Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 52–53 (2008) 194–198
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Sugar cane juice for the bioreduction of carbonyl compounds

João Carlos C. Assunção [a], Luciana L. Machado [a], Telma L.G. Lemos [a], Geoffrey A. Cordell [b], Francisco Jose Q. Monte [a,*]

[a] *Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, 60.451-970 Fortaleza, CE, Brazil*
[b] *Natural Products Inc., Evanston, IL 60203, USA*

Available online 19 January 2008

Abstract

A series of compounds including aliphatic and aromatic aldehydes and ketone were reduced using a plant cell preparation from sugar cane (*Saccharum officinarum*). The products were obtained in poor to excellent yields (2.5–100%), and with good enantiomeric excess (25–100%) measured by GC chiral column. Other functional groups were also evaluated including ester, nitrile and amide, but were not reduced. Preliminary kinetic studies are reported. This represents the first report of the use of sugar cane as a biocatalytic agent.

Keywords: *Saccharum officinarum*; Poaceae; Biocatalytic reduction; Aliphatic and aromatic aldehydes and ketones

1. Introduction

The use of plants as natural catalysts has attracted the attention of many researchers, and substantial progress has been reported [1,2]. Several studies have reported the use of microorganisms (mushrooms and bacteria) as biotransforming agents, but the use of whole cells is rarer [3–9]. A recent comprehensive review has summarized the use of cell culture systems (vegetables and microorganisms) and whole plant tissues for evaluation as potential selective biocatalytic agents [10].

The tremendous interest in the development of new and natural biocatalysts is due to the wide potential biotechnologic applications for these materials [11] mainly for the industrial sector, such as pharmaceuticals [12,13], cosmetics [14], and agricultural and environmental uses [13]. Some important characteristics of biocatalysts are their frequently lower cost, high versatility and efficiency, in addition the highly desirable chemical aspects such as chemoselectivity, regioselectivity, and enantioselectivity. The combination of these factors makes the potentiation of biocatalytic reactions very attractive for the industrial sector [10]. In addition, there is the added advantage of using reagents for organic transformations which can be used on a sustainable basis, rather than depleting resources [10].

One of the main limitations of the application of biocatalysts in their native form in the industrial sector is their stability to higher temperatures and their sensitivity to mixed (organic–aqueous) solvent systems [15–17]. However, multidisciplinary (biology–chemistry) efforts are achieving important progress in this area [18]. Genetic modifications (mutations) which generate enzymes that are more resistant to temperature variations and to diverse reaction media, as well as increased enantioselectivity, is making possible the wider application of biocatalysts in industry [19].

Plants contain a wide variety of secondary metabolic constituents and a corresponding diversity of functional group transformations which have been accomplished through selective enzymatic transformations. They, depending on substrate specificity, are therefore a potential vast source of enzymes which can conduct basic and possibly even some complex synthetic organic chemical modifications on a selective basis [10]. In this respect, a more detailed exploration is required the use of common plants and vegetables as potential sources of such new reagents which can display differential selectivity, and can effect a diversity of organic transformations.

Saccharum officinarum L., popularly known as "sugar cane", is a plant of the Poaceae family originating from the Southeast Asia [20]. This specie is very common in the tropical areas of the world, and is cultivated widely in Brazil, where it is considered the main source of combustible alcohol, an industrial sector that is growing significantly [21]. Brazil is the largest supplier of this specie in the world, with a production of approximately 430

* Corresponding author.
E-mail address: fmonte@ufc.br (F.J.Q. Monte).

1381-1177/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.molcatb.2008.01.004

million tonnes in 2006, according to data from the Ministry of Agriculture [22].

In the process of crushing the stem of sugar cane, a liquid called "sugar cane juice" is generated. Sugar cane juice is a drink which is very widely appreciated in many tropical countries, including Brazil, mainly because of its flavorful taste. The main component is sucrose, but other compounds, including flavonoids, are known to be present, although no detailed quantitative analytical data are available [23].

In previous studies, studies from this laboratory on the use of *Manihot* species [24] and coconut juice [25] as biocatalytic reducing and esterifying agents were reported. Continuing these studies as a part of a research program to examine the use of common vegetables as organic reagents for regio- and stereoselective syntheses in organic chemistry, preliminary results from studies on the use of the juice of *S. officinarum* as a potential reagent for biotransformation reactions are reported (Fig. 1).

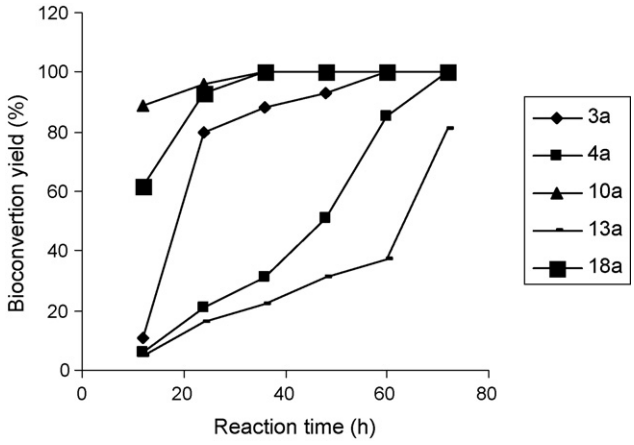
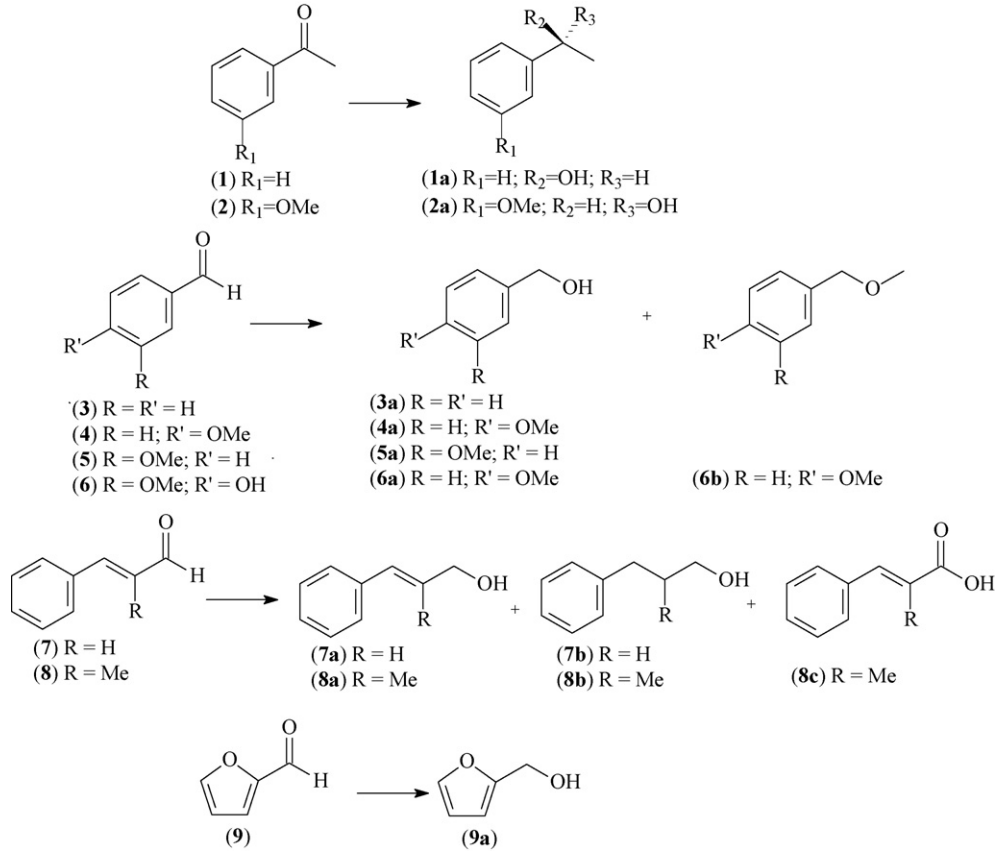


Fig. 1. Bioconversion of aldehydes benzaldehyde **3**, anisaldehyde **4**, furfuryl **9** and ketones: cyclohexanone **13** and β-keto-ethyl-butyrate **16** to the corresponding alcohols, benzyl alcohol **3a**, anisyl alcohol **4a**, furfuryl alcohol **9a** cyclohexyl alcohol **13a** and β-keto-ethyl-butyryl alcohol **16a** using sugar cane juice.

2. Results and discussion

The intact plant system, fresh sugar cane juice, was used, and the initial experiment was to determine the total protein in the enzymatic system using the Hartree method [26]. The value observed (5%) was higher than that reported previously in the literature (1%) [27]. Initially, the biotransformation reactions were carried out using a series of aromatic ketones and aldehydes (**1**–**9**) (Scheme 1) as substrates, which were treated with the fresh juice of *S. officinarum* at room temperature for 72 h [24,28,29]. The crude reaction mixtures were initially visualized by Si gel TLC using the vanillin spray reagent, and then quantitatively by GC–MS the results are presented in Table 1. These data were confirmed by the preliminary results obtained from the ^{1}H NMR experiments, with little difference in accuracy. The integration of the methyl ketone singlets of the ketones **1** and **2**, or the aldehydic proton of the aldehydes **3**–**9**, were compared with the carbinolic hydrogen resonances of **1a** and **2a**, the methylene hydrogens of



Scheme 1.

Table 1
Relative percentage yields of reduction products of **1–14** and **16** and the hydrolysis of **18** using ^{1}H NMR and GC–MS

Product	Biconvertion (%) NMR ^{1}H/GC	ee (%)
1a	34.0/39.3	56.7 (R)
2a	16.1/19.4	41.0 (S)
3a	100.0/100.0	
4a	100.0/100.0	
5a	100.0/100.0	
6a	91.0/90.0	
6a; **6b**[a]	43.4; 56.6/34.4; 65.6	
7a; **7b**	66.6; 33.4/66.5; 33.5	
8a; **8b**; **8c**	38.2; 5.7; 56.08/38.2; 5.7; 56.08	
9a	100.0/100.0	
10a	100.0/100.0	
11a	100.0/100.0	
12a	NC/34.0	
13a	NC/81.2	
15a	9.3/2.5	25.7 (S)
16a	100.0/100.0	100 (S)
18a	NC/45.3	

NC: not conclusive.
[a] In presence of DMSO.

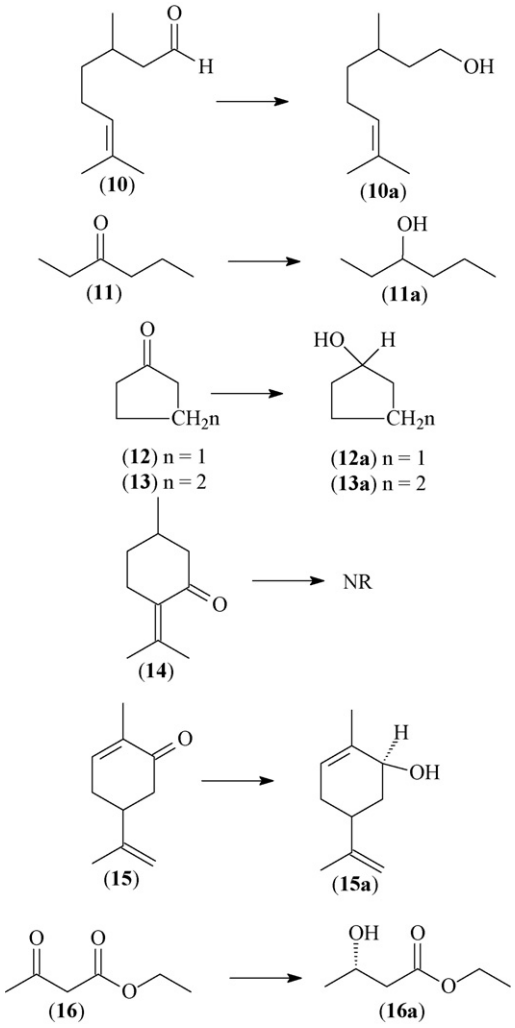
Table 2
^{1}H NMR signals used in the integration of selected protons of compounds **1–13**, **15** and **16**, compared with integration of the product alcohols **1a–13a**, **15a** and **16a** using sugar cane juice

	H-observed	δ_H
Substrate		
1	3H-2	2.62 (s)
2	3H-2	2.54 (s)
3	CHO	9.61 (s)
4	CHO	9.74 (s)
5	CHO	9.71 (s)
6	CHO	9.78 (s)
7	CHO	9.42 (s)
8	CHO	9.76 (s)
9	CHO	9.50 (s)
10	CHO	9.61 (s)
11	3H-1	2.20 (m)
12	2H-2	1.81 (m)
13	H-2	2.05 (m)
15	H-3	6.76 (qd)
16	2H-2	3.45 (s)
Product		
1a	H-1	4.96 (q)
2a	H-1	4.91 (q)
3a	2H-1	4.70 (s)
4a	2H-1	4.66 (s)
5a	2H-1	4.70 (s)
6a	2H-1	4.61 (s)
6b	2H-1	4.45 (s)
7a	2H-1	4.33 (dd)
7b	2H-1	3.68 (t)
8a	3H-4	1.93 (s)
8b	3H-4	2.18 (s)
8c	3H-4	0.94 (d)
9a	2H-5	4.65 (s)
10a	2H-1	3.58 (t)
11a		
12a		
13a		
15a	H-3	5.35 (m)
	H-1	4.34 (m)
16a	H-3	4.19 (m)

the primary alcohols of **3a–7b** and **10a**, or the methyl hydrogens for the products **8a–8c** (Table 2). The integration was corrected for hydrogen proportionality.

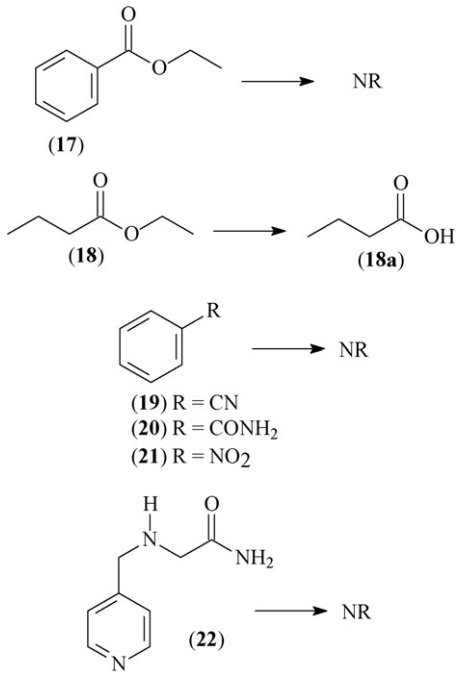
As expected, aldehydes were more reactive than ketones, and a methoxy group in the *para*- or *meta*-positions in the case of compounds **4** and **5**, respectively, had no influence on the yield of the reaction compared with **3**, having an unsubstituted aromatic ring. In the reduction process, all of the tested aldehydes, except **6**, **7**, and **8**, produced alcohols in excellent yield, superior to the reported results for *Daucus carota* [28] and comparable to those of *Manihot esculenta* and *Manihot dulcis* [24] and *Zygosaccharomyces rouxii* [30]. Previously, vanillin **6** was unaffected by the two *Manihot* species [24]. However, biocatalytic reduction of **6** with *S. officinarum* proceeded with the formation of **6a** in 90% yield. In the presence of DMSO (to enhance substrate solubility) this reaction produced the alcohol **6a** (34.4%) and the ether **6b** (65.6%). This demonstrated that DMSO could induce a transmethylation process. On the other hand, regioselectivity was not observed with cinnamaldehyde (**7**), where the reduction reaction generated two products, one (**7a**, 66.5%) resulting from selective reduction of the carbonyl group, and the other (**7b**, 33.5%) reflecting non-regioselective reduction of the carbonyl and olefinic bonds. Regioselectivity was also not observed in the reaction of α-methyl cinnamaldehyde (**8**), where a significant product reflected reduction only of the carbonyl group (**8a**, 38.2%), and the minor product displayed reduction of both the carbonyl and the olefinic bond (**8b**, 5.7%). An unexpected compound, resulting from the oxidation of the aldehyde group to a carboxylic acid (**8c**, 56.1%), was the major product. Finally, in the aldehyde series, it is important to emphasize the high yield (100%) and the regioselectivity to produce **9a** from the reaction with the aliphatic aldehyde **9**.

A commonly required functional group transformation is the reduction of a ketone to a chiral secondary alcohol for which biocatalytic strategies have been developed [10,18]. The reactions with the selected compounds having a ketone group are represented in Scheme 1. For the ketones **1** and **2**, the bioconversion afforded the corresponding secondary alcohols in relatively low yields (36.6% of **1a** and 17.7% **2a**). However, moderate enantioselectivity was obtained, with 56.7% ee for the (*R*)-isomer (**1a**) and 41.0% ee for the (*S*)-isomer (**2a**). The enantiomeric excess was calculated using a chiral column, and the *R* and *S* configurations of **1a** and **2a**, respectively, were determined using polarimetry and the application of an established formula [31]. The *S* configuration established for the alcohol **2a** was in agreement with the Prelog model for bioreduction [28]. On the other hand, the *R* configuration attributed for **1a** is in disagreement with this rule. In addition, it was observed that the least electron-deficient acetophenone **1a** was reduced to a greater extent than electron-rich acetophenone **2a**.



Scheme 2.

The enzymatic reaction was extended to the aliphatic ketones **10–16**, as represented in Scheme 2. An excellent yield (100%) was obtained with the ketone **11**, while the cyclic ketones **12** (five-membered) and **13** (six-membered) presented moderate (34.0%) and good (81.2%) yields, respectively. Reaction mixtures from **12** and **13** were quantified only by GC–MS, applying the conditions determined for aromatic ketones and aldehydes, and the results are presented in Table 2. Among the α,β-unsaturated ketones, for **14** no reactivity was observed, and a much lower yield was observed for **15** (5.9%). The lower ee (25.7%) for **15a** was calculated using a chiral column, and the *S* configuration of **15a** was established by comparison of its specific rotation with the literature value [32,33]. The *Saccharum* reductase enzyme system was also evaluated for effects on the β-keto-ester **16**, having two different carbonyl groups. The reaction mixture was quantified by GC–MS and ^{1}H NMR spectroscopy. Complete regio- and enantio-stereoselectivity was observed through the exclusive reduction of the keto group at C-3 yielding 3*S*(+)-hydroxy-ethyl-butyrate **16a** (100% yield) as product, showing an ee value of 100%. For **16** and **16a**, the integrated absorptions in the ^{1}H NMR spectrum were the CH_2 alpha to the ester carbonyl and methine hydrogen in the obtained



Scheme 3.

secondary alcohol. The *S* configuration of **16a** was established by comparison of the specific rotation with the literature value [32,33].

Finally, the enzymatic reaction was extended to other functionalized compounds (Scheme 3): esters (ethyl benzoate, **17** and ethyl butyrate, **18**), a nitrile (benzonitrile, **19**), an amide (benzamide, **20** and isoniazide, **22**), and a nitro derivative (nitrobenzene, **21**). The aromatic ester was unaffected, but the aliphatic ester produced the corresponding carboxylic acid (butyric acid in 45% yield) rather than the alcohol. No reactivity was observed for the compounds benzamide, benzonitrile, and nitrobenzene.

2.1. Conclusion

In summary, this work demonstrated that fresh sugar cane juice (*S. officinarum*) can serve as an effective reagent for reductase-catalyzed reactions with the advantage of enhancing the enantioselectivity, suggesting that this vegetable product may have interesting potential as an alternative cheap sustainable, and readily available system to be used as a chemical reagent in biotransformations. Additional experiments are underway to examine the scope of this potential as a reducing agent, and to examine the optimization of reaction yields and the stability of the system.

3. Experimental

3.1. General

The optical rotations were measured on a PerkinElmer 341 digital polarimeter using chloroform as the solvent. The pure starting materials and the products obtained were ana-

lyzed by GC–MS on a Shimadzu Model 17A/QP5050 using a (5%-phenyl)-dimethylpolysiloxane OV-5 capillary column (30 m × 0.25 mm × 0.25 μm); carrier gas helium, flow rate 1 mL/min with split mode. The injector temperature and detector temperature were 250 and 280 °C, respectively. The column temperature was programmed at 4 °C/min from 40 to 180 °C, and then at 20 °C/min from 180 to 280 °C. The enantiomeric excess was calculated using a Thermo Electron GC-FID model Trace GC Ultra instrument equipped with Varian Capillary chiral column Chirasil-Dex CB (β-cyclodextrin, 25 m × 0.25 mm × 0.25 μm); carrier gas helium, flow rate 1 mL/min with split mode. The injector temperature and detector temperature were 220 °C. The column temperature was programmed at 2 °C/min from 130 to 160 °C. NMR spectra were recorded on a Bruker Avance DRX-500 (500 MHz) using $CDCl_3$ solvent. Chemical shifts, given on the δ scale, were referenced to the residual, undeuterated portion of the deuterated $CDCl_3$ solvent (δ_H 7.27). Column chromatography was run using silica gel 60 (70–230 mesh, Vetec.). The aldehydes and ketones were purchased from Aldrich Chemical Co., Milwaukee, USA.

3.2. Plant material

The sugar cane juice was purchased in Maranguape, Ceará, Brazil. The reactions were conducted immediately after acquisition to assure the integrity of the enzymatic system.

3.3. Extraction and isolation

In separate experiments, substrates **1–22** (200 mg) were added to sugar cane juice (200 mL). The mixtures were incubated on a shaker (160 rpm) at room temperature for 72 h, using an established methodology [24,28,29].

The aqueous solutions were then extracted with EtOAc (3× 100 mL), and the solvent was evaporated under reduced pressure. The residues were filtered on a short silica gel column, using $CHCl_3$ as eluent, to afford the reduced products: **1a** (128 mg), **2a** (135 mg), **3a** (152 mg); **4a** (162 mg); **5a** (170 mg); **6a** (120 mg); **7a** (159 mg); **8a** (147 mg); **9a** (128 mg); **10a** (134 mg); **11a** (120 mg); **12a** (135 mg), **13a** (152 mg); **14** (162 mg); **15a** (170 mg); **16a** (147 mg); **17** (120 mg); **18a** (159 mg); **19** (170 mg); **20** (120 mg); **21** (159 mg); **22** (147 mg). The products were analyzed by GC/MS, and ^{1}H NMR spectral data, and in comparison with literature values [34–36].

In the kinetics experiments, the same procedure was used for the bioreduction, and samples were analyzed by GC with reaction times varied from 12, 24, 36, 48, 60, and 72 h. Experiments were performed in duplicate.

Acknowledgments

The authors thank the Brazilian agencies CNPq and CAPES for fellowships and financial support, and the CENAUREN/UFC for the NMR data.

References

[1] M. Chartrain, R. Greasham, J. Moore, P. Reider, D. Robinson, B. Buckland, J. Mol. Catal. B: Enzym. 11 (2001) 503.
[2] R. Bruni, G. Fantin, A. Médici, P. Pedrini, G. Sacchetti, Tetrahedron Lett. 43 (2002) 3377.
[3] A. Mironowicz, Phytochemistry 47 (1998) 1531.
[4] M. Takemoto, Y. Matsuoka, K. Achiwa, J.P. Kutney, Tetrahedron Lett. 41 (2000) 499.
[5] G. Roda, S. Riva, B. Danieli, H. Griengl, U. Rinner, M. Schmidt, A.M. Zabelinskaja, Tetrahedron 58 (2002) 2979.
[6] W. Stampfer, B. Kosjek, K. Faber, W. Kroutil, J. Org. Chem. 68 (2002) 402.
[7] K. Toneva, S. Vlahov, S. Boneva, S. Vassilev, K. Vassilev, Bulg. Akad. Nauk. 55 (2002) 43.
[8] T. Matsuda, K. Watanabe, T. Kamatinaka, T. Harada, K. Nakamura, Chem. Commun. (2003) 1198.
[9] K. Edegger, W. Stampfer, B. Seisser, K. Faber, S.F. Mayer, R. Oerhrlein, A. Hafner, W. Kroutil, Eur. J. Org. Chem. 8 (2006) 1904.
[10] G.A. Cordell, T.L.G. Lemos, F.J.Q. Monte, M.C. Mattos, J. Nat. Prod. 70 (2007) 478.
[11] L.H. Andrade, R.S. Utsunomiya, A.T. Omori, A.L.M. Porto, J.V. Comasseto, J. Mol. Catal. B: Enzym. 38 (2006) 84.
[12] S.K. Sharma, M. Husain, R. Kumar, L.A. Samuelson, J. Kumar, A.C. Watterson, S. Virinder, Pure Appl. Chem. 77 (2005) 209.
[13] R.N. Patel, Biocatalysis in the Pharmaceutical and Biotechnology Industries, CRC Press, Boca Raton, FL, 2006, p. 893.
[14] T. Veit, Eng. Life Sci. 4 (2004) 508.
[15] F.H. Arnold, Acc. Chem. Res. 31 (1998) 125.
[16] J.B. Jones, C.H. Wong, Curr. Opin. Chem. Biol. 2 (1998) 67.
[17] M.T. Reetz, M.H. Becker, H.W. Klein, D. Shockigl, Angew. Chem. Int. Ed. 38 (1998) 1758.
[18] R. De Conti, J.A.R. Rodrigues, P.J.S. Moran, Quim. Nova 24 (2001) 672.
[19] M.T. Reetz, M.H. Becker, K.M. Kuhling, F.H. Arnold, Angew. Chem. Int. Ed. 37 (1998) 2647.
[20] D.A. Sampietro, M.A. Vattuone, M.I. Islã, J. Plant Physiol. 163 (2006) 837.
[21] P.S.G. Magalhães, D.G.P. Cerri, Bios. Eng. 96 (2007) 1.
[22] Ministry of Agriculture, Brazil. Available www.agricultura.gov.br, accessed on March 23, 2007.
[23] R. Colombo, M.F. Lanças, J.H. Yariwake, J. Chromatogr. A 1103 (2006) 118.
[24] L.L. Machado, J.S.N. Souza, M.C. Mattos, S.K. Sakata, G.A. Cordell, T.L.G. Lemos, Phytochemistry 67 (2006) 1637.
[25] A.M. Fonseca, F.J.Q. Monte, R. Braz-Filho, M.C. Mattos, M.C.F. Oliveira, G.A. Cordell, T.L.G. Lemos, J. Mol. Catal. B: Enzym., submitted for publication.
[26] E.F. Hartree, Anal. Biochem. 48 (1972) 422.
[27] N.M.A. Nassar, Field Crops Res. 13 (1986) 177.
[28] J.S. Yadav, S. Nanda, P.T. Reddy, A.B. Rao, J. Org. Chem. 67 (2002) 3900.
[29] J.S.N. Souza, Dissertação de Mestrado, Edições UFC, Fortaleza, Brasil, 2003.
[30] B.A. Anderson, M.M. Hansen, A.R. Harkness, C.L. Henry, J.T. Vicenzi, M.J. Zmijewski, J. Am. Chem. Soc. 117 (1995) 2358.
[31] F.A. Carey, R.J. Sundberg, Advanced Organic Chemistry, Third ed., Plenum Press, New York, 1990, pp. 68–69.
[32] E.F.J. Vries, J. Brussee, C.G. Kruse, A. Van der Gen, Tetrahedron: Asymm. 5 (1994) 377.
[33] N. Blancharda, P.V. De Weghea, Org. Biomol. Chem. 4 (2006) 2348.
[34] C. Pouchert, The Aldrich Library of Infrared Spectra, Third ed., 1981, p. 1850.
[35] C. Pouchert, J. Behnke, The Aldrich Library of ^{13}C and ^{1}H FTNMR Spectra, 1, First ed., 1993, p. 4300.
[36] H.W. Lee, S.K. Ji, I.Y.C. Lee, J.H. Lee, J. Org. Chem. 61 (1996) 2542.