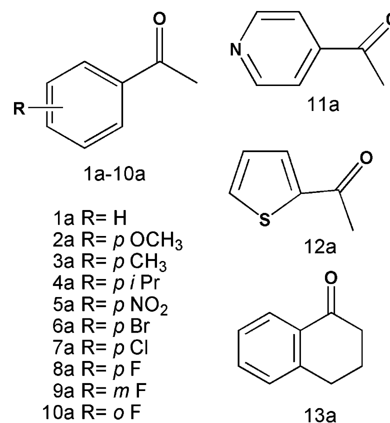

Fig. 2. Substrates for the reduction of ketones by *R. sativus* hairy roots.

3.3. Bioreduction of a series of prochiral alkylaryl-ketones

Taking into account the excellent conversion rates (98%), optical purity (*ee* >98%) and, especially the stereochemistry of the observed product from the model substrate, we decided to study the reduction of the prochiral alkylaryl-ketones series shown in Fig. 2. The results observed at day 7 of biotransformation are depicted in Table 1. Both conversion rates and stereoselectivities depended on the substrate nature and, some interesting observations can be pointed out when comparing to bioreductions carried out by other whole cell biocatalysts.

In reductions of acetophenones using *Rhodotorula* sp. AS2.2241 [25] and *D. carota* roots [8], where their enzymes obey Prelog's rule, it has been stated that electron-withdrawing substituents at *para*-position favored the rate of the reduction, but electron-donating ones slowed down the reaction rate. In our experiments, although the conversion rates of the last type of substituents (p-OCH_3, p-CH_3 and *p*-*i*Pr) resulted slighter than the ones of the model substrate acetophenone, they are actually quite good. The behavior of electron-withdrawing substituted acetophenones (p-NO_2, *p*-Br, *p*-Cl, *p*-F), was not so predictable. For example, (*R*)-1-(*p*-chlorophenyl)ethanol was obtained with excellent yields and optical purities. In concordance, in an already mentioned work when wild plant tissues were used [23], conversion rates and enantioselectivity of the (*S*)-1-(*p*-chlorophenyl)ethanol are higher than the ones obtained with acetophenone. On the other hand, nitro analogous was recovered with only 60% of *ee*. The experiments showed very good activity (conversion rate 100%) but very low stereoselectivity of the new biocatalytic system toward this particular substrate. This result is in agreement with the one reported by Comasseto et al. [26] who also showed that *p*-nitroacetophenone

Table 1
Asymmetric reduction of alkylaryl-ketones with *R. sativus* hairy roots.

Substrate	C[a] (%)	*ee*[a] (%)	Product
1a	98	>98	**(*R*)-1b**
2a	86	>99	**(*R*)-2b**
3a	92	>99	**(*R*)-3b**
4a	85	>98	**(*R*)-4b**
5a	100	60	**(*R*)-5b**
6a	98	95	**(*R*)-6b**
7a	98	>99	**(*R*)-7b**
8a	97	92	**(*R*)-8b**
9a	78	12	**(*S*)-9b**
10a	52	>99	**(*R*)-10b**
11a	100	47	**(*R*)-11b**
12a	14	96	**(*R*)-12b**
13a	16	>99	**(*R*)-13b**

[a] Measured by GC on a chiral stationary phase.

was reduced by fungal strains of *Aspergillus*, leading predominantly to the anti-Prelog product, with low stereoselectivity.

Interesting differences were observed when *ortho*-, *meta*- and *para*-fluorinated derivatives were used to test if the substituent position can influence the course of the biocatalytic transformation. *R. sativus* HR reduced the *p*-fluoroacetophenone with very good conversion rates and a significant optical purity. When the substrate was the *ortho*-derivative, although the yields were not as good as those obtained with the previous ketone, the ee_s were excellent. Interestingly, the position of the substituent had an enormous influence, not only in the conversion rates but also in the stereoselectivity of the process. Hence, we observed a significant reduction of the *m*-fluoroacetophenone (78%), but with a low stereoselection, affording the *S*-alcohol with only 12% *ee*. In a previous work carried out with these fluorinated-derivatives with three filamentous fungi, it was not either possible to find out a consistent correlation between the position of this substituent and the conversion rates or the stereoselectivity [27].

We also tested a couple of heteroaryl methyl ketones. Acetyl thiophene was reduced with good stereoselectivity into the (*R*)-*sec*-alcohol but with poor conversion rates. In this sense, other researchers also reported that moderate conversion took place when they assayed the reduction of 2-acetyl thiophene into the (*S*)-*sec*-alcohol with *Candida viswanathii* [28] and *D. carota* roots [19]. On the other hand, 4-acetylpyridine was completely transformed although with a moderate stereoselectivity. On the contrary, the cyclic alkanone, 1-tetralone was reduced exclusively into the (*R*)-*sec*-alcohol but with a low conversion rate.

Finally, in order to check isolated yields, we chose *p*-chloroacetophenone as a model substrate based on the excellent results obtained in its biotransformation screening, regarding optical purity and conversion rate. After solvent extraction and chromatographic purification, it was possible to isolate 41.5 mg of *p*-chlorophenylethanol, corresponding to a 69% of isolated yield.

4. Conclusions

R. sativus HR may be a useful biocatalyst to afford the (*R*)-*sec*-alcohols by anti-Prelog reduction of prochiral ketones. In most cases, conversion rates were very good and optical purities were excellent. HR cultures present new possibilities to synthetic chemists in terms of efficiency and cost, considering the stereoselective transfer of hydrogen without the external addition of coenzyme NAD(P)H to the media. The genetic and biochemical stability of these differentiated plant cultures and their efficient productivity offer substantial advantages over cell suspensions [13] and plant organs. They are easy handle systems that offer the possibility of scaling up [29], representing an alternative to explore and use the immense potential of plant enzymes by chemists.

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References

[1] W. Kroutil, H. Mang, K. Edegger, K. Faber, Curr. Opin. Chem. Biol. 8 (2004) 120–126.
[2] K. Faber, Biotransformations in Organic Chemistry, Springer Verlag, Berlin, Heidelberg/New York, 2004, pp. 192–193.
[3] K. Goldberg, K. Schroer, S. Lütz, A. Liese, Appl. Microbiol. Biotechnol. 76 (2007) 249–255.
[4] V. Prelog, Pure Appl. Chem. 9 (1964) 119–130.
[5] I. Lavandera, B. Höller, A. Kern, U. Ellmer, A. Glieder, S. de Wildman, W. Kroutil, Tetrahedron: Asymmetry 19 (2008) 1954–1958, and references therein.
[6] H. Sakamaki, K. Itoh, T. Taniai, S. Kitanaka, Y. Takagi, W.C. Chai, A. Horiuchi, J. Mol. Catal. B: Enzym. 32 (2005) 103–106.
[7] A. Mironowicz, Phytochemistry 47 (1998) 1531–1534.
[8] J.S. Yadav, S. Nanda, P.T. Reddy, A.B. Rao, J. Org. Chem. 67 (2002) 3900–3903.
[9] T. Olejniczak, A. Mironowicz, C. Wawrzeńczyk, Bioorg. Chem. 31 (2003) 199–205.
[10] K. Shimoda, H. Hamada, H. Hamada, Phytochemistry 69 (2008) 1135–1140.
[11] K. Matsuo, S. Kawabe, Y. Tokuda, T. Eguchi, R. Yamanaka, K. Nakamura, Tetrahedron: Asymmetry 19 (2008) 157–159.
[12] K. Ishihara, H. Hamada, T. Hirata, N. Nakajima, J. Mol. Catal. B: Enzym. 23 (2003) 145–170.
[13] S. Guillon, J. Trémouillaux-Guiller, P. Kumar Pati, M. Rideau, P. Gantet, Curr. Opin. Plant Biol. 9 (2006) 341–346.
[14] A.A. Orden, F.R. Bisogno, D. Cifuente, O.S. Giordano, M. Kurina-Sanz, J. Mol. Catal. B: Enzym. 42 (2006) 71–77.
[15] D. Caron, A.P. Coughlan, M. Simard, J. Bernier, Y. Picheť, R. Chênevert, Biotechnol. Lett. 27 (2005) 713–716.
[16] E. Agostini, S.R. Milrad de Forchetti, H.A. Tigier, Plant Cell Tiss. Org. Cult. 47 (1997) 177–182.
[17] V. Kumar, A. Sharma, B.C.N. Prasad, H.B. Gururaj, G.A. Ravishankar, Electron. J. Biotechnol. 9 (2006) 349–357, doi:10.2225/vol9-issue4-fulltext-4 (online).
[18] S. Dellaporta, J. Wood, J. Hicks, Plant Mol. Biol. Rep. 1 (1983) 19–22.
[19] V. Aldabalde, P. Arcia, A. Gonzalez, D. Gonzalez, Green Chem. Lett. Rev. 1 (2007) 25–30.
[20] E. Moyano, S. Fornalé, J. Palazón, R.M. Cusidó, M. Bonfill, C. Morales, M.T. Piñol, Phytochemistry 52 (1999) 1287–1292.
[21] V. Bonhomme, D. Laurain-Mattar, J. Lacoux, M. Fliniaux, A. Jacquin-Dubreuil, J. Biotechnol. 81 (2000) 151–158.
[22] S. Singh, J.S. Melo, S. Eapen, S.F. D' Souza, J. Biotechnol. 123 (2006) 43–49.
[23] Z.-H. Yang, R. Zeng, G. Yang, Y. Wang, L.-Z. Li, Z.-S. Lv, M. Yao, B. Lai, J. Ind. Microbial. Biotechnol. 35 (2008) 1047–1051.
[24] L.H. Andrade, R.S. Utsunomiya, A.T. Omori, A.L.M. Porto, J.V. Comasseto, J. Mol. Catal. B: Enzym. 38 (2006) 84–90.
[25] W. Yang, J. Xu, Y. Xie, Y. Xu, G. Zhaob, G. Lin, Tetrahedron: Asymmetry 17 (2006) 1769–1774.
[26] J.V. Comasseto, L. Assis, L.H. Andrade, I. Schoenlein-Crusius, A.L.M. Porto, J. Mol. Catal. B: Enzym. 39 (2006) 24–30.
[27] J.V. Comasseto, A.T. Omori, L.H. Andrade, A.L.M. Porto, Tetrahedron: Asymmetry 14 (2003) 711–715.
[28] A.L. Kamble, P. Soni, U.C. Banerjee, J. Mol. Catal. B: Enzym. 35 (2005) 1–6.
[29] R. Eibl, D. Eibl, Phytochem. Rev. 7 (2008) 593–598.

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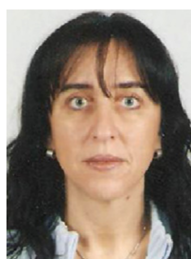
Cynthia Magallanes-Noguera is a Molecular Biologist. She is working on a research project to obtain her PhD in the generation of new biocatalyst to perform stereoselective biotransformations.



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