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

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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

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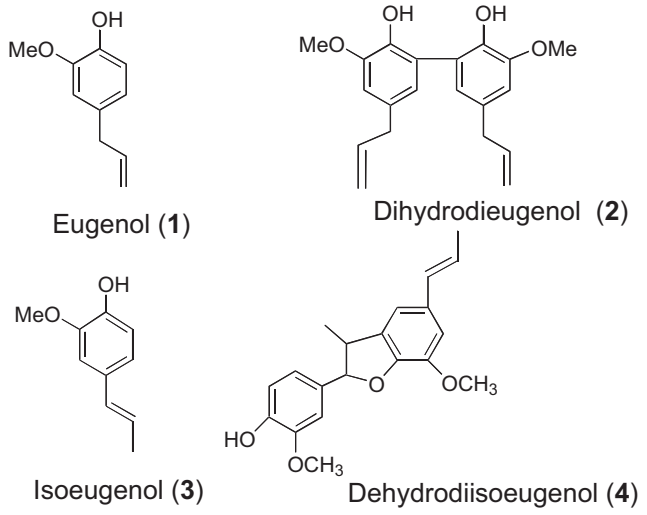

Fig. 1. Structure of monomer and dimers.

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].