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Comparative electrochemical study on monolignols and dimers relevant for the comprehension of the lignification process

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1. Introduction

Lignins are located in the secondary cell wall of higher plants and present a complex structure that might be considered as deriving from a pseudo combinatorial cross coupling of diverse delocalized *p*-hydroxycinnamic alcohol radicals. The resulting aromatic polymer gives, through interactions with cellulose and hemicelluloses, plants mechanical support and defense against pathogenic attacks. Lignins are mainly composed of three phenylpropanoïd units, *p*-hydroxyphenyl (H), guaïacyl (G) and syringyl (S) derived from monolignols *p*-coumaryl **1**, coniferyl **2** and sinapyl **3** alcohols (Vanholme et al., 2012) (Fig. 1).

Numerous studies on lignification of plant cell wall have been performed (Morreel et al., 2004; Vanholme et al., 2010; Davin et al., 1997; Jouanin and Lapierre, 2012), but many points still remain unclear despite the high number of *in vitro* and *in vivo* studies performed since several decades (for a review, see Liu, 1997). Several enzymes involved in the oxidative polymerization of the hydroxycinnamic alcohols have been characterized (mainly laccases and peroxidases) but, for instance, there is no unified scheme addressing the mechanistic features of lignification of cell wall allowing the understanding of the variations encountered in lignins structure according to plant origin or growth state. An efficient tool, pioneered by Freudenberg (1956), has been developed by different teams for lignification mimicking that consists in modeling this oxidation reaction in *in vitro* experiments with model compounds and different oxidases in various experimental conditions, leading to the formation of dehydrogenative polymers (DHP). Many factors have indeed to be considered in order to elucidate the mechanism of *in vitro* lignification, such as solvent effects, pH effects, inter- and intramolecular interactions, rates of supply of both substrates and enzymes, and the type and activity of the enzyme studied (Demont-Caulet et al., 2010). Obviously, substrates structure is also a preponderant feature accounting for the kinetics of the reaction. Fournand et al. (2003) indeed found that the oxidative activity of HRP (horseradish peroxidase) at pH 6 was reduced by a factor 3 to 30 between coniferyl alcohol and its different dimers. However, the main limitation of this methodology is that the different parameters may only be investigated independently and a general chemical model accounting for their combined effects is lacking. Nevertheless, the main question to address is to know which chemicals, derived from monolignols, can be involved in lignin growth through direct oxidation by lignification enzymes.

Two reasons can indeed be envisaged for the non-oxidation of monolignols by lignification enzymes and lie in the specificity of the enzymes in term of either accessibility of the active site (steric hindrance) or higher redox potential for the substrate than for the

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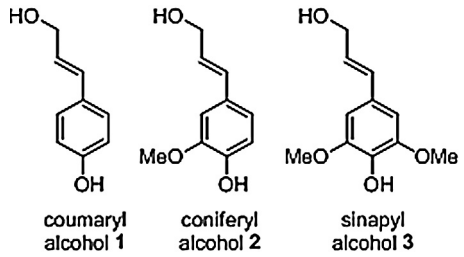

Fig. 1. Structure of the main monolignols **1**–**3** involved in the lignification process.

metallic species involved in the catalytic system of the enzyme. In the case of plant peroxidases, substrate specificities have already been observed and assigned to steric rather than electronic factors (Demont-Caulet et al., 2010). While coniferyl alcohol **2** is readily oxidized by most peroxidases, sinapyl alcohol **3** often appears as a poor substrate although having a lower redox potential (Méchin et al., 2007), but happens to be good substrate of peroxidases carrying larger active site (Barcelo et al., 2007). It was also described that a polymeric lignin can be a substrate for a poplar cell wall peroxidase (Sasaki et al., 2004). The redox potential data of peroxidases are particularly scarce and far from being fully understood. New procedure to estimate the redox potential of any peroxidase are being developed (Ayala, 2010) to understand why these enzymes are able to catalyze the oxidation of methoxy-substituted benzyl alcohols with redox potential as high as 1.39 V (Hong et al., 2006).

In the case of plant laccases (Shumakovich et al., 2006; Cambria et al., 2012), no specificity has been evidenced until now, but *in vivo* investigations suggest that a laccase identified in *Arabidopsis* (LAC17) is involved in the specific incorporation of coniferyl alcohol monomer in the polymeric structure (Berthet et al., 2011). Laccase activity is known to be mostly dependent of the redox potential difference between the T1 copper atom and the substrate but other factors such as the size and shape of the substrate apparently may also affect the reaction rate (Frasconi et al., 2010). Some recently reported molecular evolution data based on random mutagenesis leading to mutations in the region close to the substrate binding site of laccases show a more efficient phenol oxidation activity (Maté et al., 2010).

However, the non-oxidation of a compound by a specific enzyme may be overcome through electronic transfer from a radical present in the medium to the non-oxidized compound, as well as through dismutation of two radicals that can occur, as proposed in the case of flavonols (Ghidouche et al., 2008) and resveratrol (Cottyn et al., 2011), leading to carbocation equivalents offering a different chemical reactivity and allowing a reaction with non-oxidized species through nucleophilic addition. *In vitro* experiments may be performed with various model molecules for the determination of the substrate specificity of a given enzyme, but in order to discriminate between steric or electronic factors inhibiting the oxidation, we have to gather a maximum of data on the redox potential of the different species in relationship with pH conditions.

Electrochemistry technique provides a powerful tool for studying reaction mechanisms involving electron transfers. Comparison of the respective redox potentials of enzyme and substrates afford a screening tool that allowed in particular identifying potential phenolic redox mediators for a fungal laccase (Xu et al., 1996; Brunow, 1998). However, if data are available for monolignols, redox potential of higher oligomers and especially dimers are lacking. The objective of the present study is to complete previous electrochemical studies on coniferyl alcohol **2** (Hapiot et al., 1992a,b, 1993a, 1994a) by focusing on its dimers in order to bring new information on the different lignification pathways.

2. Material and methods

The chemical structures of the compounds used in this study are shown in Figs. 1 and 2. Monolignols **2** and **3** were synthesized using the methods of Ludley and Ralph (1996) and Wang et al. (2006). Compound **5** was synthesized using the method of Quideau and Ralph (1994). The other dimers **4**,**6**–**9** were synthesized according to the methods of Mouterde et al. (2013) and Pickel et al. (2010). All reagents were purchased from Sigma–Aldrich Chemical Co. and were used as received.

3. Experimental

Electrochemical experiments were performed with Voltalab Radiometer analytical instruments (PST006) using GPES electrochemical software version 4.9 (Utrecht, The Netherlands). The experiments were conducted in a 20 ml single compartment three-electrode glass cell, designed to allow the tip of the reference electrode (SCE) to come close to the working electrode, and equipped with a magnetic stirrer. The working electrode was a

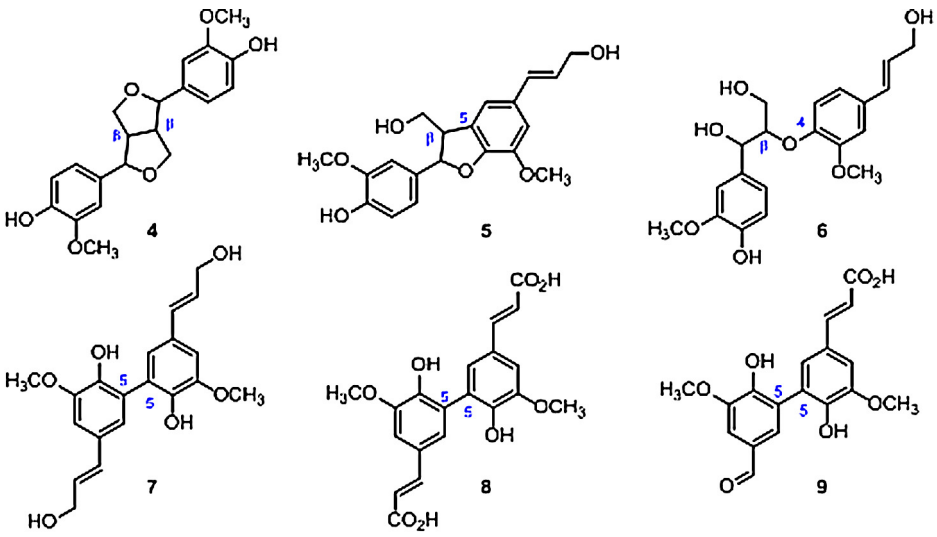

Fig. 2. Structure of the phenolic dimers **4**–**9** representative of structures found in lignin used for the electrochemical characterization.