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Laccase-catalyzed dimerization of glycosylated lignols

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ABSTRACT

Phenylpropanoid glucosides (PPGs) are naturally occurring and bioactive phenolic derivatives, largely distributed in plants. In this work different PPGs have been chemically or enzymatically synthesized from the lignols coniferyl and *p*-coumaryl alcohols as substrates for a laccase-catalyzed oxidative coupling. The biooxidation of these PPGs has been investigated here and novel dihydrobenzofuran-based structurally modified analogues have been isolated and characterized. Specifically, the presence of a carbohydrate moiety increased the water solubility of these compounds and reduced the number of dimeric products, as pinoresinol-like structures could not be formed. Looking for a possible sugar-promoted stereochemical enrichment of the obtained diastereomeric mixtures of dimers, different carbohydrate moieties (D-glucose, L-glucose and the disaccharide rutinose) were considered and the respective d.e. values of the dimeric products were measured by ^{1}H NMR and HPLC. However, it was found that the sugar substituent had a minor effect on the stereochemical outcome of the radical coupling reactions, the best measured result being a d.e. value of 21%.

1. Introduction

Laccases (benzendiol:oxygen oxidoreductases, EC 1.10.3.2) are copper-containing oxidases, distributed in plants, fungi and bacteria, that catalyze the formation of four highly reactive organic radicals by reducing molecular oxygen to water [1]. It has been reported that laccases play, *i.a.*, an important role in the formation of lignin by sequential oxidative couplings of monolignols, a family of naturally occurring phenolic compounds [2]. *p*-Coumaryl (**1**) and coniferyl (**2**) alcohols, Fig. 1, are two major examples of this class of chemicals.

From a synthetic point of view, these 'blue' enzymes are known to oxidize a wide range of organic molecules, both directly or in the presence of a low-molecular-weight redox mediator [3]. Our group has accordingly reported several examples dealing with the oxidation of sugars [4], alkaloids [5] and, more extensively, phenols [6]. Specifically, we have shown that stilbenoids and vinyl phenols are suitable substrates for these enzymes. Mixture of dimers and oligomers are always obtained, with the 2,3-dihydrobenzofuran-based β-5-type dimeric structures being isolated as the main products [7]. These compounds carry one or two stereogenic carbons and are always obtained as racemates and, when two adjacent stereocenters are present (like in the dimers obtained from resveratrol [7b]), only the *trans*-stereoisomers are formed.

The lack of enantioselectivity is a serious synthetic drawback of these biocatalyzed oxidative couplings. Nature has solved this problem developing the so-called "dirigent proteins", a group of peptides that act as chiral templates and direct the stereochemical outcome of these processes. A significant example of these "dirigent proteins" was isolated from the plant *Forsythia intermedia* and used by Davin et al. for the *in vitro* stereoselective biocatalyzed synthesis of (+)-pinoresinol (**3**) by the laccase-catalyzed oxidation of coniferyl alcohol [8]. Later on, in 2010, Pickel et al. isolated and cloned an enantiocomplementary dirigent protein, able to guide the bio-oxidative coupling toward the formation of (−)-pinoresinol [9]. However, this elegant biomimetic approach is of limited synthetic appeal as, for any substrate and desired enantiomer of a dimeric product, a specific dirigent protein should be isolated, providing that it does exist in Nature.

Abbreviations: E.I., electron ionization; Py, pyridine; DMAP, dimethylaminopyridine; THF, tetrahydrofuran; DBU, 1,5-diazabiciclo(5.4.0)undec-5-ene; TMSOTf, trimethylsilyl trifluoromethanesulfonate; DCM, dichloromethane; TBAF, tetra-*n*-butylammonium fluoride; TBDMS, *tert*-butyldimethylsilyl; d.e., diastereomeric excess.

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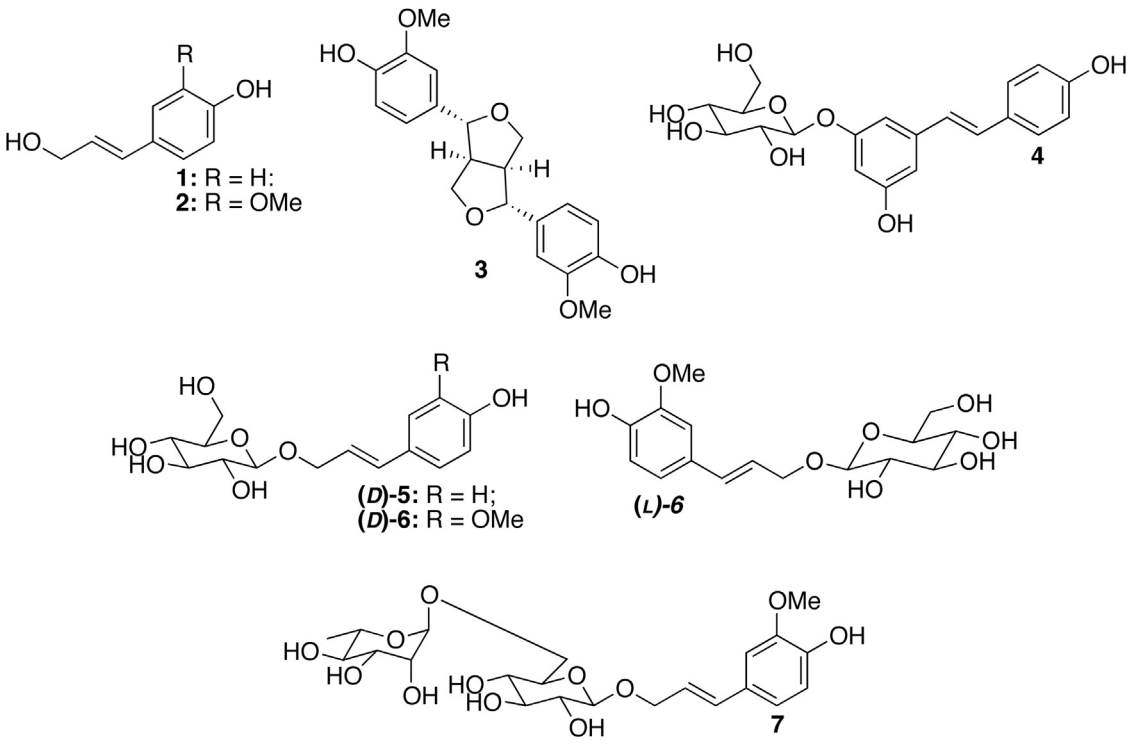

Fig. 1. Compounds **1–7**.

To overcome this limitation, Urlacher and coworkers have recently proposed to couple the laccase-mediated oxidation of coniferyl alcohol to a stereoselective reductase that is able to transform the unwanted enantiomer of pinoresinol [10].

In a different and more general approach, we have exploited the 'classical' lipase-catalyzed kinetic resolution. The racemic mixtures of β-5-type dimers derived from the laccase-mediated oxidation of different vinyl phenols were separated obtaining both the possible enantiomers as enriched species. The target products could be isolated with e.e. up to 98% [11].

More recently, both the enantiomers of the β-5-type dimers of resveratrol could be isolated on a preparative scale starting from the laccase-mediated oxidation of piceid (**4**), a natural D-glucoside of resveratrol [12]. A mixture of *trans*-2*R*,3*R* and *trans*-2*S*,3*S* glucosylated dimers was easily isolated in good yield, and the two diastereomers were separated by preparative HPLC on a chiral column. The target enantiomers of resveratrol dimers were eventually obtained with e.e. values up to 97% by glycosidases-catalyzed hydrolysis of the glucose units.

We thought that this approach could be extended to other compounds. Phenylpropanoid glucosides (PPGs, also known as glucosylated lignols) are naturally occurring in various plants. As secondary metabolites, these compounds are described as phytopharmaceuticals [13] and they are studied for their potential biological activities [14]. Specifically, their antioxidant, antidepressant and hypotensive effects have been extensively investigated [15].

In this work the laccase-catalyzed oxidative coupling of a small family of glycosylated phenylpropanoids, previously synthesized from coniferyl and *p*-coumaryl alcohol, has been investigated, and novel, structurally modified analogues of these bioactive natural compounds have been isolated and characterized. Looking for a possible sugar-induced stereochemical enrichment of the obtained diastereomeric mixtures of dimers, different carbohydrate moieties (D-glucose, L-glucose and the disaccharide rutinose) were considered.

2. Experimental

2.1. General methods and information

NMR spectra were recorded on Bruker AC400 spectrometer (400 MHz) in MeOH-d_4, DMSO-d_6 or D_2O, MS spectra were recorded on a Bruker Esquire 3000 Plus Ion Trap spectrometer NMR and the MS spectra *in extenso* are available in the Supplementary materials.

HPLC analyses were carried out using a Jasco 880-PU pump equipped with a Jasco 875-UV/vis detector. HPLC conditions: Kinetex 5 μm Biphenyl 100 Å Phenomenex 150 × 4.6 mm column, gradient of H_2O: CH_3CN as mobile phase (0–1 min 90% H_2O, 10% CH_3CN; 1–20 min 80% H_2O, 20% CH_3CN; 20–22 min 80% H_2O, 20% CH_3CN; 22–23 90% H_2O, 10% CH_3CN), flow rate 0.7 mL min^{-1} at 25 °C, detection at 270 nm. Reactions were monitored by TLC: precoated silica gel 60 F_{254} plates (Merck, DE), developed with a 20% solution of H_2SO_4 in ethanol or using the molybdate reagent ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 42 g; $Ce(SO_4)_2$, 2 g; H_2SO_4 conc., 62 mL; made up to 1 L of deionized water).

Flash chromatography: silica gel 60 (70–230 mesh, Merck, DE).

Biotransformations were carried out using a G24 Environmental Incubator New Brunswick Scientific Shaker (Edison, USA) or a Thermomixer Comfort (Eppendorf, DE).

2.2. Enzymes and chemicals

Laccase from *Trametes versicolor* was from Sigma-Aldrich. α-L-*R*hamnosyl-β-D-glucosidase (rutinosidase) from *Aspergillus niger* was produced according to the listed reference [16]. The enzymes were used based on their respective activities evaluated according to literature assays [16,6b].

TBDMS-phenol protected phenylpropanoid alcohols were prepared from their correspondent acids or aldehydes according to literature [19]. All other reagents were of the best purity grade from commercial suppliers.