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Preliminary evidence for sinapyl acetate as a lignin monomer in kenaf

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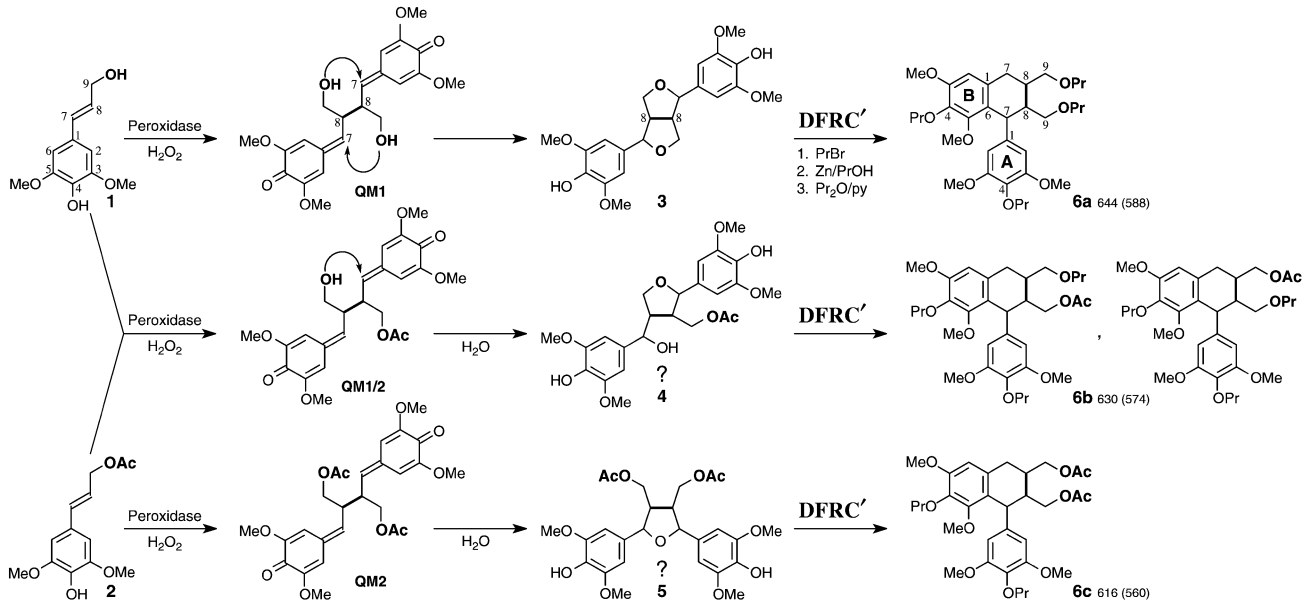
9-Acetylated syringyl 8–8-linked dehydrodimers are degradation products released from kenaf lignins, implicating sinapyl acetate as a lignin precursor.

Lignins of many agriculturally important crops and woody plants are acylated by various acids, although the biochemistry associated with such acylation remains unresolved and the genes are unknown. Nor is it known if lignin monomers (the hydroxycinnamyl alcohol monolignols) are first acylated to produce ester conjugates which are then incorporated by coupling and cross-coupling into lignin by the traditional free-radical coupling reactions, or whether acylation occurs *following* the monolignol radical coupling reactions or on the lignin polymer itself.[1,2] These issues are becoming important to resolve as genes controlling the various processes and the functions of such acylation are sought in order to improve the utilization of plant resources. Acylated components have also been found to increase (on a lignin basis) when lignification is decreased by down-regulating enzymes in the monolignol biosynthetic pathway.[3]

NMR of isolated kenaf bast fiber lignins suggested well over 50% lignin acetylation, almost entirely of the sidechains' primary aliphatic alcohols.[4] Our 'Derivatization Followed by Reductive Cleavage' ('DFRC') method, which cleaves ether linkages in lignins and releases analyzable monomers and dimers,[5,6] leaves such esters intact.[7,8] A modification (termed DFRC′), using propionyl analogs of the normal acetyl reagents, allowed us to establish that the native lignin was about 60% 9-acetylated and, more revealingly, that syringyl (3,5-dimethoxy-4-hydroxyphenyl) units were significantly acetylated whereas only traces of acetylated guaiacyl (4-hydroxy-3-methoxyphenyl) units could be detected.[1]

How is it possible to establish whether monolignols are acetylated prior to the polymerization steps of lignification? Even finding acetylated monomers in lignifying tissues will not rigorously establish that they are involved in lignification. Structural analysis of the lignin polymer can provide a reasonably definitive answer. There is one lignification pathway that is significantly altered by pre-acetylation of the monolignols. That is the pathway in which the 9-OH on the monolignol becomes involved in post-coupling reactions, *i.e.* the pathway normally leading to 8–8-coupled (resinol) units **3**, Scheme 1. The key concept is the following. With the 9-position acetylated, 8–8-coupling can still presumably occur (the 9-OH is not required for the radical coupling step; the propenyl analog isoeugenol G–CH = CH–CH_3, for example, will also undergo 8–8-coupling),[9] but the re-aromatization reactions following the radical coupling step can no longer be driven by the internal



Scheme 1 The key to establishing whether monolignols are pre-acylated lies with the 8–8-coupling products (the resulting 8–8-bonds are bolded for emphasis). The traditional monolignol sinapyl alcohol **1** will dehydro-dimerize initially forming the 8–8-coupled bis-quinone methide intermediate **QM1**, which re-aromatizes by internal 9-OH attack on each quinone methide electrophilic 7-carbon to produce syringaresinol **3** as the overwhelmingly major product. When acetylated sinapyl alcohol **2** dimerizes, it forms an analogous bis-quinone methide intermediate **QM2**. However, **QM2** can not be re-aromatized by internal trapping. The products have not yet been characterized but it is logical that structure **5** would arise from water attack on one quinone methide moiety with the resulting 7-OH attacking the other quinone methide to form a tetrahydrofuran; analogous products have been found in ferulate dehydrodimers.[14] When **1** and **2** radicals cross-couple, the intermediate bis-quinone methide **QM1/2** now has one quinone methide moiety which can be internally trapped by the single 9-OH to form a single tetrahydrofuran ring, but the other quinone methide can only be re-aromatized by attack of an external nucleophile. The resulting product, presumably **4**, therefore retains at most a single acetate. Products **6** result from DFRC′-degradation (the DFRC modification using only propionate reagents) of the non-, mono-, and di-acetylated 8–8-coupled units. See text for details. The MWs of DFRC′ products are given beside the compound numbers, with the MWs of the base peaks (used for Fig. 1) in brackets.

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attack of the 9-OH on the quinone methide intermediate **QM2**, Scheme 1. The 9-acetylation prevents such a reaction. Other pathways must therefore be in effect producing other products. The important point is that the acetyl group can remain attached in non-resinol 8–8-coupling products, products that could not have arisen from post-coupling acetylation reactions. {We recognize that there remains the remote possibility, not ruled out by the observations presented here, that processes involving subsequent opening of resinols **3** and acetylation of the opened products could occur, but that would appear to require at least two enzymatic activities. A resinol ring opening and reduction is effected, for example, by NADPH-dependent pinoresinol–lariciresinol reductase.[10]}

In seeking preliminary evidence for sinapyl acetate incorporation, it didn't appear necessary to elucidate the full coupling and cross-coupling pathways for sinapyl acetate. All that was required was to show that sinapyl acetate **2**, in coupling and cross-coupling reactions, would give acetylated products that would produce DFRC′ 8–8-linked products identical to those that are released from kenaf (lignins) and not from plants having non-acetylated lignins. Oxidation of sinapyl alcohol **1** with H_2O_2/peroxidase or metal oxidants typically gives the lignan syringaresinol **3** as the predominant dehydrodimeric product, along with a small amount of the 8–O–4-coupled product.[11] In this study, sinapyl alcohol was oxidized with H_2O_2–peroxidase in a 20 mM buffer solution containing 20% acetone; syringaresinol **3** was the only 8–8-product, produced in over 90% yield. DFRC′ treatment of syringaresinol **3** yielded aryltetralin **6a**, Scheme 1, as a major product.† Similar compounds are produced following thioacidolysis of 8–8-linked lignin units.[12] Oxidation of sinapyl acetate **2** (Scheme 1) under similar conditions yielded a mixture of currently uncharacterized compounds retaining acetate groups. DFRC′ treatment of the mixture yielded **6c**, the diacetate analog of **6a** (from sinapyl alcohol). The structural analogy, from MS spectra (Fig. 1), indicates that sinapyl acetate also undergoes 8–8-coupling, and that at least one of the products **5** (Scheme 1, although the exact structure has not yet been determined) produces the aryltetralin **6c** following DFRC′ treatment. Oxidation of a mixture of sinapyl alcohol **1** and sinapyl acetate **2** must result in cross-coupling reactions to produce crossed 8–8-coupled structures **4**, since DFRC′ degradation now produces mono-acetylated aryltetralins **6b** in addition to the non- and di-acetylated analogs **6a** and **6c** (as evidenced by GC-MS).

It would be reasonable to expect that substructures **4** and **5** exist (in phenol-etherified form) in kenaf if sinapyl acetate participates in formation of its lignin. Selected ion chromatograms of TLC-fractionated DFRC′ products from kenaf lignin (Fig. 1) or whole kenaf cell walls clearly show the presence of all three DFRC′ products, compounds **6a–c**. Comparison of GC retention times and mass spectra of DFRC′ products with those from the *in vitro* coupling reactions of sinapyl alcohol and sinapyl acetate indicates that compounds **6b** and **6c** derive from sinapyl acetate. Compound **6a** derives from normal lignins, but the acetylated analogues **6b** and **6c** do not.

Although a great deal remains to be done to detail the coupling reactions, authenticate the nature and stereochemistry of the products, and fully elucidate their DFRC′ products, the preliminary data presented here appears to us to provide rather compelling evidence that sinapyl acetate is involved in lignification in kenaf, and is the likely source of the high 9-acetylation observed in kenaf bast fiber lignins. Detection of 9-acetates in syringyl 8–8-coupled DFRC′ products **6b–c** from kenaf suggests the existence of substructures which are likely formed from dehydrogenative coupling of sinapyl acetate itself or cross-coupling with sinapyl alcohol during the lignification process and provides evidence that acetates on kenaf lignin are formed through incorporation of sinapyl acetate, as a lignin precursor, into lignin macromolecules by radical coupling. Sinapyl acetate therefore appears to be an authentic lignin monomer in kenaf.

The preliminary evidence provided here that acylation is at the monolignol stage allows researchers to seek the substrates and presumed transferases involved in the specific acylation of monolignols, and to identify the genes responsible, allowing the process to be genetically manipulated.

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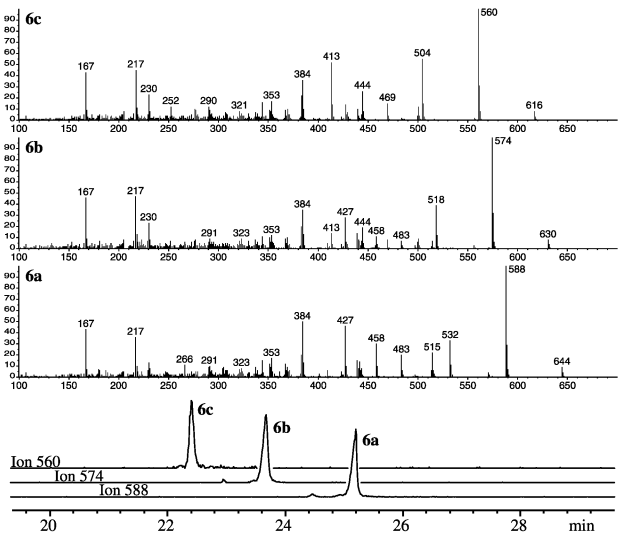

Fig. 1 Mass spectra and selected-ion chromatograms of DFRC′ products from kenaf showing the presence of aryltetralin 8–8-products containing 0, 1 and 2 acetates.

Notes and references

† In addition to the MS data in Fig. 1, NMR (360 MHz, acetone-d_6) was diagnostic: δ_H 1.06, 1.10, 1.17, 1.18 (4 × 3H, t, J = 7.5 Hz, propyl-Me), 2.03 (1H, m, B8), 2.06 (3H, s, B-OAc), 2.27 (1H, m, A8), 2.30, 2.38, 2.54, 2.56 (4 × 2H, q, J = 7.5 Hz, propyl-CH_2), 2.82 (2H, m, 7), 3.25 (3H, s, B3-OMe), 3.69 (6H, s, A-OMe's), 3.80 (3H, s, B5-OMe), 4.00 (1H, m, A9a), 4.13 (1H, m, B9a), 4.21 (1H, d, J = 6.6 Hz, A7), 4.21 (1H, m, B9b), 4.30 (1H, m, A9b), 6.45 (2H, s, A2/6), 6.71 (1H, s, B2). The parent compound, 8-(4-hydroxy-3,5-dimethoxyphenyl)-6,7-bis-hydroxymethyl-1,3-dimethoxy-5,6,7,8-tetrahydronaphthalen-2-ol, has been isolated from several plant sources according to Beilstein; the earliest reference appears to be by Weinges.[13]

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