

Synthesis of ^{13}C -labeled Coniferaldehyde and Its Incorporation into Synthetic Lignins

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Introduction

Lignins are formed in plants by peroxidase/ H_2O_2 -induced polymerization of *p*-coumaryl, coniferyl and sinapyl alcohols. In plants where CAD (cinnamyl alcohol dehydrogenase) activity is low, *p*-hydroxycinnamaldehydes presumably build up and could become a major component of the lignin produced. Cinnamaldehyde-containing lignins have been shown to severely inhibit fiber degradation in forages. To

determine lignification pathways and to explain the degradation behavior, it is necessary to determine the structure of CAD deficient lignins. Comparison of synthetic lignins (dehydrogenation polymers, DHPs) with plant lignins should identify structural features and provide an understanding of the differences in forage digestibility. A synthetic pathway to DHPs is presented using [γ - ^{13}C]coniferaldehyde **8**.

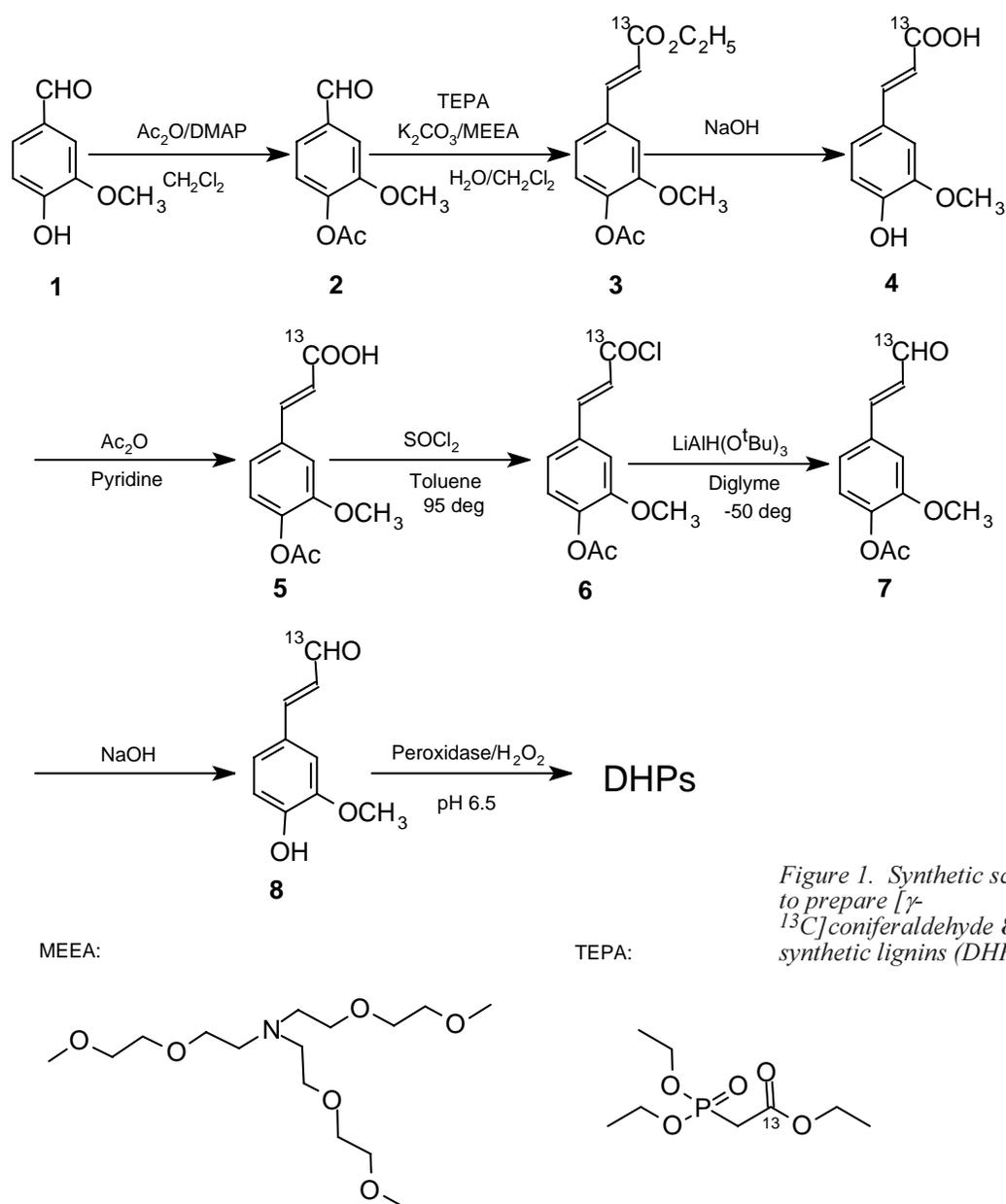


Figure 1. Synthetic scheme used to prepare [γ - ^{13}C]coniferaldehyde **8** and synthetic lignins (DHPs).

Method

Reaction of vanillin **1** with acetic anhydride/4-N,N-dimethylaminopyridine (DMAP) in methylene chloride gives 4-O-acetylvanillin **2**. The ^{13}C -label is introduced via a Wittig-Horner-Emmons reaction of **2** with [1- ^{13}C]triethylphosphonoacetate in a two-phase reaction system of water/methylenechloride using potassium carbonate as base and tris[(methoxyethoxy)ethyl]amine as a phase-transfer-catalyst. Saponification of the ester **3** under strongly alkaline conditions yields [γ - ^{13}C]ferulic acid **4** which has to be reacylated to give [γ - ^{13}C]4-O-acetylferulic acid **5**. Reaction of **5** with thionyl chloride in toluene at 95° C yields the acid chloride **6** which is not isolated but reduced immediately to [γ - ^{13}C]4-O-acetylconiferaldehyde **7** using lithium-tri-*t*-butoxyaluminum-hydride in diglyme at -50° C under inert conditions. Deacetylation of **7** with aqueous sodium hydroxide leads to the target molecule **8**. Two different dehydrogenation polymers (DHPs) were prepared using [γ - ^{13}C]coniferaldehyde **8**. The first was made by using 10% **8** and 90 % unlabeled coniferyl alcohol to elucidate cross reactions between the normal lignin monomer (coniferyl alcohol) and the aldehyde component. The second was made by reacting pure [γ - ^{13}C]coniferaldehyde **8** under lignification

conditions to elucidate homo-coupling reactions in cases where the aldehydes are in high concentration. Preparation of the DHPs was done using the “Zutropf-Method” where a solution of **8**, or **8** and coniferyl alcohol, was added dropwise to a mixture of peroxidase and H_2O_2 in a buffer solution.

Discussion and Conclusion

A method has been developed to prepare [γ - ^{13}C]coniferaldehyde **8**. The pathway utilized the previously described (Research Summaries 1991) way of easily introducing the ^{13}C label by using commercially available [1- ^{13}C]triethylphosphonoacetate in the Wittig-Horner-Emmons-reaction to produce ferulate. The aldehyde has now been used to prepare synthetic lignins (DHPs). NMR spectroscopy of these DHPs should elucidate radical coupling pathways that are accessible to coniferaldehyde and, along with the model data (see following article), provide the necessary data required to more carefully characterize lignins from CAD-deficient plants. Knowledge of which pathways and products can be selected for in plant breeding, or can be genetically altered, should ultimately result in plants that are more digestible and therefore provide greater sustainability to the farm system.