

Conundrums regarding 5–O–4-linkages in softwood MWLs

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ABSTRACT

Recent investigation of α -hydroxy 5–O–4-lignin model compounds by NMR indicates that the short-range ^{13}C – ^1H correlations for the ring 2- and 6-carbons should be distinct in HSQC NMR spectra of softwood MWLs and possibly in guaiacyl-type synthetic lignins (DHPs). The levels of 5–O–4-type linkages have been reported to be on the order of 1–7% of C9 units for MWLs. A comparison of the aromatic spectral regions from the short-range ^{13}C – ^1H correlation spectra of α -hydroxy 5–O–4-models and loblolly pine MWL suggests that 5–O–4-type units are either not present at the suggested levels or are not present with the α -hydroxy functionality. The HSQC NMR spectral data for a CAD-deficient pine MWL has interesting correlations in this region of interest potentially assignable to 5–O–4-units involving dihydroconiferyl alcohol and/or guaiacylpropane-1,3-diols.

INTRODUCTION

The investigation of wood meal and MWL (milled wood lignin) by thioacidolysis coupled with mass spectrometry has given wood chemists the most useful method to date for qualitative and quantitative information regarding the nature of the monomeric lignin units and the linkages between these units. Aside from the most prevalent β –O–4-linkages (c.a. 50%) which are cleaved during thioacidolysis, the residual dimers considered as condensed units, are dominated by the 5–5-, β –5- and β –1-linkages at 33, 31 and 25% respectively (relative % of the dimer fraction in spruce). Of special interest are the estimates for the 5–O–4- and β – β -linkages which are at 6 and 5%.¹ The estimate for the 5–O–4 contribution based on the entire MWL fraction i.e. 3% correlates well with figures for spruce MWL reported previously by Adler,² and with values recalculated from Larsson and Miksche's data (spruce lignin)³ by Parkäs, Lundquist and Brunow (personal communication).

Thioacidolysis coupled with ^{31}P NMR indicated that 71% of wood meal and 77% of MWL from P. radiata could be quantified and comparisons of the condensed and uncondensed units were made.⁴ Of

the condensed units, the 5–O–4-linkage was calculated to be about 5% of the C9 units for both MWL and wood meal.

Wood chemists have also turned to non-degradative methods of analysis such as 1D and 2D NMR of MWLs. Overlapping signals in both proton and carbon spectra and the inherent insensitivity of the ^{13}C experiment has hindered using 1D NMR for qualitative and quantitative analyses. The use of specific 2D experiments taking advantage of relatively recent advances in gradient pulses and inverse detection has clarified areas of the spectrum that were previously obscured due to overlapping signals and insensitivity. Using these experiments, much new information has been added concerning linkages such as those of the spirodienone/ β –1 and dibenzodioxocin type structures.^{5–7}

Recent efforts have begun to quantify the unit types from these 2D spectra^{8,9} and as always the preparation and analysis of appropriate models is essential for qualitative and quantitative purposes. The assignment of the C–H correlations of the 2D HSQC (short range) experiment from model compounds has diagnostically mapped the sidechain region which covers the most prevalent of the linkages such as the β –O–4, β –5 and β – β .¹⁰ The less well known β –1/spirodienone and the dibenzodioxocin linkages can also be identified.

Two of the linkages, the 5–O–4-biphenyl ether and the 5–5-biphenyl are however more difficult to identify using the HSQC experiment as only protons and their directly attached carbons are correlated—the linkage of interest involves non-protonated carbons. An observation by Smit, et al.⁴ that the 5–O–4-linked units would have similarities with syringyl components with respect to their NMR characteristics, suggests that the 2- and 6-correlations for these units could be recognized easily in the aromatic region of a guaiacyl-only lignin.

EXPERIMENTAL

Model Compounds

Models **A**, **C**, **E**, **G**, **H** and **I** were either wholly or in part isolated from reaction mixtures prepared by John Pew and William Connors.¹¹ They produced models and DHPs using various phenylpropanoid or phenylethanoid monomers via horseradish peroxidase and hydrogen peroxide.

Compound **C** was isolated from a reaction with acetovanillone as the starting monomer. Typically a reaction mixture consisted of 60% biphenyl, 16%

starting material and 24% resin. The “resin” fraction described by Pew was acetylated and column-chromatographed to yield about 4 mg of compound C from 50 mg.

The basic dimers for **E**, **H** and **I** were isolated by Pew and were acetylated for NMR purposes using equal amounts of acetic anhydride/pyridine or methylated with methyl iodide/potassium carbonate.

Compound **G** was isolated by Pew from a reaction where propioguaiacone was the starting material. The compound was reduced with NaBH₄ and then acetylated as above.

The NMR chemical shift data for compound **B** was reported by Peng.¹² The dimer was isolated from loblolly pine wood, which had been subjected to large-scale derivatization followed by reductive cleavage or “DFRC” analysis.

Compounds **D** and **F** were prepared from apocynol and vanillyl alcohol respectively using silver oxide as described by Ralph.¹¹ **D** was subsequently methylated after isolation.

Full NMR data for compounds **E**, **F**, **G**, **H** and **I** is available.¹⁴

Compound **A** was prepared by using the existing unacetylated dimer **E**. The dimer (15 mg) was combined with coniferyl alcohol (CA) (135 mg) in a 30% acetic acid/methylene chloride solvent system (20 ml) to which was added H₂O (100 μ l) and Mn(OAc)₃·2H₂O (364 mg, portion-wise over 90 minutes) followed by 60 minutes of stirring. The reaction mixture was extracted, dried, acetylated and separated by GPC chromatography to yield about 40% DHP, 11% oligomers (4–6 C₉ units) and 27% trimers and dimers.

Lignin HSQC Spectra

Acetylated milled wood lignins isolated from loblolly pine and the CAD-deficient mutant described previously¹⁵ were used for this study.

¹H-, ¹³C and 2D-NMR spectra were obtained with a Bruker DPX-250 spectrometer using standard Bruker pulse sequences. A quadrupolar 5mm probe with a Z-gradient coil was used for all samples. The samples were dissolved in 400 μ l of acetone-d₆ and the central solvent peak (δ_H 2.04, δ_C 29.83) was used as the internal reference.

RESULTS AND DISCUSSION

The results of thioacidolysis-MS analyses, as well as the isolation of 5–O–4–linked coniferyl alcohol by the DFRC method¹² suggest that the 5–O–4–dimer has, at least in part, a β –O–4–sidechain and has a phenolic ether linkage to the β -position of another C₉ unit. Other trimeric units consisting of (5–O–4)/(β –5) and (5–5)/(4–O–5) have been isolated and identified.¹⁶

A series of 5–O–4–models **A–I**, Figure 1, were isolated, borrowed or synthesized for NMR analysis (see EXPERIMENTAL.). The objective was to find a range for the 2- and 6-carbon/proton chemical shifts taking in to account different functionalities on the sidechain and phenolic-OH substitutions. The available data are presented in Table 1.

Table 1. NMR chemical shifts for C₂/H₂ and C₆/H₆ for 5–O–4–model compounds illustrated in Figure 1.

model	C ₂ /H ₂	C ₆ /H ₆
A	105.2/6.69	107.6/6.25
B	105.5/6.96	109.1/6.48
C	108.2/7.48	112.0/7.15
D	109.1/7.48	113.8/7.23
E	105.0/6.77	107.9/6.32
F	108.9/7.42	114.5/7.11
G	104.3/6.64	106.1/6.20
H	107.3/6.63	110.2/6.18
I	109.2/6.58	111.1/6.17

Figure 1 shows the short range C/H correlations in the aromatic region of an acetylated loblolly pine MWL run in d₆-acetone. Superimposed over the spectrum are the chemical shift values from Table 1. None of the 5–O–4 model correlations appear to fit with the pine spectrum. Comparing models **C**, **E** and **H** where alpha changes from a ketone to an alcohol to a methylene, still puts the C₂/H₂ correlations in a clear area of the spectrum. Comparing model **A** with **E** shows only small differences in chemical shift occur when the phenolic OH is etherified and again the correlations are in clear areas of the spectrum. This would suggest that the 5–O–4 units in loblolly pine MWL have little in common with even the best of the models studied (**A**) or that the 5–O–4 content is very low.

Two of the C/H correlations present in the pine, 105.0/6.85 and 107.0/6.95 have reasonable matches to syringyl components. The correlation at 105.0/6.85 ppm fits well with the 2,6 carbons/protons of a syringyl β –O–4, probably the threo isomer and the correlation at 107.0/6.95 may belong to a sinapinic acid end group. Small amounts

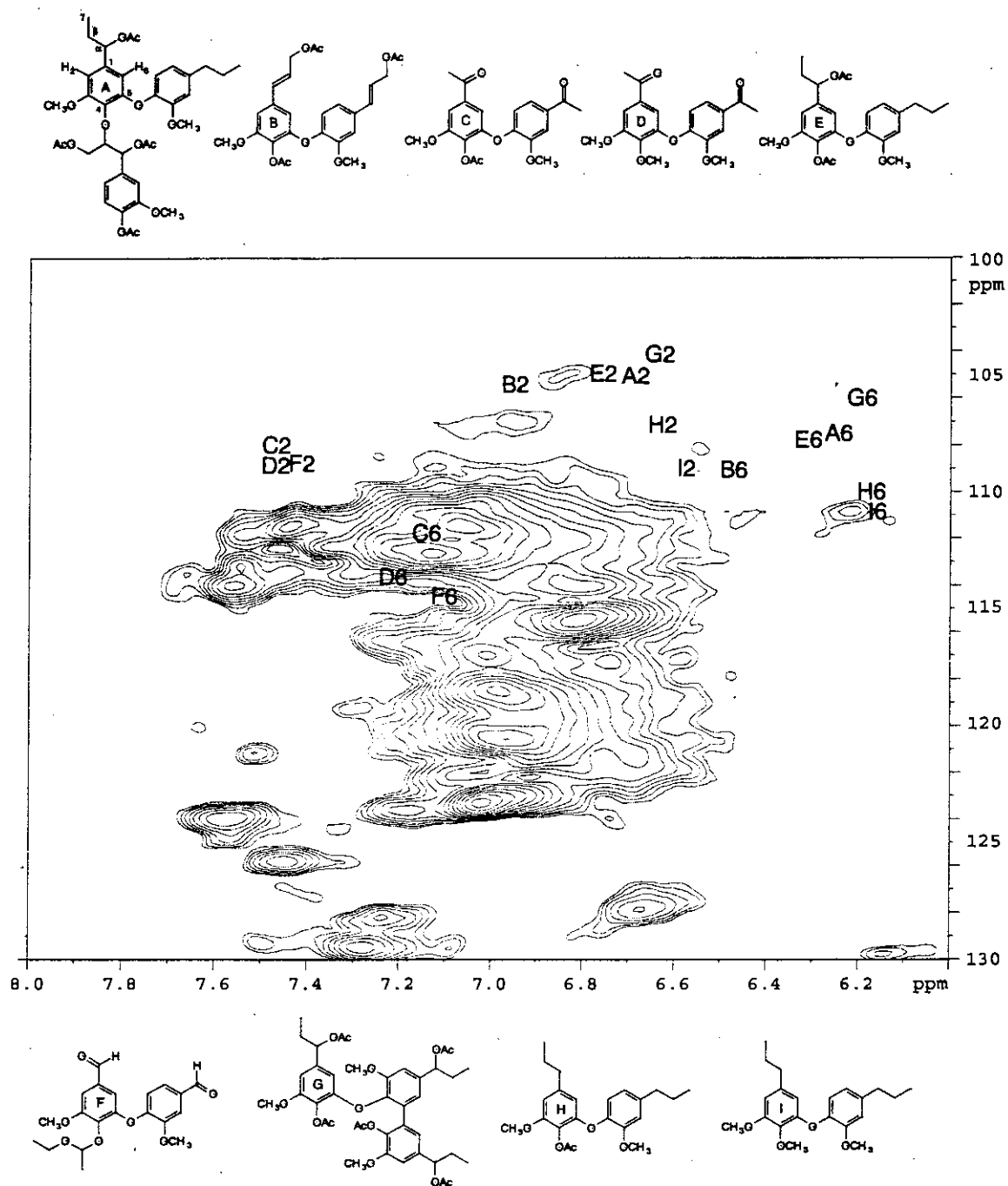


Figure 1. Model compound NMR chemical shift correlations superimposed on the aromatic region of an acetylated Loblolly pine MWL HSQC (short-range C-H correlations) NMR spectrum.

of syringyl units have been identified in loblolly pine but are reported to be less than 0.06%.¹⁷ The C/H correlation at 110.7/6.23 remains unidentified.

To evaluate the level of detection afforded by the 2D HSQC experimental conditions used, the polymer fraction from the preparation of model A was investigated. The incorporation of the 5-O-4 model compound (unacetylated E) into a coniferyl alcohol (CA) based DHP yielded 40% of a higher molecular weight fraction (generally above the hexamer region) as well as compound model A. Similar DHPs formed in this manner have been previously described by Landucci.^{18,19} Quantitative ¹³C NMR analysis of the 5-O-4 laced DHP indicates that 6 to 7% of the units in the DHP originate from the introduced dimer. The quantitation was based on one methoxyl carbon for each C9 unit and the methyl of the propyl sidechain of the introduced dimer. The propyl methyl was chosen, as it was least likely to be affected during the polymerization.

The C-H correlations for the C2/H2 and C6/H6 are clearly seen in the HSQC experiment and the very low level of β - β sidechains (<2%) was still discernable in the sidechain region for this DHP. The 5-O-4/CA DHP was diluted with an equal weight of CA only DHP prepared in a similar manner (no existing 5-O-4 correlations in the CA only DHP) to reduce the overall percentage of 5-O-4 component from 6 - 7% down to 3 - 4% which is more in line with literature values. The mixed DHPs were then re-analyzed by NMR and the results are shown in Figure 2a. The correlations for the C2/H2 and C6/H6 are clearly visible at 3-4%.

What does this all mean? Were it not for the excellent data from degradative analyses, one might likely conclude that 5-O-4-ethers are insignificant in the softwood lignins examined to date (Fig. 2b). It is difficult to imagine why they cannot be seen in this NMR experiment, which is relatively quantitative. A possibility is that the units are in fact not part of the native structure and are generated under the degradative conditions, but this seems unlikely since both acidic (e.g. acidolysis, thioacidolysis, DFRC) and basic (e.g. nitrobenzene oxidation) methods produce 5-O-4-dimeric units.

The situation is reminiscent of the case with β -1-structures. Such units were quite prevalent in acidolytic degradative analyses, and yet were nearly impossible to find in NMR spectra. The reason in this case turned out to be because the anticipated "conventional β -1-structures" were not in fact formed during lignification. Instead their precursors,

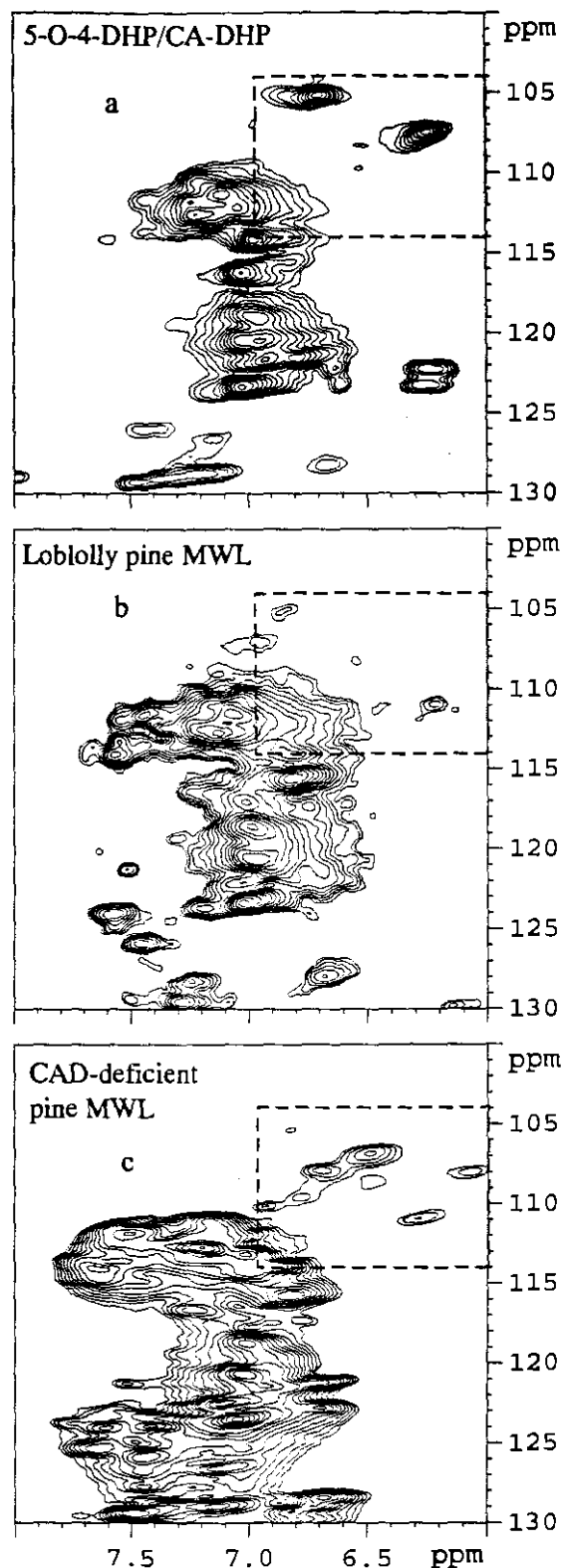


Figure 2. Aromatic regions of HSQC spectra for a) 5-O-4 loaded DHP, b) LP MWL, c) CAD-deficient pine MWL. Box indicates area of interest for 2,6 correlations.

spirodienone structures are now readily identified in lignin spectra^{5,6} Right now there is no reason to advocate such an explanation as it is difficult to envision an intermediate that would not be picked up at the 1-7% level in HSQC spectra.

The question of whether 5-O-4-structures can be seen in lignin spectra is perhaps most simply answered by examining lignins known to contain enhanced levels of such structures. A CAD-deficient pine lignin described previously¹⁵ derives in large part from dihydroconiferyl alcohol and its derived guaiacylpropan-1,3-diol. Such monomers, without the unsaturated sidechain, Cannot undergo the endwise β -O-4-coupling reactions that typify the bulk of lignin polymerization, but become heavily involved in 5-5- and 5-O-4-coupling with each other and with guaiacyl units. The CAD-deficient pine lignin therefore has a significantly larger dibenzodioxocin component²⁰ from the 5-5-coupled units. Also DFRC and thioacidolysis release substantial levels of 5-O-4-dimers. Indeed, the HSQC spectrum (Fig. 2c) from this lignin shows significant correlations in regions of the spectra where structures derived from dihydroconiferyl alcohol and guaiacylpropane-1,3-diol would likely arise.

CONCLUSIONS

The diagnostic HSQC correlations from anticipated 5-O-4-units should be readily detected if such structures were present at reported levels in the lignins. Better model compound data is still needed but the complete lack of such correlations is a conundrum for discussion and research!

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