

Isochroman Structures in Lignin: a New β -1 Pathway

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Introduction

Lignins are produced principally from a dehydrogenative polymerization of coniferyl and sinapyl alcohols. With four available coupling sites (4-O-, 1-, 5-, and β -) on the coniferyl alcohol monomer radical and at least three (4-O-, 1- and 5-) on its subsequent dimer and higher oligomer radicals, the resulting polymer is structurally and stereochemically complex. Structural details are still emerging from model studies coupled with NMR, and from closer examination of the products of various degradation methods. Only recently were dibenzodioxocins discovered in softwood lignins. They have now been found as important branch-point structures in hardwoods, grasses and legumes.

In our efforts to understand the roles and mechanisms by which lignins in forages inhibit forage digestibility by ruminants, we recently developed a new analytical method for lignin analysis, the "DFRC" method (U.S. Dairy Forage Research Center 1996). Applications of this method are beginning to reveal new details about lignin, as illustrated here. The use of pine in this study is not a move away from forages; it represents merely the study of a simplified system (pine lignin contains no sinapyl alcohol) which allows us to make observations about the general lignin pathways.

Results and Discussion

New aryl isochroman products **5** were discovered in the trimer fraction of pine wood degraded by the

DFRC procedure. These structures implicate a new pathway following β -1 coupling between a coniferyl alcohol radical **1** and a lignin oligomer radical **2**. The plausible mechanism for biosynthesis of this structural unit, Scheme 1, suggested that it might be found in native lignins. Examining *in situ* lignins with the resolution required to identify such a structure is not possible, but we report here its firm identification in isolated pine milled wood lignins.

HMQC or HSQC spectra of various pine milled wood lignin isolates showed a small but diagnostic correlation between δ_c 40.3 and δ_H 3.59 ppm (not shown). Regrettably, the other carbon/proton correlations are in congested regions of the spectra but peaks are present at the correct locations. Evidence for the entire proton coupling network was further confirmed by TOCSY experiments, Fig. 2, where correlations corresponding to all four protons on the aryl isochroman ring are clear. The correlations represent 5 simultaneous NMR chemical shifts (1 carbon, 4 proton) that correspond exactly with the shifts in the isolated trimer (as shown on Fig. 2), constituting significant proof.

Although the identification of the aryl isochroman structure in an isolated milled wood lignin can be made firmly, the apparently low amount visible in spectra does not account for the significant amounts of derived β -1 products that arise from DFRC-degradation, or various other acidolytic methods. The possibility remains that it is a product of isolation and that its precursor **4**, for example, may be the *in situ* product,

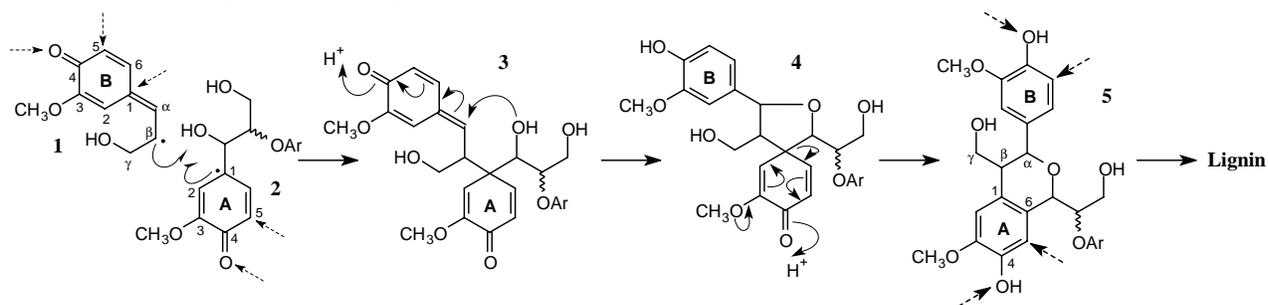


Figure 1. Proposed lignification mechanism in which β -1 coupling of a coniferyl alcohol radical **1** and a preformed lignin oligomer radical **2** produce a quinone methide intermediate **3** which is internally trapped to produce the spiro-compound **4** (rather than fragmenting via the 'normal' β -1 pathway (Lundquist 1965)). Sidechain migration produces the aryl isochroman **5**. Dashed arrows indicate potential sites of further radical coupling during lignification. Numbering follows normal lignin conventions.

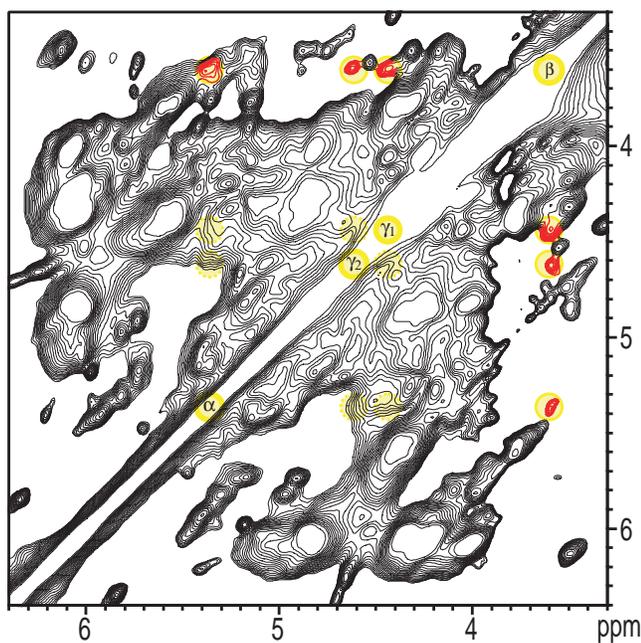


Figure 2. Sidechain region of a TOCSY (spin lock time, 100 ms) spectrum of *Pinus taeda* acetylated milled wood lignin, clearly showing the H- α /H- β /H- γ /H- δ coupling network. Correlations from the isolated compound **5** [Ar = (OMe)Ph-CH=CH-CH₂OAc], are at the center of the overlying circles. Other correlations present but not fully resolved are shown with dotted circles.

as has been proposed by Brunow. Either way, however, structure **5** provides compelling evidence of the internal cyclization pathway from β -1 intermediate **3**, where no evidence has previously been presented.

Conclusions

The isolation of compound **5** and various related dimers following DFRC-degradation of pine wood

adds further support for the occurrence of β -1-coupling during lignification. The structures suggest the existence of a plausible new pathway following the radical coupling step, a pathway which involves intramolecular trapping of the β -1 quinone methide followed by sidechain migration. Compounds assigned as having β -6-linkages have resulted from other degradative procedures; the aromatic-ring substitution patterns vary from those identified here and should be carefully re-examined in light of the more reasonable mechanism shown in Fig. 1. Products with α -6 linkages can result under acidic conditions, and it is possible that the dienone-phenol rearrangement (**4**® **5**, Fig. 1) is mediated by the acidic conditions of the DFRC procedure. The mechanistic plausibility of the reaction pathway, the isolation of expected degradation dimers, and the identification of the aryl isochroman structure in pine milled wood lignins by NMR, all suggest that such structures, or their precursors, are present in native lignins.

References

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