Revealing the fate of the phenylcoumaran linkage during lignin oxidation reactions

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Abstract

The fate of most lignin linkages, other than the β-O-4, under selective oxidation conditions is largely unknown. In this work we use advanced β-5 lignin model compounds to identify the fate of phenylcoumaran units in a softwood lignin during oxidation with DDQ. By using model compounds combined with detailed characterisation of the oxidised lignin polymer using HSQC and HMBC NMR we show that phenylcoumarones are a major product, and therefore constitute a novel non-native β-5 linkage in oxidised lignins. Additionally, the reactivity of these units in lignin led us to further investigate their connectivity in lignin, showing that they are found as both phenolic and etherified units. The findings and approach developed here will help improve the efficiency of selective oxidative lignin depolymerisation processes, particularly those aimed at the upgrading of softwood lignin in which phenylcoumarans are a major linkage.

Introduction

The depolymerisation of lignin to monomers is increasingly being targeted as a route to renewable aromatic chemicals. Currently various methodologies are being explored to achieve this including

reductive,[1–4] oxidative[5–9] and redox neutral approaches.[10–14] One general approach, which has been shown to provide a handle for selective depolymerisation, involves selective oxidation of the benzylic[5–7] or primary alcohols[15] in the β-aryl ether (β-O-4) linkage prior to a second depolymerisation step which generates relatively simple mixtures of monomeric products. The has been achieved using a number of catalytic systems including DDQ/tert-butyl nitrite/O_2,[7,16,17] 4-acetamido-TEMPO/HCl/HNO_3/O_2,[6] DDQ/NHPI/NaO_2/O_2,[8] electrochemical NHPI/2,6-lutidine[9] and [Ir(bipyridinate)Cp*].[18] These oxidative approaches all target the most abundant and labile β-O-4 linkage in lignin, with the outcome, particularly for benzylic oxidation methods, being well characterised in lignin using 2D HSQC NMR methods.[6,7,19] To date, however, little attention has been paid to the fate of the other lignin linkages, particularly phenylcoumarans, during lignin oxidation reactions. Interestingly, previous 2D NMR studies have clearly shown that that these linkages are not inert under the oxidations conditions examined to date.[6,7] Consequently, establishing their fate may hold the key to improving the efficiency of lignin depolymerisation reactions and enable the identification of new aromatic products from lignin.

This becomes particularly apparent when we consider, for example, softwood lignins where β-5 linkages make up 9-12% of all the linkages present in the native lignin.[20] Bearing in mind that each β-5 linkage results from the formation of a recalcitrant C-C bond between two C9 units, up to 24% of all aromatic groups may be considered to be part of such linkages. Therefore, it is of utmost importance to understand their fate in order to improve the efficiency of lignin valorisation processes. This inspired us to examine the behaviour of the phenylcoumaran linkage during lignin oxidation processes, in this case using DDQ which has been shown to be a versatile oxidant for lignin[7,19,21–25] and, to date, the most commonly used reagent for the selective benzylic oxidation of lignin. Here we chose to use stoichiometric DDQ for our investigations as this allows for fine control of the reaction conditions and removes some of the complexity involved in achieving catalytic oxidation reactions.

Results and Discussion

Oxidation of β-5 model compounds with DDQ

We started by considering the most appropriate models to use for the phenylcoumaran unit in lignin (Figure 1A) and applied the 'simple' models **1** and **2**, representing possible end group type units bearing dihydroconiferyl alcohol type side chains, and the previously reported more complex models **3** and **4**,[26] representing internal phenylcoumaran units next to the most abundant β-O-4 units (Figure 1B).

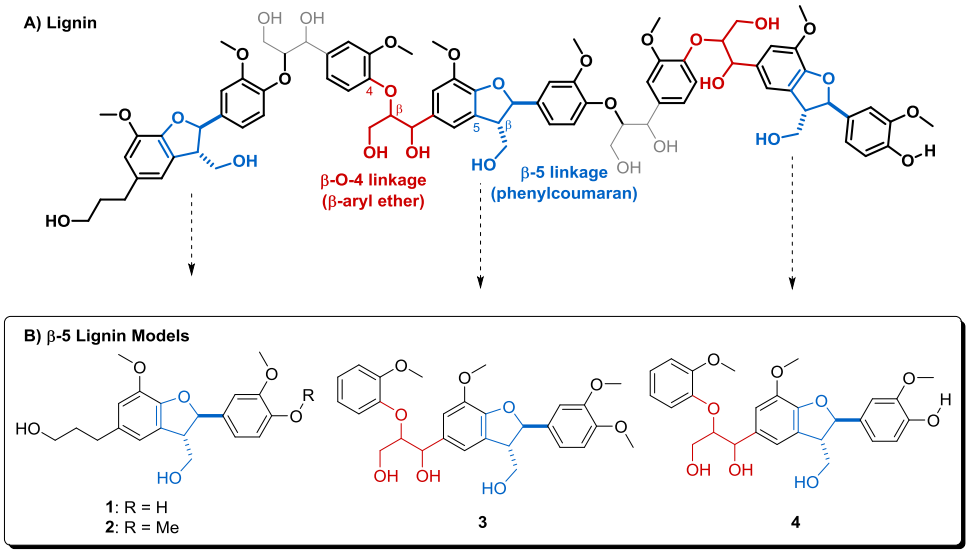
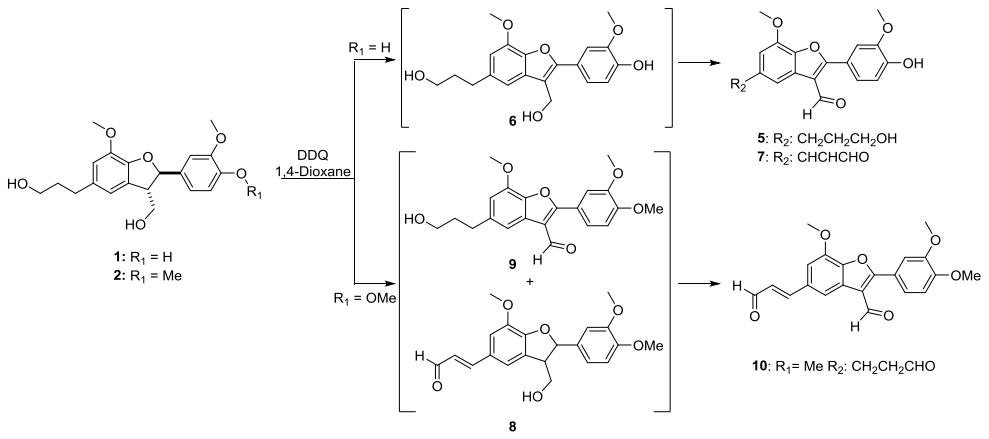


Figure 1: A) A schematic of a possible short softwood lignin molecule showing the major β-O-4 and β-5 linkages. (only intended for illustrative purposes; it does not model real linkage abundances nor does it include any of the other lignin structural units such as β-β, 4-O-5, dibenzodioxocins etc). B) Models for various different β-5 unit environments found in the lignin polymer.

Initial treatment of phenolic model **1** with 3 eq. of DDQ at room temperature showed that a clean double oxidation of the phenylcoumaran moiety occurred giving aldehyde **5** in moderate isolated yield (Scheme 1). The presumed intermediate benzofuran-alcohol **6** was not detected during these experiments, indicating rapid oxidation of this intermediate to **5** probably occurs under the reaction conditions. We also investigated the reactivity of this model at an elevated temperature of 60 °C which resulted in additional oxidation of the propanol side chain to give α,β-unsaturated aldehyde **7** as the

major product when excess DDQ was used. Similar reactivity has previously been observed for the oxidation of aryl propanes with DDQ.[27]

The reactivity of non-phenolic model **2**, i.e. a model for an 'internal' β-5 linkage in lignin, was also examined under identical conditions. This revealed some very noticeable differences between phenolic and etherified models. In particular, treatment of **2** with DDQ at room temperature yielded an intractable mixture of **8** and **9** resulting from competitive oxidation of either the propanol side chain or the phenylcoumaran ring, but not both. This indicates that oxidation of each moiety deactivates the other towards further oxidation. This is consistent with the electron withdrawing nature of the newly installed functional groups in **8** and **9** reducing the reactivity of the substrate towards further single electron oxidation by DDQ. This also shows that, relative to **1**, methylation of the phenolic group in **2** deactivates the phenylcoumaran ring towards oxidation, consistent with these reactions proceeding through initial formation of benzylic carbocation intermediates which are more effectively stabilised by phenolic groups (**1**) than methyl ethers (**2**).Furthermore, when heating the reaction to 60 °C a double oxidation of both the side chain and phenylcoumaran ring was observed,as seen for **1**, giving **10** as the major product.



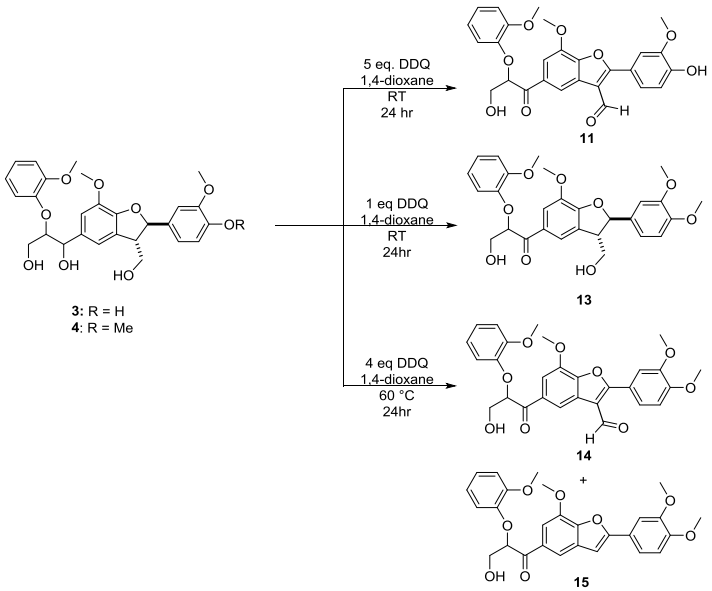
Scheme 1: Reactivity of the model compounds **1** and **2** under DDQ oxidation conditions. *Reaction Conditions and Isolated Yields*: **1** to **5**: 3 eq. DDQ, 1,4-dioxane, room temperature, 24 hours, 57%; **1** to **7**: 10 eq. DDQ, 1,4-dioxane, 60 °C, 24 hours, 17%; **2** to **10**: 10 eq. DDQ, 1,4-dioxane, 60 °C, 24 hours, 44%. The equivalents of DDQ used for each reaction was determined by sequentially increasing the equivalents of DDQ used for each oxidation until an end point was reached (see Figures S1-S4

for crude ^{1}H NMR analyses). The relatively low isolated yields for these reactions appear to reflect the difficulty in isolating and purify these products rather than extensive side products formation or poor conversion.

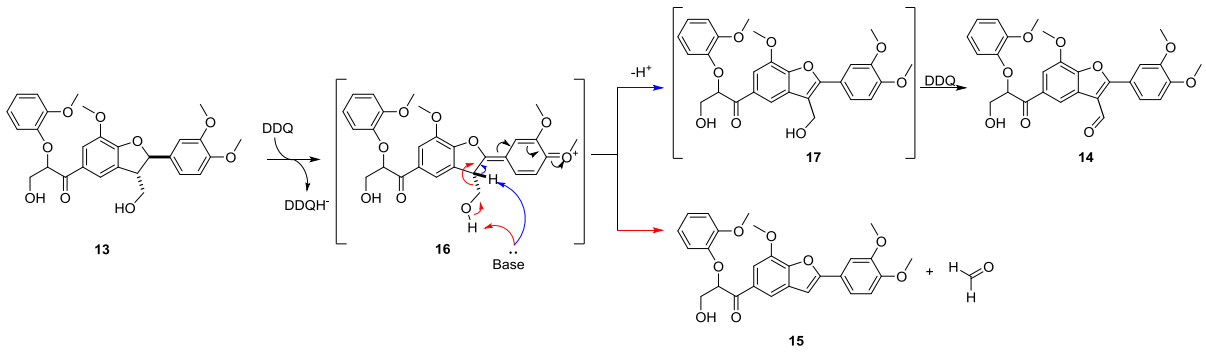
Whilst models **1** and **2** served as a convenient starting point for our investigations and closely resemble some of the end-groups that will be found in lignin, we were aware that, based on the proposed nature of lignin biosynthesis,[28] that most linkages will be 'internal' in the lignin polymer (Figure 1A). We therefore studied the reactivity of the more complex β-O-4-β-5 models **3** and **4** (Scheme 2).[26]

Treatment of phenolic model **3** with DDQ at room temperature resulted in concurrent oxidation of both the benzyl alcohol in the β-O-4 moiety and the phenylcoumaran ring, giving mixtures of oxidation products when using 1-3 eq. of DDQ (Figure S5), but **11** as the major product with 5 eq. Comparing the reactivity of non-phenolic model **4** again showed substantial differences, with a very selective oxidation of the benzylic alcohol in the β-O-4 moiety being observed at room temperature to give **13** in high yield when using 1 eq. of DDQ. This suggests, as might be expected, that oxidation of the benzyl alcohol in the β-O-4 linkage is more facile than oxidation of the propanol side chain in **2** and that both oxidations have a similar deactivating effect on the adjacent phenylcoumaran ring towards further oxidation. Indeed, treating **4** with increasing equivalents of DDQ (2-3 eq.) only gave traces of phenylcoumaran oxidation product **14**, as determined by crude NMR analysis (Figure S6).

Similar to the reactivity observed for **2**, when this reaction was heated to 60 °C oxidation of both the phenylcoumaran ring and the benzyl alcohol of the β-O-4 moiety was observed, giving **14** as the major product**.** Interestingly, an additional product **15** was identified, presumably arising from a retro-aldol type reaction of a cationic intermediate resulting from a formal hydride abstraction by DDQ during the reaction (Scheme 3).



Scheme 2: Reactivity of the model compounds **3** and **4** under DDQ oxidation conditions. *Isolated Yields:* **11**: 30%, **13**: 69%, **14**: 38%, **15**: 23%. The equivalents of DDQ used for each reaction was determined by sequentially increasing the equivalents of DDQ used for each oxidation until an end point was reached (see Figures S5-S7 for crude ^{1}H NMR analyses). The relatively low isolated yield for **11** reflects the difficulty in isolating and purify this compound rather than extensive side product formation or poor conversion.



Scheme 3: Proposed mechanism for the competitive formation of **14** and **15** during the DDQ oxidation of **4**.

Reactivity of β-5 units in Softwood Lignin

Having established the reactivity of β-5 linkages towards DDQ oxidation using our advanced model compounds, we proceeded to study the oxidation of lignin. We therefore isolated a Douglas fir lignin using a mild dioxasolv process[29] which, being a softwood lignin, contained relatively large amounts of both β-O-4 and β-5 linkages as judged by ^{1}H-^{13}C HSQC NMR (Figure 2A). We then treated this lignin

under comparable conditions to our model studies and analysed the resulting oxidised lignins using 2D NMR methods.

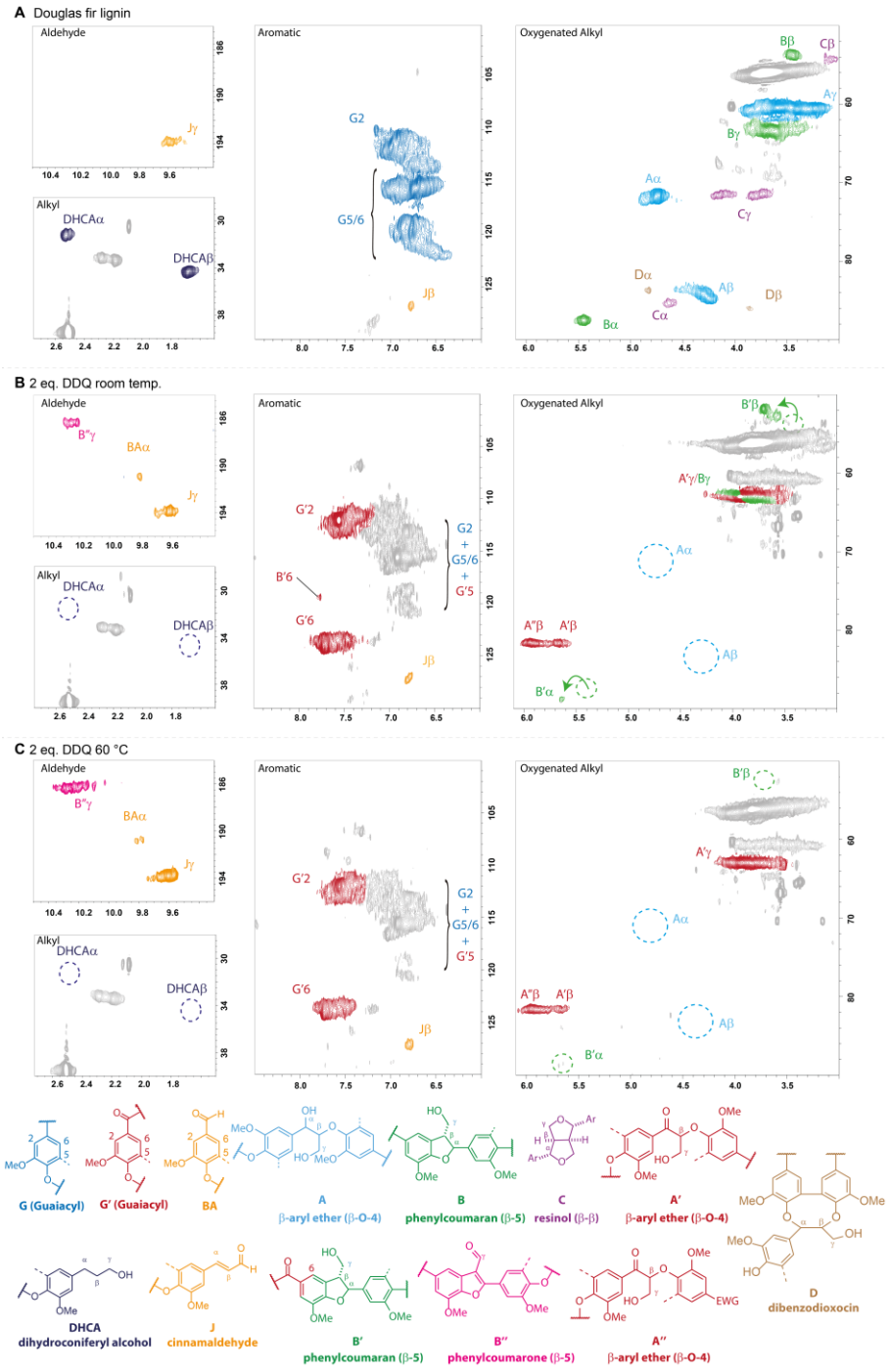


Figure 2: A) 2D HSQC analysis of dioxosolv Douglas fir lignin, (see SI for extraction procedure). The individual linkages are assigned based on relevant literature.[30] Unassigned cross peaks are shaded grey. B) 2D HSQC analysis of oxidised dioxasolv Douglas fir lignin. *Reaction conditions*: 2 weight equivalents, DDQ, 1,4-dioxane, RT, 24 hours. C) 2D HSQC analysis of oxidised

dioxasolv Douglas fir lignin. *Reaction conditions*: 2 weight equivalents DDQ, 1,4-dioxane, 60 °C, 24 hours. NMR – 700 MHz, d_6-DMSO.

HSQC NMR analysis of our Douglas fir lignin treated at room temperature with 1 weight equivalent of DDQ (~0.85 equivalents based on total lignin C_9 units) showed only partial oxidation had occurred (Figure S8) and so we focussed our attention of reactions run with 2 weight equivalents of DDQ. This resulted in near complete oxidation of the β-O-4 linkages to benzylic ketones, with two characteristic cross peaks observed for the β-position in the HSQC resulting from different degrees of oxidation of adjacent groups (**Figure 2B**).[7,19] We could additionally identify that some phenylcoumaran units were retained in this lignin. Interestingly though, a shift in the α- and β- cross peaks of this linkage was observed which we could assign, based on our model compound studies, to structures similar to **13**, i.e. phenylcoumarans adjacent to an electron withdrawing group (Figure S9). Furthermore, we could also observe a diagnostic aromatic cross peak for such structures in the oxidised lignin (B´6 in Figure 2B), further supporting their assignment in lignin. Additionally, the same cross peaks could clearly be observed after DDQ oxidation of a model polymer containing only β-O-4 and β-5 units (See Figure S10).[31] Given this observation, it is interesting to note the potential future utility of selective oxidation reactions in establishing bonding patterns in lignin which are otherwise difficult to access via other methods, specifically β-O-4 units adjacent to β-5 units. Gratifyingly, when we examined the aldehyde region of the HSQC spectrum we could observe small amounts of a new aldehyde species which, based on our model studies, could be assigned to structures similar to models **5**/**14**, i.e. oxidised phenylcoumaran units (phenylcoumarones).

Analysis of the alkyl region of the HSQC spectrum also revealed a complete disappearance of cross peaks assigned to the propanol end groups (dihydroconiferyl alcohol units) in lignin. Based on the reactivity of these structural units in models **1** and **2** it seems likely that these are being oxidised to α,β-unsaturated aldehydes (cinnamaldehydes) during the reaction, however the presence of such units in the starting lignin and small amounts of cinnamyl alcohol units, which may also be oxidised to such aldehydes, makes confirmation of this reactivity in lignin difficult.

Additionally, reactions performed at 60 °C resulted in a relatively more intense aldehyde cross peak corresponding to the phenylcoumarones and the disappearance of all cross peaks assigned to unoxidised phenylcoumarans (**Figure 2C**). In line with our model studies, this indicated a more efficient oxidation of this structural unit at 60 °C. As the assignment of phenylcoumarones in lignin was made based only on one aldehyde cross peak, HMBC experiments were used to further support this assignment (**Figure 3**). This HMBC experiment showed, by comparison of lignin spectra to those obtained for model **14**, that the aldehyde cross peak observed in the HSQC NMR does indeed correspond to oxidised phenylcoumarones.

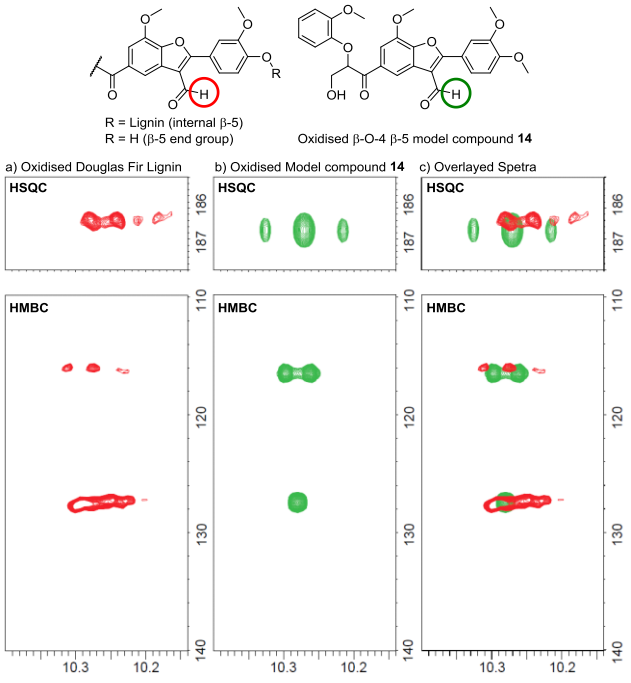


Figure 3: Comparison of the appropriate regions of the HSQC and HMBC spectra from oxidisied Douglas fir lignin with the oxidised β-5 model compound **14**. NMR – 700 MHz, *d6*-DMSO. For further comparison see Figure S11.

Taken all together these results point towards two general types of phenylcoumarans being present in lignin, which can be defined based on their propensity towards oxidation with DDQ; namely those that are oxidised at room temperature and those which are only oxidised at higher temperatures. There have been some recent suggestions, based on DFT calculations, that phenylcoumarans should only be found as phenolic end groups in lignin.[32] Based on our model studies all such units should be

oxidised even at room temperature, which is clearly not the case. In order to probe this further we analysed an acetylated sample of our Douglas fir lignin. This showed that two different types of phenylcoumarans are present in the lignin corresponding to aromatic ethers and phenolic groups (**Figure 4**), as has previously been suggested but now confirmed.[33] Furthermore quantitative ^{1}H NMR analysis of this acetylated lignin suggests that the ratio of etherified to phenolic phenylcoumarans is approximately 3 : 1 (See Figure S12). In order to try to understand better how different phenylcoumarans are incorporated into lignins, a survey of trilignols was undertaken (Supporting Information Section 8.0). This suggested that the propensity of different phenylcoumaran units to undergo oxidative coupling reactions varies considerably depending on their structure, however none were found to be completely excluded from oxidative coupling reactions. Consequently, as during lignification monolignols are delivered only slowly to the growing lignin chain, oxidation of phenolic phenylcoumaran end group units is unlikely to be a limiting factor and resulting in both phenolic and etherified structures in natural lignins.

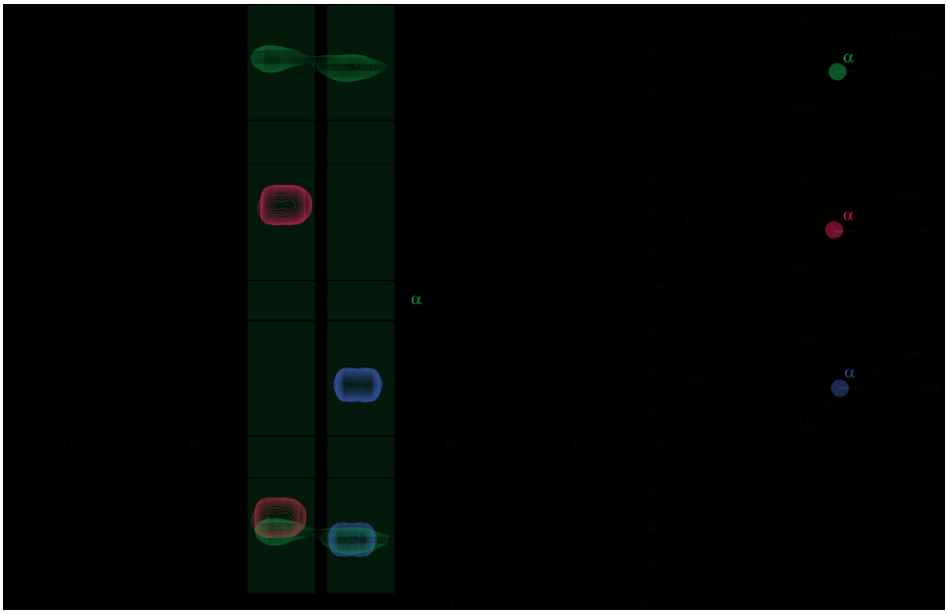


Figure 4: A comparison of the key α ^{1}H-^{13}C cross peaks of the β-5 linkage in A) an acetylated Douglas fir lignin; B) an acetylated phenolic model (**18**); C) an acetylated etherified model (**19**) and D) and an overlaid spectrum of all three. NMR all in $CDCl_3$.

Conclusions

Using both simple and more complex β-5 model compounds we have shown, for the first time, that the reactivity of this structural unit in lignin can be traced during oxidation with DDQ. In particular we showed that the formation of novel β-5 derived phenylcoumarone-aldehydes analogous to e.g. model **11** could be identified in lignin, the assignment of which was confirmed through detailed HSQC and HMBC NMR studies. We also showed how the effects of oxidation of structural units adjacent to phenylcoumarans can subtlety, but diagnostically, affect the chemical shifts of cross peaks observed for this unit in the HSQC NMR. The results of these studies were then related back to the structural characteristics of these units in lignin and hence how these units are incorporated into lignin during its biosynthesis.

Given that the β-5 linkage is the second most abundant structural unit in softwood lignin after the β-O-4 linkages, being able to establish and track its reactivity will be very important in realising efficient utilisation of such lignins, either through depolymerisation reactions or in material applications. The work presented here is the first step towards a comprehensive understanding of the chemistry of this

linkage during oxidation reactions and shows that by using appropriately complex models the chemistry of these units in lignin can be traced.

Acknowledgements

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