Journal of Molecular Catalysis B: Enzymatic 72 (2011) 102–106

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Screening of plant cell cultures for their capacity to dimerize eugenol and isoeugenol: Preparation of dehydrodieugenol

Liliana Hernández-Vázquez[a], Ma. Teresa de Jesús Olivera-Flores[b], Francisco Ruíz-Terán[c], Ivon Ayala[c], Arturo Navarro-Ocaña[c,*]

[a] Departamento de Sistemas Biológicos, Universidad Autónoma Metropolitana, Unidad Xochimilco, A.P. 23/181 México, D.F., Mexico
[b] Departamento de Bioquímica, Laboratorio de Cultivos Vegetales, Facultad de Química, UNAM, Ciudad Universitaria, México, D.F. 04510, Mexico
[c] Departamento de Alimentos y Biotecnología, Facultad de Química, Universidad Nacional Autónoma de México (UNAM), Ciudad Universitaria, México, D.F. 04510, Mexico

ARTICLE INFO

Article history:
Received 1 December 2010
Received in revised form 5 May 2011
Accepted 16 May 2011
Available online 7 June 2011

Keywords:
Biotransformation
Oxidative coupling
Plant cell culture
Eugenol
Dehydrodieugenol

ABSTRACT

Nine plant cell cultures were used to catalyze the oxidative coupling reaction of eugenol and isoeugenol. All the evaluated plant cell cultures carried out the oxidative reaction. The calli of *Medicago sativa* and the cell suspension of *Coriandrum sativum* produced the highest yield of dehydrodieugenol.

1. Introduction

Plant cell cultures exhibit a vast biochemical potential for the production of specific secondary metabolites. The plant cell culture may also retain the ability to transform exogenous substrates into products of interest [1]. Biotransformation, in which the cell culture acts as a bioreactor [2], is considered to be an important method to turn cheap and simple substrates into rare and expensive products [1–3]. Reactions catalyzed by cell cultures include: hydroxylation, oxidation, reduction, hydrogenation, glycosylation, and hydrolysis [3].

The development of biocatalysts for oxidation reactions is a versatile and very important area of research, with enzymes and cell cultures playing a particularly important role. This approach constitutes a cleaner and greener alternative to traditional chemical methods in which catalysts such as $FeCl_3$, $K_3Fe(CN)_6$ and Cu(OH)Cl are used for the oxidative coupling of phenolic compounds [4,5].

Horseradish peroxidase (HRP), a commercially available enzyme, has been established as an effective biocatalyst for organic and inorganic oxidation reactions [6]. Kutney et al. [7] reported HRP catalyzed carbon–carbon bond formation of suitable dibenzylbutanolides for biotransformation to lignans. HRP has also been used for the enantioselective oxidation of 2-naphthols to the 1,1-binaphthyl-2,2-diols [8]. In addition, this enzyme has been used in the biotransformation of phenolic compounds, abundant in essential oils, such as eugenol and isoeugenol [9], leading to the formation of coupled products linked through the aromatic ring, that are interest for the biogenesis of neolignans. However, the addition of H_2O_2 to the reaction mixture decreases the chemical yield in some cases [6]. An alternative is the use of plant cell cultures in which cell wall peroxidases rapidly metabolize a huge amount of the H_2O_2 produced by the addition of foreign substrates.

Recently, Takemoto et al. have found that *Camellia sinensis* cell culture is an efficient source of peroxidase (POD) [10]. This cell culture has been used for the oxidative coupling of dibenzylbutanolides and for the enantioselective oxidative coupling of 2-naphthol derivatives, resulting in moderate to good ee values [11].

O-Methoxyphenols, such as eugenol (4-allyl-2-methoxy phenol) (**1**), isoeugenol (4-propenyl-2-methoxyphenol) (**3**) and the dimeric compounds are constituents of essential oils in a great diversity of medicinal plants (Fig. 1) [12]. Consequently, these compounds have attracted considerable attention in the flavour and food industry.

On the other hand eugenol (**1**, Fig. 1), the main component in clove oil, is a valuable starting compound for several drugs. It has been used in cosmetics and food products as a flavouring additive, antimicrobial and antioxidant agent [13], and in dentistry, for instance, in combination with zinc oxide

* Corresponding author. Tel.: +52 55 56225346; fax: +52 55 56225309.
E-mail address: arturono@servidor.unam.mx (A. Navarro-Ocaña).

1381-1177/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2011.05.005

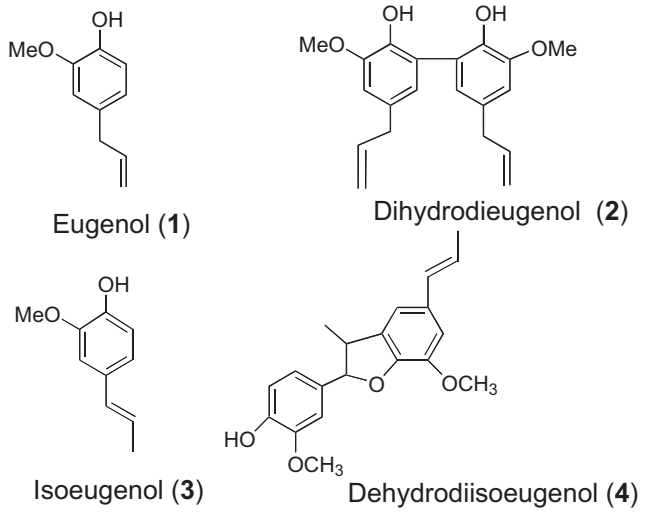

Fig. 1. Structure of monomer and dimers.

it is used as a pulp capping agent, temporary filling and root conduit sealer [14]. However, eugenol causes allergenic contact dermatitis [15] and high concentrations of this compound have been reported to have some cytotoxic properties [16]. Dehydrodieugenol (**2**, Fig. 1) (biseugenol, 3,3′-dimethoxy-5,5′-di-2-propenyl-1,1′-biphenyl-2,2′diol) the symmetrical dimer of eugenol, is a natural O,O′-dihydroxy biphenyl [17], which shows biological activity comparable with that observed in eugenol. This dimer has shown greater antioxidant [4] and anti-inflammatory activity than eugenol [18] and less cytotoxicity [19].

The aim of this work was to evaluate the capacity of nine plant cell cultures to catalyze the dimerization of eugenol and isoeugenol via the oxidative coupling reaction, as well as to develop an alternative to chemical methods for the synthesis of the bioactive compound biseugenol.

2. Materials and methods

2.1. General

Reagents and solvents were purchased from Organic Research, Baker or Aldrich, and were used without any additional purification. ^{1}H NMR spectra were recorded on a Varian 400 MHz instrument in $CDCl_3$, using tetramethylsilane (TMS) as an internal reference. Thin Layer Chromatography (TLC) was used for a preliminary qualitative analysis of the biotransformation products and this was performed on silica gel plates. Alugram® SIL G/UV 254 0.2 mm (Macherey-Nagel) and hexane–AcOEt (4:6) was used as the eluent. The quantitative analysis of dehydrodieugenol produced after the biotransformation was performed on a Waters-1525 High Pressure Liquid Chromatography (HPLC) equipment with a UV detector (Waters 2487) under the following conditions: Symmetry ® C-18 column at 30 °C, a flow rate of 1 mL/min, UV wave length at 230 and 280 nm, and a H_2O–MeOH (70:30) solvent system with 0.1% trifluoroacetic acid.

2.2. Substrate and products

Dehydrodieugenol (**2**) was prepared from eugenol following a procedure previously reported [5]. ^{1}H NMR ($CDCl_3$/TMS): δ 3.35 (d, 4H, J = 6.8 Hz), 3.91 (s, 6H), 5.04–5.14 (m, 4H), 5.91–6.04 (m, 2H), 6.72 (d, 2H, J = 2.1 Hz), 6.75 (d, 1H, J = 2.1 Hz), spectroscopy data were consistent with those reported in the literature [5,4b]. Dehydrodiisoeugenol (**4**) was obtained from isoeugenol (**3**) by a reported procedure [4b]. ^{1}H NMR ($CDCl_3$/TMS): δ 1.37 (d, 3H, J = 6.8 Hz), 1.86 (dd, 3H, J = 6.8, 1.6 Hz, 3.40–3.50 (m, 1H), 3.84 (s, 3H), 3.88 (s, 3H), 5.09 (d, 1H, J = 9.6 Hz), 5.80 (s, 1H), 6.11 (dq, 1H, J = 15.6, 6.4 Hz), 6.36 (dd, 1H, J = 15.6, 1.2 Hz), 6.76 (s, 1H), 6.78 (s, 1H), 6.86–6.91 (m, 2H), 6.96 (s, 1H), spectroscopy data were consistent with those reported in the literature [4]. TLC and HPLC analysis used the dimeric compounds **2** as reference samples for determinations. The dimeric compound **4** was used as reference sample for monitoring the biotransformation on TLC, and this was isolated from biotransformation with *Bouvardia ternifolia*.

2.3. Biological material

Nine plant species were used: alfalfa (*Medicago sativa* L.), bean (*Phaseolus vulgaris*), coriander (*Coriandrum sativum*), matarique (*Psacalium peltatum*), melon (*Cucumis melo*), carrot (*Dacus carota*) capulín (*Prunus serotina*), mammillaria (*Mammillaria huitzilopochtli*) and *B. ternifolia*.

2.3.1. Calli

The calli of alfalfa, bean, coriander, matarique, melon and carrot were obtained from using a method previously reported [20]. The alfalfa and bean calli were maintained in Schenk and Hildebrant (SH) medium [21] supplemented with sucrose (3%), 2,4-dichlorophenoxyacetic acid (2,4-D), 2 and 3 mg L^{-1} respectively, glycine (20 mg L^{-1}), kinetine (0.2 and 1 mg L^{-1} for each respectively), and vitamins (myo-inositol 100 mg L^{-1}, thiamine–HCl 5 mg L^{-1}, nicotinic acid 5.0 mg L^{-1}, pyridoxine–HCl 5 mg L^{-1}). In the case of bean calli, ascorbic acid (0.75 mg L^{-1})–citric acid (0.75 mg L^{-1}) were also added as antioxidants.

The calli of melon and carrot were maintained in Murashige and Skoog (MS) medium [21] supplemented with sucrose (3%), 2,4-D (3 mg L^{-1}), glycine (20 mg L^{-1}), kinetine (6 mg L^{-1}), and ascorbic acid (0.4 mg L^{-1})–citric acid (0.4 mg L^{-1}). Coriander and matarique calli were subcultured in MS medium supplemented with sucrose (3%), benzylaminopurine (BA), 0.3 mg L^{-1}. In the case of coriander 2,4-D (3 mg L^{-1}) and ascorbic acid (1.5 mg L^{-1})–citric acid (1.5 mg L^{-1}) were used, and for matarique, indol-3-acetic acid (IAA), 1 mg L^{-1} and ascorbic acid (100 mg L^{-1})–citric acid (100 mg L^{-1}). For all media 2.5 g L^{-1} of gelzan™ CM were added as a setting agent and the pH fixed at 5.7. All calli were incubated at 25 °C with a photoperiod of 16 h of light and 8 h of darkness, except for matarique calli that were incubated in darkness at the same temperature.

The mammillaria (*M. huitzilopochtli*) calli was obtained from *in vitro* grown plants which were cut in 1 cm sections and five of these explants were introduced to MS medium supplemented with sucrose (3%), 2,4-D (1 mg L^{-1}), glycine (10 mg L^{-1}), kinetine (1 mg L^{-1}), vitamins (myo-inositol 100 mg L^{-1}, thiamine–HCl (B1) 10 mg L^{-1}, nicotinic acid (B3) 1 mg L^{-1}, pyridoxine–HCl (B6) 1 mg L^{-1} and glycine 2 mg L^{-1}), and gelzan™ CM (2.5 g L^{-1}), at pH 5.7 to develop the calli [22].

For capulin *P. serotina* seeds were obtained from the fruit of a wild tree, which were germinated in the following manner, the seeds were scarified and surface-sterilized by immersion for 10 min in a 30% aqueous solution of a commercial bleach with 0.1% of tween and 10 μL of mycrodyn®, followed by five washings with sterile water. The seeds were germinated on Murashige and Skoog (MS) medium and after three weeks the plants were dissected. Explants from petioles were cut in 0.5 cm sections and transferred to MS medium supplemented with sucrose (3%), 2,4-D (2 mg L^{-1}), ascorbic acid (10 mg L^{-1})–citric acid (10 mg L^{-1}), and gelzan™ CM (2.5 g L^{-1}), at pH 5.7 to develop the calli. The conditions for the incubation were similar to those described above. The first subculture took place after three weeks; the calli were maintained on solid MS medium and subcultured every three weeks [23].

Calli of *B. ternifolia* was established from young leaves of a wild plant of *B. ternifolia* (personal communication). The cultures were maintained in MS medium supplemented with sucrose (3%), 2,4-D (1 mg L^{-1}), glycine (2 mg L^{-1}), kinetine (0.05 mg L^{-1}), vitamins (myo-inositol 100 mg L^{-1}, thiamine–HCl (B1) 10 mg L^{-1}, nicotinic acid (B3) 1 mg L^{-1}, pyridoxine–HCl (B6) 1 mg L^{-1}) and gelzan™ (2.5 g L^{-1}). The conditions of the incubation were similar to those described above.

2.3.2. Production of plant cell suspensions

The medium used was the same as that used to obtain calli from the different species but without gelzan™ .Ten grams of calli were added to an Erlenmeyer flask containing 250 mL of medium and then subcultured at 14 days intervals and kept in a rotatory shaker at 25 °C and 125 rpm, with a photoperiod of 16 h of light and 8 h of darkness.

2.4. Screening of the biocatalytic capacity of the plant cell cultures to dimerize 1 and 3 (oxidative coupling)

2.4.1. Dimerization of 1 and 3 using calli monitored by TLC analysis

Ten grams of a 14-day-old calli of the different plants were disrupted (with a vortex) in 50 mL of phosphate buffer, at pH 6.5 and 8000 rpm. The substrates, **1** and **3** (50 mg), were dissolved in 1.0 mL of acetone and then added to each flask containing the homogenized calli. The flasks were incubated in the dark at 25 °C for five days on a rotator shaker (120 rpm). As a control the same amount of calli without the substrate but with the same amount of acetone was set under the same conditions. After five days, the cells were filtered under reduced pressure. The filtrate was collected, extracted with ethyl acetate (3 × 25 mL) and concentrated at vacuum. The residues were dissolved in acetone (1 mL) and spotted onto TLC plates. A mixture of hexane–AcOEt (4:6) was used as the eluent. Samples of eugenol (**1**), isoeugenol (**3**), dehydrodieugenol (**2**) were used as references.

When *B. ternifolia* was used to perform the biotransformation the mixture of compounds, obtained during the reaction, was purified using TLC using as eluent hexane–methanol–ethylacetate (7:1:2, Rf 0.6). The yield recovered was 23% and the spectroscopy data coincided with those previously reported for dehydrodiisoeugenol (**4**) obtained from isoeugenol (**3**).

2.4.2. Dimerization of compounds 1 and 3 using cell suspensions monitored by TLC analysis

The substrates **1** and **3** (50 mg) were dissolved in 1.0 mL of acetone and then these were added to each flask containing a cell culture suspension of 14 days old. The mixtures were incubated for additional five days at 25 °C and 125 rpm, with a photoperiod of 16 h of light and 8 h of darkness. The analysis of the biotransformation showed a similar composition of product as the calli.

2.5. Preparation of dehydrodieugenol for further analysis by HPLC

Dehydrodieugenol (**2**) was prepared under similar conditions to those described for the calli and cell culture suspensions, using 150 mg of eugenol distributed in three flasks with calli or a cell suspension. The incubation conditions and product extraction were similar to those described above. The extract was dissolved in methanol (2 mL) and analyzed by HPLC. The yield of dehydrodieugenol was calculated from a calibration curve.

3. Results and discussion

3.1. Cell culture

All the evaluated calli were friable, except that of mamillaria, which was compact. The cell suspension cultures of *B. ternifolia*, *Medicago sativa*, *Psacalium peltratum*, *P. vulgaris* and *D. carota* were easily disaggregated, whereas *P. serotina*, *C. sativum*, and *C. melo* were less disaggregated. All showed a low oxidation during the propagation of them.

3.2. Dimerization of 1 and 3 catalyzed by calli and cell suspensions monitored by TLC

TLC analysis of the mixture of compounds, in each biotransformation reaction of eugenol (**1**) using calli, showed the presence of a compound more polar than the substrate (Fig. 2). This was identified as dehydrodieugenol (**2**) by comparison with a reference sample, which confirmed the biotransformation of eugenol by the biocatalyst.

For capulin (C), matarique (G) and bean (E) the biotransformation of **1** seemed to be complete because eugenol was not observed. In the plant cell culture, used as control, where the substrate was not added (Fig. 2), some compounds were observed in the controls (C′ and E′), these did not interfere on the biotransformation reaction. In this work, we focused on determine which plant cell culture could be used to transform eugenol no attention was done on the isolation of the products formed in the controls of capulin and bean (C′, E′). Since the TLC analysis was performed on samples of similar concentration, the size of the spots observed was a reliable indication of the progress on the transformation of eugenol to product.

The results for the biotransformation of **1** with cell suspension (data not shown) were similar to those obtained for calli. However, the total transformation of the substrate was not observed in the bean cell suspension and for capulin **2** was not the sole product.

The TLC analysis of the reaction mixtures from the biotransformation of isoeugenol using calli presented a mixture of products (Fig. 3). In all cases, at least three reaction products were observed, the main product was identified as dehydrodiisoeugenol (**4**) by comparing the colour and Rf spot values observed with the reference compound previously obtained by the chemical method.

In all cases a complete transformation of the substrate was observed, except for *M. hutzilopozchtli* calli (F). We may say, from the spot intensity observed in TLC, that the highest transformation was obtained with the calli of *B. ternifolia* and *P. vulgaris*, taking into consideration that the reaction mixtures analyzed were at similar concentration. The mixture of compounds obtained from the biotransformation using *B. ternifolia* were chosen in order to isolate the main product from which 23% of yield was obtained. The spectroscopy data are in concordance to those previously described for dehydrodiisoeugenol (**4**) obtained from **3**.

When cell suspensions were used as a biocatalyst (data not shown), the results were similar to those obtained with, calli except in the case of melon, where the substrate was not totally transformed and capulin, where dehydrodiisoeugenol (**4**) was observed as the sole product.

In the case of isoeugenol no less than three compounds were observed in the reaction where dehydrodiisoeugenol was obtained in higher amount than the other compounds (Fig. 3). The quantitative analysis on eugenol (**1**) transformation was done leaving the compounds obtained on the transformation of isoeugenol for further analysis.

The calli and cell suspensions evaluated in this work catalyzed the dimerization of eugenol and isoeugenol via oxidative coupling. However, under the conditions studied, the reactivity was different

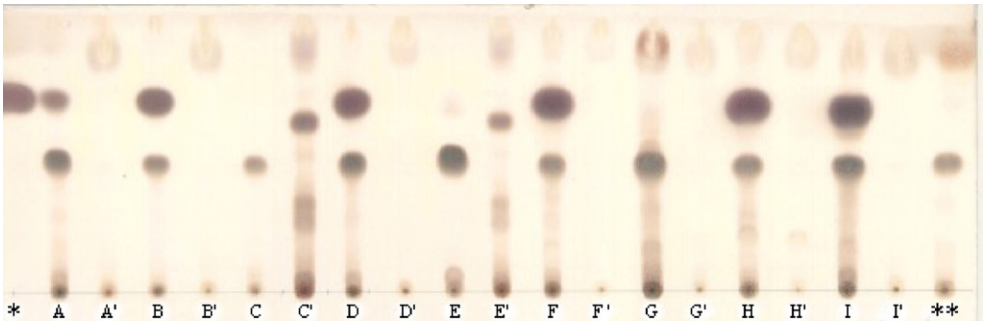

Fig. 2. TLC chromatogram of the biotransformation of eugenol biocatalyzed by calli. The letters refer to the plants listed in Table 1. *, Eugenol; A, *M. sativa*; B, *B. ternifolia*; C, *P. serotina*; D, *C. sativum*; E, *P. vulgaris*; F, *M. hutzilopochtli*; G, *Matarique*; H; *C. melo*; I, *D. carota*; **, Dihydrodieugenol. The letters A′–I′ are the control reactions for the same plants (without substrate).

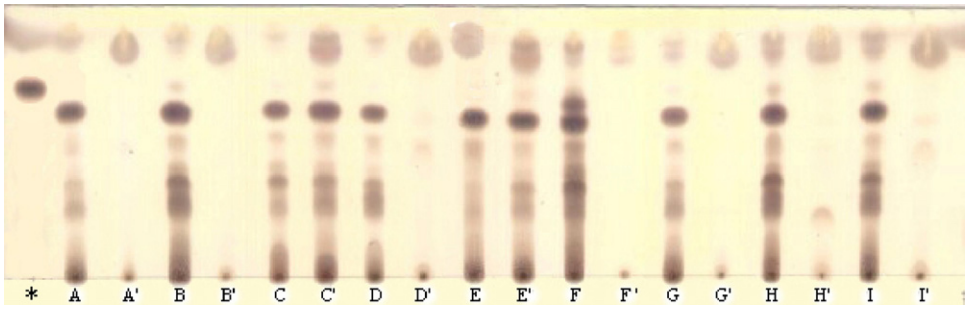

Fig. 3. TLC chromatogram of the biotransformation of isoeugenol biocatalyzed by calli. The letters refer to the plants listed in Table 1. *, isoeugenol; A, *M. sativa*; B, *B. ternifolia*; C, *P. serotina*; D, *C. sativum*; E, *P. vulgaris*; F, *M. hutzilopochtli*; G, *Matarique*; H, *C. melo*; I, *D. carota*. The letters A′–I′ are the control reactions for the same plants (without substrates).

for each culture. All the assayed cell cultures would be suitable for carrying out oxidative coupling of other phenols.

3.3. Dimerization of eugenol (1): preparation of dihydrodieugenol (2)

Fig. 4 shows the obtained yield from the biotransformations of eugenol (**1**) to dehydrodieugenol (**2**) with calli and cell suspensions of the evaluated plants and Table 1 shows the percentage of recovered eugenol in each experiment.

The dehydrodieugenol obtained using calli ranged from 3.2 to 35.8% yield. The highest yield was obtained from the reaction with alfalfa calli (35.8%), from which 18.28% of eugenol was recovered. This result was an improvement on that reported for the reaction catalyzed by HRPO, which yielded only 22% of **2** [9]. The lowest yield was observed with capulin calli, which produced only 3.2% of **2** and 19.3% recovery of **1**.

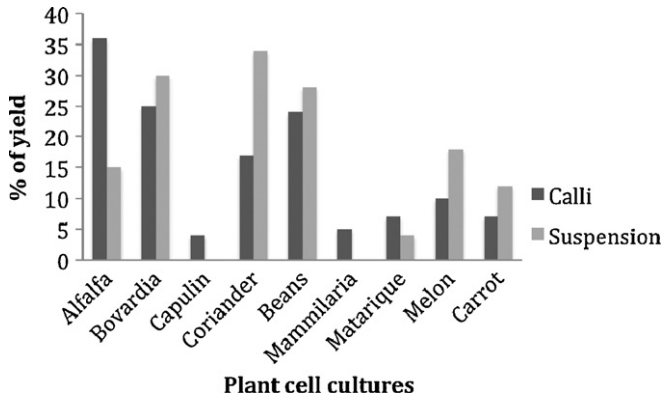

Fig. 4. Yield of dehydrodieugenol from the cell culture reactions.

Table 1
Yields of dehydrodieugenol and eugenol recovered after the cell culture reaction.

Plant	Recovered eugenol (%)		Yield of dehydrodieugenol (%)[a]	
	Calli	Cell suspension	Calli	Cell suspension
Medicago sativa	18.28	44.79	35.81	15.02
Bouvardia ternifolia	36.38	35.18	24.45	30.35
Prunus serótina	19.33	0.86	3.22	0.24
Coriandrum sativum	32.02	15.24	16.79	33.14
Phaseolus vulgaris	0.44	15.28	23.21	27.61
Mammillaria hutzilopochtli	59.75	78.09	4.39	0.27
Psacalium peltratum	0.29	1.29	5.53	3.50
Cucumis melo	40.14	37.14	8.29	15.99
Dacus carota	34.15	63.13	5.83	10.17

[a] HPLC: Symmetry® C-18 column at 30 °C, a flow rate of 1 mL/min, UV wave length at 230 and 280 nm, and a H_2O–MeOH (70:30) solvent system with 0.1% trifluoroacetic acid.

When cell suspensions were used, the highest yield was observed with coriander (33.1%), with a 15.2% recovery of eugenol. The lowest conversion was observed using capulin, whose yield was less than 1%, with a recovered eugenol being less than 1%.

Although *Bovardia* and *P. vulgaris* produced lower yields than alfalfa (30.3 and 27.6% respectively), the amount of biseugenol obtained did not differ substantially between calli and cell suspensions. Liquid or solid media used in the growth of the cells was not an important factor in the yield of these plants.

The lowest yields were obtained with both calli or cell suspensions of capulin, mammillaria and matarique, in each case being less than 10%. In the case of coriander, melon and carrot, the highest yields were obtained with cell suspensions.

In summary, the yield of dehydrodieugenol appears to depend on the plant species and the type of the plant cell culture used, whether callus or cell suspension. Although the yields of **2** were not very high, it is important to pointing out that they could be

improved by modifying the reaction conditions and optimization. Chemical methodology produces higher yields, employing oxidizing agents such as the iron compounds $K_3Fe(CN)_6$ (98%), CuCl(OH) and triethylenediamine (72%) [4,5] however, the methodology used in this work presents a cleaner and greener approach compared to traditional methods of oxidative coupling of eugenol.

4. Conclusion

TLC analysis showed that the nine plant species tested as biocatalysts were able to successfully biotransform isoeugenol and eugenol to their dimers. Consequently, these cultures can be used for oxidative coupling of phenolic compounds. HPLC analysis of the biotransformation of eugenol showed that the highest yield of compound **2** was obtained with the calli of alfalfa (35.8%). However, this yield was lower when cell suspensions were used.

References

[1] A. Giri, V. Dhingra, C.C. Giri, A. Singh, O.P. Ward, M.L. Narasu, Biotechnol. Adv. 19 (2001) 175–199.
[2] T. Suga, T. Hirata, Phytochemistry 29 (1990) 2393–2406.
[3] K. Shihara, H. Hamadab, T. Hirata, N. Nakajima, J. Mol. Catal. B: Enzym. 23 (2003) 145–170.
[4] (a) M. Ogata, M. Hoshi, S. Urano, T. Endo, Chem. Pharm. Bull. 48 (2000) 1467–1469;
(b) R. Bortolomeazzi, G. Verardo, A. Liessi, A. Callea, Food Chem. 118 (2010) 256–265.
[5] A. Farias-Dias, Phytochemistry 27 (1988) 3008–3009.
[6] M. Takemoto, Y. Suzuki, K. Tanaka, Tetrahedron Lett. 43 (2002) 8499–8501, and reference cited here.
[7] J.P. Kutney, X. Du, R. Naidu, N.M. Stoynov, M. Takemoto, Heterocycles 42 (1996) 479–484.
[8] M. Sridhar, S. Vadivel, U.T. Bhalerao, Tetrahedron Lett. 38 (1997) 5695–5696.
[9] A. Krawczyk, E. Lipkowska, T. Wróbel, Collect. Czech. Chem. Commun. 56 (1991) 1147–1150.
[10] M. Takemoto, Y. Aoshima, N. Stoynov, J.P. Kutney, Tetrahedron Lett. 43 (2002) 6915–6917.
[11] M. Takemoto, Y. Suzuki, K. Tanaka, Tetrahedron Lett. 43 (2002) 8499–8501.
[12] (a) G. Wenqiang, L. Shufen, Y. Ruixiang, T. Shaoku, Q. Can, Food Chem. 101 (2007) 1558–1564;
(b) L. Juhász, L. Kürti, S. Antus, J. Nat. Prod. 63 (2000) 866–870.
[13] (a) G. Delogu, D. Fabbri, M.A. Dettori, A. Forni, G. Casalone, Tetrahedron: Asymmetry 15 (2004) 275–282.
[14] S. Fujisawa, Y. Kashiwagi, T. Atsumi, I. Iwakura, T. Ueha, Y. Hibino, I. Yokoe, J. Dent. 27 (1999) 291–295.
[15] (a) M.D. Barrat, D.A. Basketter, Contact Dermatitis 27 (1992) 98–104;
(b) M. Takeyoshi, S. Noda, S. Yamazaki, H. Kakishima, K. Yamasaki, I. Kimber, J. Appl. Toxicol. 24 (2004) 77–81.
[16] R. Gerosa, M. Borin, G. Megazzi, M. Puttini, G. Cavalleri, J. Endod. 22 (1996) 532–534.
[17] A De Diaz, M.H.E. Gottlieb, Phytochemistry 19 (1980) 681–682.
[18] (a) Y. Murakami, M. shoji, S. Hanazawa, S. Tanaka, Biochem. Pharmacol. 66 (2003) 1061–1066;
(b) Y. Murakami, M. Shoji, A. Hirata, S. Tanaka, I. Yokoe, S. Fujisawa, Arch. Biochem. Biophys. 434 (2005) 326–332.
[19] S. Fujisawa, M. Ishihara, I. Yokoe, J. Mol. Des. 3 (2004) 241–246.
[20] (a) S. Rosales-Mendoza, R. Soria-Guerra, Ma.T. Olivera-Flores, R. López-Revilla, G.R. Argüello-Astorga, J.F. Jiménez-Bremont, R.F. García-De la Cruz, J.P. Loyola-Rodríguez, A.G. Alpuche-Solís, Plant Cells Rep. 26 (2007) 969–976;
(b) J. Cortés-Eslava, S. Gómez-Arroyo, R. Villalobos-Pietrini, J.J. Espinosa-Aguirre, Toxicol. Lett. 153 (2004) 283–292;
(c) E. Rojas-Anaya, E. Loza-Rubio, Ma.T. Olivera-Flores, M. Gomez-Limm, Transgenic Res. (2009) 911–919.
[21] (a) T. Murashige, F. Skoog, Physiol. Plant. 15 (1962) 473–497;
(b) R.U. Schenk, A.C. Hildebrandt, Can. J. Bot. 50 (1972) 199–204.
[22] R.E. Robles-Zepeda, M. Jiménez-Estrada, A. Navarro-Ocaña, I. Saad-Villegas, I. Brunner, E. Ruiz-Bustos, Afr. J. Biotechnol. 8 (2009) 3874–3878.
[23] L Hernández, H. Luna, A. Navarro-Ocaña, Ma.T. Olivera-Flores, I. Ayala, Chin. J. Biotechnol. 24 (2008) 1199–2001.