OXIDATIVE COUPLING OF NATURAL PHENOLS BIOCATALYZED BY PLANT ENZYMATIC PREPARATIONS WITH PEROXIDASE ACTIVITY

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Introduction. The use of plant cultured cells as biocatalysts has the advantage of act as polyenzimatic systems biocatalyzing the transformation of substrates in product through more than one reaction involving one or more enzymes (1).The interest for the use of peroxydases in organic synthesis has increased, because they are an efficient and green alternative. Among the reaction promoted by these enzymes, the dimerization of phenols is particularly interesting. Reports indicate that the dimers of bioactive phenols show greater activity than the corresponding monomers; as some hydroxycinamic acids and allylbenzenes (2). Eugenol is the main component of the clove oil, also present in cinammon and nutmeg (3); and the dieugenol shows greater antioxidant activity (4).

Methods. Calli of alfalfa, bean, *Centella asiática* and arnica (*Heterotbeca inuloides)* were prepared by the usual procedure for plant cultured cells (4). Enzymatic preparations from all calli were prepared homogenizing them with phosphate buffer (pH 7).
The peroxidase activity of each preparation was determined, qualitatively by the reaction with anisidine and guaiacol. The reactions of dimerization of eugenol (**1**) (Fig 1) and vainillin (**3**) (Fig. 2) were performed using the enzymatic preparation and hydrogen peroxide, at room temperature. In the case of eugenol, the reaction mixture was purified by column chromatography (CC). For vainillin, the analysis of the reaction was done by thin layer chromatography and ^{1}H-NMR.

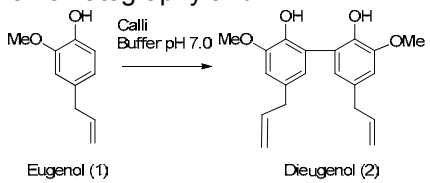

Figure 1. Dimerization reaction of eugenol with plant cultured cells.

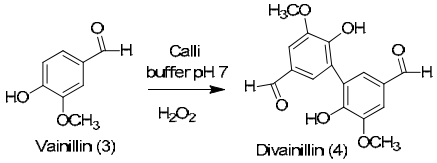

Figure 2. Dimerization reaction of vainillin with plant cultured cells.

Results. Table 1 shows the isolated yields of dieugenol after CC purification with the enzymatic preparation from the plant cultured cells; although the yield are moderate, the reactions can be optimized. Arnica shows the best yield.

Table 1. Results from the dimerization of eugenol .

Enzymatic preparation	Isolated yield
Alfalfa	11 %
Arnica	30 %
Centella asiática	14 %
Bean	30 %

The analysis of the reaction mixtures from the dimerización of vanillin, by thin layer chromatography, shows the same main product in all the biotransformations, and the analysis by 1H-NMR indicates that the compound correspond to divainillin (**4**)

Conclusions.
The results indicate that the plant cell cultures retain the peroxidase activity and vanillin and eugenol are suitable substrates for the enzymes present in those enzymatic preparations.

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