Incorporation of 5,5-Coupled Diferulate Into Lignins

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Introduction

In past Research Summaries, we have presented studies that produced compelling evidence that plant cell wall ferulates actively incorporate into lignins via radical processes, rather than by an 'opportunistic' mechanism that has been widely held. Finally, last year, two-dimensional NMR spectra from a uniformly ^{13}C-enriched ryegrass provided unambiguous proof that this was indeed occurring in plants. Even more importantly, and somewhat unexpectedly, the NMR spectra also revealed that ferulates were acting as nucleation sites for the lignification process and therefore play a crucial role in directing the growth and development of the plant cell wall, ultimately impacting its digestibility. Ferulate dimers were also shown to be far more important in the cell wall than had been thought — prior work has neglected most of the diferulate isomers and identified only the 5,5-coupled dimer. Ferulate dimers, diferulates, are particularly important in the wall since they are capable of simultaneously cross-linking polysaccharide chains to each other and then to lignin, effecting powerful wall cross-linking.

Unfortunately, with the sole 5,5-diferulate that had been reported and quantitated for the past 20 years, it has been assumed to cross-link to lignin via the same old 'opportunistic' mechanism. Although it is almost absurd now to think that any diferulate will not also become actively involved in the free radical coupling processes that occur during lignification, there were still questions about what products would result from such radical coupling. Unfortunately, directly observing the fate of diferulates from NMR of plant isolates is an even more foreboding task than identifying the role of ferulates because of the number of ferulate dimers, their lower levels, and the problem that the ferulate cross-linked to lignin in a dimer is difficult to resolve from the structurally similar ferulate-lignin cross-link itself. Consequently, in a manner similar to that used previously to identify how ferulate could be incorporated into lignin, strategically ^{13}C-labeled 5,5-diferulate was biomimetically incorporated into a synthetic lignin and its incorporation profile determined by NMR methods.

Experimental

A ^{13}C-labeled model compound (Fig. 1) which mimics the 5,5-diferulate as it is attached to the C5-position of arabinofuranosyl residues on arabinoxylans in grasses was synthesized by a route far too complex to be described here. At the 5% level, it was biomimetically incorporated into a synthetic lignin from coniferyl alcohol. 2D-long range correlation (HMBC) spectra of the lignin-diferulate complex in 9:1:1 acetone-d_6:D_2O:dmso-d_6 were run to determine the incorporation profile (the structures produced by incorporation of the diferulate into the lignin).

Results and Discussion

In addition to 4-O-coupled products that can arise from either mechanism, diferulate produced 8-coupled products (8-O-4', 8-5', and 8-β') that can only arise by the radical mechanism. The incorporation profile, Fig. 2, is strikingly similar to that of ferulate. Clearly, diferulates and ferulates can each undergo radical coupling into lignin to produce the full range of expected structures. It is important to note that only the 4-O-products are capable of releasing identifiable ferulate or diferulate and this becomes particularly complex for diferulate which has two (joined) ferulates which may incorporate somewhat independently. Clearly, as we have warned and demonstrated with ferulate, 5,5-coupled diferulate (and in fact all of the

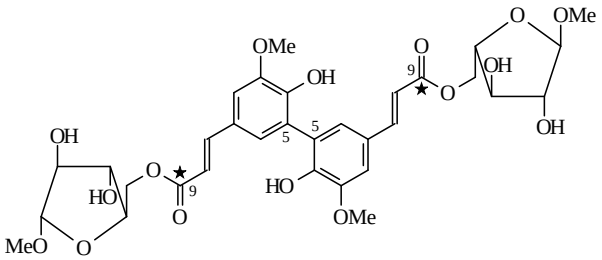

Figure 1. The ^{13}C-labeled 5,5-diferulate dimer which mimics the structure in arabinoxylans.

isomeric diferulates) are, and will continue to be, severely under-quantitated because it is impossible to release them from their intimately associated lignin. They therefore have a greater role in cell wall cross-linking and consequent indigestibility than is usually assumed.

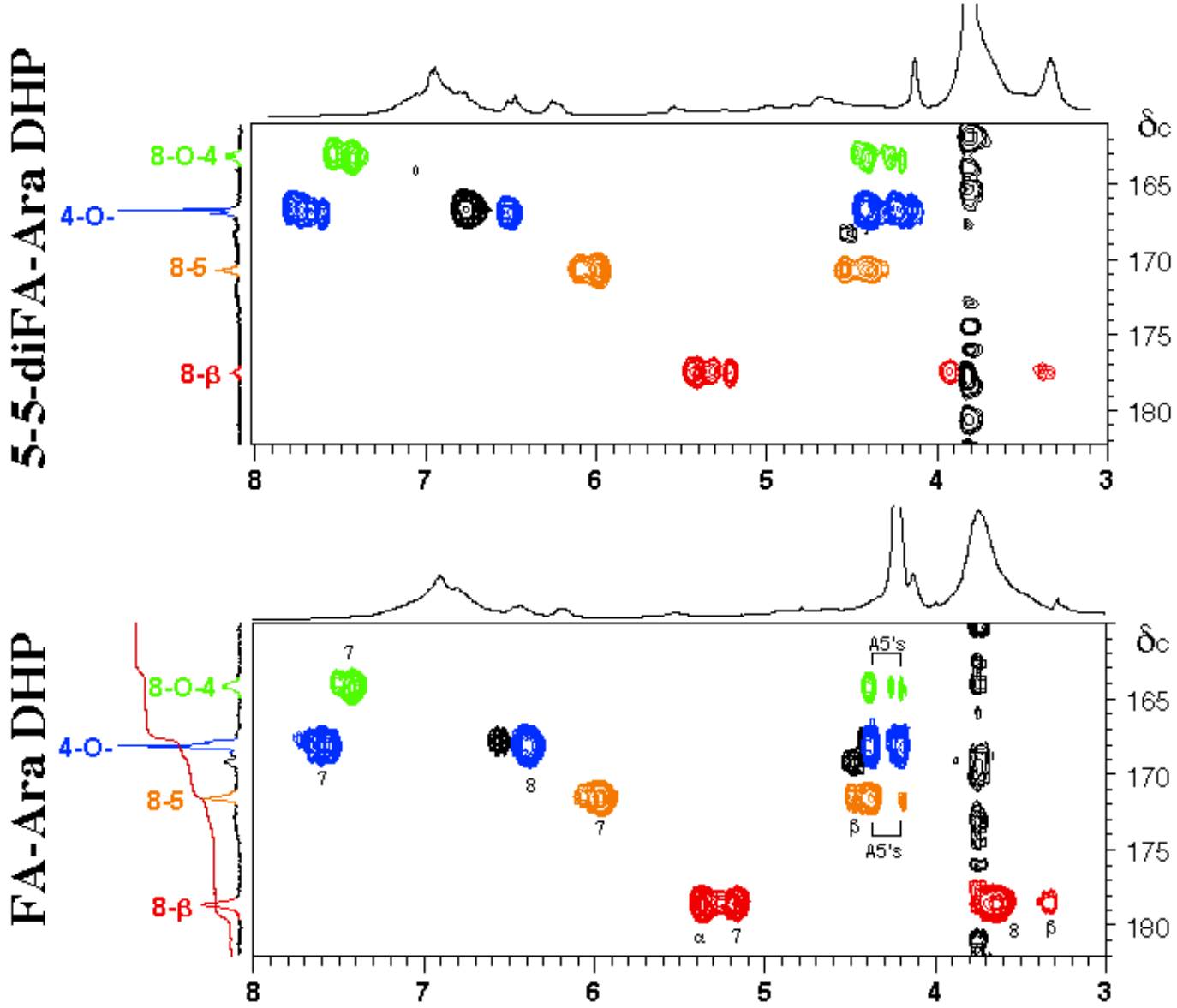

Figure 2. HMBC subspectra (carbonyl region only) of the product resulting when the labeled diferulate model (upper) or the corresponding ferulate model (lower) is biomimetically incorporated into a DHP. Similar incorporation profiles are seen. Radical coupling to 8-O-4', 4-O-X' (including phenylcoumarans), 8-5' and 8-β' products are readily identified.