Green Chemistry

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/c7gc01812f

On the structure of softwood kraft lignin†

Claudia Crestini, *[a] Heiko Lange, [a] Marco Sette [a] and Dimitris S. Argyropoulos *[b]

Two constitutional structural schemes are proposed attempting to unify and rationalize a series of focused NMR and chromatographic determinations aimed at providing an integrated picture for the structure of softwood kraft lignin. The complexity of native softwood lignin when coupled with the complexity of the kraft pulping process is known to lead to a rather heterogeneous material that has eluted us to date. The present work embarks at applying state-of-the-art quantitative 1D and 2D NMR methods on carefully isolated softwood kraft lignin samples and fractions. The accumulated data, when coupled with size exclusion chromatography, mass spectrometric analyses and literature accounts that pertain to the chemistry of kraft pulping, provide the following picture for softwood kraft lignin. Softwood kraft lignin is composed of two distinct fractions that can be separated by using anhydrous acetone. The acetone insoluble fraction is a somewhat branched polymeric material that still contains a variety of native wood lignin bonding patterns, albeit in significantly reduced abundance, as well as new structures induced during the process. The acetone soluble fraction is a significantly more branched and less polymeric material with an abundance of chemical structures that may be created when oligomeric phenols react under kraft pulping conditions. To account for the presence of the various moieties in these two fractions, kraft pulping fragmentation and repolymerization chemistries are extensively invoked, including radical processes initiated by sulfur.

Received 18th June 2017,
Accepted 20th July 2017

DOI: 10.1039/c7gc01812f

rsc.li/greenchem

Introduction

Lignin and in particular technical lignins are attractive feedstocks for a number of industrial operations with significant sustainability and green chemical connotations. Their complex and ill-defined structure, however, represents a major constraint toward developing lignin based technologies, materials and chemicals. The inherent heterogeneity of native lignin is further compounded by the diversity and the complex chemistries of the applied delignification processes with the kraft pulping process being of no exception. Overall, materials termed "technical lignins" possess a highly variable structure that depends on the species and its seasonal and geographical location, the delignification or extraction method applied and the intensity of the delignification process. A given sample of a technical lignin possesses significant variability in terms of its constituent aromatic units, interunit bonding patterns and frequencies as well as molecular weights and molecular weight distributions.

Therefore, considering such technical lignins as having uniform characteristics is an oversimplification that not only precludes for an in depth insight into the specific structural features of a given sample but, most importantly, also seriously prevents the development of chemical strategies for its upgrade and value addition. A detailed structural definition and understanding of different technical lignins is, therefore, of pivotal significance if such materials are to become valuable chemical feedstocks.

It is without a doubt that the development of novel NMR techniques has dramatically altered the lignin landscape. Consequently, it is imperative that dated structural assignments need critical revisions, using modern tools and our updated understanding of the lignin structure and physicochemical behavior.

NMR techniques, and in particular hetero-correlated pulse sequences allow the facile resolution of a plethora of signals as they emerge due to the different lignin subunits. To date such NMR methods represent the most powerful approach at our disposal for deciphering different lignin structures.[1–6] Recently the use of QQ-HSQC has offered unprecedented clarification and allowed for a critical review of the old accepted paradigms regarding the structure of milled wood lignin (MWL).[7–10]

The development of integrated biorefinery processes and their position within the context of a circular economy cannot prescind from the full valorization of lignin residues from pulping, *in primis*, among them, kraft lignin. While milled

[a]Department of Chemical Sciences and Technologies, University of Rome 'Tor Vergata', Via della Ricerca Scientifica, 00133 Rome, Italy. E-mail: crestini@stc.uniroma2.it; Tel: +39 067259 4734
[b]Organic Chemistry of Wood Components Laboratory, Departments of Forest Biomaterials and Chemistry, North Carolina State University, 2820 Faucette Drive, Raleigh, North Carolina 27695, USA

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c7gc01812f

wood lignins are by definition nearly pure and extracted under mild experimental conditions,[11] the situation becomes dramatically different when attempts are made to characterize lignins that emerge from the kraft pulping process. It is noteworthy to mention here that the kraft process is characterized by an extended (2–3 hours) exposure of the wood at elevated pH values and temperatures exceeding 160 °C. Under such conditions a large variety of reactions occur within the lignin structure, creating a material that is vastly different from the native lignin in the starting wood.

To date, the only structural model for softwood kraft lignin that we have at our disposal is that of Marton proposed in 1971.[12] It is based on the studies of the chemical behavior of lignin model compounds under kraft pulping conditions and on wet chemical characterization protocols.[8]

Overall, there is surprisingly little modern understanding of the detailed structure of softwood kraft lignin, despite its excessive abundance and many of efforts devoted to its structure and valorization.[13–26] Unfortunately, none of these efforts offers a detailed, current structure for softwood kraft lignin, with little attention being paid to the fate of its aliphatic units and the role of the extractives on them. These issues represent distinct novelty features of this work promoting our understanding of this complex and valuable resource.

Fortunately, the advent of quantitative ^{31}P NMR has offered a facile and detailed means to identify the different functional groups (various phenolic units, aliphatic OH's and carboxylic acids) present in various kraft lignins[27–29] and to quantify their abundances. In this respect, valorization efforts based on utilizing labile protons in kraft lignin have been significantly aided by structural characterisation based on ^{31}P NMR.

The situation is, however, a lot more limited as it pertains to the carbon framework of kraft lignin. While some HSQC NMR studies[19,30] have been carried out on such lignins and a number of their aromatic subunits have been qualitatively determined, there is a surprising lack of knowledge with respect to a more detailed insight of the structural features of the actual backbone and its aliphatic character.

One aspect of kraft lignin that has never been considered during the efforts made toward the detailed structural elucidation (other than some limited recent efforts on functional group distributions of its fractions[32]) is its heterogeneity and polydisperse nature. Numerous process variations including seasonal feedstock variability, *H*-factor and related delignification alterations create an inherently heterogeneous, complex and highly variable mixture in kraft lignin. Consequently, any efforts to precisely define such a mixture can only be considered on the basis of its individual well defined component fractions.

Our work, in this contribution, attempts to address the enumerated limitations with the aim to offer an in depth understanding to such a complex, heterogeneous and elusive material. Our effort embarks from examining whole kraft lignin and then continues by isolating well defined fractions of the same material. We then concertedly apply quick-quantitative QQ-HSQC,[7] quantitative ^{13}C NMR and quantitative ^{31}P NMR offering quantitative structural information of the whole lignin and its various fractions. The interpretation of the derived data, on the basis of interunit bonding patterns and their respective abundances, is critically examined and discussed on the basis of our knowledge of fundamental kraft pulping chemistry. Our overall effort is finally integrated by proposing much-needed, structural schemes for two main fractions of softwood kraft lignin.

Experimental

Materials and chemicals

The examined lignin was the product of softwood chips being pulped in a continuous digester using the kraft pulping process with a target kappa number of about 22 and a kraft pulping intensity, as necessitated by an *H* factor of about 1500–1700. Carbon dioxide precipitation technology was used to precipitate the lignin from the black liquor.[31] Prior to use, the lignin was thoroughly washed with deionized water to an approximate pH of the washing liquor being nearly neutral. It was then air dried and eventually thoroughly dried in a vacuum oven set at 40 °C for at least 48–72 hours.

The hexane used for the fractionation of this lignin was of technical grade, purchased from Fisher Scientific (N320) and the acetone was purchased from Sigma-Aldrich (reagent grade). All solvents and reagents were used as received.

Lignin fractionation

The fractional precipitation procedure to isolate the enumerated fractions of softwood kraft lignin used in this work was as per the "Detailed Fractional Precipitation of KL1" protocol described elsewhere.[32] The overall yield of the acetone soluble fraction was about 70%, while that of the acetone insoluble fraction was about 30%, and the yields of the individual fractions obtained in the work of Cui *et al.* are described in Table 2.[32] It is to be noted here that the distinct nature of these two fractions is reflected in their glass transition values (T_g) determined earlier[55] (**ASKL** T_g was 114 °C and **AIKL** T_g was 170 °C).

Extraction of pine sawdust

Approximately 70 g of southern pine sawdust were placed in a cellulose thimble for subsequent extraction in a standard 500 mL Soxhlet extractor. A first round of extraction was achieved using 600 mL of dichloromethane for 18 hours; this was followed by a second round of extraction using 600 mL of a 1/1 (v/v) toluene/ethanol mixture for 18 hours. For the last round of extraction, 600 mL of acetone were used for 18 hours.

The extractants in dichloromethane, toluene/ethanol and acetone delivered 1.4%, 0.4% and 0.1% of extractives, respectively, after evaporation and concentration *in vacuo*.

Purification of kraft lignin

Approximately 70 g of southern pine kraft lignin, produced as described earlier, were weighed in a cellulose thimble for

extraction in a standard 150 mL Soxhlet extractor using 600 mL of hexane for 18 hours. A total of 1.2% extractives were isolated.

Identification of extractives *via* GC-MS

Immediately after an extraction process was completed an aliquot of the extract, typically 500 μL, was taken and diluted with 200 μL of ethyl acetate, before 100 μL pyridine and 100 μl of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide were added. After 30 min at room temperature, the silylated extractives were analyzed by gas chromatography coupled with mass spectrometry. These analyses were carried out using a Shimadzu GCMS QP2010 Ultra equipped with an AOi20 autosampler unit. A SLB®-5 ms capillary GC column (L × I.D. 30 m × 0.32 mm, df 0.50 μm) was used as the stationary phase and ultrapure helium as the mobile phase. The Shimadzu LabSolutions GCMS Solution software (Version 2.61) was used. The various components were identified by comparison against the NIST11 library. The identified extractives are listed in Table 1 (ESI†).

Kraft-pulping of sawdust

To 50 g of non-extracted or extracted (as the protocol required) southern pine sawdust suspended in 57.3 mL of aqueous sodium hydroxide (192 g L^{-1}), 33.3 mL of an aqueous solution of sodium sulfide nonahydrate (329 g L^{-1}) are added. To this suspension are added 209 mL of distilled water. This mixture, which comprises an active alkalinity of 17%, and sulfidity of 25% at a liquor/wood ratio of 6/1, was subsequently subjected to pulping conditions using a heated Parr reactor for the time needed to reach an *H*-factor of 1750 (calculated based on experiment-specifics according to http://www.knowpulp.com). When *H*-factor 1750 was reached (*ca.* 4.5 h in the set-up used for this experiment), the heating was removed, and the reaction mixture was cooled down to *ca.* 70 °C, before it was filtered in order to remove unreacted wood particles. The filtrate was then acidified by means of gaseous carbon dioxide that was bubbled through the solution until a pH of 9 was reached. Working still with the rather warm solution, the new precipitate was filtered off, and then re-suspended in water. This suspension was subsequently acidified to pH 2 by means of aqueous sulfuric acid (50% v/v), and filtered. The filter cake was washed with water, and finally air-dried.

5.7–5.9 g (11.3–11.8%) of kraft lignin were obtained.

Quantitative ^{31}P NMR analyses

Quantitative ^{31}P NMR analysis was performed as reported before:[28,29] in brief, an accurately weighed amount of lignin (about 30 mg) was phosphitylated using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (Cl-TMDP) and the spectra were recorded on a Bruker 300 MHz spectrophotometer (256 scans at 20 °C). All chemical shifts reported are relative to the reaction product of water with Cl-TMDP, which gives a sharp signal in pyridine/$CDCl_3$ at 132.2 ppm. NMR data were processed with MestreNova (Version 8.1.1, Mestrelab Research).

Quick quantitative HSQC measurements

Spectra were acquired at 303 K with a Bruker Avance 600 spectrometer equipped with a cryoprobe. The sample consisted of approx. 80 mg of (non-acetylated) lignin dissolved in 600 μL of DMSO-d_6. A matrix consisting of 256 × 2048 points was obtained in eight scans. QQ-HSQC measurements were performed in accordance with the original reference as reported before.[7,8]

Data processing: NMR data were processed with MestreNova (Version 8.1.1, Mestrelab Research) by using a 60°-shifted square sine-bell apodisation window; after Fourier transformation and phase correction a baseline correction was applied in both dimensions. The final matrix consisted of 1024 × 1024 points, and cross-peaks were integrated with the same software that allows the typical shape of peaks present in the spectrum to be taken into account.

Quantitative ^{13}C NMR measurements

Samples of approximately 60–80 mg of (non-acetylated) lignin were dissolved in 500 μL DMSO-d_6; 50 μL chromium acetyl acetonate in DMSO-d_6 (approx. 1.5 mg mL^{-1}) were added as a spin-relaxation agent; 50 μL of trioxane in DMSO-d_6 (approx. 15 mg mL^{-1}) was used as an internal standard. The spectra were recorded at room temperature on a Bruker AVANCE 500 MHz spectrometer equipped with a 5 mm double resonance broadband BBI inverse probe. An inverse-gated proton decoupling pulse sequence was applied with a 90° pulse width, a relaxation delay of 1.7 s and an acquisition time of 1.2 s. A total of 20–25 K scans were acquired for each spectrum. NMR data were processed with MestreNova (Version 8.1.1, Mestrelab Research).

Derivatization & gel permeation chromatography

Acetobromination derivatization:[33] a lignin sample (10 mg) was weighed into a pre-dried reaction vessel, where 2.3 mL of glacial (anhydrous) acetic acid was added. The reaction mixture was stirred for 30 min, and then, 0.25 mL (3.38 mmol) of acetyl bromide was added. The reaction mixture was finally stirred at room temperature for 4 h. Acetic acid and excess of acetyl bromide were evaporated with an efficient rotary evaporator followed by high vacuum drying at 25–30 °C for 30–45 min. The acetobrominated lignin products were completely soluble in the GPC mobile phase (THF).

GPC measurements[34] were carried out using a Waters GPC instrument equipped with UV (set at 280 nm) and RI detectors using tetrahydrofuran (THF) as the eluent at a flow rate of 0.7 mL min^{-1} at 35 °C. An injection volume of 50 μL and a sample concentration of 0.3 mg mL^{-1} were used. Two ultra Styragel linear columns (Styragel HR2 and Styragel HR 5E) were linked in series. A series of polystyrene narrow standards were used for calibration purposes (the actual series was: M_w; 820, 2330, 3680, 18 700, 31 600, 44 000, 212 400, 382 100, 570 000, 994 000, and 1 860 000 g mol^{-1}). Analyses were run in duplicate.

Results and discussion

Lignin is efficiently depolymerized during kraft pulping,[13] with the resulting monomeric and oligomeric fragments being subjected to a number of additional reactions that are eventually reflected within the structure of the final material. Evidently, the lignin that emerges does not have much in common with the native lignin present in the starting wood.

Perhaps one of the most critical aspects and questions that surrounds the softwood kraft lignin structure is the detailed elucidation of its aliphatic side-chains.[13,19] While we can affirm that they are significantly depleted during the pulping process, to date there is no clear picture related to the nature of the aliphatic side chains in kraft lignin.[13] In an effort to shed light on this important issue we devised an experimental pulping protocol aimed at specifically addressing the elucidation and origin of the aliphatic NMR signals received from softwood kraft lignin. In view of these considerations, it is necessary to re-visit and carefully evaluate the difficulties and limitations that are posed when one attempts to embark in defining the various structural elements of lignin.

Aromatic ring (Ar) and internal standard (IS) as quantification references

The QQ-HSQC NMR sequence[35] has been proposed as the analytical technique of choice for the quantitative determination of the different interunit linkages in lignin.[7] The pulse sequence is designed in such a way that the different C–H correlation volumes are actually proportional to the absolute amount of such moieties in the sample. The method rests, amongst others, on the fact that the different lignin signals can be easily normalized to what is accepted to be a constant and an invariant structural feature in lignin: the guaiacyl C_2–H bond. In fact this position in the aromatic ring has been documented *via* wet chemical enquiries to be completely unsubstituted in milled wood lignin (MWL).[7] As such, the corresponding clean C2–C_2–H correlation can be taken as an internal standard for the determination of all the interunit linkages, offering the possibility to report the data on the basis of aromatic units. Unfortunately, while this approach is acceptable for native wood and milled wood lignins, the situation is clearly different when technical lignins, subjected to severe chemical delignification protocols, are considered. The intensive delignification chemistry applied causes the enumerated correlation to become highly unreliable for quantification purposes. Consequently, QQ-HSQC cannot be referenced to this correlation peak. In order to overcome this obstacle, a direct comparison of quantitative ^{13}C NMR spectra with QQ-HSQC data for the same sample was devised as a promising alternative approach allowing the quantitative elucidation of the softwood kraft lignin structure. The quantitative ^{13}C NMR spectrum was acquired in the presence of a precise amount of the internal standard 1,3,5-trioxane. The latter has been shown before to offer a clean, *i.e.*, isolated and thus readily integratable signal at 93 ppm in the ^{13}C NMR spectrum.

Notably, the 100–160 ppm region contains all the aromatic carbons plus the carbons belonging to conjugated double bonds, such as stilbenes, aryl enol ethers and cinnamyl moieties. All such unsaturated structures are known to emerge during the kraft pulping process.[2] Unfortunately, the ^{13}C-NMR spectrum of softwood kraft lignin is not sufficiently resolved to allow for the accurate integration of all these different moieties. However, the QQ-HSQC that has the distinct advantage of detecting only C–H correlation peaks offers excellent signal dispersion in both the carbon and the proton dimensions. This allows for the sought resolution and as such stilbenes, aryl enol ethers and cinnamyl groups can now be deciphered and integrated as distinct cross peaks.[36] Once this is done, the area of the ^{13}C NMR spectrum from 100 to 160 ppm can then be safely normalized by subtracting the enumerated unsaturated moieties as obtained by the QQ-HSQC. The values obtained during the present effort are averaged over three different experiments[2] and the standard deviation associated

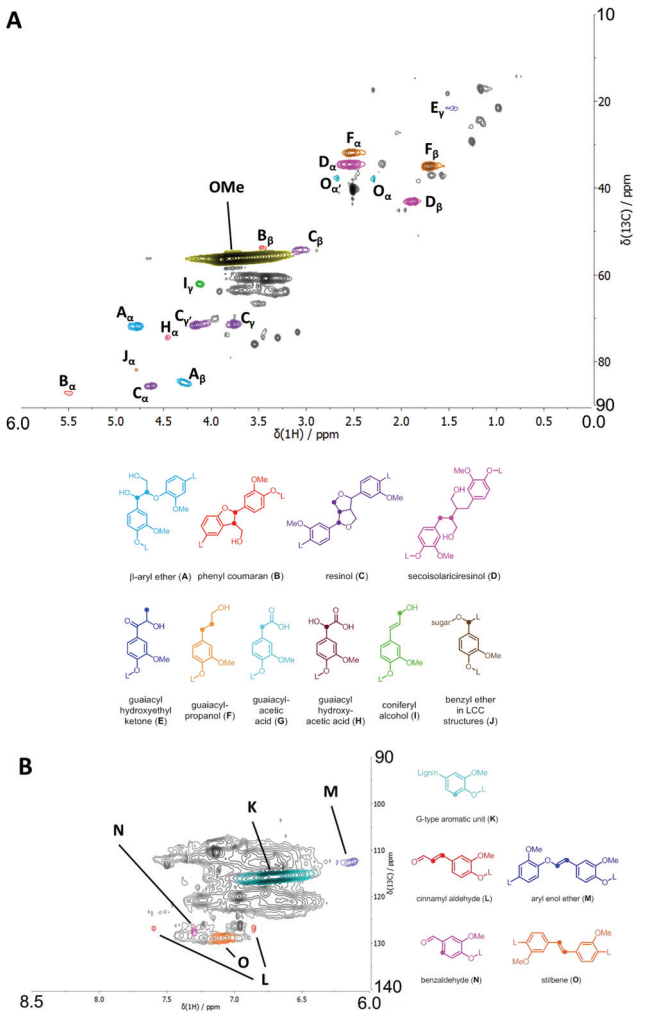


Fig. 1 HSQC spectrum of softwood kraft lignin and assignments of selected structural features: (A) aliphatic region; (B) aromatic region.

Table 1 Quantitative assignment of structural features present in softwood kraft lignin: interunit linkages, endgroups, functional groups and molecular weights, as determined by QQ-HSQC/quantitative[f] ^{13}C NMR, quantitative ^{31}P NMR[g] and GPC[h] analyses, respectively

Lignin interunit bonds	Abundance (per 100 Ar[a])[b]	Chemical shift (cross peak (δ_C/δ_H) used for integration)
Arylglycerol-β-aryl ethers (β-*O*-4′)	3.2	71.0/4.8 (C_α–H)
Phenyl coumaran (β-5′)	0.8	86.5/5.5 (C_α–H)
Pinoresinols (β–β′)	2.4	84.8/4.6 (C_α–H)
Lignin carbohydrate α-benzyl ethers	0.1	81.3/4.7 (C_α–H)
Stilbenes	4.8	128.2/7.2 (C_α–H, C_β–H)
		128.0/7.1 (C_α–H, C_β–H)
Aryl enol ethers	1.3	112.1/6.2 (C_α–H)
		109.0/5.6 (C_α–H)
Secoisolariciresinols	3.2	42.3/1.9 (C_β–H)
Lignin end groups	**Abundance (per 100 Ar[a])[b]**	**Chemical shift (cross peak (δ_C/δ_H) used for integration)**
Cinnamyl alcohols	0.8	61.4/4.1 (C_γ–H)
Arylacetic acid	0.6	39.5/2.4 (C_α–H)
		39.5/2.7–2.9 (C_α–H)
Aryl-hydroxy-acetic acid	0.7	74.3/4.4 (C_α–H)
Aryl ethyl ketones	0.6	31.3/2.5 (C_β–H)
Aryl propanols	3.4	34.4/1.7 (C_α–H)
Aryl hydroxyethyl ketone	0.6	21.0/1.4 (C_γ–H)
Aromatic aldehydes	<0.1	126.5/6.9 (C_6–H)
Cinnamyl aldehydes	0.2	126.3/7.3 ($C_{\alpha/\beta}$–H)
Lignin functional groups	**Abundance (per 100 Ar[a])[b]**	**Chemical shift (cross peak (δ_C/δ_H) used for integration)**
Aromatic C–H	216	110–160/0–11
Methoxy	79.5	55.6/3.8
Quinones	0.1	122.3/5.7
Lignin hydroxyl groups	**Amount[c] (mmol g^{-1})**	**Chemical shift ^{31}P NMR (δ_P)**
Carboxylic OH	0.5	135.5–134.0
Aliphatic OH	2.6	149.0–146.0
o-Disubstituted phenols (including *o*-substituted catechols)[e]	2.1	144.3–140.3
o-Monosubstituted phenols (guaiacyl units)	1.7 (of which unsubstituted catechols 0.2)[d]	140.2–138.8
Total phenolic OH	3.8	144.3–137.4
Molecular weight[e]		
M_n (g mol^{-1})	1400	
M_w (g mol^{-1})	6000	
Polydisperisty index (M_w/M_n)	6.2	

[a] Ar = aromatic units. [b] From QQ-HSQC and quantitative ^{13}C NMR. [c] From QQ-HSQC, quantitative ^{31}P NMR and quantitative ^{13}C NMR. [d] This datum accounts for *o*-unsubstituted catecholic moieties only. Whenever a position *ortho* to a phenolic group is substituted (*i.e.*, carrying aliphatic, aromatic or alkoxy-substituents), the signal is additionally shifted upfield. [e] From GPC analysis. [f] Error analyses apply as per ref. 7, 9 and 10. [g] Error analyses apply as per ref. 28 and 29. [h] Error analyses apply as per ref. 34.

with the determined weakest signals (lignin interunit frequencies) was found to be <10%.

This approach unequivocally offers the overall amount of aromatic carbons present in a given lignin sample. Furthermore, the concerted use of such quantitative data, as derived from both ^{13}C NMR and QQ-HSQC, allows expressing the results in terms of units per aromatic ring. This is a significant advance and allows for a clear insight into the kraft lignin structure thus offering the possibility to arrive at a constitutional molecular scheme.

Oxygenated aliphatic moieties

The detailed analysis of the oxygenated aliphatic region of the QQ-HSQC spectrum shown in Fig. 1 allows one to rapidly arrive at the conclusion that the simple integration of a quantitative ^{13}C-NMR spectrum of technical lignins is comple-

tely inadequate to provide any meaningful information but, at the most, only approximate values for the aliphatic units. This is due to the fact that the ^{13}C NMR signals in this area seriously overlap. Consequently, their integration and the estimation of the different interunit frequencies require tremendous assumptions, oversimplifications and/or signal addition and subtraction operations. Furthermore, distinct to MWL, technical lignins are often impure containing carbohydrate contaminants. Finally, the notoriously low abundance of many of the interunit bondings further compounds critical issues of signal to noise ratios, thus reducing the reliability of the integration of the spectra for both milled wood[37] and technical lignins.[2]

Fortunately, QQ-HSQC has instead been proven to be the analytical technique of choice for the quantitative estimation of oxygenated aliphatic moieties in lignins.[7,8] More specifically, as opposed to the limitations enumerated above, the peaks are well resolved since the QQ-HSQC spectra offer three distinct correlation contours for each lignin subunit that can be readily quantified (see Fig. 1).

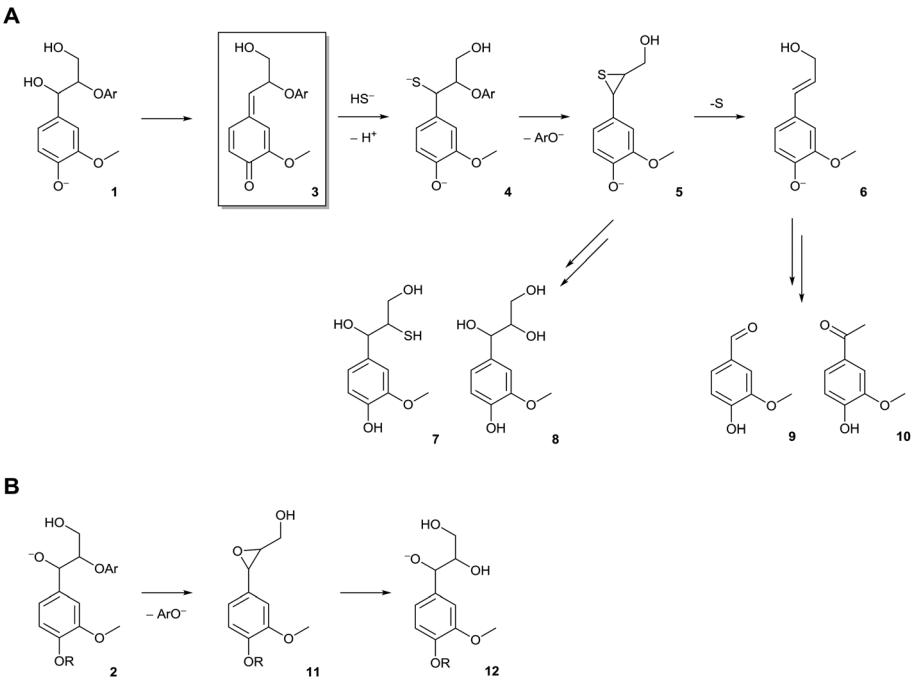
In particular, using the aforementioned advantages of the two-dimensional resolution of HSQC spectra, arylglycerol-β-aryl ether (β-*O*-4′), phenyl coumaran (β-5′) and pinoresinol (β–β′); diphenyl ethane (β-1′); spirodienone (SD) and dibenzodioxocin (DBDO) units can all be identified based on literature chemical shift assignments,[38] and quantified. The described approach for quantification, is now executed in the absence of any of the aforementioned interference that add to errors, as per earlier efforts.[1–3,37,39]

It is of significant importance to note here that the sample of softwood kraft lignin examined in this effort was produced from a kraft pulping process aimed to produce bleachable grade pulp. This has subjected the wood to an *H* factor of approximately 1700 which is quite severe but necessary to reach high degrees of delignification for a bleachable grade cellulosic material. Consequently, this kraft lignin is characterised by the complete absence of diphenyl ethane (β-1′); spirodienone (SD) and dibenzodioxocin (DBDO) units. Remarkably, however, this sample is actually found to contain arylglycerol-β-aryl ether (β-*O*-4′), phenyl coumaran (β-5′) and pinoresinol (β–β′) units albeit in trace amounts; the significance of which will be further discussed in latter parts of this paper.

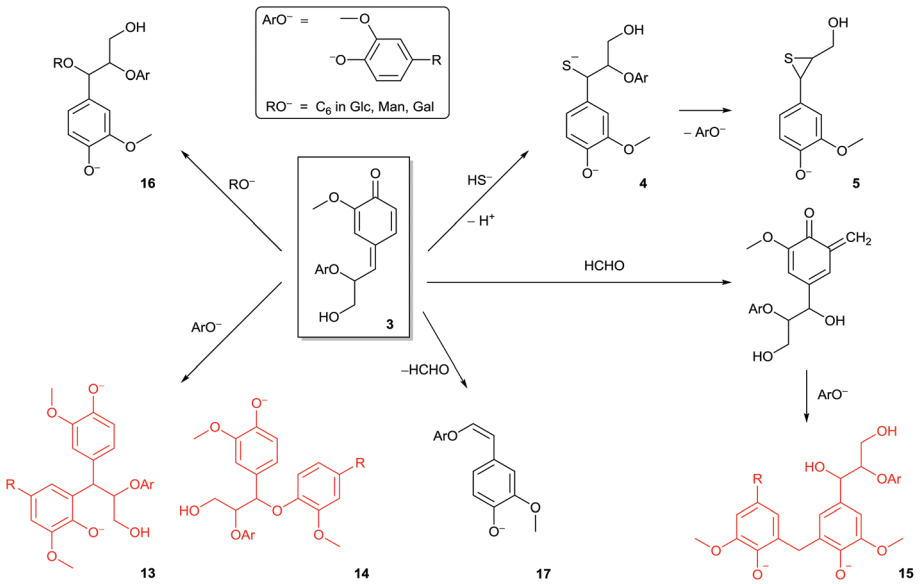
Table 1 shows the determined structural interunit linkages and frequencies together with the chemical shifts of the signals used for the QQ-HSQC integration and quantification. As anticipated, the QQ-HSQC spectrum of softwood kraft lignin is significantly different from that of milled wood lignin.[7,8] Hence, kraft pulping induces specific structural alterations in the lignin that can be easily identified using the HSQC NMR acquisition protocol.

In accordance with the current state of our knowledge, the key chemical intermediate of kraft pulping is the quinone methide **3** (Scheme 1). The electropositive Cα character of **3** invites nucleophilic addition on it by the large abundance of nucleophiles present in the kraft reaction medium; namely hydrosulfide and hydroxide anions, OH groups from carbohydrates and lignin side chains; phenolic OH groups from lignin terminal units and released phenolic fragments.[13,21,40]

Scheme 1 shows an array of the possible products formed by the depolymerization of phenolic (**1**) and non-phenolic (**2**)



Scheme 1 (A) Main lignin depolymerization pathways during kraft pulping. (A) Nucleophilic attack of hydrosulfide on quinone methide intermediates (**3**) during kraft pulping leading to fragments containing thiol groups (**7**) arylglycerols (**8**), vanillin (**9**) and acetovanillone (**10**); (B) deprotonation followed by an internal nucleophilic substitution of non-phenolic lignin subunits.



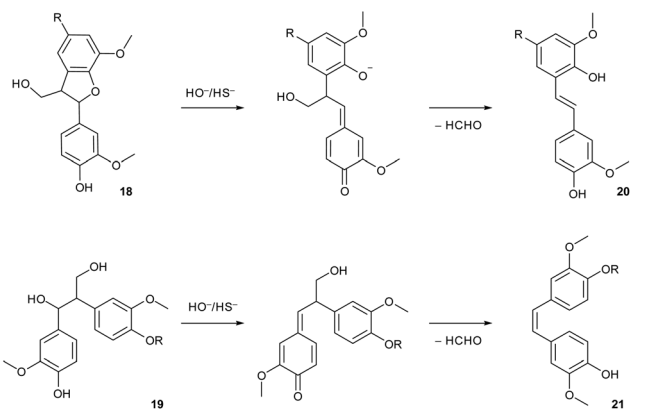
Scheme 2 Reaction pathway of quinone methide intermediates during kraft pulping. In red: products that are not detected in significant amounts.

lignin subunits during kraft pulping upon hydrosulfide attack at the quinone methide (Scheme 1A) or alternatively by deprotonation followed by an internal nucleophilic substitution of non-phenolic lignin subunits (Scheme 1B). The attack of hydrosulfide yields the fragmentation of the lignin side chain and the formation of an episulfide intermediate (**5**) that in turn can be opened by the addition of a second nucleophile (Scheme 1A, **7** and **8**). The cinnamyl alcohol (**6**), the aryl glycerol (**8**) and the vanillin (**9**) and acetovanillone (**10**) side chains formed were all identified in the HSQC spectra of kraft lignin in the form of their derivatives. Table 1 shows the specific amounts of the lignin end groups identified, expressed in percentages per aromatic unit.

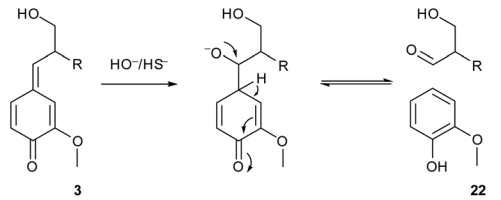
Scheme 2 shows various additions and elimination reactions on the quinone methide intermediate (**3**) invoked to occur during kraft pulping.[13] Among them, Cα–C5 (**13**), Cα-*O*-4 (**14**) and diphenyl methane (**15**) units (Scheme 2, structures shown in red). However, this effort, in accordance with previous accounts,[19,30] cannot confirm that such units are present in significant amounts in kraft lignins since the HSQC spectrum does not show such signals (Fig. 1). Consequently, it is to be concluded that such condensation reactions do not occur to a significant extent. On the contrary, small amounts of benzyl ether linkages (**16**) between lignin and carbohydrates were apparent in the HSQC spectra shown in Fig. 1 (81.3/4.7 (Cα–C_α–H)). This is not surprising since there is a large abundance of mobile carbohydrate moieties present in the kraft pulping media that can readily ionize at the elevated pH and temperatures of the process. Such nucleophiles can thus readily attack the Cα of the lignin based quinone methide creating the detected lignin–carbohydrate linkages. As depicted in Scheme 2, the loss of formaldehyde is thought to generate aryl enol ethers (**17**)[13,16] that were actually detected, albeit in low amounts (Table 1).

Phenyl coumaran (**18**) and β-1′ (**19**) lignin subunits do not undergo depolymerization upon kraft delignification, instead they generate stilbene subunits[13] (**20** and **21**) that were actually detected within the isolated softwood kraft lignin examined in this work (Fig. 1, Scheme 3, and Table 1).

A close examination of the determined amounts of interunit linkages and functional groups present in softwood kraft lignin (Table 1) indicates that this material has been heavily altered, with many interunit linkages heavily depleted when compared to a typical softwood MWL.[7] In accordance with previous efforts for *Eucalyptus grandis* kraft lignin,[19] a reduction in the intensity of the oxygenated aliphatic moieties is apparent. Even if one adds to the oxygenated aliphatics determined, the terminal aliphatic side chains that include aryl enol ethers, stilbenes, arylglycerol units and LCC-groups, the overall amount of oxygenated aliphatics is significantly lower than the



Scheme 3 Attack of hydrosulfide anions on phenylcoumaran and diphenylethane lignin subunits during kraft pulping, yielding stilbenes.



Scheme 4 C1–Cα cleavage of the lignin backbone by the retroaldol reaction and the formation of phenolic monomers.

aromatic units (Table 1). The kraft pulping process is thought to dissolve lignin *via* the cleavage of the arylglycerol aryl ether bonds (β-*O*-4′). If kraft lignin were to be formed by such a lignin fragmentation process and these were simply released in the liquor, then a significant amount of the original side chains should still be present. As anticipated, and, more importantly, as the accumulated data testify (Table 1), this is not the case. Scheme 4 shows the retroaldol reaction that was first invoked by Gierer for the removal of the lignin side chain during kraft pulping.[13] Our data point toward the possibility that this reaction is more significant than previously thought, since it yields the release of phenolic compounds that are readily detected in the precipitated black liquor and as per this effort.

Further insight into the occurrence of the retro-aldol reaction can be gained by critically evaluating the literature GC-MS pyrolysis data of softwood MWL and the respective kraft lignin.[19,41,42] While the main products from GC-MS pyrolysis arising from MWL are always the possible cinnamyl alcohols according to their lignin botanical origin, this is not the case when kraft lignins are examined under such conditions. The percentage of pyrolysis products containing 0 or 1 carbon atoms on the side chain are prevailing over the others in the case of kraft lignins. This implies that the side chain is extensively lost during pulping. Among the possible reaction pathways active during kraft treatment the only process that could yield loss of the side chain is the retro-aldol reaction. Any other reaction pathway, from the loss of formaldehyde to the formation of aryl ether bonds (Schemes 1–3) would retain at least part of the lignin side chain. Consequently, it is likely that during kraft pulping the released phenols undergo retroaldol reactions in significant amounts able to generate phenolic compounds. In turn, such phenols would now be able to undergo a plethora of different polymerization reactions likely initiated by radical species[43,44] to yield the final structure of isolated kraft lignin.

Saturated aliphatic moieties & the role of extractives

Saturated aliphatic moieties have been documented to exist within kraft lignins[43] and almost invariably attributed to the presence and accumulation of wood extractives in them. This is a logical explanation since it is possible that extractives could be enriching the kraft lignin either *via* simple precipitation effects or *via* their reactive covalent incorporation. No detailed studies, however, exist that further delve into the detailed understanding of these important structures present in softwood kraft lignin in significant amounts.

Beyond the extractive related explanation, the presence of aliphatic moieties may also be due to radical-induced reduction products present in kraft lignin as side chains. This is because detailed pulping chemistry studies have shown the formation of sulfur mediated radical species.[44] More specifically, redox reactions have been invoked, but yet not fully clarified, to occur during kraft pulping, producing molecular sulfur as the final oxidized product.[40,44,45] Furthermore, model compounds subjected to the kraft pulping conditions were shown to offer reduction products on their side chain. Therefore, reductive chemistry is a distinct possibility as it takes place during kraft pulping in tandem with the radical oxidation of the lignocellulosic network.

In an effort to offer better insight that pertains to the precise nature and origin of the saturated aliphatic moieties present in softwood kraft lignin, the following extraction and pulping experiments were carried out, followed by detailed GC-MS and NMR studies:

a. Exhaustive extraction of the isolated and already examined softwood kraft lignin with hexane.

b. Exhaustive extraction of southern pine sawdust of the same softwood used in the process from which the initial kraft lignin was isolated. The following solvents in sequence were used: dichloromethane (DCM), toluene/ethanol, acetone; this was followed by kraft pulping at an *H*-factor and a CO_2-based precipitation protocol similar to the industrial process used to isolate the examined lignin; samples are labelled as Extracted Southern Pine Kraft Lignin (**ESP-KL**).

c. Kraft pulping of the same sawdust used in (b) without any solvent extraction followed by precipitation of the lignin using the same CO_2-based precipitation protocol. This lignin was labelled as Non-Extracted Southern Pine Kraft Lignin (**NESP-KL**).

Our initial work with the sample of kraft lignin, examined and structurally described in the earlier sections of this work (Table 1), showed that the amount of hexane-soluble extractives was 1.2%. This was done in complete awareness that some literature accounts exist that detail the presence of extractives as such, and that also detail that certain extractives are not quantitatively removed by the extraction of simple hexanes.[2,46]

The HSQC spectra of the extracted kraft lignin and the isolated hexane extractives were overlapped to identify the peaks (present in the aliphatic region) that are related to the extractives. Fig. 2 shows the aliphatic region of the HSQC of the extracted kraft lignin. The peaks in green correspond to the isolated extractives while the red peaks are related to the non-extractable material. As such, we were able to conclude that the most intense peaks in the aliphatic region of these spectra were not due to extractives but to reduced lignin side chains. More specifically they were tentatively assigned to aryl propanol and secoisolariciresinol substructures on the basis of previous literature reports.[30] Low amounts of aromatic alpha-keto 2-propanols and aromatic ethanoic acid bearing side chains ($ArCOCHOHCH_3$, ArCHOHCOOH and $ArCH_2COOH$) were also

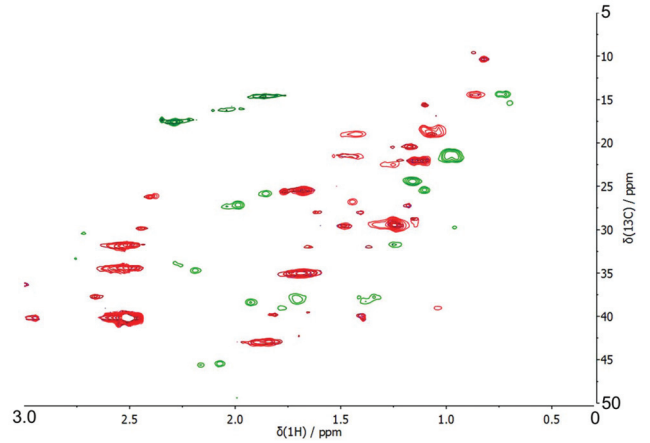


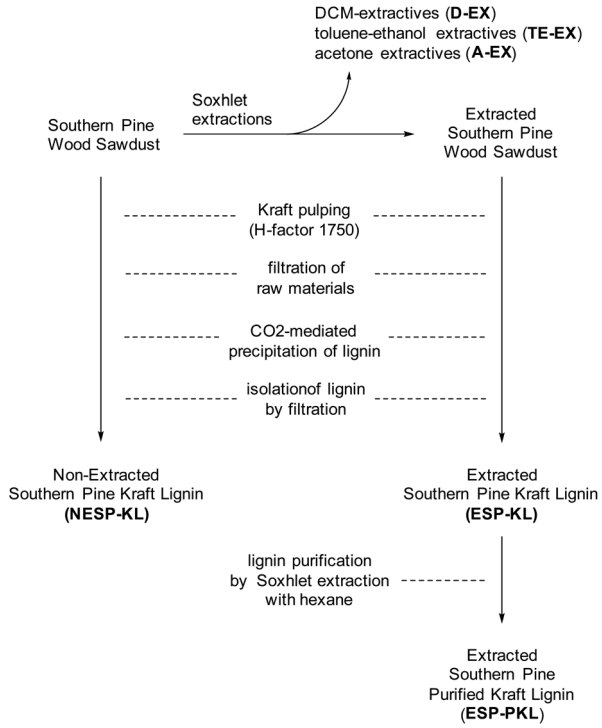
Fig. 2 HSQC spectrum for non-oxygenated aliphatic region of kraft lignin (red) overlapped with the corresponding kraft lignin extractives (green).

identified, most likely, present as end-groups (see Table 1). The assignment of these signals was carried out on the basis of earlier literature accounts.[19,30] Such aryl glycerol, aryl acetic and propionic acids, aryl propane, aryl ethyl ketone and aryl methyl ketones are seen to appear and likely be formed during kraft pulping, possibly *via* redox processes already invoked. However, the elucidation of the precise chemistries leading to their formation requires thorough experimental verification and documentation. Their amounts are reported in Table 1.

Additional minor and yet unidentified not-extractable peaks remain unassigned in the aliphatic region of the HSQC spectrum (Fig. 2). As already discussed, it has been hypothesized that, during kraft pulping, lignin side chains could crosslink with unsaturated centers present in certain extractive compounds thus covalently incorporating themselves in lignin generating complex structures.[40]

The experiments described under points (b) and (c) above were indispensable to further delve into the validity of this hypothesis. More specifically, the HSQC spectra of kraft lignins obtained as outlined in the Experimental section and roughly depicted in Scheme 5 from exhaustively extracted southern pine sawdust (**ESP**) (b) and from non-extracted southern pine sawdust (**NESP**) (c), respectively, were compared. Additionally, kraft lignin obtained from the previously extracted sawdust, *i.e.*, **ESP-KL** (b) was isolated in order to eliminate any possible traces of the remaining and/or newly formed soluble extractives to yield **ESP-PKL**. The overall amount of the wood extractives was found to be 1.9%, whose composition is shown in Table 1 (ESI†): GC-MS analyses of the extractives showed the occurrence of about 50 structures.

The isolated kraft lignins, namely **NESP-KL** and **ESP-KL**, as well as the extractives were all subjected to detailed HSQC analyses. Interestingly, non-oxygenated aliphatic signals were seen to be present in all spectra. By careful overlapping of these spectra it became possible to identify and distinguish the correlation peaks whose origin is the extractable material from those which originate from the products of kraft pulping and are present on the lignin backbone (Fig. 3).



Scheme 5 Indication of the experimental flow for the creation of the various softwood kraft lignin samples identified in the text as **ESP-KL**, **NESP-KL**, and **ESP-PKL**.

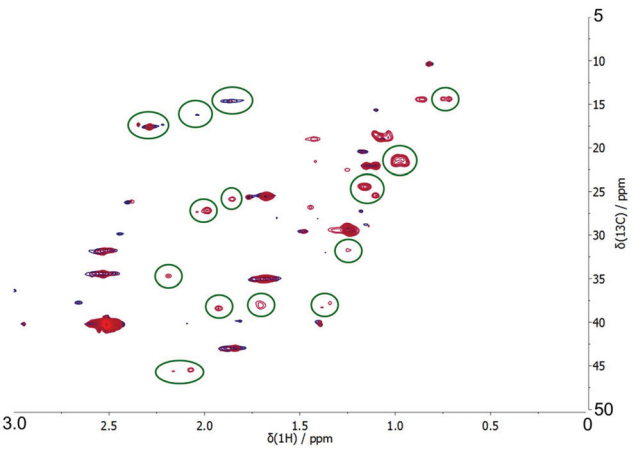


Fig. 3 Overlapped HSQC spectra of **NESP-KL** and **ESP-KL**. Blue peaks: present in the kraft lignin from extracted wood. Red peaks: present in the kraft lignin obtained from non-extracted wood. Green circled peaks: extractable species.

As anticipated, the **NESP-KL** showed more aliphatic peaks than the **ESP-KL**. No new peaks, possibly originating from the covalent incorporation of extractives with lignin were evident. This is well illustrated in Fig. 3 where the blue peaks are due to the **ESP-KL** and the red peaks to the **NESP-KL**. All the peaks additionally circled in green were seen to be present in the HSQC spectrum of the extractives and are mostly absent in the spectrum of **ESP-KL**. In other words, all the peaks that were

only present in the **NESP-KL**, correspond to peaks found in the HSQC spectrum of the isolated extractives. This indicates that these are due to the extractable material that is accumulated during kraft pulping and is precipitated together with lignin (Fig. 4). Most noteworthy is that these experiments further point out that no significant reactions seem to occur during kraft pulping between lignin and the extractable material, since no new peaks were apparent in the **NESP-KL** (and, of course, being absent from both the spectra of **ESP-KL** and from the spectrum of the extractives, Fig. 3).

On the contrary, it can be confirmed that the "exhaustive" extraction was not quantitative since the HSQC spectrum of **ESP-KL** still shows a few peaks due to residual extractives as being present. This is clear from a comparison of Fig. 2 and 3: in Fig. 3, some of the blue circled peaks indicate that extractives are present in traces also in the blue-labelled spectrum of **ESP-KL**.

Evaluation of the degree of branching in softwood kraft lignin & aromatic ring substitution

The determination of the branching in lignin has been traditionally very cumbersome. Previous attempts at determining the extent of branching in kraft lignin did not yield quantitative data. However, the occurrence of extensive branching has been qualitatively shown by permanganate oxidation studies.[23] While, in fact, the main oxidation products in native milled wood lignin are the methyl esters of the benzoic acids arising from the lignin side chains, in the case of kraft lignins significant amounts of products have been identified that indicate crosslinking in positions 5 and 6.[47] Permanganate oxidation allows, in principle, the determination of the extent of branching. However, the poor mass balance, the propensity of the procedure toward introducing experimental errors and the lack of normalization factors for the integration of individual peaks identified by GC-analysis, *etc*. make this, at most, a semi-quantitative analytical technique. Furthermore, under the conditions traditionally used for permanganate oxidation, significant decarboxylation reactions, on the resulting carboxylic acids that emerge from the reaction, can occur. This will fundamentally alter the detected degree of branching of a given lignin.[23]

Due to these reasons, we have embarked at detailed enquiries for determining the degree of branching of softwood kraft lignin and its fractions by novel means. The integration of the QQ-HSQC area of the aromatic 100–160 ppm C–H region (when used in combination with the quantitative ^{13}C NMR) allows the determination of the total amount of Ar–H bonds per aromatic ring, which eventually allows one to arrive at the degree of branching as it emerges *via* aromatic substitution patterns.

More specifically, since this approach is founded on the concerted use of quantitative ^{13}C NMR and QQ-HSQC[7,8] and despite the relatively high relative errors involved in the traditional ^{13}C NMR acquisitions, a semi-quantitative determination of the branching degree in kraft lignin is now possible.

This approach applied to the softwood kraft lignin examined in this study revealed 216 Ar–H bonds per 100 aromatic units (Table 1). Since each aromatic ring in kraft lignin is supposed to have a carbon chain at position C1 and at least two substituents in positions C3 and C4 of the aromatic ring, respectively (namely a methoxy group and a phenolic or an arylether bonding) this implies that at least 3 of the six carbon centers of the aromatic rings are functionalised. If lignin were purely linked in a β-*O*-4′ aryl ether bonding, each aromatic ring would have 3 C–H bonds in the C2, C5 and C6 positions, respectively. The presence of phenyl coumaran linkages would reduce the amount of C_5–H bonds. This is not, however, the case since the presence of such interunit bonds was shown to be negligible (Table 1). Therefore, a hypothetic completely linear lignin structure should show 3 C–H bonding patterns per aromatic ring (or 300 per 100 Ar). The occurrence of branching would imply a bonding between aromatic rings in the form of biphenyl or biarylether bonds.[48] In both cases, the presence of such linkages would decrease the amount of aromatic C–H bonds per ring. The presence of 216 Ar–H bonds per 100 aromatic units (Table 1) indicates that about 84% of the aromatic rings are involved in branching. Of note here is that the calculation of the degree of branching stands for "single-linked branching on average".

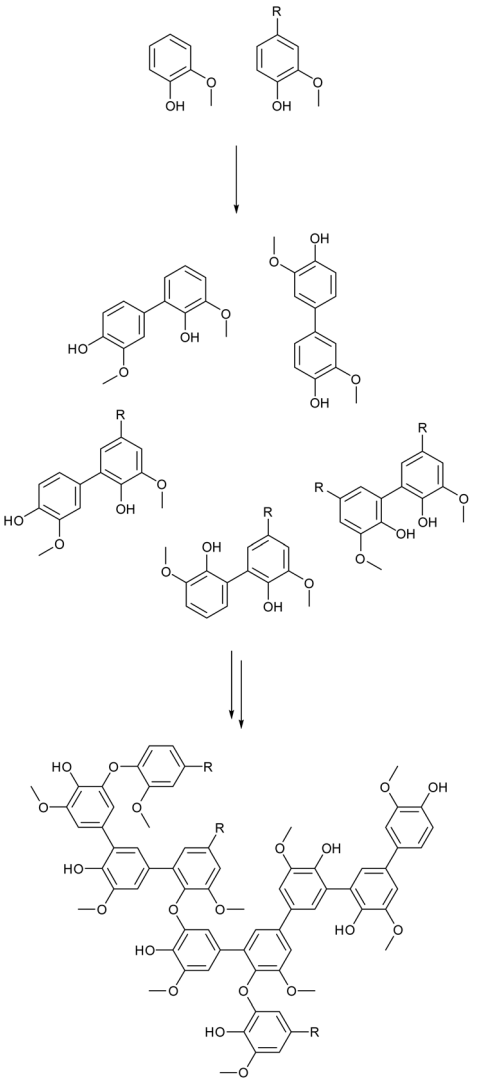
The origin of branching in softwood kraft lignin

In an effort to rationalize the findings of the extensive aromatic substitution discussed in the previous section, the following account is warranted. At the onset one needs to be aware of the fact that the lignin (in the form of milled wood lignin) in the starting softwood has been found not to be significantly branched.[8]

Under the harsh kraft pulping reaction conditions, part of the hydrosulfide is converted into polysulfide, sulfur, thiosulfate and sulfate.[44] These transformations appear to be mediated by the presence of multiple redox systems present in wood.[44] Thus the kraft pulping chemistry has to be evaluated not only from the ionic reactions point of view, but also from the possibility of the occurrence of a wide variety of redox reactions.

More specifically, model compound studies have shown that sulfur, under kraft pulping conditions, is able to mediate one-electron reactions with the concomitant formation of phenoxy radicals.[43,44] Elemental sulfur is known to be formed during kraft pulping.[43] Upon heating, the octa-cyclic sulfur can be transformed into a biradical. Such species have been shown to be responsible for oxidative coupling reactions of phenols,[49] or to alternatively initiate cross-linking in polymeric systems and rubbers.[43] In turn, the sulfur-centered radical evolves into polysulfide.[43] Once phenoxy radicals are formed on phenolic monomeric or oligomeric compounds, the main radical reactive pathway is the coupling at positions *ortho* and *para* to the phenolic group with the formation of biphenyl and biphenyl ether structures.[43]

Such a pathway offers an explanation for the occurrence of 4-*O*-5′ lignin subunits and for the high degrees of aromatic branching detected. These events likely occur with particular severity during the final delignification phases when the concentration of released phenolates is increased causing the



Scheme 6 Possible products arising from the radical coupling pathways of fragments released during kraft pulping.

released lignin fragments to undergo radical repolymerization. At the final stages of delignification and since the side chains have already been largely depleted, extensive aromatic ring substitution and coupling becomes the prevailing set of reactions that eventually becomes a permanent feature in the structure of the softwood kraft lignin. This is supported by the gradual decrease of the polysulfide content during pulping.[43] Scheme 6 shows possible involved radical coupling pathways of fragments released during kraft pulping.

Functional groups

Carboxylic acids, phenolic groups & aldehydes. Carboxylic acids are present in softwood kraft lignin not only as components in extractives but are also present in quantities as high as 5% of the overall aromatic units as shown by quantitative ^{31}P NMR analyses. With respect to phenolic units, data presented in Table 1 show that about 46% of the aromatic rings of softwood kraft lignin used in this study possess a free phenolic OH-group, with 21% of them being *ortho*-substituted and 25% being *ortho*-disubstituted (condensed). This amount is much higher than in MWL[8,50] and has been widely reported before for other kraft lignins.[2,50] In contrast, the aliphatic OH-groups determined by quantitative ^{31}P NMR (average 31 aliphatic OH-groups per 100 Ar) were found to be considerably reduced when compared to MWL.[8,50] This is coherent with the significant depletion of the aliphatic lignin side chains already discussed.

Aldehydes are also produced during kraft pulping, albeit in low amounts. The signals relative to both benzaldehydes (126.5/6.9 (C_6–H)) and cinnamyl aldehydes (126.3/7.3 (C_7–H)) have been identified in the QQ-HSQC spectra, and these were quantified in low abundance (Table 1).

Degree of demethylation reactions occurring during kraft pulping. During kraft pulping, hydrosulphide anions nucleophilically attack the methoxyl groups in lignin causing demethylation and yielding odoriferous methyl hydrosulfide and dimethyl sulfide.[13] Integration of the methoxy group signals in the quantitative ^{13}C NMR and QQ-HSQC spectra offered the direct determination of the degree of demethylation occurring during kraft pulping. If one considers that the initial softwood lignin was composed entirely of guaiacyl units (which is a reasonable approximation) then its degree of methoxylation would be 100%. Our work showed that the degree of methoxylation present in softwood kraft lignin was only about 79%. As such this implies that 21% of the aromatic units were demethylated during kraft pulping. Consequently, at various points along the delignification reaction, a relatively large fraction, (about 21% of the aromatic units) was converted into reactive catecholic intermediates that eventually reacted further. Only modest amounts of these moieties are apparent in the ^{31}P NMR spectra (0.23 mmol g^{-1}, Table 1). However, these data only account for non-substituted catechol moieties; in fact, the presence of *ortho*-substituents, *i.e.*, in positions 2 or 5 of the aromatic rings, would change their chemical shift and overlap with the condensed phenolic units (Table 1).

As a side note, catechols may oxidize to *ortho* quinones, functional groups in general elusive to most analytical protocols. On the basis of previous literature accounts,[51] signals for quinones were detected and determined to be present in softwood kraft lignin in trace amounts (Table 1).

The presence of free phenolic OH groups in position 3 of the aromatic ring significantly alters the orientating character of the other substituents and thus dramatically affects the ring reactivity. Such effects may promote possible oxidative coupling reactions in positions C2 and C6, offering additional avenues further promoting complex ring functionalization patterns.

Overall, the well documented demethylation reactions are not only the reason for the odoriferous nature of kraft pulping. Most significantly, their extensive occurrence offers the generation of reactive intermediates that promote complex substitution patterns to emerge on the aromatic rings of softwood kraft lignin.

Kraft lignin fractionation

Factors causing the complexity & heterogeneity of lignin & the need for fractionation. In order to allow further insight

into the structure of kraft lignin, it is of pivotal importance to consider the fact that kraft lignin is a highly heterogeneous material. The experimental data obtained so far show the presence of a highly complex material whose composition contains a variety of structures that range from "classical" lignin subunits to units of high degree of branching as well as newly generated aliphatic side chains. Our data also clearly indicate that the product 'kraft lignin' is the result of a process of cleavage and repolymerization of lignin-based fragments, which appear under the harsh kraft pulping conditions. While specific attention has been paid to the processes yielding depolymerization products and their kinetic behavior, the archival literature does not report much regarding the repolymerization of phenolic fragments that in principle, is an unwanted process during pulping.[43,44]

As such the inherent heterogeneity of the starting technical lignin, so far examined in its totality, needs to be addressed next. It is imperative that if one is to arrive at a structural scheme for softwood kraft lignin, then one needs to examine individual components of it. For this purpose during this effort we applied fractional precipitation followed by the detailed characterization of specific fractions. Fractional precipitation was selected as the approach of choice since a continuum of narrow lignin fractions can be isolated by the incremental addition of a non-polar solvent (hexane) in a polar solution (acetone) of softwood kraft lignin, in accordance with our previous work.[32,52]

The fundamental reason behind the fact that such uniform fractions of softwood kraft lignin are obtained by fractional precipitation is based on the following: fractional precipitation offers the possibility to gradually alter the polarity of the environment specific lignin molecules that are solvated with and form a condition of complete dissolution. Lignin molecules of even minor polarity differences are incompletely solvated and thus precipitated. As such, theoretically, the polydispersity of lignin fractions approaches unity, the finer the selection of the solvent gradient.

While acetone seems to be a good polar solvent for certain components of softwood kraft lignin, it offers a significant amount of a solid insoluble residue termed acetone insoluble softwood kraft lignin (**AIKL**). It is to be noted here that the whole process is valid for anhydrous acetone containing less than 0.1% water.[32,52] Consequently, the first step of fractionation consists of the separation of kraft lignin into an acetone insoluble and an acetone soluble part, **AIKL** and **ASKL**, respectively. The acetone soluble portion, about 70% of the starting material, is then subjected to fractional precipitation by addition of increasing amounts of hexane. The lignin is thus divided into an acetone insoluble fraction (**AIKL**) and four different acetone/hexane soluble fractions were selected to be further examined, namely **H300IKL**, **H500IKL**, **H900IKL** and **H1500IKL** whose details of isolation are shown in Table 2.

Molecular weight & molecular weight distribution of the lignin fractions. Size exclusion analyses of the original, unfractionated softwood kraft lignin showed a M_n value of 1400 g mol^{-1} and a M_w of 6000 g mol^{-1} with a ratio of about M_w/M_n = 4.3 (Table 1).

The large heterogeneity apparent, indicated by the ratio of M_w/M_n, is to be anticipated on the basis of early literature accounts[53,54] and the repolymerization and degradation reactions enumerated above.

Overall, the average degree of polymerization is impossible to be determined in light of the numerous limitations and

Table 2 Isolation information and structural characterization of kraft lignin fractions as obtained by QQ-HSQC[d], quantitative ^{13}C NMR, quantitative ^{31}P NMR[e] and molecular weight distribution measurements as evaluated by GPC[f] analyses

		ASKL-derived **HIKL** fractions			
Fraction information	**AIKL**	**H300**	**H500**	**H900**	**H1500**
Volume fraction of hexanes	0	0.231	0.333	0.474	0.600
Lignin interunit bonds – abundance (per 100 Ar)[a]					
Arylglycerol-β-aryl ethers (β-*O*-4′)	12.7	4.4	4.6	2.5	1.4
Phenyl coumaran (β-5′)	2.6	1.8	1.8	0.9	0.4
Pinoresinols (β–β′)	3.5	2.4	2.9	1.7	1.6
Lignin carbohydrate α-benzyl ethers	0.1	0.1	0.1	0.1	0.1
Stilbene	5.5	3.8	3.4	4.4	4.2
Aryl enol ethers	0.6	2.2	2.6	2.4	2.4
Seicolariciresinol	3.1	2.6	2.6	2.3	2.1
Lignin end groups – abundance (per 100 Ar)					
Cinnamyl alcohols	1.0	0.9	0.8	0.7	0.7
Arylacetic acid	<0.1	0.3	0.3	0.2	0.2
Aryl ethyl ketones	0.7	0.6	0.5	0.6	0.4
Aryl propanols	3.9	0.3	0.5	0.2	1.4
Aryl hydroxyethyl ketone	0.4	0.1	0.3	0.4	0.4
Aromatic aldehydes	<0.1	<0.1	0.1	0.1	0.1
Cinnamyl aldehydes	0.1	0.1	0.1	0.1	0.1
Lignin functional groups – abundance (per 100 Ar)					
Aromatic C–H	249	223	214	205	203
Methoxy	79.7	68.1	67.8	84.3	89.8
Quinones	<0.1	<0.1	<0.1	<0.1	<0.1
Lignin hydroxyl groups – amount (mmol g^{-1})[b]					
Carboxylic OH	0.5	0.6	0.7	0.8	0.6
Aliphatic OH	3.0	2.4	2.1	2.0	1.7
o-Disubstituted phenols (including *o*-substituted catechols)	1.8	2.5	2.4	2.5	2.7
o-Monosubstituted phenols (guaiacyl units)	1.7	2.7	2.9	2.9	3.0
Total phenolic OH	3.5	5.2	5.3	5.5	5.7
Molecular weight[c]					
M_n (g mol^{-1})	3300	2000	1400	1100	900
M_w (g mol^{-1})	12 200	3300	2000	1300	1000
Polydisperisty index (M_w/M_n)	3.7	1.6	1.4	1.2	1.1

[a] From QQ-HSQC and quantitative ^{13}C NMR. [b] From QQ-HSQC, quantitative ^{31}P NMR and quantitative ^{13}C NMR. [c] From GPC analysis. [d] Error analyses apply as per ref. 7, 9 and 10. [e] Error analyses apply as per ref. 28 and 29. [f] Error analyses apply as per ref. 34.

findings discussed earlier with the most significant being that a unit formula for kraft lignin cannot be established since the C9 formula (used for native lignin) is no longer applicable due to the depletion of aliphatic side chains. However, assuming a unit MW ranging from 100 to 200 g mol^{-1} (a wide enough range to include any possible fragment with or without the presence of a side chain), it is possible to hypothesize an average degree of polymerization ranging from 14 to 30.

Similar molecular weight measurements when conducted for the acetone insoluble (**AIKL**) and acetone soluble (**ASKL**) fractions of the same softwood kraft lignin showed a considerable different picture. The acetone insoluble kraft lignin fraction showed an M_n value of 3300 g mol^{-1} and an M_w value of about 12 000 g mol^{-1} (Table 2). This larger molecular weight is also supported by the presence of bonding patterns in it, as well as a lower number of phenolic end groups (see the ensuing discussion of Table 2 below) that corroborate to longer polymeric chains being present. Recent reports pertaining to this issue have also suggested the possibility that the higher molecular weight of this fraction may be a manifestation of extensive π-stacking within it.[55–57]

In contrast to **AIKL**, any of the acetone soluble kraft lignin fractions (**ASKL**) showed M_n values ranging from about 1000 to 2000 g mol^{-1} and M_w values ranging from about 1000 to 3000 g mol^{-1} (Table 2). The lower molecular weights are further supported by the absence of specific bonding patterns in it that may sustain a polymeric structure and the considerably higher number of phenolic end groups (see the ensuing discussion of Table 2 below) that corroborate to smaller polymeric chains being present.

Structural analyses of the softwood lignin fractions. In a manner identical to our efforts carried out for the whole softwood kraft lignin, the different fractions were also subjected to structural analyses using QQ-HSQC, quantitative ^{13}C NMR and quantitative ^{31}P NMR. Table 2 gives an overview of the obtained quantitative data. Notably, the data have been organized into numbers for lignin side chains, lignin end groups and functional groups, as it was done for the whole kraft lignin starting material already reported in Table 1.

There is a clear and significant difference between the **AIKL** fraction and the acetone/hexane fractions. **AIKL** shows a higher content for all native lignin interunit bondings when compared to the acetone soluble, **ASKL**-derived fractions. Furthermore, an overall higher content of aliphatic hydroxy groups and a lower degree of aromatic substitution (2.49 Ar–H/Ar in **AIKL** *vs.* 2.03–2.20 Ar–H/Ar in the hexane precipitated fractions) are also apparent in **ASKL**-based fractions (Table 2). These data indicate that the **AIKL** contains lignin fragments that are not completely depolymerized. The increased aliphatic OH-group content corroborates to the presence of non-degraded lignin side chains in **AIKL**. The occurrence of a lower degree of branching in **AIKL** further suggests that elements of this fraction did not undergo extensive repolymerization, in agreement with the higher molecular weights already discussed. Overall, **AIKL** seems to represent a less severely degraded lignin structure when compared to **ASKL**.

In contrast, the hexane precipitated **ASKL** fractions are rather homogeneous and do not show significant structural differences among them. More specifically, they show a very low content of lignin side chains, end group units and aliphatic OH groups as compared to **AIKL**. This is associated with a significantly higher degree of branching (lower frequencies of aromatic CH groups) compared to **AIKL**, a high content in phenolic groups and significantly lower molecular weights. It is interesting to note that the various interunit lignin bonds and the amount of aliphatic OH groups progressively decrease in the **ASKL** fractions as the volume fraction of hexane was increased while a progressive increase in branching and phenolic OH group content was apparent (Table 2).

It can thus be concluded that softwood kraft lignin is constituted of two main fractions that may be easily isolated by acetone dissolution. An acetone insoluble fraction displays a higher molecular weight, containing approximately one branched aromatic ring for every two, a set of typical lignin interunit bondings and additional moieties that are introduced by kraft pulping such as stilbenes, aryl enol ethers and reduced side chains. One can hypothesize that this fraction is possibly composed of lignin fragments partially crosslinked and repolymerized from fragments generated during pulping. The acetone soluble fraction is composed of significantly more branched lower molecular weight polyphenolic oligomers fundamentally depleted of 'typical' lignin side chains. It contains only traces of the typical lignin interunit bondings and additional moieties such as carboxylic acids, stilbenes, aryl enol ethers and reduced side chains. The absence of side chains suggests that this fraction could have been generated from radical repolymerization of monomeric phenolic fragments released during pulping.

Proposed constitutional schemes for softwood kraft lignin. In accordance with our primary objective, the accumulated information presented so far was used to construct a new structural scheme for softwood kraft lignin. Based on our findings it became obvious that two different structures needed to be created; namely, one for the acetone insoluble fraction (**AIKL**) and one for the acetone soluble fraction (**ASKL**) due to their distinct characteristics as revealed during this effort.

It is to be noted that despite all our efforts, the proposed structures, shown in Fig. 4, need to be considered as qualitative mainly due to the fact that a variety of minor moieties are shown but their frequencies are not accurately reflected since their low abundance would require a much greater number of aromatic units to be included.

Fig. 4A evidently displays the macromolecular structure of the acetone insoluble fraction of softwood kraft lignin (**AIKL**). This is distinct to the oligomeric fragments of the acetone soluble softwood kraft lignin (**ASKL**) displayed in Fig. 4B.

The construction of these two distinct components of softwood kraft lignin was based on the analytical data collected in this effort and the ensuing discussion. More specifically the rationale for constructing the structure of **AIKL** as displayed in Fig. 4A is as follows:

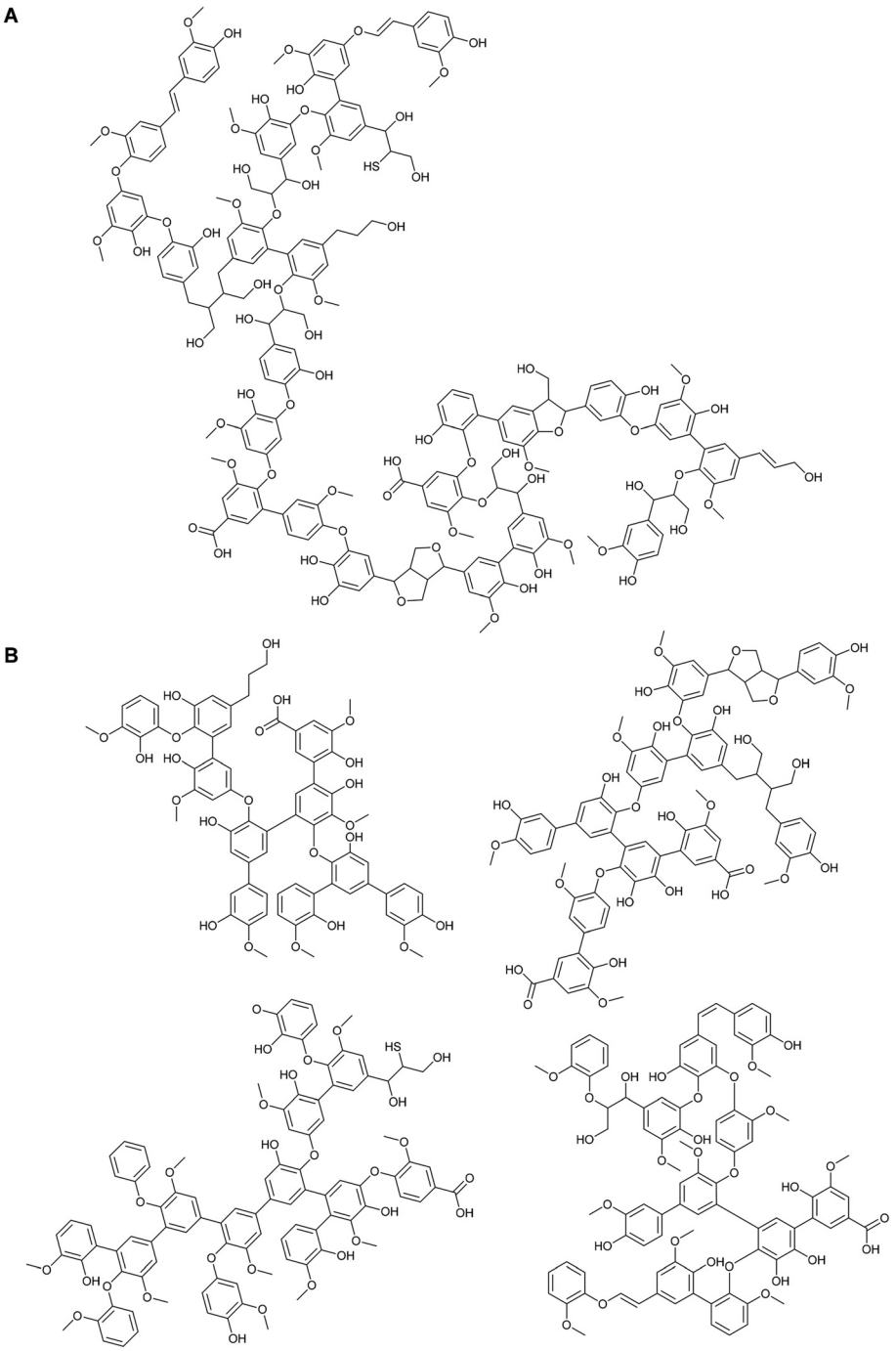
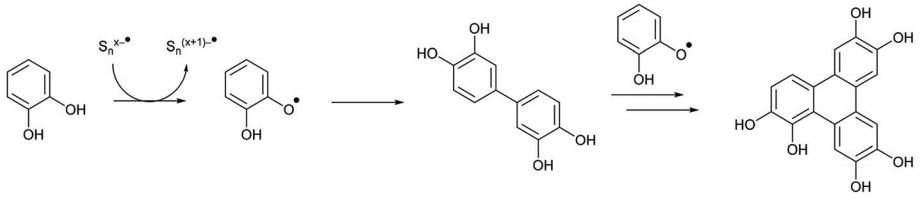


Fig. 4 Proposed constitutional structural schemes for softwood kraft lignin. (A) Acetone insoluble fraction (**AIKL**); (B) acetone soluble fraction (**ASKL**).

The main feature of **AIKL** is the need for significant contributions of typical native lignin bonding patterns such as β-*O*-4′, phenyl coumaran (β-5′) and pinoresinols (β–β′), as well as newly formed reduced aliphatic chains such as enol ethers and stilbenes. **AIKL** is also shown to contain significant branching as displayed by 4-*O*-5′ and 5–5′ structural units. The former presumably arise from oxidative coupling with phenolic fragments released during pulping. These fragments, upon interaction with sulfur radical species, may undergo oxidative coupling. 5–5′ structural units may also be the result of dibenzodioxocin ring opening reactions as well as 5–5′ units (albeit in small amounts) originally present in the native lignin.

In contrast to **AIKL**, the acetone soluble fraction of softwood kraft lignin (**ASKL**), shown in Fig. 4B, is considerably enriched with phenolic units and thus in need to be represented by the four oligomeric fragments displayed. The obvious higher degree of branching of **ASKL** compared to **AIKL**, together with the significantly lower number average



Scheme 7 Polycyclic aromatic moieties possibly invoked to occur in kraft pulping *via* a radical polymerization pathway of catechol.

molecular weight of the oligomeric fragments shown, was essential so that the accumulated experimental data can be accurately represented (Table 2). The lack of typical native lignin side chains is also apparent in **ASKL**.

In relation to the degree of condensation that was determined and eventually required to be accommodated in the structures of Fig. 4, the following comparison with native wood lignin needs to be made. This comparison is aimed at revealing the salient features of kraft pulping chemistry that is to be further discussed. Based on the detailed quantitative ^{31}P NMR measurements of our earlier work,[8] **AIKL** and **ASKL** were determined to contain 4 and 6 times, respectively, more condensed phenolic hydroxy units when compared to native wood lignin.

The possible hypothesis that may be offered to account for the particularly elevated condensed phenolics and the low amount of side chains, particularly present in **ASKL**, is that this fraction of kraft lignin might be formed upon repolymerization of phenolic fragments. These fragments are released from the wood lignin during pulping that notably operates at about 160 °C and at pH values exceeding 12.5–13.0 over a period of several hours.

This contention is supported by early model compound studies that show that under kraft pulping conditions, side chain elimination occurs and activated C1 and C5 positions couple with each other since both of them are less hindered and rather reactive.[43,44]

It is also to be noted that the proposed structures contain one atom of sulfur as per our actual determinations of sulfur contents in the original lignin and the fractions. These were determined to be about 1.5% ± 0.1 by weight. The sulfur was thus placed in the proposed structures as per the suggestion of Marton,[12] being in the Cβ carbon of an alkylated side chain.

Our efforts to completely unify and accommodate the accumulated structural information with the measured elevated degrees of branching, phenolic moieties and the extensive biphenyl nature of the fractions were met with limitations when attempting to create truly representative structural models. For this reason, the ensuing discussion follows in order to provide further insight into these limitations paving the way towards further advances and eventually a deeper understanding of the structure of softwood kraft lignin.

As already demonstrated and discussed in earlier sections of this manuscript, the powerful nucleophilic character of the hydrosulfide anion present in kraft media causes extensive (10%) demethylation chemistry to operate on the methoxylated units of the lignin. Once demethylation and depolymerisation take place, the released products are substituted catechols.

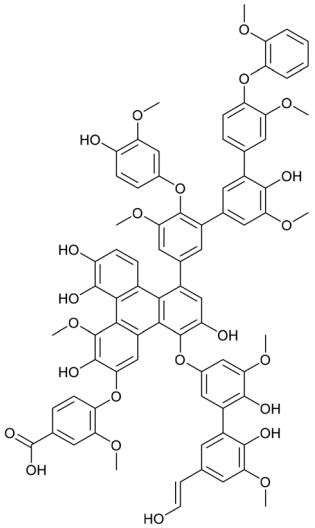


Fig. 5 A segment of softwood kraft lignin containing a possible polycyclic aromatic structure.

Catechols have been documented to polymerize *via* radical chemistry, creating, amongst others, carbon–carbon coupling products in the activated positions of the rings.[58] Unfortunately, the literature contains no detailed structural elucidation of polymerized catechols. The main reactivity difference between a guaiacyl (3-methoxy-4 hydroxyl) and a catechol structure has its origin in the substituent effects. In the guaiacols, the *meta* positions to the phenolic groups are deactivated towards radical coupling, while in catechols both the *ortho–para* and the *meta* positions are activated. Therefore, in principle, besides dimerization, further radical addition reactions with other catechol groups might occur with the formation of polycyclic aromatic rings. Scheme 7 attempts to display such a possible simplified reaction pathway. Admittedly, the reactions and the role of quinones, semiquinones and of quinidrone intermediates are not taken into account in Scheme 6 whose underlying mechanistic details should be considerably more complex.

Such transformations offer the means to rationalize and unify the overall data with the measured elevated degrees of branching, phenolic moieties and the extensive biphenyl character of the fractions and in particular the **ASKL** fraction. While only hypothetical, Fig. 5 attempts to show a segment of the acetone soluble fraction for softwood kraft lignin that might contain a polycyclic aromatic structure. Ongoing efforts

in our laboratories, involving pertinent methods for the detection of such moieties are currently underway.

Conclusions

The complex and highly heterogeneous nature and structure of softwood kraft lignin can be resolved by the careful application of quantitative 1D and 2D NMR, as well as size exclusion and mass spectrometric methods. Over the past several decades a large body of work has been carried out to account for the multitude of reactions that operate during kraft pulping. These accounts were used to further understand the structure of a carefully isolated softwood kraft lignin sample and its constituent fractions. With the exception of some small amounts of β-*O*-4′ and some minor amounts of stable carbon–carbon moieties such as phenyl coumaran (β-5′) and pinoresinols (β–β′), softwood kraft lignin and its fractions contain most of the structures invoked by past literature accounts such as stilbenes, aryl enol ethers as well as lignin–carbohydrate α-benzyl ethers and secoisolariciresinols. When this information was coupled to the determination of the molecular weights and the various other functional groups present in softwood kraft lignin, it was concluded that the structure of an isolated softwood kraft lignin has very little in common with the material it originated from. It is composed of a mixture of various oligomeric and polymeric fractions and as such, any effort to define a structural scheme for it will be nothing but an average oversimplification. For this reason we embarked at clearly defining the two major fractions of softwood kraft lignin, namely the acetone insoluble and the acetone soluble fractions. These fractions showed major differences amongst them thus providing important compositional information that significantly advanced our understanding of the overall starting material. The acetone soluble fraction is a significantly more branched and less polymeric material than the acetone insoluble fraction. The acetone insoluble fraction is a less branched polymeric material containing small amounts of native wood lignin bonding patterns. The quantitative bonding frequencies obtained, as well as all other relevant data, such as molecular weights, functional groups and degrees of branching allowed for two constitutional structural schemes to be proposed for the two distinct fractions of softwood kraft lignin. Overall, these structural schemes offer a much needed compilation of such information based on the application of state-of-the-art analytical methodologies.

Acknowledgements

The authors would like to gratefully acknowledge the EU NMR facility of Utrecht for technical assistance in the frame of the European Union project “EU-NMR-European Network of Research Infrastructures for Providing Access & Technological Advancement in Bio-NMR” FP-2005-RII3, Contract 026145.

References

1 G. Gellerstedt, D. Robert, V. D. Parker, M. Oivanen and L. Eberson, Quantitative 13C NMR Analysis of Kraft Lignins, *Acta Chem. Scand.*, 1987, **41b**, 541–546.
2 M. Y. Balakshin and E. A. Capanema, Comprehensive structural analysis of biorefinery lignins with a quantitative 13C NMR approach, *RSC Adv.*, 2015, **5**(106), 87187–87199.
3 L. Zhang and G. Gellerstedt, 2D Heteronuclear (1H–13C) Singe Quantum Correlation (HSQC) NMR Analysis of Norway Spruce Bark Components, in *Characterization of Lignocellulosic Materials*, ed. T. Q. Hu., Blackwell Publishing Ltd., Oxford, UK, 2008, pp. 1–16.
4 H. Kim and J. Ralph, Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO-d6/pyridine-d5, *Org. Biomol. Chem.*, 2010, **8**(3), 576–591.
5 J. C. del Río, P. Prinsen, J. Rencoret, L. Nieto, J. Jiménez-Barbero, J. Ralph, Á. T. Martínez and A. Gutiérrez, Structural Characterization of the Lignin in the Cortex and Pith of Elephant Grass (Pennisetum purpureum) Stems, *J. Agric. Food Chem.*, 2012, **60**(14), 3619–3634.
6 J. C. del Río, J. Rencoret, P. Prinsen, Á. T. Martínez, J. Ralph and A. Gutiérrez, Structural Characterization of Wheat Straw Lignin as Revealed by Analytical Pyrolysis. 2D-NMR. and Reductive Cleavage Methods, *J. Agric. Food Chem.*, 2012, **60**(23), 5922–5935.
7 M. Sette, R. Wechselberger and C. Crestini, Elucidation of Lignin Structure by Quantitative 2D NMR, *Chem. – Eur. J.*, 2011, **17**(34), 9529–9535.
8 C. Crestini, F. Melone, M. Sette and R. Saladino, Milled Wood Lignin: A Linear Oligomer, *Biomacromolecules*, 2011, **12**(11), 3928–3935.
9 M. Sette, H. Lange and C. Crestini, Quantitative HSQC Analyses of Lignin: A Practical Comparison, *Comput. Struct. Biotechnol. J.*, 2013, **6**, e201303016–e201303016.
10 C. Crestini and M. Sette, In depth structural elucidation of milled wood lignin by QQ-HSQC and ^{31}P-NMR, 16th International Symposium on wood Fiber and Pulping Chemistry Proceedings, ISWPC, 2011, vol. **1**, pp. 281–284.
11 A. Björkman, Isolation of Lignin from Finely Divided Wood with Neutral Solvents, *Nature*, 1954, **174**(4440), 1057–1058.
12 K. V. Sarkanen and C. H. Ludwig, joint author, *Lignins: occurrence. formation. structure and reactions*, Wiley-Interscience, New York, 1971, ch. 10, pp. 125–144.
13 J. Gierer, Chemical aspects of kraft pulping, *Wood Sci. Technol.*, 1980, **14**(4), 241–266.
14 T. Nakamura, H. Kawamoto and S. Saka, Condensation Reactions of Some Lignin Related Compounds at Relatively Low Pyrolysis Temperature, *J. Wood Chem. Technol.*, 2007, **27**(2), 121–133.
15 F. Berthold, E.-L. Lindfors and G. Gellerstedt, Degradation of Guaiacylglycerol-β-Guaiacyl Ether in the Presence of NaHS or Polysulphide at Various Alkalinities. Part II. Liberation of Coniferyl Alcohol and Sulphur, *Holzforschung*, 2009, **52**(5), 481–489.

16 J. Gierer and S. Wännström, Formation of Alkali-Stahle C-C-Bonds Between Lignin and Carbohydrate Fragments During Kraft Pulping, *Holzforschung*, 2009, **38**(4), 181–184.
17 K. Niemelä, GLC-MS Studies on Pine Kraft Black Liquors Part I. Identification of Monomeric Compounds, *Holzforschung*, 2009, **42**(3), 169–173.
18 S. Sarkanen, D. C. Teller, C. R. Stevens and J. L. McCarthy, Lignin. 20. Associative interactions between kraft lignin components, *Macromolecules*, 1984, **17**(12), 2588–2597.
19 D. Ibarra, M. I. Chávez, J. Rencoret, J. C. Del Río, A. Gutiérrez, J. Romero, S. Camarero, M. J. Martínez, J. Jiménez-Barbero and A. T. Martínez, Lignin Modification during Eucalyptus globulus Kraft Pulping Followed by Totally Chlorine-Free Bleaching: A Two-Dimensional Nuclear Magnetic Resonance. Fourier Transform Infrared. and Pyrolysis–Gas Chromatography/Mass Spectrometry Study, *J. Agric. Food Chem.*, 2007, **55**(9), 3477–3490.
20 D. Kanungo, R. C. Francis and N.-H. Shin, Mechanistic Differences Between Kraft and Soda/AQ Pulping. Part 2: Results from Lignin Model Compounds, *J. Wood Chem. Technol.*, 2009, **29**(3), 227–240.
21 P. M. Froass and A. J. Ragauskas, NMR Studies Part 3: Analysis of Lignins from Modern Kraft Pulping Technologies, *Holzforschung*, 1998, **52**(4), 385–390.
22 On the formation of enol ether structures in lignin during kraft cooking [structural changes in lignin during kraft cooking. 6. acidolysis. Hibbert ketones. homovallin. ···] http://www.academia.edu/18129808/On_the_formation_of_enol_ether_structures_in_lignin_during_kraft_cooking_structural_changes_in_lignin_during_kraft_cooking_6_acidolysis_Hibbert_ketones_homovallin_ (accessed Jan 10. 2016).
23 J. Parkås, G. Brunow and K. Lundquist, Quantitative Lignin Analysis Based on Lignin Degradation Products: Permanganate Oxidation, *BioResources*, 2007, **2**(2), 169–178.
24 D. R. Robert, M. Bardet, G. Gellerstedt and E. L. Lindfors, Structural Changes in Lignin During Kraft Cooking Part 3. On the Structure of Dissolved Lignins, *J. Wood Chem. Technol.*, 1984, **4**(3), 239–263.
25 G. Gellerstedt and K. Gustafsson, Structural Changes in Lignin During Kraft Cooking. Part 5. Analysis of Dissolved Lignin by Oxidative Degradation, *J. Wood Chem. Technol.*, 1987, **7**(1), 65–80.
26 G. Gellerstedt and E.-L. Lindfors, Structural Changes in Lignin During Kraft Pulping, *Holzforschung*, 2009, **38**(3), 151–158.
27 D. S. Argyropoulos, Heteronuclear NMR Spectroscopy of Lignins, in *Lignin and Lignans*, CRC Press, 2010, pp. 245–265.
28 A. Granata and D. S. Argyropoulos, 2-Chloro-4.4.5.5-tetramethyl-1.3.2-dioxaphospholane. a Reagent for the Accurate Determination of the Uncondensed and Condensed Phenolic Moieties in Lignins, *J. Agric. Food Chem.*, 1995, **43**(6), 1538–1544.
29 D. S. Argyropoulos, ^{31}P NMR in Wood Chemistry: a Review of Recent Progress, *Res. Chem. Intermed.*, 1995, **21**(3), 373–395.
30 M. Y. Balakshin, E. A. Capanema, C.-L. Chen and H. S. Gracz, Elucidation of the structures of residual and dissolved pine kraft lignins using an HMQC NMR technique, *J. Agric. Food Chem.*, 2003, **51**(21), 6116–6127.
31 P. Tomani, The Lignoboost Process, *Cellul. Chem. Technol.*, 2010, **44**(1–3), 53–58.
32 C. Cui, R. Sun and D. S. Argyropoulos, Fractional Precipitation of Softwood Kraft Lignin: Isolation of Narrow Fractions Common to a Variety of Lignins, *ACS Sustainable Chem. Eng.*, 2014, **2**(4), 959–968.
33 J. Asikkala, T. Tamminen and D. S. Argyropoulos, Accurate and Reproducible Determination of Lignin Molar Mass by Acetobromination, *J. Agric. Food Chem.*, 2012, **60**(36), 8968–8973.
34 H. Lange, F. Rulli and C. Crestini, Gel Permeation Chromatography in Determining Molecular Weights of Lignins: Critical Aspects Revisited for Improved Utility in the Development of Novel Materials, *ACS Sustainable Chem. Eng.*, 2016, **4**(10), 5136–5151.
35 D. J. Peterson and N. M. Loening, QQ-HSQC: a quick. quantitative heteronuclear correlation experiment for NMR spectroscopy, *Magn. Reson. Chem.*, 2007, **45**(11), 937–941.
36 H. Lange, P. Schiffels, M. Sette, O. Sevastyanova and C. Crestini, Fractional Precipitation of Wheat Straw Organosolv Lignin: Macroscopic Properties and Structural Insights, *ACS Sustainable Chem. Eng.*, 2016, **4**(10), 5136–5151.
37 E. A. Capanema, M. Y. Balakshin and J. F. Kadla, Quantitative Characterization of a Hardwood Milled Wood Lignin by Nuclear Magnetic Resonance Spectroscopy, *J. Agric. Food Chem.*, 2005, **53**(25), 9639–9649.
38 *Lignin and Lignans: Advances in Chemistry*, ed. C. Heitner, D. Dimmel and J. Schmidt, CRC Press, 2010.
39 A. Berlin and M. Balakshin, Industrial lignins: analysis, properties, and applications, *Bioenergy Res.: Adv. Appl.*, 2014, 500.
40 G. Gellerstedt, Softwood kraft lignin: Raw material for the future, *Ind. Crops Prod.*, 2015, **77**, 845–854.
41 L. Reina, A. Galetta, V. Vinciguerra, F. Resquin and P. Menéndez, The relationship between Eucalyptus grandis lignin structure and kraft pulping parameters, *J. Anal. Appl. Pyrolysis*, 2014, **107**, 284–288.
42 A. Oudia, E. Mészáros, E. Jakab, R. Simões, J. Queiroz, A. Ragauskas and L. Novák, Analytical pyrolysis study of biodelignification of cloned Eucalyptus globulus (EG) clone and Pinus pinaster Aiton kraft pulp and residual lignins, *J. Anal. Appl. Pyrolysis*, 2009, **85**(1–2), 19–29.
43 G. Gellerstedt, A. Majtnerova and L. Zhang, Towards a new concept of lignin condensation in kraft pulping. Initial results, *C. R. Biol.*, 2004, **327**(9–10), 817–826.
44 A. Majtnerova and G. Gellerstedt, Radical coupling - A major obstacle to delignification in kraft pulping, *Nord. Pulp Pap. Res. J.*, 2006, **21**(1), 129–134.
45 D. R. Dimmel, L. F. Bovee and B. N. Brogdon, Electron Transfer Reactions in Pulping Systems (VII): Degradation Reactions of β-Methoxy Lignin Models, *J. Wood Chem. Technol.*, 1994, **14**(1), 1–15.

46 K. Koljonen, M. Österberg, M. Kleen, A. Fuhrmann and P. Stenius, Precipitation of lignin and extractives on kraft pulp: effect on surface chemistry. surface morphology and paper strength, *Cellulose*, 2004, **11**(2), 209–224.
47 S. C. Fox and A. G. McDonald, Chemical and Thermal Characterization of three Industrial Lignins and their Corresponding Lignin Esters, *BioResources*, 2010, **5**(2), 990–1009.
48 F. Yue, F. Lu, S. Ralph and J. Ralph, Identification of 4-O-5-Units in Softwood Lignins via Definitive Lignin Models and NMR, *Biomacromolecules*, 2016, **17**(6), 1909–1920.
49 A. S. Hay and B. M. Boulette, Reaction of sulfur with 2.6-di-substituted phenols, *J. Org. Chem.*, 1976, **41**(10), 1710–1712.
50 F. S. Chakar and A. J. Ragauskas, Review of current and future softwood kraft lignin process chemistry, *Ind. Crops Prod.*, 2004, **20**(2), 131–141.
51 Lignin and Lignans: Advances in Chemistry https://www.crcpress.com/Lignin-and-Lignans-Advances-in-Chemistry/Heitner-Dimmel-Schmidt/p/book/9781574444865 (accessed May 12. 2017).
52 D. S. Argyropoulos, The Emerging Bio-Refinery Industry Needs to Refine Lignin Prior to Use, *J. Biotechnol. Biomater.*, 2014, **S6**, e001.
53 D. S. Argyropoulos and H. I. Bolker, The Gel Degradation Theory. Part III. An Experimental Kinetic Verification, *J. Wood Chem. Technol.*, 1987, **7**(4), 499–511.
54 D. S. Argyropoulos and H. I. Bolker, Gel degradation theory. 1. An experimental verification with a model tri-functional network, *Macromolecules*, 1987, **20**(11), 2915–2922.
55 H. Sadeghifar and D. S. Argyropoulos, Macroscopic Behavior of Kraft Lignin Fractions: Melt Stability Considerations for Lignin–Polyethylene Blends, *ACS Sustainable Chem. Eng.*, 2016, **4**(10), 5160–5166.
56 A. Guerra, A. R. Gaspar, S. Contreras, L. A. Lucia, C. Crestini and D. S. Argyropoulos, On the propensity of lignin to associate: A size exclusion chromatography study with lignin derivatives isolated from different plant species, *Phytochemistry*, 2007, **68**(20), 2570–2583.
57 S. Contreras, A. R. Gaspar, A. Guerra, L. A. Lucia and D. S. Argyropoulos, Propensity of Lignin to Associate: Light Scattering Photometry Study with Native Lignins, *Biomacromolecules*, 2008, **9**(12), 3362–3369.
58 P. Conte, Structural Characterization of Isomeric Dimers from the Oxidative Oligomerization of Catechol with a Biomimetic Catalyst, *Biomacromolecules*, 2007, **8**, 737–743.

