Designing biomass lignins for the biorefinery

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ABSTRACT

As ever more component monomers are discovered, lignin can no longer be regarded as deriving from just the three canonical monolignols. Pathway intermediates and additional products of truncated biosynthesis are now established lignin monomers. The array of acylated monolignols continues to expand. Game-changing findings have demonstrated that phenolics from alternative pathways, including flavonoids and hydroxystilbenes, are also involved in lignification, expanding the traditional concept. Beyond the basic science intrigue, these findings propound exciting new avenues for valorizing lignins, or for producing more readily extractable or depolymerizable lignins, in crop and bioenergy plants.

Keywords: *Lignin, Lignification, Lignin monomers, Combinatorial radical coupling, Flavonoids, Hydroxystilbenes, Monolignol conjugates*

INTRODUCTION
Lignin biosynthesis is highly malleable, allowing a variety of phenolics to be incorporated as lignin monomers. Phenolics from beyond the monolignol pathway, such as flavonoids and stilbenes, have been shown to be monomers in some plants. Any phenolic transported to the lignifying zone of the cell wall can, subject to simple chemical compatibility, be incorporated into the polymer. Researchers are now able to contemplate strategic tailoring of lignin's structure, reactivity, and value, and muse over what might constitute a lignin that is ideally suited for conversion to phenolic monomers, adding value to the biorefinery.

RESULTS AND DISCUSSION
Various approaches toward lignin modification are covered in the following sections.

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Zip-lignins

Lignins can be designed to deconstruct more readily during processing (the so-called 'zip-lignin' approach). It is possible to engineer weak bonds (esters) into the lignin backbone,[1] facilitating lignin depolymerization during pretreatment or processing (such as in pulping).[2] It turns out that Nature is already naturally biosynthesizing such lignins at low levels in a variety of plants.[3] We may have even inadvertently selected for this trait in targeting woody species that pulp most easily, for example.
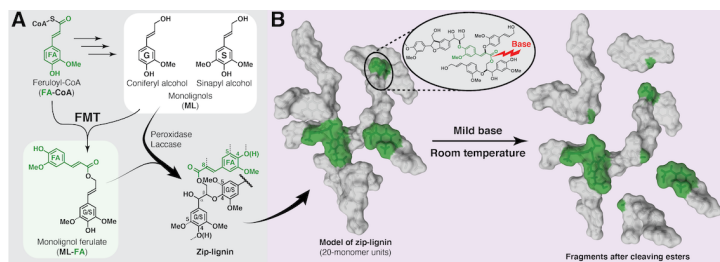

Fig. 1. Zip-Lignins from lignification with monolignol ferulate conjugates. A feruloyl-CoA: monolignol transferase (FMT) is used to produce monolignol ferulate conjugates that can serve as monomers for lignification.[1,4] The resultant lignin has esterified units (green highlighting) in the polymer backbone that can be readily chemically cleaved to enhance delignification and improve access to poly-saccharides.[1]

An "ideal lignin"

Based on the plethora of information stemming from the lignin biosynthetic research community over the last decade, it is now a realistic juncture to posit characteristics for an "ideal lignin" archetype for biomass processing. For the depolymerization of the polymer to monomers, one ideotype is a lignin that has at least the following three characteristics. First, it should be stable under acidic conditions to prevent condensation and the generation of undesired additional C–C bonds, during pretreatment. Second, it should contain only ether (C–O) inter-unit linkages in its backbone so that it can be fully depolymerized. Finally, it should be generated *in planta* from a single phenylpropanoid monomer to allow the production of the simplest array of monomeric compounds. C-lignin, such as that found in vanilla seed coats,[5] is one such example, as this lignin is essentially a homopolymer synthesized almost purely by β–O–4-coupling of caffeyl alcohol with the growing polymer chain, producing benzodioxanes as the dominant unit in the polymer; it is an example of such an "ideal lignin" that can be depolymerized to a single monomeric product in high yield.[6]
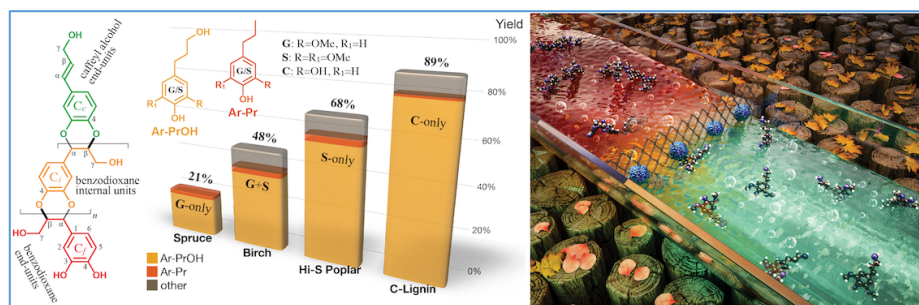

Fig. 2. C-lignin, derived from caffeyl alcohol. Lignification using caffeyl alcohol as the sole mono-mer results in an almost pure homopolymer of benzodioxane units arising from β–O–4-coupling; al-though C-units can, in principle, couple with all the options open to G-units, the β–O–4-coupling pro-pensity is simply overwhelming such that only low levels of other structures can be found in the polymer.[7] Yield and selectivity of Pd/C-catalyzed hydrogenolysis to produce arylpropanol, arylpropane, and other monomers, for the vanilla seedcoat C-lignin is graphed here (in which the arylpropanol monomer is catechylpropanol and the minor arylpropane monomer is catechylpropane) *vs* spruce, birch, and high-S poplar. The C-lignin delivers almost 90% yields of monomers in a product that is 90% one compound, dihydrocaffeyl alcohol (= catechylpropanol); the prior record monomer yield had been from the high-S lignin.[8] Conditions: 5% Pd/C, 473 K, 15 h, 4 MPa H_2. Picture in the bottom right is part of a picture prepared for a cover issue.[6]

Introducing new pathways into lignification, new units into lignins

With the discovery of the flavone tricin in monocot (and a few other) lignins, it is logical that

various other phenolics from flavonoid pathways are beginning to be discovered in natural lignins. For example, naringenin chalcone, naringenin, and dihydrotricin (in addition to tricin), from different flavonoid families, have been discovered and authenticated in papyrus,[9] paving the way to consider other classes of flavonoids as lignin monomers. There is evidence that naringenin, for example, has already been incorporated into the lignins in poplar and rice transgenics.[10-11]

Lignin Conjugates, 'clip-offs' – new discoveries, and enhancing levels

Various acids have long been known to acylate cell walls in general and lignins in particular.[4] Monolignol acetates, *p*-hydroxybenzoates, *p*-coumarates and, more recently, ferulates have therefore become recognized as authentic lignin precursors, produced by extensions of the monolignol biosynthetic pathway. More recently, various other conjugates have been identified, including simple benzoates and vanillates.[12-13]

Phenolic acids that can be easily clipped off biomass have established markets and significant value. We illustrate the near-absurdity of deriving the common commodity chemical *p*-aminophenol, and the chemical and pharmaceutical acetaminophen (= paracetamol, = Tylenol®) from benzene by comparing the process with that from natural *p*-hydroxybenzoate that can be clipped off various biomass sources and straightforwardly and efficiently converted to these compounds, Fig. 3.[14] The required functionalization of the aromatic in *p*-hydroxybenzoate is already in place, allowing ready access to the amide that is then easily converted to the amine by the Hoffman rearrangement, and trivially acetylated in the final step as in the industrial process. By comparison, just producing phenol from benzene is a major 3-step process, albeit benefitting from the clever Hock reaction and the massive scale of the petrochemical industry. Then nitration is not regiospecific, and only one of the isomers is used for reduction to the amine. In the absence of cheap benzene, sourcing *p*-aminophenol and acetaminophen from it would be imprudent.
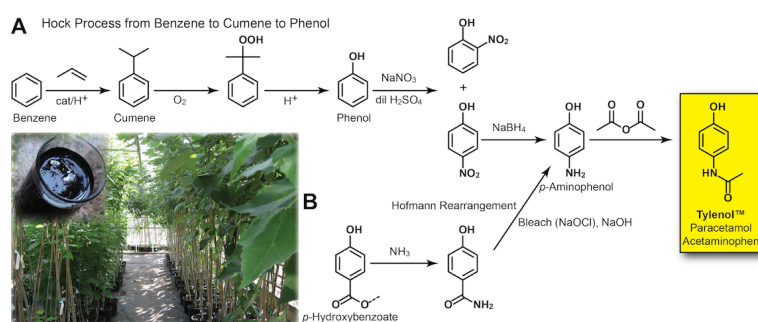

Fig. 3. Tylenol **Synthesis of acetaminophen (paracetamol, Tylenol®), from fossil *vs* biomass sources. A)** Synthesis from fossil-based benzene requires, for example, a several-step hydroxylation to produce phenol via the Hock process.[15] nitration followed by separation to produce *p*-nitrophenol, reduction to *p*-aminophenol, and final acetylation to produce acetaminophen. **B)** Because the *p*-hydroxybenzoate on poplar, willow, or palm lignins naturally has the correct substitution pattern,[4,16] it is simply a matter of producing the amide from the ester followed by a classical Hofmann rearrangement[17] to produce the *p*-aminophenol followed by the same acetylation step, a significantly shorter and more rational scheme.[14] The picture inset is of greenhouse-grown poplar (courtesy of Shawn Mansfield, UBC) *vs* one fossil source, coal tar for sourcing the starting materials. https://www.himadri.com/images/coal_tarpitch_cover_2.jpg)

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