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Mild hydroxypropylation of polyflavonoids obtained under pilot-plant scale

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ABSTRACT

Condensed tannins from pine (*Pinus radiata*), mimosa (*Acacia mearnsii*), and quebracho (*Schinopsis lorenzii*) were modified with propylene oxide (PO) *via* a mild derivatization (20 °C, 24 h). The hydroxypropylation to three PO/C_{15} ratio (1, 2, 3) produced polyphenolic derivatives in reasonable yields (60–90%). Evidense of modification was described by functional group analysis, and tannin biological properties (binding-, and antioxidant-capacity). Isolated products were characterized by physicochemical methods (solubility test, molecular weight distribution, and thermal analysis). ^{1}H NMR provided evidence of strong structure-properties relationship in function of the A-ring type, and C→C linkage features. Pine tannin fractions exhibited the best PO-uptake. Acetoxy signal intensity of acetylated derivatives revealed that mimosa and quebracho tannins are less reactive polyflavonoids toward hydroxypropylation at room temperature. Thermal behavior, solubility, and antioxidant-, and binding-capacity were affected by the modification. However, pine tannins behavior differ from those highly purified polyflavonoids.

1. Introduction

Polyphenols are the most diverse and abundant secondary metabolites in vascular plants. This group comprises low molecular weight (*Mw*) compounds (*e.g.* phenolic acids, flavonoids and stilbenes), and further high *Mw* molecules such as polyflavonoids (commonly named condensed tannins (CTs), Fig. 1), catechol's melanin, phlorotannins, and lignin. The polyphenolic fraction of woody plants has been mainly used in alternative medicine, for tanning the leather, and for the developing of adhesive resins (García et al., 2013). However, the structure of the CTs in term of monomer unit, and the linkage type, affects the chemical and biological properties of such natural compounds.

Tannins from mimosa (*Acacia mearnsii* formerly *mollissima*, De Wildt), quebracho (*Schinopsis balansae*, and *Schinopsi lorentzii*), and pine (*Pinus pinaster* Ait.) are commercializable products (Pizzi, 2008). Recently procedures for bark tannin extraction from spruce (*Picea abies*) in Finland (Kemppainen et al., 2014), and for *Pinus radiata* in Chile (Berg et al., 2009) have been also validated in view of envisaged commercialization.

However, CT from pine bark species exhibit limitations as building-blocks for polymer formulation. Structural constraints, high reactivity, and low solubility in organic solvents are recognized drawbacks (García, 2014). Despite limitations, natural aromatic biopolymers are valuable renewable chemical for replacing conventional fossil-carbon phenols in a wide range of applications.

In order to tailor physicochemical properties of tannins and lignin chemical modification is a valuable strategy (Hadley, 2007). Among many derivatization reactions O-alkylation have been widely utilized (Wu and Glasser, 1984; Hadley, 2007; Arbenz and Avérous, 2015). Improved properties of oxyalkyled derivatives point out the feasibility of modification with propylene oxide (PO) in order to expand applications. Hydroxypropyl tannin (HPT) are synthesized in a reactor (7.0–17.1 bar) at high temperature (150–261 °C) which favors extended-chain grafting (Arbenz and

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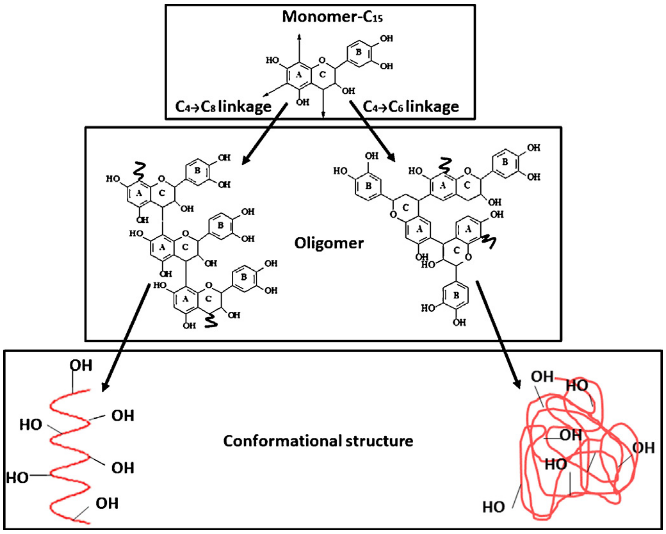

Fig. 1. Chemical structure of monomer unit (1), oligomer-chain (2), and conformational features (3) of condensed tannin in function of the C → C linkages. Arrows in monomer-C_{15} unit illustrate the most common C → C linkages. $C_4 \rightarrow C_6$ (*Mimosa tannin*), $C_4 \rightarrow C_6/C_4 \rightarrow C_8$ (*Quebracho tannin*), $C_4 \rightarrow C_8$ (*Pine tannins*).

Avérous, 2014). On the other hand, a modified protocol was developed in order to synthesize HPTs at room temperature (20–24 °C) (García et al., 2014a (Patent WO/2014/187754)). Synthetized HPT exhibited recognized advantages as polyol in polyurethane chemistry (García et al., 2015a), and as nucleating agent for polylactic acid (García et al., 2015b). HPT obtained at 22 °C provide advantages in term of high thermal stability, tailored reactivity, and enhanced solubility in comparison to tannin-derivatives based on chain-extended moieties (García et al., 2014b).

On the other hand, the Technological Development Unit (UDT) from University of Concepción-Chile, has been developed an extraction procedure for bark tannin under pilot-plant conditions (Berg et al., 2009 (Patent US 20090077871 A1)) in order to utilize such polyphenols for material applications. Considering that the valorization of bark polyphenols is an important task nowaday, the aim of this work deals about the chemical modification of two *P. radiata* tannin fractions obtained as pilot-plant feedstock. Isolated tannin were modified with PO at room temperature, and the derivatives characterized by physicochemical, and biological methods. Commercial tannin such as mimosa and quebracho were modified as well in order to establish comparative results.

2. Materials and methods

2.1. Tannins

Two A-ring type tannins were utilized. Tannin from *P. radiata* (phloroglucinol type A-ring), and commercial tannin from mimosa and quebracho (resorcinol type A-ring) were used.

Commercial tannin from Mimosa and Quebracho supplied by Seta Sun (China) and Unitán s.a.i.c.a (Argentina) were used as references, respectively. Mimosa tannin is mostly 4 → 6 linked (Pizzi and Stephanou, 1993) which confers a so-called "angular" typology (see Fig. 1), quebracho possess a mixture of 4 → 6/4 → 8 linkages, while Pine tannin is mainly 4 → 8 linked structure oligomers. Mimosa tannin is predominantly a prorobinetinidin-based olygomer/polymer, while quebracho tannin is a profisetinidin-type polyphenol (Pizzi, 2008).

Tannins were stored over silica gel in a vacuum desiccator (20 °C) prior to use. All chemicals were utilized without further purification.

Pine bark tannin extractions were performed in pilot-scale conditions at 120 °C during 120 min as described by Bocalandro et al. (2012). Extractions were carried out in a reactor (volume 4 m^3) using a methanol/water solution (1:20, w/v). Once the tannin extract had been obtained, it was evaporated under vacuum (absolute pressure 0.05 bar) to remove the methanol, thus obtaining two fractions (*1*) the water-soluble (PRS), and (*2*) the water-insoluble (PRI) one obtained after decanting. The extracted tannins are considered as a mixture of oligomers mostly 4 → 8 linked (Jeréz et al., 2007), providing a so-called "helical"-type topology (see Fig. 1) (Pizzi and Stephanou, 1993).

PRI fraction (5 kg) was washed exhaustively with distilled water in order to remove solubles and oven-dried, while 20 kg of PRS-aqueous solution (45–50 wt.%) was spray-dried in a dryer (Büttner–Schilde–Hass AG).

2.2. Hydroxypropyl tannin (HPT)

Tannins (100 g, ca. 80 mmol) were dissolved in 500 mL aq. 2 M NaOH and the pH was adjusted to 12. One-third, each, was combined with different molar equivalents of PO in order to establish three degree of substitution (DS_{HPT}: 1.0, 2.0, 3.0). The reaction was carried out for 24 h while stirring at room temperature (~20 °C). Adjusting the pH to 2 using conc. HCl (40%, v/v) produced a precipitate that was centrifuged. The precipitate (Frac. I) was collected by decantation, and washed five times with distilled water and oven-dried (40 °C, 48 h). The HPT from the supernatant was recovered by solvent evaporation (roto-evaporation at 60 °C) till one-third of the initial volume. The induced precipitate (Frac. II) was collected and washed three times with cold distilled water (5 °C) and oven-dried (40 °C, 72 h). Derivatives were stored over silica gel in a vacuum desiccator and freeze-dried prior to analysis. All other reagents were used as received.

2.3. Acetylation

Tannins and derivatives were acetylated with a mixture of pyridine and acetic anhydride (1:1, v/v) (Sigma-Aldrich, Germany) at 60 °C for 72 h. The acetates were collected by precipitation in distilled water and freeze-drying. Peracetylated products were used for proton Nuclear Magnetic Resonance (^{1}H NMR), and Gel Permeation Chromatography (GPC) analysis (García et al., 2015a).

2.4. Total phenols content (TP)

Polyphenols and derivatives were dissolved in methanol (1 gL^{-1}) and 0.5 mL of Folin-Ciocalteu reagent added (Sigma Aldrich). After 3 min, 1.0 mL saturated solution of Na_2CO_3 (332 gL^{-1}) was added and the sample was diluted with methanol up to 10 mL. The absorption band at 725 nm was measured after 1 h (Folin and Ciocalteu, 1927).

2.5. Antioxidant activity (AA)

Free radical scavenging power was assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Brand-Williams et al., 1995). Polyphenols and derivatives were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of dissolved sample, 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution (0.5 mM in ethanol). The changes in color were monitor at 517 nm after 100 min of reaction. The scavenging activity percentage was determined according to Mensor et al. (2001).