

# Evidence for the Role of Sinapyl *p*-Coumarate in Maize Lignification

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## Introduction

Maize lignin contains up to 20% *p*-coumaric acid (**1**, Fig. 1) which is esterified at the  $\gamma$ -position of phenylpropane side-chains. Attachment at the  $\gamma$ -position indicates that *p*-coumaric acid is esterified intracellularly to *p*-hydroxycinnamyl alcohols (**2**); the resulting *p*-hydroxycinnamyl *p*-coumarates (**3**) are exported to the cell wall where they participate in oxidative coupling processes associated with lignification. This summary describes the successful use of thioacidolysis/desulfurization experiments to determine whether *p*-coumaroylated lignins in maize are formed primarily with coniferyl or sinapyl *p*-coumarate.

## Methods

Thioacidolysis was performed on 10 mg of lignin, 40 mg of cell walls, and 2 to 5 mg of model compounds. Thioethylated products were hydrogenated with Raney nickel, trimethylsilylated, and analyzed by GC-MS and by GC-FID. The synthesis of model compounds has been previously reported (1989 and 1993 Research Summaries).

## Results and Discussion

Ether linkages in maize lignin were cleaved by thioacidolysis to release syringyl and guaiacyl units acylated with *p*-coumaric acid. After hydrogenation with Raney nickel, GC-MS analysis of thioacidolysis products revealed two previously unidentified peaks with mass spectra consistent with compounds **6a** and **6b**, hydrogenated forms of coniferyl and sinapyl *p*-coumarate (Figs. 1 and 2). The identity of these peaks was confirmed by comparing their GC retention times and mass spectra with authentic compounds. Recovery of *p*-hydroxycinnamyl *p*-coumarates was low due to ester cleavage and incomplete  $\beta$ -ether cleavage by thioacidolysis. Yields were not improved when thioacidolysis was conducted under reduced duration or temperature. Hydrogenation of phenylpropane side chains with Pd/C prior to thioacidolysis also did not improve the recovery of *p*-coumarate esters. The possibility of sinapyl *p*-

coumarate being an artifact formed during thioacidolysis was discounted by subjecting a mixture of willow lignin (a syringyl-guaiacyl lignin with no *p*-coumarate esters) and a model of  $\beta$ -ether **5a** to thioacidolysis/desulfurization. Although small quantities of coniferyl *p*-coumarate (**6a**) were recovered, sinapyl *p*-coumarate (**6b**) was not, confirming that sinapyl *p*-coumarate was a component of maize lignin and not an artifact formed by transesterification reactions. Peak areas of sinapyl *p*-coumarate (**6b**) were eight to nine times greater than that of coniferyl *p*-coumarate (**6a**), indicating that *p*-coumarate moieties were primarily attached to syringyl units. Since acylation should not differentially affect the release of syringyl or guaiacyl units from  $\beta$ -ether linked structures, our results strongly suggest that sinapyl *p*-coumarate (**3b**) was the major precursor of *p*-coumaroylated maize lignin.

## Conclusions

Analysis of thioacidolysis/Raney nickel products has demonstrated that *p*-coumaric acid is esterified primarily by syringyl units in maize lignin, providing compelling evidence that sinapyl *p*-coumarate is the major precursor of *p*-coumaroylated lignins in grasses. Recovery of *p*-hydroxycinnamyl *p*-coumarates was low. Modifications of the analytical procedure did not significantly improve yields. Current work in our laboratory is focused on understanding the biosynthesis, transport, and oxidative coupling reactions of *p*-hydroxycinnamyl *p*-coumarates.

*Figures on next page.*

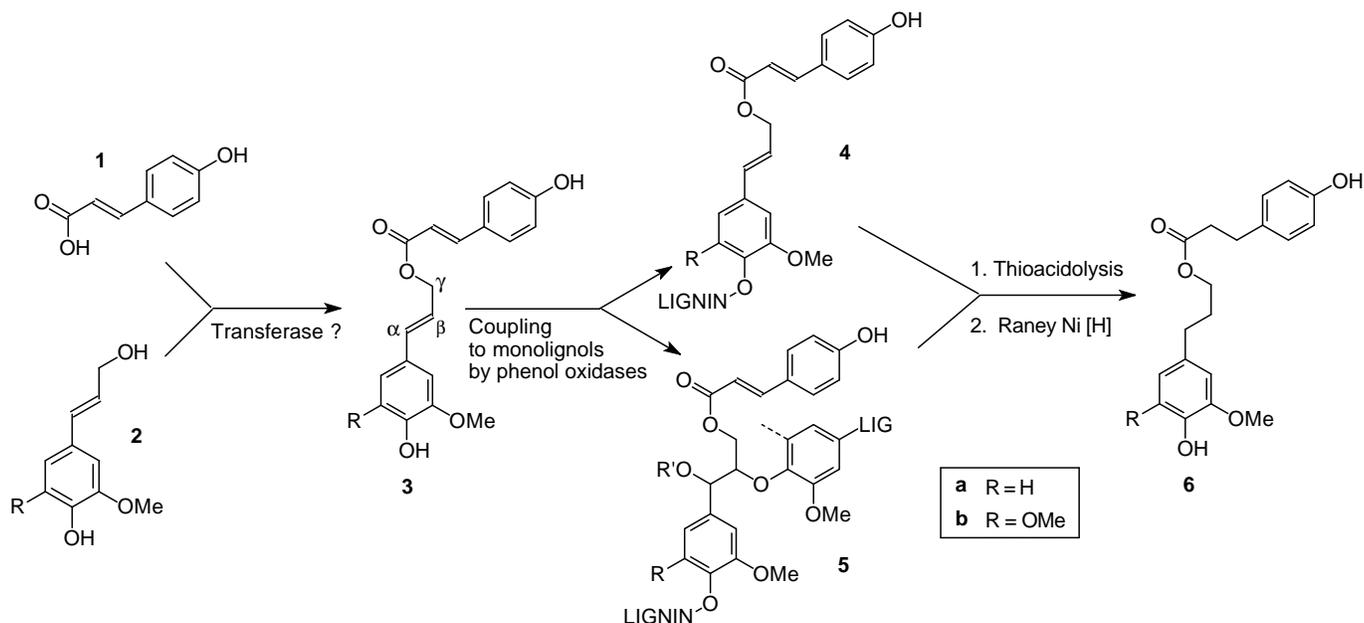


Figure 1. *p*-Coumaric acid (1) esterification by *p*-hydroxycinnamyl alcohols (2) forms *p*-hydroxycinnamyl *p*-coumarates (3) which are incorporated into lignin to give, among other products, two types of  $\beta$ -ether structures: the hydroxycinnamyl *p*-coumarate end group 4 and the  $\beta$ -ether 5. Thioacidolysis cleaves  $\beta$ -ethers while leaving a small proportion of the *p*-coumarate esters intact. After Raney nickel treatment, products 6a and 6b result, providing a distinction between coniferyl *p*-coumarate and sinapyl *p*-coumarate involvement in lignification.

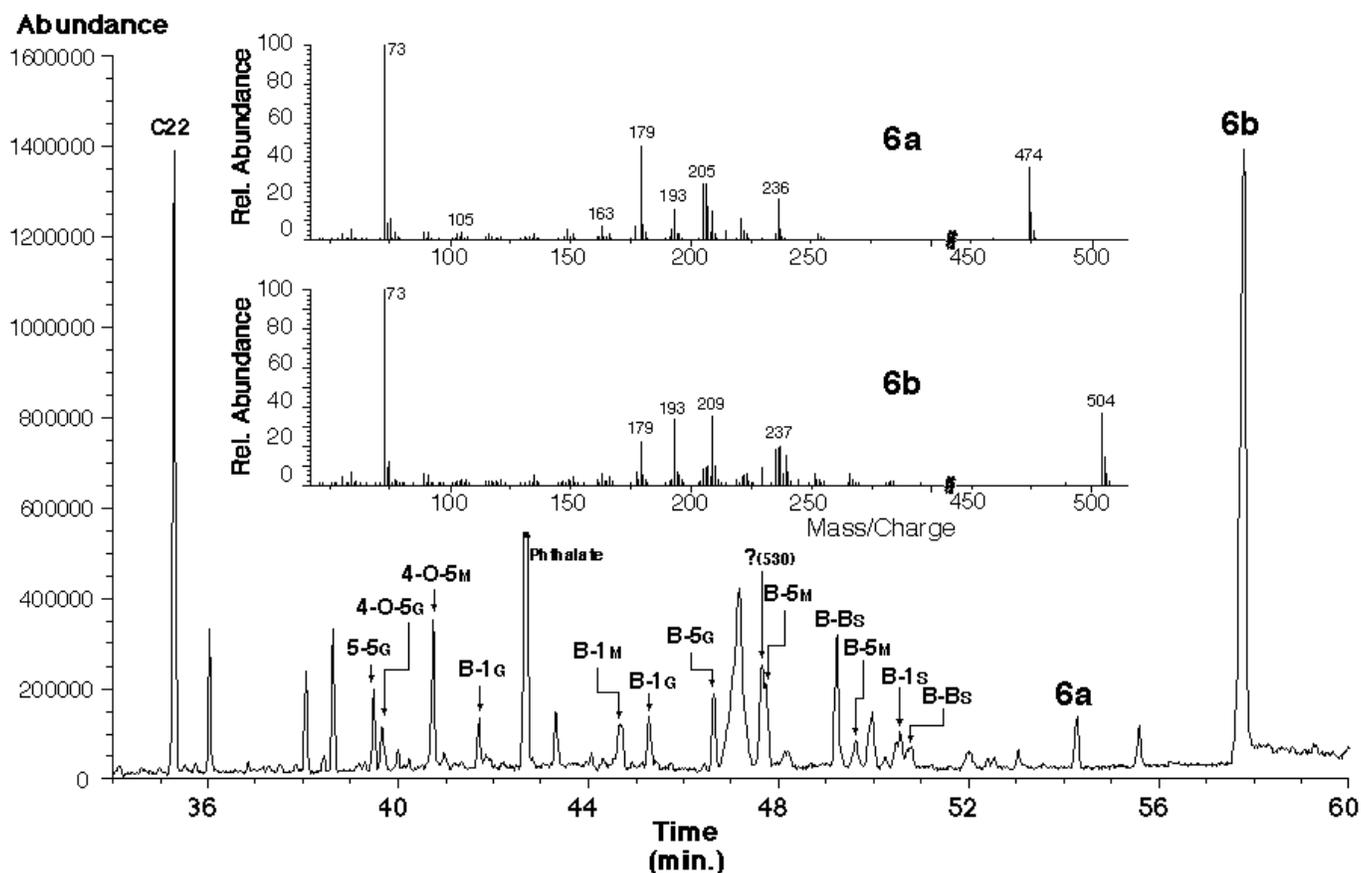


Figure 2. GC-MS of dimers recovered after thioacidolysis of maize lignin. Dimers were trimethylsilylated prior to analysis. *p*-Coumarate esters (6a and 6b) were observed later in the total ion chromatogram than previously identified S-G products.