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An Unusual Lignin from Kenaf

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Abstract: A novel (possibly unique) lignin isolated from kenaf bast fibers had a high syringyl:guaiacyl ratio, was highly etherified, and was high in β-ether units with predominantly *erythro* stereochemistry. Moreover, it was extensively side-chain acetylated (primarily at γ), implicating 4-hydroxycinnamyl acetates as monomers in its biosynthesis.

Lignins have been characterized from such a variety of plant materials that it seems unlikely for a new type of lignin to be revealed. The lignin from kenaf is, however, strikingly different from any other lignin that has been characterized.[1] Its uniqueness is revealed both in terms of its high syringyl content and in the extensive acetylation of the side-chain hydroxyls. Acetate groups have been recognized as being associated with lignin in kenaf[2] as well as in hardwoods,[3] although they do not appear to have been unambiguously identified on isolated lignins. NMR studies have not suggested that lignins were acetylated, although this could have been missed previously because of the common practice of peracetylating lignin (for improved solubility and spectral dispersion) prior to NMR. Certainly, the regiochemistry of such acetates has not been addressed.

Kenaf lignin isolated by traditional procedures[4] from bast fibers from the core of Tainung kenaf stems was initially intriguing because of its high syringyl content. Dicots typically have a rather even syringyl:guaiacyl distribution, whereas nitrobenzene oxidation of the kenaf dioxane lignin gave a molar syringaldehyde:vanillin ratio of 6.0 (without loss corrections). Syringaldehyde:vanillin ratios in hardwoods have been reported up to ca. 5.2,[3,5] and a thesis describes an 8.4 ratio.[6] The dominance of syringyl units in this kenaf leads to a very simple lignin as seen from the ^{13}C-NMR of the acetylated kenaf dioxane lignin, Figure 1a. What is striking, in addition to the obviously high syringyl:guaiacyl ratio, is that β-ethers predominate significantly over other interunit linkage types (cf. the $\beta-\beta$ units at ca. 55.5 ppm) and that the material is highly etherified (since the proportion of phenolic end groups, seen from the aromatic acetate carbonyl peaks, is low). These values have been estimated from quantitative ^{13}C NMR (not shown) at ~80% β-ether units and 14% phenols. The aliphatic region of the 2D HMQC—TOCSY[7] spectrum, Figure 2, confirms the assignment of side chain protons, again shows that syringyl β-ether units predominate, and beautifully disperses the isomers. The prior observations[8] that *erythro* β-ether isomers predominate in syringyl units is strikingly revealed in this spectrum; there is little diastereoselectivity in guaiacyl lignins.

The NMR spectrum of the underivatized lignin, Figure 1b, provided even more startling information—the lignin was extensively acetylated (ca. 50%). The spectrum shows that acetylation was almost entirely (ca. 95%) at the primary γ-position of the side chain, as was confirmed by an HMBC experiment correlating the acetate carbonyl carbon with γ-protons on lignin (not shown). Although acylation of dicot lignins by hydroxycinnamic acids has previously been reported to be almost insignificant, grasses have proportions of side-chain alcohol groups esterified by *p*-coumaric acid; such esterification has recently been shown[4] to be exclusively at the γ-position in a maize lignin isolate and indications are that other grasses, both C_3 and C_4, display the same regiochemistry (Ralph, J. Unpublished results). The logical conclusion was that grasses pre-esterify lignin monomers to produce the hydroxycinnamyl *p*-coumarates which are then exported and incorporated into a lignin complex by the traditional oxidative coupling mechanisms.[4,9] Presumably, the same can be concluded in the case of the kenaf in this study; the acetate is not so cleanly at the γ-position (ca. 95%), but we have previously observed acetate migration in lignin model compounds.[10] We suscept that, in kenaf, sinapyl (and perhaps coniferyl) acetates are enzymatically produced

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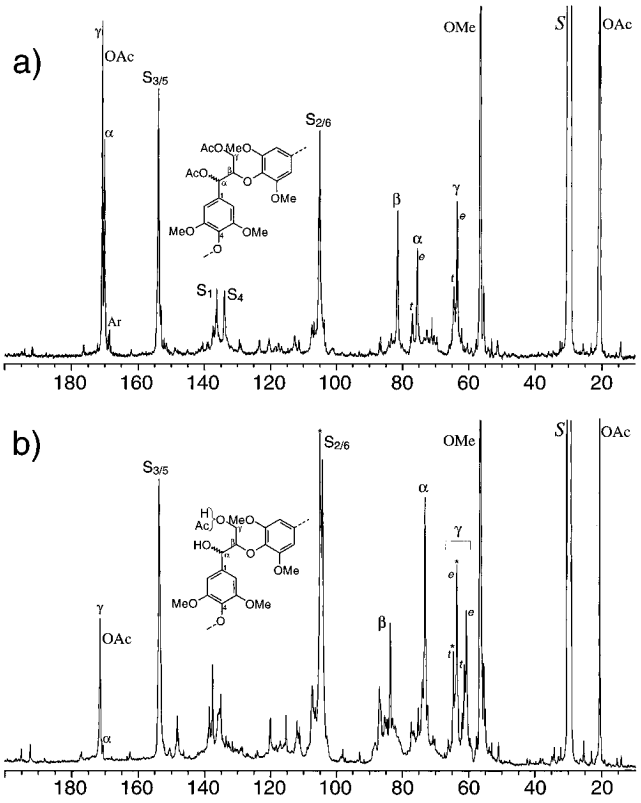

Figure 1. ^{13}C NMR spectra of the isolated kenaf lignins showing the preponderance of syringyl peaks. Assignments are for β-ethers: (a) acetylated, (b) underivatized. * indicates easily identifiable peaks that result from acetate substitution. NMR conditions (qualitative): Bruker AMX-360, 10 mm tube/probe, temperature 300 K, 70° pulse width, acquisition time 0.7 s, relaxation delay 0.3 s (total recycle delay, 1 s), ca. 50 000 scans, Waltz-16 decoupling: (a) 373 mg in 2.4 mL of acetone-d_6, (b) 330 mg in 2.4 mL of 9:1 acetone-d_6:D_2O.

and exported for lignification with sinapyl and coniferyl alcohols. It is most likely that the small amount of α-acetate derives from internal migration rather than from the alternate mechanism of acetate addition to quinone methide lignin intermediates, a mechanism over which the plant has no direct control.[4,11] The presence of such large amounts of acetate on lignin was sufficiently novel that our procedure for the lignin isolation came under close scrutiny.[1] The isolation was repeated under conditions in which no reagents or solvents could conceivably produce acetylation artifacts; the lignin isolated in this manner retained the extensive acetylation.

We are currently intrigued by what might be the function of such an acetylated lignin for the plant, and if somehow (and for some reason) this is a mechanism to achieve high syringyl lignins—syringyl rich synthetic lignins with high molecular weights are difficult to prepare *in vitro*.[12−14] Of practical relevance is that the presence of acetate groups on kenaf lignin will consume base during alkaline delignification, but that delignification of this lignin fraction should be relatively straightforward and extensive because the high-syringyl lignin is relatively unbranched and, as seen from the ^{13}C-NMR spectra, contains a substantial proportion of high-temperature base-cleavable β-aryl ether units.

In summary, kenaf lignin is unusual and perhap unique. It actually represents a new lignin type implicating new monomers (the 4-hydroxycinnamyl acetates) in its synthesis. A more extensive manuscript detailing the composition, structure, and fascinating NMR spectra of kenaf lignins is in preparation.

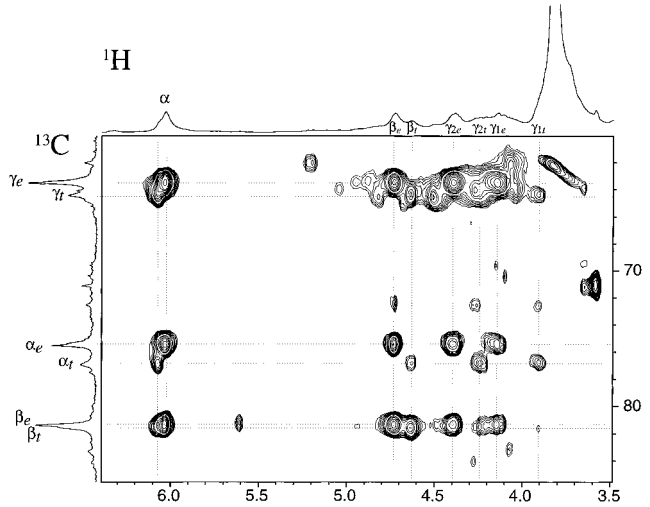

Figure 2. Aliphatic region of a 2D HMQC−TOCSY experiment showing syringyl β-ether units and the excellent dispersion of *erythro*- and *threo*-isomers. NMR conditions: Bruker AMX-360, 5 mm tube, 5 mm normal mode probe (proton coil outermost), Bruker pulse program "invbmltp" [phase-sensitive, inverse-detected C−H correlation using a BIRD sequence for minimizing protons bound to ^{12}C-carbons (300 ms inversion-recovery delay), and MLEV-17 Hartman−Hahn mixing (100 ms)], spectral widths 4000 Hz (^1H) and 13 100 Hz (^{13}C), acquisition time 0.26 s, relaxation delay 1 s, 256 increments of 520-scan 2K FIDs, total acqusition time 62 h. Processing: cosine-bell apodization in both dimensions, phase-sensitive (TPPI) Fourier transform with zero-filling to 1K by 1K real data points resulting in 3.9 (^1H) and 12.8 (^{13}C) Hz/pt resolutions.

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