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Preparation and relevance of a cross-coupling product between sinapyl alcohol and sinapyl *p*-hydroxybenzoate

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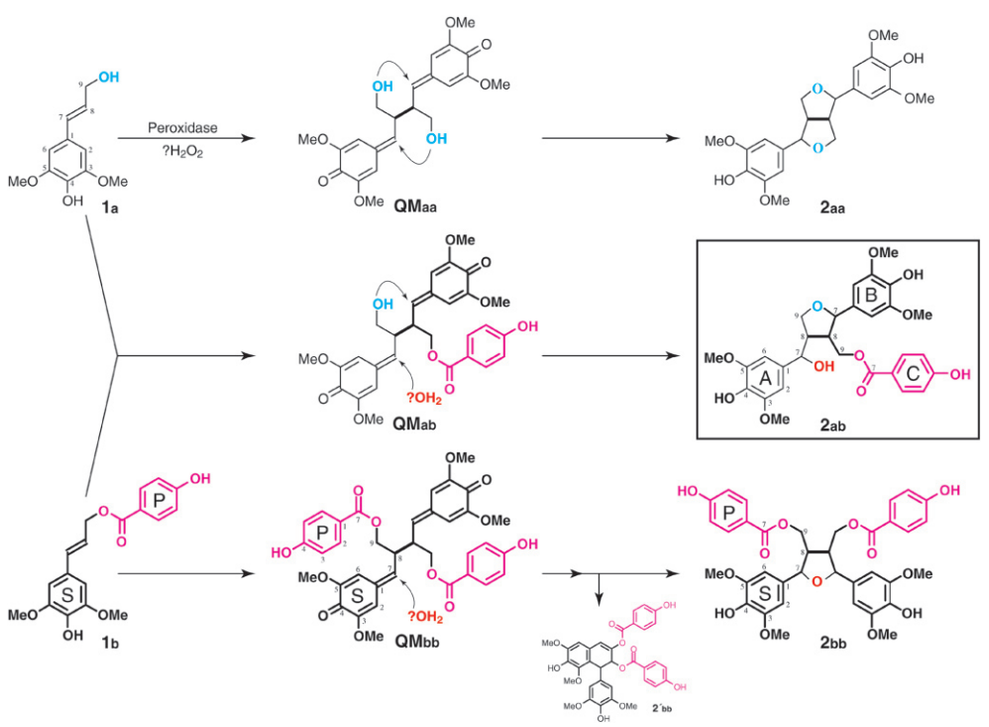
Cross-coupling of sinapyl *p*-hydroxybenzoate and sinapyl alcohol produces an 8-8-cross-coupled product that is also detected in lignifying poplar tissues, implicating sinapyl *p*-hydroxybenzoate as a lignin precursor.

The primary lignin monomers are the three monolignols, *p*-coumaryl, coniferyl, and sinapyl alcohols (4-hydroxycinnamyl, 4-hydroxy-3-methoxycinnamyl, and 3,5-dimethoxy-4-hydroxycinnamyl alcohols). Lignins of many agriculturally important crops and woody plants have their sidechain primary alcohol (the so-called 9- or γ-OH) acetylated, *p*-hydroxybenzoylated or *p*-coumaroylated.[1–3] The biochemistry of such acylation remains unresolved and the genes associated with the presumably involved transferase enzymes are unknown. As genes controlling various processes and the functions of such acylation are sought in order to improve the utilization of plant resources, it has become important to resolve whether monolignols are first acylated to produce ester conjugates which are then incorporated by coupling and cross-coupling into lignin by the conventional free-radical coupling reactions, or whether acylation occurs *following* the monolignol radical coupling reactions or on the lignin polymer itself.[4,5] We have provided preliminary evidence (to be more fully documented this year, Lu and Ralph unpublished) that the high level of 9-acetylation in kenaf lignins arises, at least in significant part, from pre-acetylated sinapyl alcohol,[6] and have provided at least good circumstantial evidence for monolignol *p*-coumarates in grasses.[7,8]

During recent studies profiling phenolic metabolite differences between normal poplar plants and transgenics deficient in genes/enzymes on the lignin biosynthetic pathway,[9,10] we discovered a novel 8-8-coupling product **2ab** bearing a *p*-hydroxybenzoate substituent.[9] This product was readily detected in normal plants but decreased to below detectable levels in plants heavily down-regulated by caffeic acid *O*-methyltransferase (COMT). The structural proof of the crucial product is *via* the independent preparation of the compound here.

Coupling reactions involving monolignols strongly favor homo-dehydrodimerization; cross-coupling reactions can be difficult to achieve.[2,11] Nevertheless, peroxidase–H_2O_2 oxidation of equimolar amounts of sinapyl *p*-hydroxybenzoate and sinapyl alcohol produced a satisfactory 25% isolated yield of the cross-coupled product **2ab**, Scheme 1.† The ratio of products **2aa** : **2ab** : **2bb** was essentially 1 : 2 : 1 suggesting that the coupling reactions were insensitive to the acylation of the 9-OH.

Sinapyl alcohol **1a** radical 8-8-coupling produces an intermediate *bis*-quinone methide **QMaa**, Scheme 1, which



Scheme 1 Coupling reactions of sinapyl alcohol **1a** and sinapyl *p*-hydroxybenzoate **1b**. Cross-coupled product **2ab** has been found in actively lignifying xylem tissue in poplar. Intermediate **QMbb** is a surprisingly stable, isolable product in homo-coupling reactions of **1b**.

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rearomatizes to afford syringaresinol **2aa** by internal trapping *via* the two 9-OH groups.[13]‡ When the 9-OH group is acylated, as in the "dimerization" of sinapyl *p*-hydroxybenzoate **1b**, however, it is obviously incapable of trapping the quinone methide. Consequently, one quinone methide rearomatizes by external water addition, as is typically seen following 8-*O*-4-dehydrodimerization.[13] At this point there is an internal OH capable of trapping the other quinone methide moiety. The resulting product is therefore not a dehydrodimer, but the product **2bb**, with a molecular mass 16 units higher. The cross-coupling reaction between **1a** and **1b** also produces an intermediary bis-quinone methide, but one such moiety is internally trapped by the single 9-OH. Rearomatization of the other requires water addition, and produces product **2ab**. Finding the cross-coupling product **2ab** in plants is compelling evidence that sinapyl *p*-hydroxybenzoate must therefore be an authentic "monomer" in the lignin (or lignan) pathway; it is unlikely to have arisen from the dehydrodimer of sinapyl alcohol, **2aa**.

Indeed product **2ab** was identified in the actively lignifying xylem tissue of 3 month old poplar.[9] The product synthesized here was shown to be identical by its UV and mass (including MS-MS) spectral data, and HPLC retention time (including by co-injection). Its detection in actively lignifying tissue suggests that it may be a compound destined for the lignin polymer. More importantly, it demonstrates that sinapyl *p*-hydroxybenzoate is the "monomer" in the coupling reaction *in planta.* A *p*-hydroxybenzoyl transferase is therefore implicated for acylating the monolignol **1a** to feed the acylated substrate **1b** into the lignification process in poplar, where the lignin contains *p*-hydroxybenzoylated units.[15] The product **2ab** has also been isolated previously, without comment, from *Salix sachalinensis.*[16]

One of the more intriguing and unexpected chemical aspects of the homo-coupling reaction using sinapyl *p*-hydroxybenzoate **1b** in peroxidase–H_2O_2 at pH 5 to independently generate compound **2bb** was that the intermediary quinone methide **QMbb** was isolable and stable.§ A pale yellow precipitate formed that was difficult to dissolve in common organic solvents. NMR in DMSO-d_6 revealed it to be the quinone methide **QMbb**. Although certain syringyl and guaiacyl quinone methides were sufficiently stable in solution to allow NMR spectra to be recorded in 1983,[17] they have generally been considered unstable, although crystalline bromo analogs have been long known.[18] **QMbb** in the solid state did not degrade after over 8 months in a freezer. Regrettably, it has not yet been possible to obtain sufficiently good crystals for an X-ray crystal structure. Extracting the entire reaction product into EtOAc and washing with saturated aqueous NH_4Cl acidified with HCl produces mainly compound **2bb** (along with the tetrahydronaphthalene **2′bb**) demonstrating that compound **2bb** derives from this quinone methide intermediate.

In conclusion, demonstration that sinapyl *p*-hydroxybenzoate **1b** is an authentic precursor of lignification augments the finding that sinapyl acetate is implicated in kenaf bast fiber lignification[6] and the evidence for sinapyl and coniferyl *p*-coumarate in maize (and other grasses).[7,8] The mechanism therefore appears to be a general one for the three types of natural lignin acylation observed in nature. Future research will be aimed at determining if low levels of structures resulting from incorporation of **2ab** and **2bb** can be detected in lignins. The ultimate goals will be to find the transferases and their genes, and to attempt to understand the role of lignin acylation in these plants.

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Notes and references

† Synthesis of the cross-coupled product from sinapyl alcohol and sinapyl *p*-hydroxybenzoate: sinapyl *p*-hydroxybenzoate[5] (700 mg, 2.12 mmol) and sinapyl alcohol[12] (460 mg, 2.19 mmol) were dissolved in acetone (100 ml) and added into phosphate buffer (20 mM sodium dihydrogen phosphate, pH 4.5, 400 ml) in 500 ml flask. Horseradish peroxidase (Sigma, Type II, 150–250 units mg^{-1}, 8 mg) was added followed by addition of the hydrogen peroxide urea complex (200 mg, 2.13 mmol) dissolved in 10 ml water. This mixture was stirred for 1 h when TLC indicated complete starting material disappearance. The mixture was saturated with NH_4Cl and acidified with dilute aqueous HCl (3%) to pH around 3–4 and extracted with EtOAc (300 ml × 2). The organic phase was dried over $MgSO_4$ and filtered. The crude product (pale white foam) was obtained after evaporation of the organic phase. The cross-coupled compound **2ab** (280 mg, 25% yield) was isolated by silica gel chromatography with cyclohexane–EtOAc, 1 : 1 as eluting solvent. UV and MS data have been reported separately.[9] NMR (acetone-d_6) δ_H 4.96 (1H, d, J = 5.0 Hz, A7), 3.99 (1H, m, A8), 4.14 (2H, m, A9s), 3.78* (6H, s, A3/5-OMe), 6.72 (2H, s, A2/6); 4.90 (1H, d, J = 6.3 Hz, B7), 2.55 (1H, m, B8), 4.41 (1H, m, B9a), 4.68 (1H, m, B9b), 3.74* (6H, s, B3/5-OMe), 6.66 (2H, s. B2/6); 7.77 (2H, m, C2/6), 6.87 (2H, m, C3/5) (* assignments may be interchanged); δ_C 136.0 (A1), 104.53 (A2/6), 148.5 (A3/5), 135.9 (A4), 72.6 (A7), 48.4 (A8), 70.0 (A9); 134.5 (B1), 104.45 (B2/6), 148.5 (B3/5), 136.0 (B4), 85.3 (B7), 49.8 (B8), 64.0 (B9); 122.4 (C1), 132.5 (C2/6), 116.0 (C3/5), 162.6 (C4), 166.4 (C7). Small amounts of other products such as 8-*O*-4-dehydrodimers and aryltetralin 8-8-products were not further examined.

‡ In reactions with sinapyl alcohol alone, the 8-*O*-4-dehydrodimer is also produced but typically at less than 9% yield.[14]

§ **QMbb**: NMR (DMSO-d_6) δ_H 3.66 (3H, s, S3-OMe), 3.70 (3H, s, S5-OMe), 4.02 (1H, m, S8), 4.42 (2H, m, S9), 6.56 (1H, d, J = 10.3, S7), 6.56 (1H, d, J = 1.9, S6), 6.82 (2H, m, P3/5), 6.93 (1H, d, J = 1.9, S2), 7.82 (2H, m, P2/6); δ_C 40.7 (S8), 55.55 (S5-OMe), 55.62 (S3-OMe), 65.9 (S9), 104.8 (S6), 112.9 (S2), 115.9 (P3/5), 121.1 (P1), 132.3 (P2/6), 133.5 (S1), 140.4 (S7), 152.2 (S5), 153.7 (S3), 163.3 (P4), 166.2 (P7), 175.29 (S4).

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