Exploring Allylation and Claisen Rearrangement as a Novel Chemical Modification of Lignin

Luca Zoia,* Anika Salanti, Paola Frigerio, and Marco Orlandi

The conversion of lignin into value-added products is traditionally hampered by its stochastic structure and its complex reactivity. The allylation reaction and the aromatic Claisen rearrangement of the allyl group on lignin as chemical modifications are reported for the first time in this work. This approach is aimed at the development of new lignin-based materials and the improvement of its compatibility and ease of processing. In particular, the Claisen rearrangement of lignin is foreseen as a valuable approach to release phenolic groups in an already chemically modified lignin, giving additional reactive sites for further transformation. These reactions were carried out on a purely guaiacylic lignin (TMP), taken as reference material due to its simplicity, and on a more structurally complex herbaceous lignin (P1000®). The Claisen rearrangement of the allylic chain was successfully achieved by treatment in dimethylformamide at reflux temperature for 15 hours. Finally, a screening of the antioxidant activity of reference, allylated, and Claisen rearranged lignins was carried out. Rearranged lignins exhibited satisfactory antioxidant activities if compared to the reference ones.

Keywords: Allylation; Biorefinery; Claisen rearrangement; ^{13}C NMR; Lignin; ^{31}P NMR

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INTRODUCTION

Lignin is the second most abundant polymer in the world after cellulose, and it constitutes about 15 to 25% of the dry weight of woody plants. Lignin biosynthesis starts from the oxidative coupling of mesomeric phenoxy radicals originating from three *p*-hydroxycinnamic alcohols (*p*-coumaryl, coniferyl, and sinapyl alcohol, respectively), which differ in their degree of methoxylation. The respective aromatic constituents in lignin are called *p*-hydroxyphenyl (P), guaiacyl (G), and syringyl (S) units. The biochemical pathway leads to the formation of a number of intermonomeric linkages providing a highly functionalized, three-dimensional racemic polymer of extremely complex structure.

Between 40 and 50 million tons/year of lignin are produced worldwide as a mostly non-commercialized waste product (www.ili-lignin.com The International Lignin Institute). Even if a major part of industrial lignin is still incinerated for the production of process steam and energy, attempts to valorize lignin waste date back to the first half of 1900s (Harris 1940). Examples of its use as a road binder, as a bonding agent in several new plastics (Howard and Sandborn 1937; Carroll and Plunguian 1939), and as a source of vanillin and other phenolic substances (Tomlinson *et al.* 1936) are in fact broadly documented.

Large quantities of lignin are made available yearly from pulping processes as well as bio-ethanol digestion and saccharification processes (Ragauskas *et al.* 2006).

Even if the hydrolytic process causes a modification of the polymer characteristics, the abundance of lignins of different types as waste products has made such material an attractive proposition for the preparation of value-added products.

In a recent review, Vishtal and Kraslawski (2011) identified the main limiting factors for a sustainable utilization and conversion of lignin. These are: recovery, purification, heterogeneous structure, and unique reactivity. Major concerns are associated with the last two problems. In fact, the relatively low molecular weight and the variable chemical behavior of industrial lignins often make them unsuitable for the employment in the most promising and explored fields of application: the incorporation into polymer compositions as physical additives (filler) and the use as macromonomers as precursors to carbon-based materials (thermoplastic biocomposites) (Gandini and Belgacem 2008).

Lignin as a filler has attracted much attention with regards to polymer stabilization against degradation processes involving free radicals. Given the large phenolic content of all lignins and the role of these structures as radical traps, a huge amount of papers dealing with the incorporation of lignin into poly(olefins) and rubber are found in literature (Alexy *et al.* 2000; Gregorova *et al.* 2006; Chaochanchaikul *et al.* 2012).

Lignin blends and their rational exploitation in materials science and technology have been the subject of numerous studies and have been widely reviewed (Feldman 2002; Thakur *et al.* 2014). Thus, lignin has been used as a filler in different elastomeric matrices (Ciemniecki and Glasser 1988a,b) and also incorporated as either a dispersant, a binder, or an additive in different industrial products (Belgacem *et al.* 2003; Stewart *et al.* 2008). Great research efforts have focused on lignin exploitation as a filler to be added to rubber composites for total or partial replacement of carbon black, while achieving similar reinforcement (Kumaran and De 1978; Setua *et al.* 2000; Benko *et al.* 2010; Frigerio *et al.* 2014). On the other hand, lignin as a macromonomer has been introduced in phenol-formaldehyde and epoxy resins, as well as in polyurethane and natural polyesters formulations.

Traditionally, when lignin is copolymerized in phenol-formaldehyde resins, it first needs to be purified and activated by chemical modification, increasing its potential reactive sites towards formaldehyde. The most studied strategies in this category are: hydroxymethylation, phenolation, and demethylation (Hu *et al.* 2011).

The effect of lignin blending with epoxy resins is strongly affected by the type of lignin used. In general, different performances could be related to differences in molecular weight and type and amount of functional groups (Zhao *et al.* 2001; Feldman 2002; Koike 2012).

Insertion of lignin derivatives as copolymers in polyurethane resins is another widely explored field, where lignin serves as the hard segment in the polyurethane structure (Ciobanu *et al.* 2004). Hydroxyl groups on lignin readily react with isocyanates to form polyurethanes, but the resulting highly networked polymer is barely reprocessable or recyclable. Through oxypropylation, the hydroxyl groups are liberated from steric constrains and, at the same time, the solid lignin becomes a more versatile liquid to viscous polyol (Glasser and Wu 1984; Nadji *et al.* 2005; Cateto *et al.* 2009).

More recently, greater environmental concern has prompted the academic and industrial interest towards the development of fully sustainable thermoplastics (poly(hydroxyalkanotaes)) that mimic the properties and functions of traditional materials. According to this objective, lignin could act either as a filler, enhancing the

thermal and mechanical properties of the composite (Bertini *et al.* 2012), as a macromolecular copolymer (Chung *et al.* 2013), or as a source of the constituting monomers (Mialon *et al.* 2010, 2011).

Within these research topics, the present work is focused on the study of lignin allylation reaction and subsequent Claisen rearrangement of the allyl group as potential chemical modifications aimed at the development of new lignin-based materials. In particular, the Claisen rearrangement of lignin was foreseen as a valuable approach to release phenolic groups in an already chemically modified lignin, giving additional reactive sites for further transformation. To the best of our knowledge, the introduction of allyl chains, as well as their Claisen rearrangement, has never been performed on the lignin macromolecule. These reactions were carried out on a purely guaiacylic lignin (TMP), taken as reference material for its simplicity, and then on a more structurally complex herbaceous lignin (P1000®) to investigate the influence of lignin structure and monomeric composition on the reactivity. Allylation is a straightforward reaction and provides a versatile product, prone to several chemical modifications, as typical of double bonds. On the other hand, the Claisen rearrangement can be used as a simple reaction to introduce carbon–carbon bonds and release phenolic groups, resulting in a chemically modified lignin having in addition a certain degree of antioxidant activity.

Lignin samples after allylation and Claisen rearrangement were fully characterized by means of ^{31}P NMR, ^{13}C NMR, and GPC analyses. Moreover, to demonstrate the presence of free phenolic moiety after Claisen rearrangement, a screening of the antioxidant activity of reference, allylated, and rearranged lignin was performed.

EXPERIMENTAL

The reaction steps (lignin allylation and Claisen rearrangement) and the chemical characterizations performed on the reaction products have been summarized in the flow diagram reported in Fig. 1.

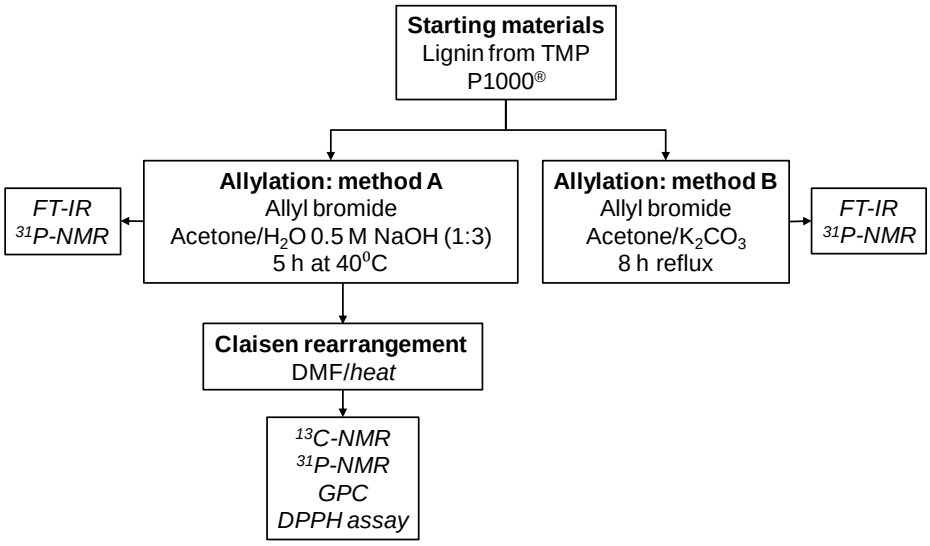

Fig. 1. Experimental flow diagram summarizing the reaction steps (lignin allylation and Claisen rearrangement) and the reaction product characterizations

Reagents and Materials

All the reagents, acetone (99.5%), allyl bromide (99.0%), N,N-dimethyl-formamide (99%), sodium hydroxide (98%), and potassium carbonate (anhydrous 99%) were purchased from Sigma-Aldrich and used as received without further purification. Protobind1000® (P1000®), an industrial lignin obtained from agricultural fibrous feedstocks, was provided by Green Value. Softwood lignin was isolated from TMP fibers according to the acidolysis extraction procedure (Salanti *et al.* 2012).

Lignin Allylation: Method A

In a round bottom flask, oven-dried lignin (1 g) was solubilized in 40 mL of an acetone/0.5 M aqueous NaOH (1:3) solution. Excess allyl bromide (1.5 eq with respect to total OH content in lignin) was then added, and the mixture was reacted, under stirring, at 40 °C. After 5 h, acetone was rotary evaporated and the crude was acidified to pH 2, adding 37% HCl dropwise. Allylated lignin was recovered by Buchner filtration or ethyl acetate extraction, depending on the kind of lignin treated, and oven-dried. The reaction yield was comprised between 80 and 90%, accounting for water-soluble low molecular weight compounds leached during the recovery operations.

Lignin Allylation: Method B

In a round bottomed flask equipped with a condenser, oven-dried lignin (100 mg) was refluxed in acetone under stirring in the presence of excess allyl bromide (1.5 eq with respect to total OH content in lignin) and potassium carbonate. After 8 h, the mixture was poured dropwise, under vigorous stirring, into an Erlenmeyer flask containing cool acidic water (pH=1). Allylated lignin was collected by Buchner filtration and air dried. The reaction yield was comprised between 80 and 90%, accounting for water-soluble low molecular weight compounds leached during the recovery operations.

Claisen Rearrangement

In a round bottom flask equipped with a magnetic stirrer and a condenser, 100 mg of allylated lignin was refluxed in 10 mL of dimethylformamide (DMF) for 15 h. DMF was then removed by freeze-drying.

Radical Scavenging Activity Evaluation

The radical scavenging activity of pristine, allylated, and Claisen rearranged TMP and Protobind1000® lignin was determined by means of a spectroscopic assay involving the consumption of the stable free radical originated by DPPH (2,2-diphenyl-1-picrylhydrazyl) in a methanolic solution. The colorimetric assay was performed according to a published method (Salanti *et al.* 2010). Different dosages of a 0.5 mg/mL dioxane solution (100, 200, 300, 400, 500 μL) of the appropriate lignin sample were added into 3 mL of a DPPH methanolic solution (6.1×10^{-5} M, daily prepared). The mixtures were stored in the dark for 15 min, and then their absorbance (A) was measured at 515 nm using a Shimadzu UV-2101PC spectrophotometer. The inhibition percentage (I %) of the free radical DPPH• was calculated according to the following formula,

$$I\,\% = [(A_0\text{-}A) / A_0] \times 100 \quad (1)$$

where A_0 is the absorbance of the DPPH reference solution. The obtained data were plotted on a log dose-inhibition curve and the resulting linear calibration curves (TMP

lignin: R^2 = 0.98; TMP allylated: R^2 = 0.88; TMP rearranged: R^2 = 0.98; P1000® lignin: R^2 = 0.93; P1000® allylated: R^2 = 0.97; P1000® rearranged: R^2 = 0.94) were used to derive the half maximal inhibitory concentration (IC_{50}).

Lignin Acetylation

100 mg of the appropriate allylated lignin sample was acetylated in a pyridine-acetic anhydride solution (1:1 v/v, 4 mL) kept overnight at 40 °C. Samples were stripped with ethanol, toluene, and chloroform (25 mL x 3 times, each solvent) and then dried in vacuum. Acetylated lignins were solubilized in THF or DMSO-d_6 for GPC and ^{13}C NMR analysis, respectively.

^{31}P NMR Derivatization

Accurately weighed, treated, and untreated lignin samples (20 mg) were dissolved in a pyridine-deuterated chloroform solution (1.6:1 v/v, 700 µL) containing 1 mg/mL of chromium(III) acetylacetonate, [$Cr(acac)_3$]. 100 µL of an internal standard solution of endo-N-hydroxy-5-norbornene-2,3-dicarboximide (e-HNDI, 121.5 mM, CDCl3/pyridine 4.5:0.5) was then added, along with 100 µL of 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane as the phosphorus derivatizing agent (Granata and Argyropoulos 1995). ^{31}P-NMR spectra were recorded on a Bruker Avance 500 MHz instrument.

FTIR-ATR Analysis

FTIR-ATR spectroscopy was used for a rapid screen of the reaction outcome. The presence of signals at 990 and 920 cm^{-1} was diagnostic for the presence of allyl groups on lignin.

^{13}C NMR Analysis

^{13}C-NMR spectra were run in DMSO-d_6 on acetylated samples, to increase both solubility and chemical shift dispersion. Spectra were recorded on a Bruker Avance 500 MHz spectrometer at room temperature. The chemical shifts were referred to the solvent signal at 39.5 ppm (DMSO-d_6). A relaxation delay of 4 to 5 s was used between the scans (90° pulse angle). Line broadening of 1 to 5 Hz was applied to FIDs before Fourier transform. For each spectrum, typically about 10,000 scans were accumulated. The assignment of predominant signals was based on the chemical shift data of lignin model compounds (Ralph *et al.* 2004).

GPC Analyses

The analyses were performed on an Agilent 1100 liquid chromatography device connected to a VWD G1314 UV detector set at 280 nm. The injection port was a Rheodyne loop valve equipped with a 20 µL loop. The GP-column system was composed by a sequence of an Agilent PL gel 5 µm, 500 Å and an Agilent PL gel 5 µm, 10^4 Å. The solvent used was tetrahydrofuran (THF, Fluka 99.8%). Acetylated samples were dissolved in THF (1 mg/mL) and analyzed at a flow rate of 1 mL/min. PL Polymer Standards of Polystyrene from Polymer Laboratories were used for calibration. The evaluation of the number-average molecular weight (M_n) and the weight-average molecular weight (M_w) for the examined lignin samples was performed according to the methodology developed by Himmel *et al.* (1989). Besides, the peak molecular weight M_p, defined as the molecular weight of the species with maximum absorbance, and the ratio $I = M_w / M_n$, defined as Polydispersity Index, were calculated. The M_n, M_w, and M_p values

reported are the average of three analyses (M_w: 1000 g/mol; M_n, M_p: 70 g/mol, P = 0.05, n = 3).

RESULTS AND DISCUSSION

Lignin Allylation

In the early 1990s, a Japanese research group found that allylated wood meals were given thermoplastic properties by blending with appropriate synthetic polymers or low molecular weight plasticizers. In particular, they tried to elucidate the mechanism of plasticization of wood, considering that decrystallization of cellulose within wood during allylation permits the wood to soften thermally, and the allylated lignin within wood increases the softening through acting as a plasticizer (Ohkoshi *et al.* 1992). Nevertheless, the allylation of a purely lignin specimen and a detailed characterization of the obtained product has not been reported in the literature. The aim of the first part of the present work is to fill this gap.

Two different strategies for hydroxyls allylation were tested on P1000® lignin. Allylated lignins were then analyzed by FT-IR and ^{31}P NMR. The presence of signals at 990 and 920 cm^{-1} in the IR spectra (data not showed) was considered diagnostic for the presence of allyl groups on lignin in preliminary experiments. ^{31}P NMR spectroscopy was used to evaluate the amount of labile hydroxyls functionalized. ^{31}P-NMR is clearly an indirect tool to evaluate the occurring allylation on lignin as long as what could be actually detected are the residual hydroxyl groups after functionalization.

Table 1 reports the distribution of aliphatic hydroxyls, condensed (Cond.), syringyl (S-OH), guaiacyl (G-OH), *p*-hydroxycoumaryl (P-OH) phenols, and carboxylic acids (COOH) for reference and allylated P1000® lignin with Method A and B.

Table 1. Distribution of Labile Hydroxyl Groups in Reference and Allylated P1000® and TMP Lignin, as Found by ^{31}P NMR Quantitative Analysis

Hydroxyl Groups (mmol/g)	Assignment (ppm)	P1000® Ref.	P1000® Allylated Met. A	P1000® Allylated Met. B
Aliphatic OH	150-145	1.70	1.18	0.89
Cond. + S-OH	145-141	2.00	n.d.	0.38
G-OH	141-139	1.12	n.d.	0.20
P-OH	139-137	0.48	n.d.	0.12
COOH	136-134	1.07	0.80	0.02

It is worth noticing the different outcomes of the applied allylation strategies. When Method A was employed (acetone/0.5 M aqueous NaOH), a thorough allylation of the phenolic moiety was accomplished, whereas Method B (acetone/K_2CO_3) resulted in a good but incomplete functionalization of phenols (around 80%) but achieved a whole coverage of carboxylic acids (Xin *et al.* 2014). On the contrary, with Method A, the carboxylic acids were allylated in low yield. It could be hypothesized that allylation occurred also at the carboxylic acids, but, in aqueous NaOH condition, the formed allyl

esters were easily hydrolyzed. A partial allylation of aliphatic hydroxyl groups was obtained with either method A or B. Given the absence of free phenols and considered the overall reaction conditions, the allylated lignin obtained according to Method A was selected for further investigation and, especially, as a substrate for the Claisen rearrangement. Ensuing phenolic groups from allyl rearrangement are indeed easily detected by ^{31}P NMR. From now on, if not otherwise specified, all lignins are to be intended as allylated according to Method A. The allylation reaction was then performed on a TMP acidolysis lignin, which was set as a reference substrate on account of its simple structure as a purely guaiacylic lignin.

Direct evidence regarding allyl-functionalized lignin could be obtained by ^{13}C NMR spectroscopy. ^{13}C NMR is usually involved in the elucidation of lignin side chains structure and in discerning among primary, secondary, and phenolic hydroxyl groups after acetylation (Faix *et al.* 1994). Figures 2 and 3 show the overlapped ^{13}C NMR spectra of reference (black line) and allylated (grey line) TMP and P1000® lignin after acetylation, respectively. The spectra are normalized on the methoxyls signal.

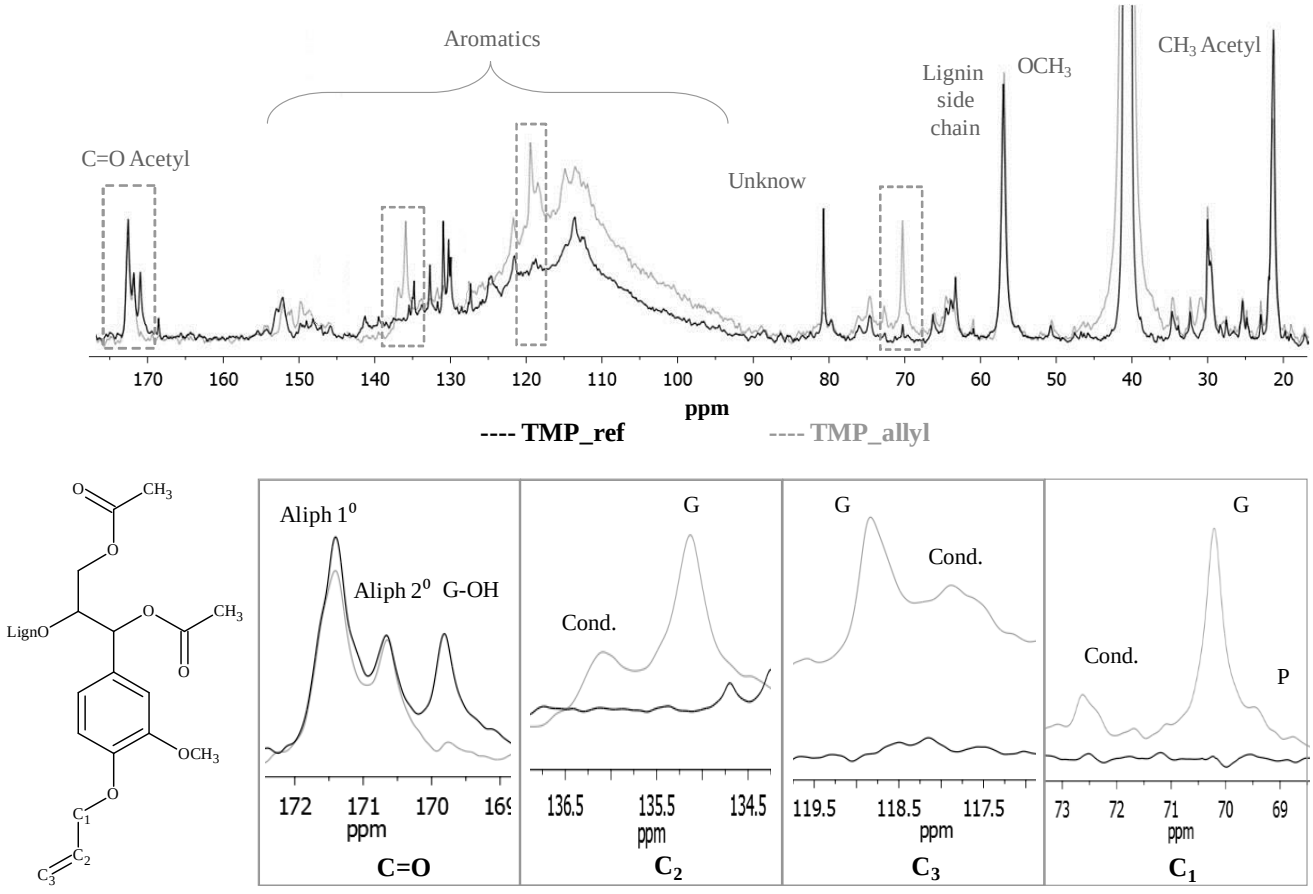

Fig. 2. Overlapped ^{13}C NMR spectra of acetylated samples of reference (black) and allylated (grey) TMP lignin registered in DMSO-d_6 and normalized on the methoxyls signal. Magnification areas of acetyl groups, carbon skeletons of the allylic functionality, and corresponding assignment to aliphatic and guaiacyl structures are reported below.

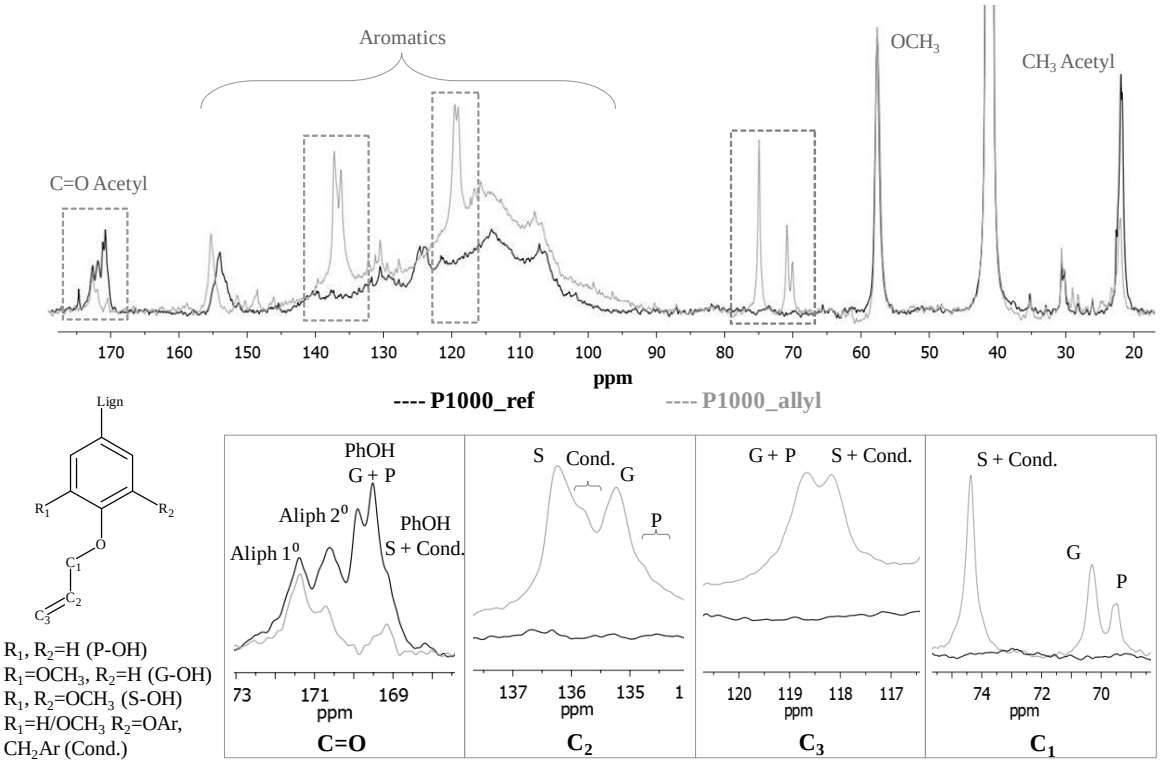

Fig. 3. Overlapped ^{13}C NMR spectra of acetylated samples of reference (black) and allylated (grey) P1000® lignin registered in DMSO-d_6 and normalized on the methoxyls signal. Magnification areas of acetyl groups, carbon skeletons of the allylic functionality, and corresponding assignment to aliphatic, condensed, syringyl, guaiacyl, and *p*-hydroxyphenyl structures are reported below.

Concerning both reference TMP and P1000® lignins, clearly recognizable signals were obtained pertaining to: acetyl carbonyl groups between 169 and 172 ppm, lignin side chains between 63 and 68 ppm (visible only for TMP on account of its simpler structure), methoxyl groups at around 57 ppm, and acetyl methyl groups at 21.8 ppm. After allylation, new signals around 135, 118, and 71 ppm were detected.

Detailed information about ^{13}C-NMR chemical shifts for the C_1, C_2, and C_3 positions of the allyl chain on characteristic lignin subunits were obtained from phenolic model compounds modified, according to Method B, as allyl ethers (Table 2). It could be observed that the chemical shift of the C_1-position of the allyl ether chain was the most affected by the surrounding chemical environment. Characteristic absorbance frequencies were found around 68 ppm for the *p*-hydroxyphenyl unit, around 70 ppm for guaiacyl unit, and around 73 ppm for syringyl and condensed unit, respectively ($\Delta \approx 5$ ppm). On the other hand, the chemical shift range of these same units was more restricted when the C_2 and C_3 positions of the allyl ether chain were concerned. The chemical shifts of the different phenol subunits were comprised between 133 and 134.5 ppm for the C_2-position ($\Delta \approx 1.3$ ppm) and between 117 and 118 ppm for the C_3-position ($\Delta \approx 0.8$ ppm). Aliphatic allyl ethers of lignin model compound were not prepared. However, according to literature data, these signals fall within the range previously reported for aromatic allyl ethers (Ralph *et al.* 2004).

Table 2. ^{13}C NMR Assignment of the Allylic Side Chain in Representative Model Compounds of Lignin Functionalized as Allyl Ethers

Allylated model compound	–O–***C₁***–C₂–C₃ (ppm)	–O–C₁–***C₂***–C₃ (ppm)	–O–C₁–C₂–***C₃*** (ppm)
Me-*p*-coumarate (P-OH)	68.30	133.42	117.84
4-propylguaiacol (G-OH)	70.20	133.81	117.80
Sinapic acid (S-OH)	73.16	134.69	117.40
Sinapic acid (COOH)	64.56	138.11	117.13
Dihydroanisole (cond. PhOH)	73.01	134.10	117.00

Magnified portions of overlapped ^{13}C NMR spectra of TMP lignin before and after allylation displaying the acetyl carbonyl region and the carbon atoms of the allyl chain are reported in Fig. 2. For clarity, an exhaustive list of the ^{13}C NMR attributions for reference and allylated TMP lignin is provided in Table 3.

Table 3. ^{13}C NMR Characteristic Absorbances of Acetyl Carbons, O-Linked Allylic Chains, and C-Linked, Claisen Rearranged Allylic Chains for Reference, Allylated, and Rearranged TMP Lignin (Acetylated Samples)

	TMP Ref	**TMP Allylated**	**TMP Rearranged**
C=O			
Aliph. -OH 1° (ppm)	171.5	171.5	171.5
Aliph. -OH 2° (ppm)	170.6	170.6	170.6
G-OH (ppm)	169.8		
S-OH type (ppm)			169.5
G-OH (hydrolysis) (ppm)			169.8
O-allylation			
C1 Aliph. –OH (ppm)		72.6	72.6
C2 Aliph. –OH (ppm)		136.1	136.1
C3 Aliph. –OH (ppm)		117.8	117.8
C1 G-OH (ppm)		70.3	70.3
C2 G-OH (ppm)		135.2	135.2
C3 G-OH (ppm)		118.8	118.8
C-allylation (rearr.)			
C1 S-OH type (ppm)			not detected
C2 S-OH type (ppm)			137.6 – 137.3
C3 S-OH type (ppm)			117.5

Between 172 and 169 ppm, in the acetyl carbonyl region, after allylation a substantial absence of phenols was apparent (reference TMP - black line, allylated TMP - grey line), as opposed to a slight decrease in the intensity of the signals related to aliphatic hydroxyls, in particular detected at the expense of primary aliphatic alcohols. Between 136.5 and 134.5 ppm, 119.5 and 117 ppm, and between 73 and 69 ppm, signals pertaining to Carbon 2 (C_2), Carbon 3 (C_3), and Carbon 1 (C_1) of the allyl ether chain were evident, respectively. In each case, the magnified portions clearly show the appearance of two distinct peaks after allylation. NMR peaks at 135.2, 118.8, and 70.3 ppm were unambiguously attributed to allylated guaiacyl phenols. Peaks placed at 136.1, 117.8, and 72.6 ppm were assigned to allylated condensed units, not detected by ^{31}P NMR. Concerning aliphatic alcohols, it should be noted that a minor to negligible extent of allylation as expected due to adverse reaction conditions involving aqueous NaOH, which obviously does not favour the deprotonation of these groups (pK_a aliphatic alcohols ≈16-18, pK_a NaOH ≈ 14).

Even when a more structurally complex lignin such as the herbaceous P1000® was analyzed, an astonishing amount of information could be obtained. Magnified portions of the overlapped ^{13}C NMR spectra of reference (black line) and allylated (grey line) P1000® concerning the acetyl carbonyl region and the Carbon atoms of the allyl ether chain are reported in Fig. 3. A number of partially resolved peaks are observed reflecting the different, acetyl- and allyl-functionalized phenolic subunits encountered in a typical herbaceous lignin. An exhaustive list of the ^{13}C NMR attributions for reference and allylated P1000® lignin is reported in Table 4.

Between 173 and 168 ppm, in the acetyl carbonyl area, partially resolved peaks associated with coalescent guaiacyl and p-hydrophenyl phenols (170 ppm) and coalescent syringyl and condensed phenols (169.6 ppm) were detected. The evident shoulder placed at 169.1 ppm was related to other condensed phenolic structure. After allylation, the absorbance of acetylated phenols plunged down; only a weak residual peak related to condensed phenols was left. A recent work demonstrated the minor reactivity of biphenylic phenols compared to other condensed structures such as diphenylether and diphenylmethane (Sen *et al.* 2013). In agreement with this study, the residual peak observed at 169.1 ppm after allylation and the corresponding shoulder in the reference lignin spectrum, was related to recalcitrant biphenylic moieties.

Moreover, concerning the aliphatic region, a strong reduction at the expense of acetylated secondary hydroxyls was detected after allylation. However, it is worth noting the absence of signals associated with aliphatic allyl ether, unequivocally established by the lack of any peaks around 72.6 ppm in the C_1 allyl carbons region. As previously mentioned, allylation of aliphatic alcohols was not expected since aqueous NaOH was involved in the reaction. However, when information about aliphatic –OHs, as attained from ^{13}C NMR analysis, was compared to the ^{31}P NMR quantification, as reported in Table 1, a kind of contradiction is encountered. The content of labile hydroxyls associated with aliphatic alcohol decreased by about 30% after lignin allylation according to ^{31}P NMR quantification. This variation in the aliphatic hydroxyls content was imputed to the loss of low molecular weight fractions during the reaction work-up, as demonstrated by a blank experiment (not reported) in which, according to Method A, a lignin sample is treated in the absence of allyl bromide, recovered, and then subjected to ^{31}P NMR analysis.

Table 4. ^{13}C NMR Characteristic Absorbances of Acetyl Carbons, O-Linked Allylic Chains, and C-Linked, Claisen Rearranged Allylic Chains for Reference, Allylated, and Rearranged P1000® Lignin (Acetylated Samples)

	P1000® Ref	P1000® Allylated	P1000® Rearranged
C=O			
Aliph. -OH 1° (ppm)	171.5	171.5	171.5
Aliph. -OH 2° (ppm)	170.6	170.6	170.6
P-OH + G-OH (ppm)	170.0		
S-OH + Cond. (ppm)	169.6		
Other Condensed (ppm)	169.1	169.1	169.1
S-OH type (ppm)			169.5
O-allylation			
C1 Cond. (ppm)		74.4*	74.4*
C1 S-OH (ppm)			
C1 G-OH (ppm)		70.3	70.3
C1 P-OH (ppm)		69.5	69.5
C2 Cond. (ppm)		135.9	136.2*
C2 S-OH (ppm)		136.3	
C2 G-OH (ppm)		135.2	135.1*
C2 P-OH (ppm)		134.7	
C3 Cond. (ppm)		118.2*	118.2*
C3 S-OH (ppm)			
C3 G-OH (ppm)		118.7*	118.7*
C3 P-OH (ppm)			
C-allylation (rearr.)			
C1 S-OH type (ppm)			35.8
C2 S-OH type (ppm)			137.3
C3 S-OH type (ppm)			117.5

* overlapped signals

Magnified portions associated with Carbon 2 (C_2), Carbon 3 (C_3), and Carbon 1 (C_1) of the allyl ether chain display a variety of signals, some of them overlapped (C_3 Carbon), others partially resolved (C_2 Carbon), and some others fully resolved (C_1 Carbon). This completely reflects the ppm range distributions discussed earlier for allylated model compound (C_1 $\Delta \approx 5$ ppm, C_2 $\Delta \approx 1.3$ ppm, C_3 $\Delta \approx 0.8$ ppm). Between 137 and 134 ppm, in the C_2-allyl region, peaks associated with syringyl and guaiacyl allylated phenols, respectively, are clearly identifiable at 136.3 and 135.2 ppm. Condensed and *p*-hydrophenyl allylated moieties are recognized at 135.9 and 134.7 ppm as a shoulder.

Between 120 and 117 ppm, in the C_3-allyl region, overlapped signals of guaiacyl and *p*-hydroxyphenyl allylated phenols fall at 118.7 ppm, whereas overlapped signals of syringyl and condensed phenols are displayed at 118.2 ppm. In the end, between 75 and 69 ppm, in the C_1-allyl region, overlapped signals of syringyl and condensed allylated phenols, and resolved peaks related to guaiacyl and *p*-hydroxycoumaryl allylated phenols, are placed at 74.4, 70.3, and 69.5 ppm, respectively.

Claisen Rearrangement.

The Claisen rearrangement is a [3,3]-sigmatropic reaction in which an allyl ether is thermally converted to an unsaturated carbonyl compound. In the case of the aromatic Claisen rearrangement it is accompanied by a rearomatization and subsequently the formation of free phenolic groups.

The Claisen rearrangement, in the form of one of its several variations known as the tandem Claisen rearrangement, has been broadly applied in the synthesis of building blocks for polybenzoxazoles and supramolecular chemistry (Hiratani and Albrecht 2008). In this work, the Claisen rearrangement of allylated lignin was foreseen as a valuable approach to release phenols in an already chemically modified lignin. Available phenols could act both as potential chain extension or cross-linking sites as well as antioxidants, supplying a tailor-made lignin derivative of controlled properties.

Substantial solvent effects interfere in the Claisen rearrangement. Rate and yield of rearrangement are affected both by the hydrogen-bonding abilities and by the polar character of the solvent. In particular, rate-accelerations derived from water are well-documented for aliphatic systems, due to the stabilization of the transition state (Severance and Jorgensen 1992). However, the solvent dependence of rates for aromatic Claisen rearrangement is complex and does not show simple increases with increasing solvent polarity (White and Wolfarth 1970).

In this work, after a screening of some suitable high-boiling solvents, *i.e*. those able to solubilize allylated lignin, non-anhydrified dimethylformamide was selected as the preferred reaction medium. For a start, rearranged TMP and P1000® lignin were subjected to GPC analysis to evaluate eventual modifications of their molecular weight. Given the reaction conditions (15 h at reflux temperature) both depolymerization and cross-linking phenomena were plausible. Calculated average molecular weight indexes (peak molecular weight - M_p, number average molecular weight - M_n, and weight average molecular weight - M_w) and polydispersion indexes (I) for reference, allylated, and rearranged TMP and P1000® lignin are reported in Table 5. The findings suggest a substantial uniformity in terms of stability and retained structure after rearrangement. It should be noted that rearranged lignin samples were recovered by bulk lyophilization of the crude material. Therefore, the representative character of the analyzed specimens is fully assured. Thus, the calculation of molecular weight indexes was not affected by the loss of degraded lignin fragment.

Afterwards, the amounts (mmol/g) of hydroxyl groups after Claisen rearranged were quantified by ^{31}P NMR spectroscopy. ^{31}P NMR spectra of reference, allylated, and Claisen rearranged TMP and P1000® lignin, along with the general structure of detected units are reported in Figs. 4 and 5, respectively.

Table 5. Peak Molecular Weight, Number Average Molecular Weight, Weight Average Molecular Weight, and Polydispersity Index of Reference, Allylated, and Claisen Rearranged P1000® and TMP Lignin

	M_p (g/mol)	M_n (g/mol)	M_w (g/mol)	I
TMP Ref	2900	3500	6400	1.8
TMP Allylated	3900	6700	13800	2.3
TMP Rearranged	4600	6700	11800	1.7
P1000® Ref	1700	2700	4000	1.5
P1000® Allylated	2800	4600	8200	1.8
P1000® Rearranged	2700	3900	6200	1.6

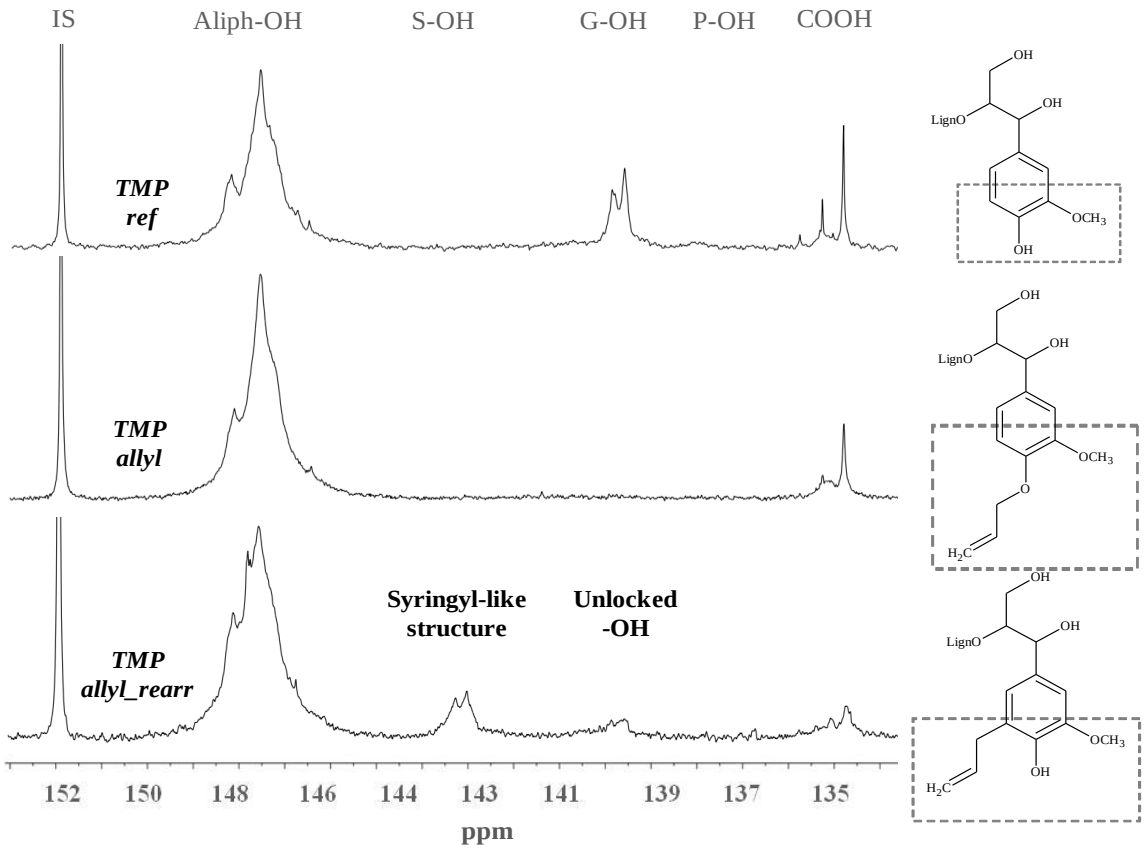

Fig. 4. ^{31}P NMR spectra of reference, allylated, and rearranged TMP lignin showing the interchange of guaiacyl phenols into syringyl-like phenols after Claisen rearrangement. The appearance of a signal related to guaiacyl phenols after rearrangement was ascribed to the cleavage of residual glycosidic linkages in TMP lignin due to the harsh reaction conditions.

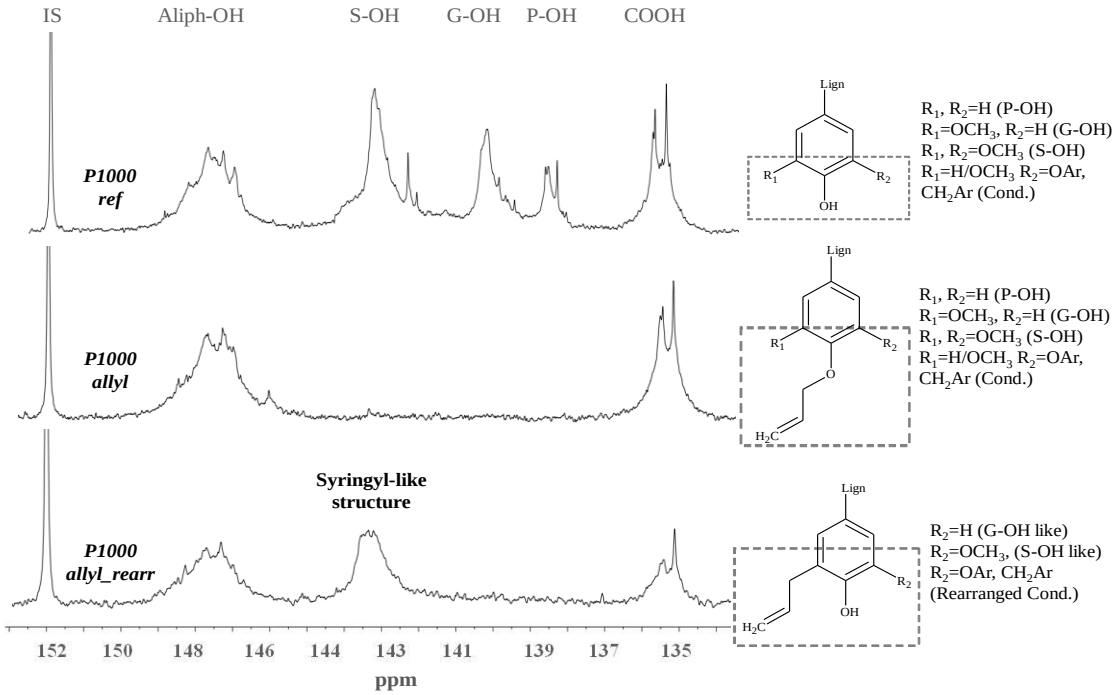

Fig. 5. ^{31}P NMR spectra of reference, allylated, and rearranged P1000® lignin showing the interchange of guaiacyl phenols into syringyl-like phenols after Claisen rearrangement. The bewildered lack of guaiacyl-like phenols originating from the rearrangement of allylated *p*-hydroxyphenyl structure was ascribed to detrimental stereoelectronic effects operating on the aromatic ring.

At this point, it should be mentioned that the shift of the oxygen-linked allyl chain to one of the free ortho-positions of the aromatic ring led to the formation of either guaiacyl-like units (when the allylic rearrangements takes place on a *p*-hydroxyphenyl moiety) or syringyl-like units (when the allylic rearrangements takes place on a guaiacyl moiety). Claisen rearrangement involving allylated syringyl units is obviously not permitted. According to this logic, the ^{31}P spectrum of the purely guaiacylic TMP lignin (Fig. 4) exhibits the appearance of a peak in the syringyl region after rearrangement. A weak signal in the guaiacyl region is also observed, presumably due to the hydrolysis of residual glycosidic linkages in the acidolytic TMP lignin. Similarly, the ^{31}P NMR spectrum of the Claisen rearranged P1000® lignin showed the presence of free phenols (Fig. 5). Surprisingly, despite its variegated structure (in terms of represented phenylpropanoidic units), only one signal, specifically in the syringyl region, was detected. It was therefore inferred that the migration of the allylic chain was limited to the allylated guayacil moiety, resulting in a signal in the syringyl area. Recent studies (Gozzo *et al.* 2003; Martin Castro 2004) demonstrated that stereoelectronic, substituent effects, and related resonance energy changes have a relevant influence on the stereoelectronic and stabilization of the incipient transition state. In particular, the presence of electron-donating substituents (as is the case of the methoxyl group in guaiacyl units) was found to accelerate Claisen rearrangement through stabilization of the intermediate.

Table 6 reports the quantification of labile hydroxyl groups in mmol/g after Claisen rearrangement, along with a percentage evaluation of the rearranged units for

both TMP and P1000® lignin. Reference and allylated lignin quantification are also reported. The amount of rearranged allylic group was calculated for each phenolic unit. This was done by dividing the mmol/g of released phenols after rearrangement, as obtained by ^{31}P NMR measurement, by the corresponding –OHs content of the reference lignin normalized on the weight percentage gain determined by allylation.

Table 6. ^{31}P NMR Quantification of hydroxyl groups after Claisen Rearrangement for P1000® and TMP Lignin

Hydroxyl Groups (mmol/g)	Aliphatic OH	Cond. + S-OH	G-OH	P-OH	COOH
TMP Ref.	5.07	n.d.	0.95	trace	0.47
TMP Allylated	4.19	n.d.	n.d.	n.d.	0.41
TMP Allyl Rearr.	4.02	0.33	trace *	n.d.	0.21
% Rearr.	-	38	/	/	-
P1000® Ref.	1.70	2.00	1.12	0.48	1.07
P1000® Allylated	1.18	n.d.	n.d.	n.d.	0.80
P1000® Allyl Rearr.	1.03	1.15	n.d.	n.d.	0.46
% Rearr.	-	>100	/	/	-

* guaiacyl units likely released after hydrolysis of residual glycosidic linkages.

As reported in Table 6, concerning aliphatic alcohols, after allylation and Claisen rearrangement on TMP and P1000®, a slight decrease was detected. As already observed in ^{13}C-NMR spectra, a negligible extent of aliphatic alcohols allylation was expected due to adverse reaction conditions involving aqueous NaOH, which obviously does not favor the deprotonation of these groups. Moreover, it should be empathized that the variation in the aliphatic hydroxyls content could be imputed to the loss of low molecular weight fractions during the reaction work-up. Also the carboxylic acids were allylated in low yield: it could be hypothesized that allylation occurred also at the carboxylic acids, but, in aqueous NaOH condition, the formed allyl esters were easily hydrolyzed. It should be noted anyways that after Claisen rearrangement the amount of carboxylic acids decreased in a significant way, maybe due to the high temperature treatment that could favor the decarboxylation reaction.

Concerning phenols, as already reported, a thorough allylation of the phenolic moiety was accomplished. After DMF thermal reaction, allylated syringyl units, obviously, could not undergo Claisen rearrangement. As previously discussed, *p*-hydroxyphenyl units showed no tendency to rearrange according to the applied conditions, so no guaiacyl-like phenols were detected. It is reminded that, concerning TMP lignin, trace amounts of guaiacyl phenols were attributed to the hydrolysis of residual glycosidic linkages (on the other hand, it should also be stressed that *p*-hydroxyphenyl structures were not represented in the reference TMP lignin). Syringyl-like phenols were quite well represented for the TMP lignin, accounting for about 38% of

rearranged guaiacylic units in the allylated lignin. In light of the characteristic low yield associated to thermic Claisen rearrangement and the high molecular weight of TMP lignin, which prevent the attainment of a favourable conformation of the allyl groups for rearrangement to take place due to steric hindrance, the result was satisfying. The use of a different solvent addressed to the achievement of higher reaction temperature, as well as longer reaction periods, are viable approaches to be explored to increase the yield.

Probably due to its lower molecular weight, the amount of syringyl-like structures detected for P1000® lignin after Claisen rearrangement was greater or, rather, even larger than empirically expected. In fact, the calculated percentage of rearrangement exceeded 100%. To rationalize this behavior, the existence of condensed phenolic structure in which at least one of the constituting residues is a *p*-hydroxyphenyl unit was assumed. According to Fig. 6, representing the hypothesized rearrangement reaction for the biphenyl and diaryl ether unit, at least one ortho-position of a phenol is still available for the allyl group to migrate after allylation.

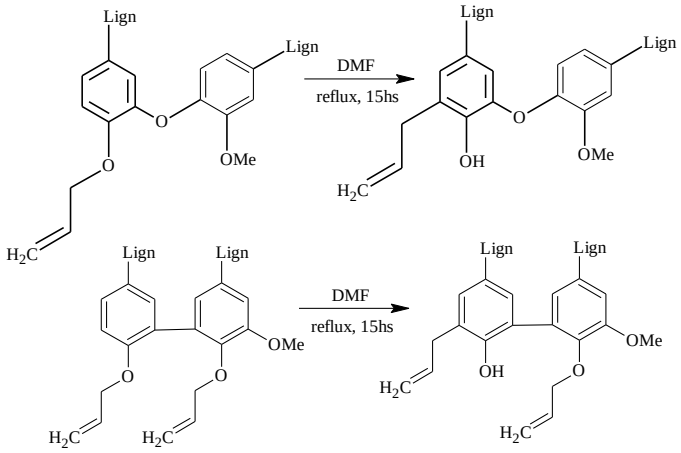

Fig. 6. Hypothesized structure of condensed phenols containing at least one rearrangeable *p*-hydroxyphenyl unit, before and after Claisen rearrangement

The occurrence of condensed structures containing *p*-hydroxyphenyl units has long been a matter of debate in the literature, and a number of papers during the years have dealt with the subject. Even though the guaiacylic nature of condensed structures in the residues is generally accepted (Erickson *et al.* 1973; Lundquist and Parkas 2011), the scientific community has also produced evidence of the existence of different types of condensed phenolic units containing non-methoxylated residues (Westermark 1985; Terashima and Fukushima 1988). Several factors induced the authors to consider the presence of *p*-hydrophenylic residues in the condensed structures of P1000® lignin. First, there was no persuasive reason to believe that allyl ether bonds involving condensed phenolic units were more prone to hydrolysis than uncondensed allylated phenols. In fact, uncondensed phenols consistent with hydrolysis processes were not detected by ^{31}P NMR. Moreover, the occurrence of condensed phenolics containing *p*-hydroxyphenyl units support the evidence about allylated guaiacyl moiety to rearrange more easily, discussed above, on account of their partial resemblance to guaiacyl residues (as could be appreciated from Fig. 6, the non-methoxylated ring of the condensed structure is assimilable to a guaiacyl unit).

Rearranged TMP and P1000® lignins were finally analyzed by ^{13}C NMR spectroscopy to evaluate changes in the peaks distribution in the acetyl and allyl region. Figures 7 and 8 report the overlapped ^{13}C NMR spectra of allylated (black line) and Claisen rearranged (grey line) for TMP and P1000® lignin, respectively, in the principal areas of interest.

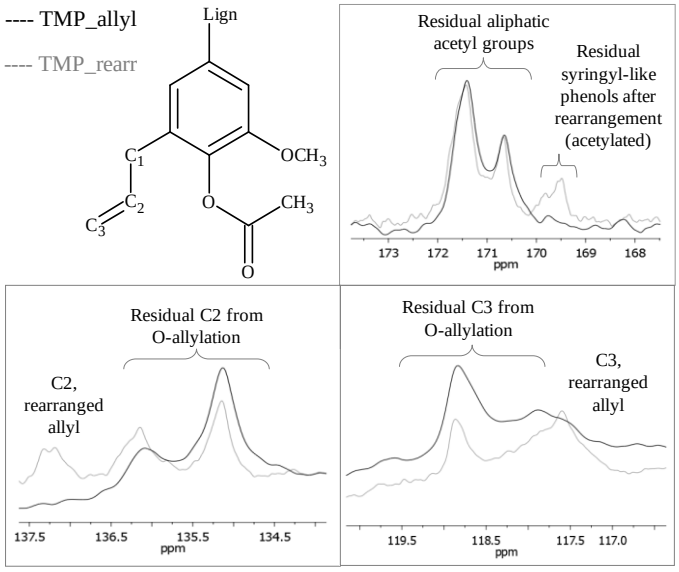

Fig. 7. ^{13}C NMR magnification areas of overlapped acetyl groups, C2-carbon, and C3-carbon of the allyl chain before (black) and after (grey) Claisen rearrangement of TMP lignin. Relative assignment to aliphatic and syringyl-like structure of both the O-allylation and the C-allylation resulting from the rearrangement are also reported. Acetylated samples acquired in DMSO-d_6 and normalized on the methoxyls signal.

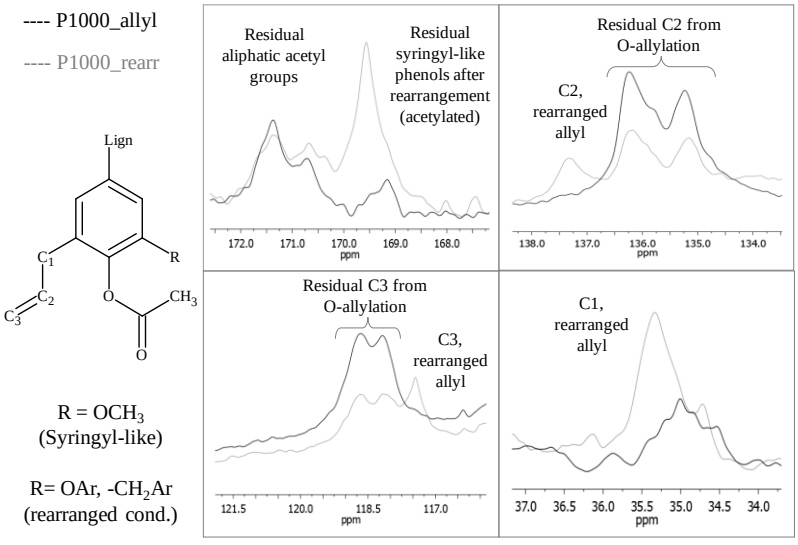

Fig. 8. ^{13}C NMR magnification areas of overlapped acetyl groups, C1-carbon, C2-carbon, and C3-carbon of the allyl chain before (black) and after (grey) Claisen rearrangement of P1000® lignin. Relative assignment to aliphatic and aromatic structure of both the O-allylation and the C-allylation resulting from the rearrangement are also reported. Acetylated samples acquired in DMSO-d_6 and normalized on the methoxyls signal.

Along with the residual absorbances of allyl ether groups, the magnification regions associated with the allyl chains clearly show the appearance of signals related to C-allylation, that is, to rearrangement of the allyl groups, at 137.3 ppm (Carbon C_2, after rearrangement), 117.5 ppm (Carbon C_3, after rearrangement), and 35.8 ppm (Carbon C_1, after rearrangement). Detailed lists of the ^{13}C NMR absorptions after Claisen rearrangement for TMP and P1000® lignin are provided in Tables 3 and 4, respectively. In the case of TMP lignin, due to the reduced extent of rearrangement, the C_1 signal disappeared in the background noise. Concerning the acetyl carbonyl region, the appearance of a signal at 169.5 ppm, associated with released syringyl-like phenols after Claisen rearrangement, was observed for both the lignins. According to the outlined chemical structure in Fig. 8, two different rearranged structures could have been expected for P1000®: the syringyl-like and the condensed, resulting from the rearrangement of the allylated *p*-hydroxyphenyl residue. However, just one signal for each Carbon atom of the C-linked allyl chain was observed. As previously discussed, the non-methoxylated ring of a condensed structure resembles a guaiacyl unit in its chemical structure (Fig. 6). Rearranged allyl chains of condensed and guaiacyl moiety have really close chemical neighborhoods and, as a result, the signals associated with syringyl-like and rearranged condensed units are not, even partially, resolved. In the light of these remarks, it is evidently the same situation encountered for allylated lignin samples, in which the signals associated with syringyl and condensed phenols were completely overlapped (a partial resolution was recognized only for the C_2 Carbon).

Radical Scavenging Activity

To screen the ability of Claisen rearranged lignins to act as a radical scavenger, a DPPH assay was performed on reference, allylated, and rearranged TMP and P1000® (Table 7).

Table 7. Radical Scavenging Activity of Reference, Allylated, and Claisen Rearranged P1000® and TMP Lignin, Expressed as IC_{50} Concentration

	IC_{50} (μg/mL)	PhOH (mmol/g)	IC_{50} (nmol PhOH/mL)
TMP ref	79.4	0.95	75.4
TMP allyl	2511.9	0.00	-
TMP rearr	125.9	0.33	41.5
P1000® ref	10.0	3.60	36.0
P1000® allyl	398.1	0.00	-
P1000® rearr	50.1	1.15	57.6

As expected, the IC_{50} value was smaller for reference TMP (79.4 μg/mL) than for reference P1000® (10.0 μg/mL), on account of the larger phenolic content of the latter, while it was really high for both the allylated samples (TMP: 2511.9 μg/mL, P1000®: 398.1 μg/mL), in agreement with the absence of active phenols. The IC_{50} values were higher for Claisen rearranged samples than for reference lignins but of the same order of

magnitude (TMP: 125.9 μg/mL, P1000®: 50.1 μg/mL), confirming that a certain amount of phenolic hydroxyls was available after the migration of allyl groups. When the radical scavenging activity was expressed as a function of the total phenolic content, the IC_{50} values of rearranged lignin samples were found to be almost the same as the reference lignin ones. Surprisingly, the IC_{50} value was lower for rearranged TMP lignin than for the reference lignin (TMP ref: 75.4 nmol PhOH/mL, TMP rearranged: 41.5 nmol PhOH/mL), *i.e.*, a minor number of phenolic groups exert the same antioxidant effect, as if the radical scavenging activity of the resulting syringyl-like phenols was greater than that of guaiacyl phenols in the pristine lignin. This interpretation would be in agreement with a study concerning the radical scavenging activity of different syringyl derivatives isolated from sugarcane molasses compared to the analogue guaiacyl derivatives (Takara *et al.* 2007). In this work, the major activity of syringyl derivatives is demonstrated. In the case of Claisen rearranged P1000® lignin, where this effect was not observed (P1000® ref: 36.0 nmol PhOH/mL, P1000® rearranged: 67.6 nmol PhOH/mL), it was supposed that the concomitant presence of condensed structure resulting from the rearrangement of an allylated *p*-hydroxyphenyl residue, besides syringyl-like units, had to some extent lowered the total antioxidant activity.

CONCLUSION

1. Lignin samples allylated in the presence of aqueous NaOH and excess allyl bromide were selected as starting materials to be subjected to the Claisen ortho rearrangement reaction. The Claisen rearrangement of the allylic chain was successfully achieved by treatment in dimethylformamide at reflux temperature for 15 hours.
2. A screening of the antioxidant activity of reference, allylated, and Claisen rearranged lignins demonstrated the presence of active free phenols on rearranged specimens.

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