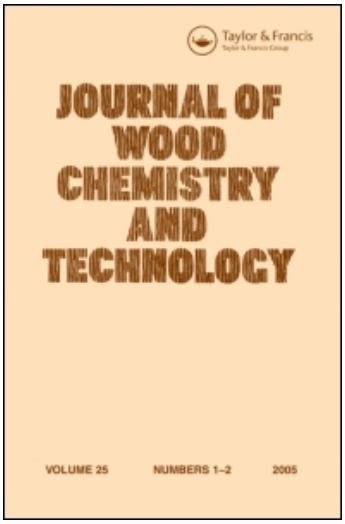

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The Syringyl Content of Softwood Lignin

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THE SYRINGYL CONTENT OF SOFTWOOD LIGNIN

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ABSTRACT

Representations of softwood lignins typically include small amounts, 2 to 5%, of syringylpropane units. Carbon-13 nuclear magnetic resonance and pyrolysis-gas chromatography of loblolly pine milled wood lignin indicated a much lower value. Pyrolysis-gas chromatography/mass spectrometry of whole softwoods also gave lower than expected yields of syringyl products. One chemical method, alkaline nitrobenzene oxidation, was also used to determine syringyl content. Results obtained using these methods indicate that the syringyl content of lignin in typical softwoods appears to be less than 0.1% of the total phenylpropane units. Ginkgo was also examined, and its lignin was found to contain only a very small amount of syringylpropane units. Cambial extracts of three softwoods were analyzed by pyrolysis-gas chromatography/mass spectrometry, and the data indicated the possibility of the presence of a slight amount of syringin. These results suggest that lignin in typical softwoods is formed almost exclusively from coniferyl alcohol.

INTRODUCTION

Softwood lignin is composed of phenylpropane units derived primarily from coniferyl alcohol. However, schematic models of softwood lignins often include a significant proportion of units

derived from sinapyl alcohol, ranging from 5% in Freudenberg's structure[1] to about 2% in Glasser and Glasser's structure.[2] Essentially, two arguments favor inclusion of these small amounts of syringyl units in softwood lignin. First, syringin, as well as coniferin, has been isolated from the sap of softwoods.[3] If these glycosides are considered to be lignin precursors, it seems plausible that sinapyl alcohol is incorporated into softwood lignin. Second, reconstruction of the ratio of monomer types from the methoxyl substitution patterns of oxidative analyses of lignins indicates a syringyl content of softwood lignin between 1 and 4%.[4,5]

In contrast, it was recently reported that syringin could not be detected in the cambial sap of six gymnosperms examined.[6] Also, analytical pyrolysis[7] and C-13 nuclear magnetic resonance (NMR) spectroscopy[8] indicated that the syringyl content of softwood lignin might be much less than that previously estimated. Also, a structural representation of softwood lignin by Sakakibara,[9] based mainly upon hydrogenolysis and hydrolysis data, does not contain any syringyl units.

Reexamination of the oxidative analyses reveals the possibility of production of artifacts which might mask the true syringyl content. For example, oxidative analysis of two "dehydrogenation polymers" (DHPs)[10] synthesized from coniferyl alcohol indicated 2 and 4% syringyl content.[5] One possible explanation for this is that some hydroxylation of guaiacyl units may occur during dehydrogenative polymerization to give 3,4-dihydroxy-5-methoxyphenyl units. 3,4-Dihydroxy-5-methoxyphenyl units have also been suggested as forming from coupling of guaiacyl units upon oxidative treatment of lignin.[11] Subsequent methylation and oxidation procedures would then afford a degradation product which might be construed to result from a syringylpropane unit. Also, if lignin contains 4-O-5 diphenyl ether linkages, these could possibly give rise to artifacts which would be indistinguishable from the oxidation product of syringyl units.

Syringyl structures among the pyrolysis products from softwood lignins have been identified by Fullerton and Franich.[12] However, they made no quantitative determinations. Recently, pyrolysis-gas chromatography (PY-GC) of HCl lignin from southern yellow pine indicated that the yield of 2,6-dimethoxyphenol was 23% as much as that that from mixed hardwood HCl lignin.[13] However, this study employed neither capillary gas chromatography nor identification of components by mass spectrometry (MS), and the pyrograms of lignins may be too complex for product identification and quantitation by packed-column gas chromatography.

The goal of the present work was to obtain a realistic value of the syringyl content of typical softwood lignin by refining the methods of C-13 NMR spectroscopy and PY-GC. We also examined a chemical method, alkaline nitrobenzene oxidation, which is not likely to create syringyl artifacts.

EXPERIMENTAL

Proton decoupled C-13 NMR spectra of acetylated loblolly pine MWLs, 300 mg in 2.0 ml of acetone-d6, were determined with a Bruker WM-250 spectrometer equipped with a 10-mm single-frequency (62.9 MHz) probe and an Aspect 2000A minicomputer. Spectra were obtained at 310°K with 10,000 to 20,000 8K Free Induction Decays accumulated over a spectral width of 20,000 Hz and zero-filled to 16K. A pulse angle of 75 degrees and a relaxation delay of 0.50 second were used. In double resonance experiments, an irradiation power of 0.2 watt was used in the Continuous Wave mode at a frequency in the proton region corresponding to 6.9 ppm.

The pyrolyzer used was a Pyroprobe 120 (Chemical Data Systems). A ribbon probe was used for MWLs which were applied as solutions in 90% dioxane. Wood samples, small slivers or powder in the case of the aspen-pine mixture, were pyrolzyed in quartz tubes using a coil probe. The rate of temperature rise was the maximum (ramp off) to a final pyrolysis temperature of 700°C, which was held for 10 seconds. The interface temperature was 220°C. For PY-GC, a split ratio of 10:1 was used. The sample stream was not

split for PY-GC/MS. Capillary columns (J&W Scientific, Rancho Cordova, California) used were: 0.254 mm x 30 m DX-4, maintained at 60°C for 1 minute then temperature programmed at 4°C/minute to 220°C, and 0.329 mm x 60 m DB-5, temperature programmed from 80°C at 2°C/minute to a final temperature of 220°C. PY-GC analyses were obtained with a Varian Vista 44 gas chromatograph and PY-GC/MS analyses were obtained with a Finnigan 4510 mass spectrometer at 70 electron volts.

The difficulties of quantitative analysis of lignin by PY-GC have been indicated previously.[7] However, this technique for determining syringyl content was improved by adding known amounts of white birch MWL, which is assumed to contain about 60% syringylpropane units, to loblolly pine MWL which was then pyrolyzed. If the yield of syringyl pyrolysis products, based on the original syringylpropane content of the lignin, is the same for hardwood and softwood lignins, this modification of "standard addition" should allow quantitative estimation of the syringyl content of loblolly pine MWL. An important consideration in analytical PY-GC is measuring syringyl pyrolysis products which are completely resolved from all other products. After careful examination of many pyrograms, the syringyl pyrolysis products chosen for quantitation were trans-1-(2,6-dimethoxyphenyl)propene and sinapaldehyde, because of the absence of interfering peaks. These products were previously identified by PY-GC/MS.[7] For PY-GC on a DB-1 capillary column, these products can be identified only by retention times, compared to those for authentic compounds. Therefore, PY-GC quantitation gives a maximum estimate of syringyl content.

Oxidations were conducted with 2.5 g of wood in 30 ml of 2N NaOH and 5 ml of nitrobenzene in stainless steel bombs heated at 175°C for 2.5 h. The alkaline solution was extracted with chloroform, neutralized and again extracted with chloroform. Gas chromatography analysis of the products was on a 30-m DX-4 capillary column but p-hydroxybenzaldehyde and syringaldehyde were not resolved. Therefore, the mass spectrometer was used as a specific detector for ion of mass 182, which is the molecular ion of syringaldehyde.

Among the products of alkaline nitrobenzene oxidation of lignin are vanillin from guaiacyl units and syringaldehyde from syringyl units. Syringaldehyde was first identified as a product from the nitrobenzene oxidation of spruce lignin by Leopold.[14] The yields of vanillin and syringaldehyde were 27.5 and 0.06%, respectively.

Milled wood lignins (MWLs) from loblolly pine (Pinus taeda) and white birch (Betula papyrifera) were isolated by 90% dioxane/water extraction of extractives-free vibratory ball milled wood. These crude MWLs were obtained in about 25% yield based upon the original lignin in the wood. The carbohydrate content of the pine MWL was lowered by the method of Lundquist and Simonson.[15] The yield of this fractionated MWL was about 3% based upon the lignin in the wood.

All wood samples used were from sapwood. Radiata pine (Pinus radiata) used for pyrolysis was obtained from the Forest Products Laboratory Wood Collection. Radiata pine used for nitrobenzene oxidation was from Kaingaroa State Forest, New Zealand. All loblolly pine samples were from North Carolina, whereas all norway spruce (Picea abies), white spruce (Picea glauca), red pine (Pinus resinosa), ginkgo (Ginkgo biloba), and lilac (Syringa) samples were collected locally.

Hot-water extracts of the cambial tissue of the woods (see Table 3) were by a method similar to that described by Freudenberg and Harkin[3] but on a much smaller scale. The woods were collected in June 1984 and extracted within 1 day of cutting.

RESULTS

C-13 NMR

A carbon-13 NMR spectrum of spruce lignin[16] under incoherent irradiation (broadband decoupling) did not show any appreciable signals which could be ascribed to carbons of syringyl units. However, the C-13 NMR spectra of crude MWLs which have not been

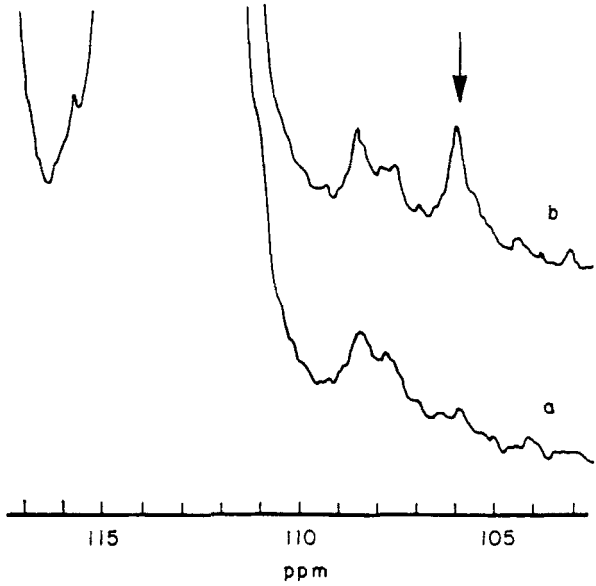

FIGURE 1. Partial carbon-13 nuclear magnetic resonance spectra of (a) low-carbohydrate acetylated loblolly pine milled wood lignins (MWL) and (b) the same MWL with American elm MWL. The arrow shows the signal caused by the 2,6-syringyl carbons. (ML85 5509)

fractionated to reduce carbohydrate content often contain resonances in the 106 ppm region. Although these resonances could be caused by the 2 and 6 aromatic carbons of syringyl units,[17] a loblolly pine MWL purified to remove carbohydrates[15] did not give any significant peaks in this region (Fig. 1a).

American elm MWL was added to the low-carbohydrate loblolly pine MWL to give a syringyl content of about 1.5% to the mixture. The C-13 NMR spectrum of this mixture clearly showed a resonance at

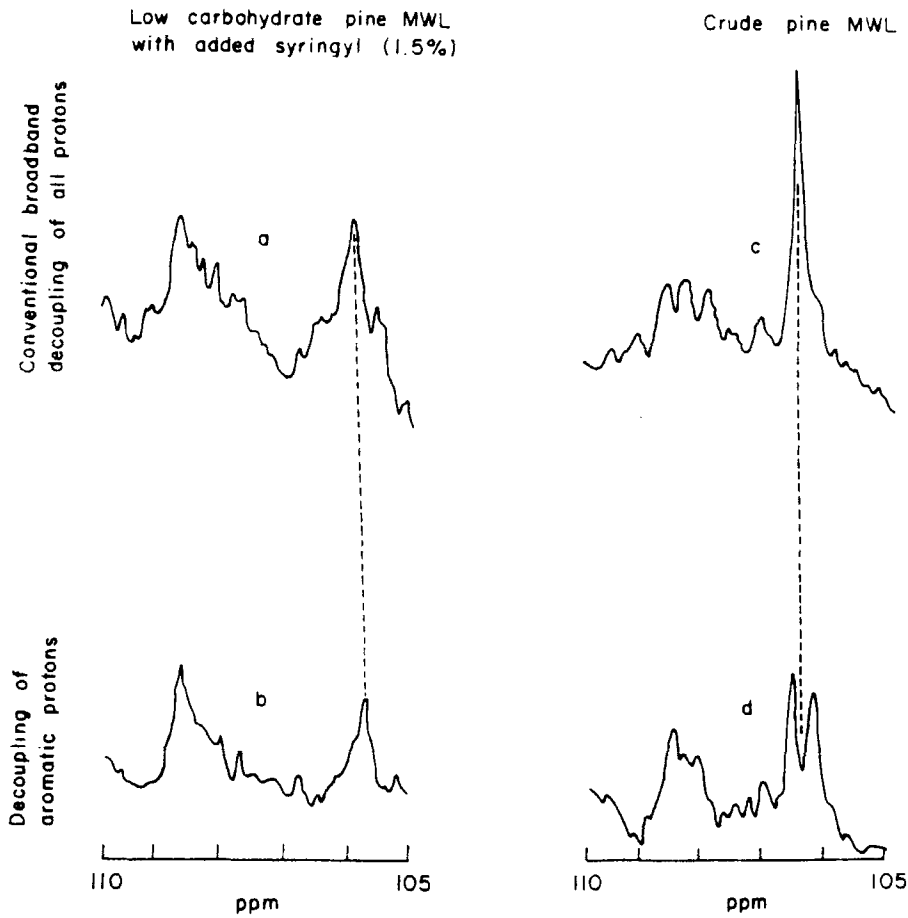

FIGURE 2. Partial carbon-13 nuclear magnetic resonance spectra of low-carbohydrate acetylated loblolly pine milled wood lignins (MWL) with American elm MWL (a and b), and of crude acetylated loblolly pine MWL (c and d) which contains about 7% carbohydrate. Spectra a c were acquired under conventional broadband decoupling conditions and spectra b and d, during coherent irradiation of the aromatic protons. (ML85 5510)

105.8 ppm because of the added syringyl 2 and 6 aromatic carbons of the elm MWL (Fig. 1b). Hence, we estimate that a syringyl content of as little as 0.25% could be detected by C-13 NMR spectroscopy. While this suggests an absence of syringyl constituents in this preparation, an important question remains: Did the fractionation procedure remove both carbohydrates and "syringyl lignin"?

A "double resonance experiment" was conducted using coherent irradiation at the frequency of the aromatic protons (coherent irradiation causes only the aromatic carbon signals to be singlets; other carbons with bonds to hydrogen will show some residual coupling and yield multiplets). In the C-13 NMR spectrum of the mixture of elm and pine MWLs, the aromatic syringyl carbon resonance at 105.8 ppm remains a singlet upon coherent irradiation of the aromatic protons (Figs. 2a and 2b). However, the crude loblolly pine MWL, which has not been mixed with hardwood lignin, gives a doublet in the syringyl region upon coherent irradiation of the aromatic protons (compare Figs. 2c and 2d). Thus, this large absorbance in the crude loblolly pine MWL cannot be caused by aromatic carbons and therefore does not represent an appreciable syringyl content in this MWL preparation.

Analytical Pyrolysis

Results from the analytical pyrolysis of loblolly pine and white birch MWLs, and known mixtures of the two are given in Table 1. Although the detector response was linear in the range of the standard additions, extrapolation to very low amounts of the syringyl products may introduce errors. However, from the data obtained by this method, we are confident that the syringylpropane content of loblolly pine MWL cannot be more than 0.01%.

The argument may be presented that small amounts of syringylpropane units in loblolly pine lignin may not yield significant amounts of pyrolysis products with the more complex side chains such as those in sinapaldehyde and trans-1-(2,6-dimethoxyphenyl)-propene. It would be more desirable to determine the amount of a simpler product, such as 2,6-dimethoxyphenol, because virtually

TABLE 1
Syringylpropane Content of Loblolly Pine Milled Wood Lignin Determined By Pyrolysis-Gas Chromatography Using a Flame Ionization Detector (FID).

Milled Wood Lignin	Syringyl-propane Content	Trans-1-(2,6-dimethoxyphenyl)-Propane	Sinapaldehyde
	%	FID counts	
Loblolly pine (6.45 μg)	<0.003[a]	<10[b]	<10[b]
White birch (5.14 μg)	~60	198,347	166,148
Loblolly pine (6.45 μg) + White birch (0.257 μg)	2.4	8,688	8,795
Loblolly pine (6.45 μg) + White birch (0.128 μg)	1.2	4,605	5,325
Loblolly pine (6.45 μg) + White birch (0.064 μg)	0.6	2,151	1,994

[a]The syringyl content of the loblolly pine MWL is calculated from the yields of the two pyrolysis products compared to that obtained from the birch/pine MWL mixtures. The syringyl content of the mixtures are minimum values. It is assumed that birch MWL has about a 60% syringyl content.

[b]These products were not detected. A peak reject of 10 counts was used. The calculated syringyl content of the loblolly pine therefore represents a maximum value.

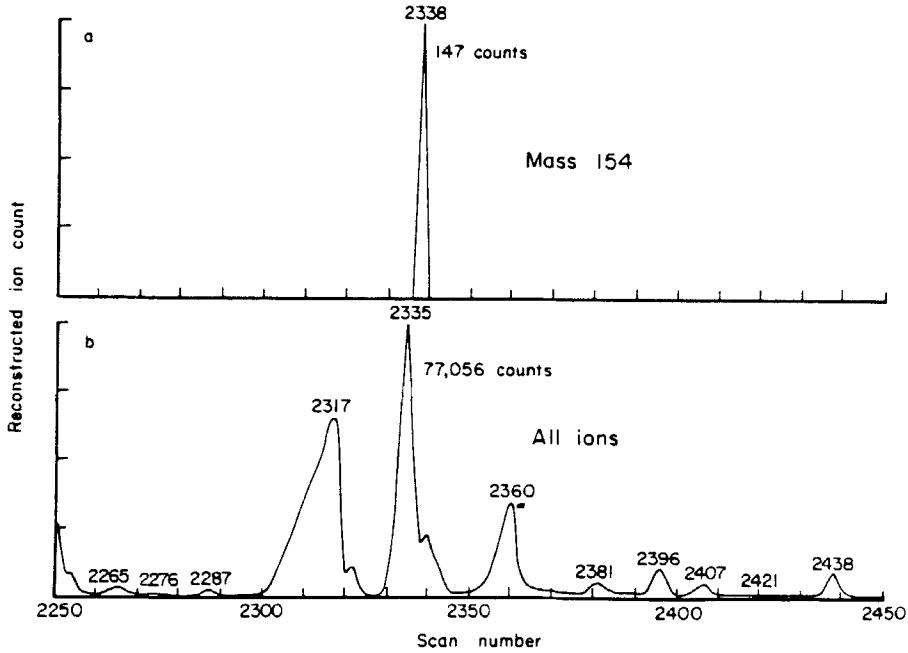

FIGURE 3. (A) A portion of the pyrogram of red pine wood, plotting only mass 154; authentic 2,6-dimethoxyphenol elutes at scan 2338. (B) The same portion of the red pine pyrogram with all ions plotted. The 2,6-dimethoxyphenol is contained in the much larger peak of scan 2335 (isoeugenol). (ML85 5511)

all syringyl units in lignin should give 2,6-dimethoxyphenol as a pyrolysis product. Additionally, it might be argued that MWLs are not representative of softwood lignins: fractionation may occur in their preparation so that lignin containing syringyl units is not obtained. Therefore, analytical PY-GC was performed on wood samples using a mass spectrometer as a quantitative and qualitative detector.

The advantage of PY-GC/MS is illustrated by Figure 3. The relative retention time of authentic 2,6-dimethoxyphenol, as well as that formed upon pyrolysis of hardwood samples, was measured to be 1.38, on a 60-meter DX-4 capillary column. When only mass 154, the molecular ion of 2,6-dimethoxyphenol, was plotted in the pyrogram of red pine wood, a retention time of 1.379, relative to the retention time of guaiacol, was calculated for the small peak at scan 2338 (Fig. 3a). However, when all ions were plotted (Fig. 3b), a large peak, isoeugenol, at scan 2335 with a relative retention of 1.377 was observed. Most PY-GC analytical systems would not be able to distinguish between these very similar retentions and the large peak might be misidentified, and wrongly quantified, as 2,6-dimethoxyphenol. Because the height count of the 154 peak is only 147, while the height count of scan 2335 is 77,056, the error would be approximately two orders of magnitude. In the pyrogram of a hardwood, obtained under the same conditions, the peak at 1.38 is mainly 2,6-dimethoxyphenol with a small, underlying peak due to isoeugenol. Other capillary columns could be sought to resolve these two compounds, but the PY-GC/MS technique makes it unneccessary, even if one is a minor component. It seems unlikely that packed-column gas chromatography analyses would be suitable for determining small amounts of syringyl products upon pyrolysis of softwood lignin or wood. Although it would seem that the 154 peak in the pyrogram of red pine wood (Fig. 3) is caused by 2,6-dimethoxyphenol, because of interfering masses no positive assignment may be made solely upon the basis of its mass spectrum. However, the amount is so slight, that it may be concluded that the syringyl content of red pine lignin is very low.

Quantitation of the PY-GC/MS method was analogous to that for the PY-GC method as a known amount of a hardwood was added to the loblolly pine to be pyrolyzed. In this case, vibratory ball milled wood samples were used to assure proper mixing and reproducible sampling. A 100:1 mixture of loblolly pine and aspen woods contains at least 0.34% of the total lignin as syringylpropane units. Using this value as a reference, estimates of the syringyl content of the

TABLE 2
Syringyl Content of Various Softwood Lignins Determined By Pyrolysis-Gas Chromatography/Mass Spectrometry on the Whole Wood Samples.

Wood	Ion Counts[a] of Mass 154 (2,6-dimethoxy-phenol)	Ion Counts[a] of Mass 124 (guaiacol)	Ratio of Mass 154 to Mass 124 Counts	Syringyl Content of Lignin
				%
L. Pine:Aspen 100:1 (w/w)	1,680	100,096	0.0169	0.033[b]
Loblolly Pine	<20[c]	117,632	<0.00016	0.003[c]
Radiata Pine	<20[c]	100,608	<0.00018	0.004[c]
White Spruce	236	142,080	0.00166	0.033
Norway Spruce	574	177,152	0.00324	0.064
Red Pine	147	79,616	0.00185	0.036
Gingko	77	119,296	0.00065	0.013

[a]Mass 154 is the molecular ion of 2,6-dimethoxyphenol and mass 124 is the molecular ion of guaiacol. The height of the peaks of the molecular ions at the respective retention times of the authentic compounds is reported. If the areas of these peaks were used, a somewhat lower syringyl content would result.

[b]The syringyl content of the aspen/loblolly pine mixture is calculated on the basis of the syringyl content of the aspen lignin, which is assumed to be about 50%, and therefore represents a minimum value. The remaining syringyl values are calculated based upon the data from this mixture.

[c]No 2,6-dimethoxyphenol was detected. Because only peaks with counts of 20 or greater were recorded, the calculated syringyl content for these samples represents a maximum value.

TABLE 3
Pyrolysis-Gas Chromatography/Mass Spectrometry of Cambial Extracts.

Cambial Extract	Ion Counts[a] of Mass 154 (2,6-dimethoxy-phenol)[b]	Ion Counts[a] of Mass 124 (guaiacol)	Ratio of Mass 154 to Mass 124 Counts
Norway Spruce	272	151,552	0.0002
Red Pine	175	25,152	0.007
White Spruce	394	58,432	0.007
Lilac	125,696	17,056	7.4

[a]Mass 154 is the molecular ion of 2,6-dimethoxyphenol and mass 124 is the molecular ion of guaiacol. The height of the peaks of the molecular ions at the respective retention times of the authentic compounds is reported. If the areas of these peaks were used, a lower syringyl content would result.

[b]The presence of 2,6-dimethoxyphenol (mass 154) could result from the pyrolysis of syringin or some other syringyl-type material. The value of the 154/124 ratio can only qualitatively indicate a syringyl content.

lignins from five softwoods and gingko, which gave a pyrogram typical of softwoods, were all below 0.07% (Table 2). The syringyl content of loblolly pine, sitka spruce, and white spruce MWLs, assayed by PY-GC/MS determinations of 2,6-dimethoxyphenol and 4-methyl-2,6-dimethoxyphenol, were all below 0.01%.

PY-GC/MS was also performed on aqueous extracts of the cambial tissues of three softwoods and lilac. Reinvestigation of the cambial extracts of softwoods had not indicated the presence of syringin.[6] No attempt was made to isolate syringin in our work; however, it seems likely that syringin would give 2,6-dimethoxyphenol upon pyrolysis. All three softwood cambial extracts did give some 2,6-dimethoxyphenol, identified by relative retention time and mass spectra (Table 3). However, the amounts were very small and comparable to that found upon pyrolysis of the whole wood. The

occurrence of 2,6-dimethoxyphenol in the pyrogram of the extracts does not, of course, prove the presence of syringin. Additionally, the presence of syringin in the cambium is not necessarily associated with incorporation of syringylpropane units into softwood lignin if the final steps of precursor biosynthesis occur within the lignifying xylem cells.

Nitrobenzene Oxidation

The syringyl content, based on nitrobenzene oxidation of wood samples, of the lignins of loblolly and radiata pines and gingko

TABLE 4
Syringyl Content of Pine and Ginkgo Lignins as Measured By Pyrolysis-Gas Chromatography/Mass Spectometry of the Syringaldehyde Formed Upon Nitrobenzene Oxidation.

Wood	Area of Mass 182 Peak[a] (syringaldehyde)	Area of Mass 152 Peak[a] (vanillin)	Ratio of Mass 182 to Mass 152 Areas	Syringyl
				%
L. pine	450	2,099,030	0.000214	0.015
L. pine + 1% Birch	8,846	1,502,250	0.00589	0.41
Radiata pine	<20[b]	1,700,680	0.000011	0.001[b]
Ginkgo	2,057	2,367,540	0.000869	0.060

[a]The 182 and 152 ion peaks are the molecular ions of syringaldehyde and vanillin, respectively. The syringyl content of the L. pine/birch mixture is based upon the syringyl content of the birch lignin and therefore is a minimum value. The remaining syringyl contents are calculated based upon the data for the mixture.

[b]No syringaldehyde was detected. Only peaks with counts of 20 or greater were recorded and the calculated syringyl content represents a maximum value.

are given in Table 4. Loblolly pine lignin appeared to contain only about 0.015% syringylpropane units. No syringaldehyde was detected among the oxidation products of radiata pine. Gingko wood gave a somewhat higher amount of syringaldehyde relative to vanillin, but the amount was low enough to suggest that its lignin is composed mainly of guaiacyl units.

SUMMARY AND CONCLUSIONS

The syringylpropane content of several softwood lignins was investigated using two instrumental methods and one chemical assay. C-13 nuclear magnetic resonance spectra did not reveal any syringyl 2 and 6 aryl carbons. We estimate that 0.25% syringyl content should be detectable by this method. Analytical pyrolysis-gas chromatography of loblolly pine did not indicate any syringyl pyrolysis products, at a detectability limit of about 0.003%. In addition to isolated lignins, whole wood was analyzed by pyrolysis-gas chromatography using a mass spectrometer to identify and quantify pyrolysis products. 2,6-Dimethoxyphenol was considered to be the most likely pyrolysis product formed from syringylpropane units in softwood lignins. No 2,6-dimethoxyphenol was detected upon the pyrolysis of loblolly pine and radiata pine woods. The detectable limit for syringylpropane content by PY-GC/MS was about 0.004%. By this method, white spruce, norway spruce, red pine and gingko lignins had detectable syringyl contents, but all were below 0.07%. Alkaline nitrobenzene oxidation of loblolly pine, radiata pine, and gingko woods also indicated syringyl contents not greater than 0.06%.

Thus the results obtained all strongly indicate that the syringylpropane content of typical softwood lignins, and gingko, is very low. Because the syringylpropane contents are so low it is difficult to determine their value accurately. However, we estimate that the syringyl content of the lignins analyzed is probably less than 0.1% of the total number of phenylpropane units. One implication of this conclusion is that if the syringyl

content of softwood lignin is 1/20th to 1/50th of that shown in some lignin structural formulas, then, in order to reflect the analytically determined methoxyl/C9 values, the p-hydroxyphenylpropane contents have to be lowered. This suggests that softwood lignin is composed almost exclusively of guaiacylpropane units.

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