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Summary

The unprotonated aromatic regions of the solid-state ^{13}C NMR spectra of several hardwoods and softwoods are examined. Spectra are acquired with cross polarization, magic-angle spinning, and delayed proton decoupling. The hardwood spectra are decomposed into syringyl and guaiacyl components with the aid of a softwood spectrum, which is assumed to be the same as the guaiacyl component of the hardwood spectrum. The molar ratio of syringylpropanoid units to guaiacylpropanoid units (S/G) in hardwood is determined from the intensities of their respective component spectra. Ratios for elm (0.45), basswood (0.70), oak (0.70), maple (0.85), cherry (1.00), birch (1.00), and poplar (1.20) are reported. These results were comparable to literature values that were obtained by a combination of methoxyl and elemental analyses. Nitrobenzene oxidation gave syringaldehyde/vanillin ratios that were approximately three times greater than the corresponding NMR S/G ratios.

Introduction

A fundamental structural characteristic of hardwood lignin is the molar ratio of syringylpropanoid (S) monomer units to guaiacylpropanoid (G) monomer units, represented by S/G. The two commonly used methods for determining S/G
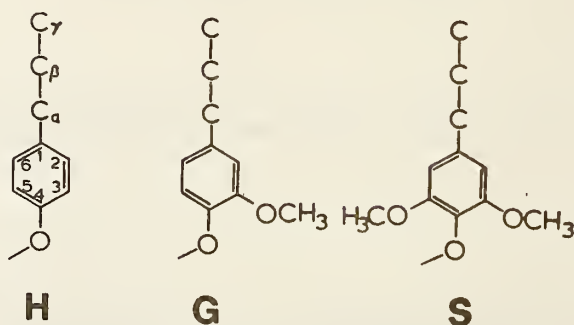
are the chromotographic analysis of nitrobenzene-oxidation products (Creighton et al. 1944), and a combination of methoxyl and elemental analyses on isolated lignin (Sarkanen et al. 1967). The ranges of S/G for hardwoods that are obtained from the two methods, namely, from 2.3 to 3.3 for the oxidative degradation method and from 0.3 to 1.5 for the methoxyl/elemental analysis, are markedly different. Explaining the high ratios for the former, Sarkanen and Hergert (1971) cited the more frequent involvement of G units in condensed structures and, in turn, the resistance of condensed structures to nitrobenzene oxidation. In view of the above ranges, they suggested that the nitrobenzene oxidation ratios were, as a rule of thumb, three times greater than the actual S/G ratios in hardwood. Physical methods of analysis, for example, IR (Sarkanen et al. 1967), UV (Sarkanen et al. 1967) and solution ^{13}C NMR (Obst and Ralph 1983), provide semiquantitative results although a quantitative determination of S/G with solution ^{13}C NMR spectra of milled wood lignins was described recently (Landucci 1985). The S/G values in that study are comparable to those obtained from the methoxyl/elemental method.

Described here is a quantitative method for determining S/G in whole hardwood with solid-state ^{13}C NMR techniques including cross polarization (CP) and magic-angle spinning (MAS) (Schaefer and Stejskal 1976; Yannoni 1982). The potential of the solid-state ^{13}C NMR experiment is indicated by (1) the broad range of ^{13}C chemical shifts for lignin (Lüdemann and Nimz 1973), (2) the quantitativeness of the CP/MAS wood spectra (Haw et al. 1984a), and (3) the convenience and simplicity of whole-wood sampling. Seven genera of hardwood are represented in order to test the universality of the method and permit multiple comparisons with S/G values obtained by other techniques.

Experimental

Samples of the following woods were obtained from commercial lumber stock: spruce (_Picea_ spp.), western red cedar (_Thuja plicata_), southern pine (_Pinus_ spp.), maple (_Acer_ spp.), yellow poplar (_Liriodendron tulipifera_), red oak (_Quercus_ spp.), basswood (_Tilia_ spp.), and cherry (_Prunus_ spp.). The white birch (_Betula papyrifera_) and american elm (_Ulmus americana_) samples were taken directly from trunk wood. Samples were ground in a Wiley mill and the wood meal (40 mesh) was extracted with benzene/ethanol (2:1) in a Soxhlet apparatus for 8 hr and air dried. The extracted ground wood (320 mg) was mixed with adamantane (10 mg), a secondary ^{13}C chemical shift reference (δ = 29.50 ppm and 38.56 ppm), and pressed into pellets. Because of weak C-H dipolar couplings, the adamantane signal survives the delay-decoupled experiment.

The ^{13}C NMR spectra were obtained at 15.08 MHz on a homebuilt spectro-meter. Delay-decoupled spectra were acquired with 2 ms of cross polarization with the ^{1}H and ^{13}C fields matched at 57 kHz, high-power proton decoupling that was begun 45 μs after the conclusion of cross polarization, and magic-

angle spinning at 2300 Hz. 90,000 scans were averaged with a repetition rate of 1.5 s. The signal-to-noise ratio was improved by exponential apodization, which introduced 12-Hz line broadening. Baseline corrections and phasing were carefully maintained in order to ensure reproducible intensity measurements of the unprotonated aromatic region, which is measured from 115 to 163 ppm. Phasing parameters were obtained from the delay-decoupled spectrum of hexamethylbenzene.

Results and Discussion

Analysis of Wood NMR Spectra

The chemical shift assignments in the CP/MAS ^{13}C NMR spectra of softwoods (Fig. 1a) and hardwoods (Fig. 1c) have been discussed previously (Kolodziejski et al. 1982; Taylor et al. 1983; Haw et al. 1984; Haw et al. 1984a); the distinguishable features of the wood spectrum are assigned as follows: from 60 to 110 ppm, cellulose and hemicelluloses; from 110 to 160 ppm and 56 ppm, aromatic and methoxyl carbons, respectively, in lignin; and 20 ppm and 172 ppm, methyl and carboxylate carbons, respectively, in acetyl groups of hemicelluloses. Delayed decoupling (Opella and Frey 1979) is employed in order to select certain lignin resonances in the CP/MAS spectrum. With this technique, the intensities due to carbons with one or two directly bonded protons, which include the principal carbohydrate carbons, are reduced to an insignificant fraction of their original intensity. Accordingly, resonances due to methoxyl (56 ppm) and unprotonated aromatic (115-163 ppm; C-1, C-3, and C-4 in G; C-1, C-3, C-4, and C-5 in S) carbons appear in the delay-decoupled softwood (Fig. 1b) and hardwood (Fig. 1d) spectra. The relative intensities of the unprotonated aromatics are unaltered (Schmitt and Sheppard 1984), but the usefulness of the methoxyl resonance is reduced by its nonquantitative

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intensity (Schmitt and Sheppard 1984) and overlap with residual carbohydrate signal. The unprotonated aromatic regions of the delay-decoupled spectra of softwood (spruce, cedar, and pine) and hardwood (poplar, birch, cherry, maple, oak, basswood, and elm) are shown in Figures 2 and 3, respectively.

Lignin high-resolution ^{13}C NMR data (Lüdemann and Nimz 1973, 1974), with which most of the following assignments are made, include multiple chemical shifts for many of the unprotonated aromatic carbons, owing to inter-unit structural variations. This fine structure is generally not resolved in the solid-state spectra. Unless specified, an assignment to a particular carbon in either an S or G unit pertains to all structures of the unit.

Peaks in the softwood spectrum (Fig. 2) are assigned to unprotonated aromatic carbons in G units; only trace amounts of p-hydroxyphenylpropanoid (H) units (Westermark 1985) and S units (Obst and Landucci 1986) are reported for softwoods. The absence of significant intensity from 160 to 162 ppm, a region assigned to C-4 in H units (Lüdemann and Nimz 1973, 1974; Nimz et al. 1981) is consistent with this assignment. The broad, structured peak centered at 148 ppm is composed of resonances due to C-3 and C-4 (Lüdemann and Nimz 1973); the peak at 134 ppm is assigned to C-1 (Lüdemann and Nimz 1973). Slight differences in the structure of the downfield resonance distinguish the three softwood spectra.

The hardwood spectrum (Fig. 3) comprises resonances due to unprotonated aromatic carbons in either S or G units; the absence of significant intensity from 160 to 162 ppm is consistent with reports (Sarkanen and Hergert 1971; Nimz et al. 1981) of there being only trace amounts of H units in hardwoods. The broad resonance at 136 ppm is assigned to C-1 in G units and to C-1 and C-4 in S units (Lüdemann and Nimz 1973). The sharp peak at 153.5 ppm is assigned to C-3 and C-5 in S units which are O-alkylated at C-4 (Haw et al.

1984). The upfield wing of the 153.5 ppm peak is assigned to C-3 and C-4 in G units (Lüdemann and Nimz 1973). C-3 and C-5 in S units that are not O-alkylated at C-4 appear at 148 ppm (Haw et al. 1984) and also contribute intensity to the wing, but the total number of S and G phenols in hardwood lignin is small, about 15% (Nimz 1974). In addition, it is expected that S phenols are the minority in view of the limited ability of the dimethoxy-substituted S unit to form condensed structures. In the cherry spectrum (Fig. 3c), the peak at 145 ppm and the intensity from 155-160 ppm are due to condensed tannins (Newman et al. 1985). The possibility of removing these impurities by means of additional extractive methods (Newman et al. 1985) was not investigated.

Determination of S/G

Large differences in the magnitude of S/G are apparent in the delay-decoupled CP/MAS ^{13}C NMR spectra of the hardwoods (Fig. 3). As S/G increases, the peak at 153.5 ppm (C-3 and C-5 in S units) will increase in intensity relative to its upfield wing (C-3 and C-4 in G units). Poplar (Fig. 3a) clearly exhibits the greatest S/G, and elm (Fig. 3g) the smallest; the others are placed intermediately but are not readily distinguishable.

In order to obtain S/G, the unprotonated aromatic region of the hardwood spectrum is decomposed into individual spectra of the S and G components. The decomposition is based upon two assumptions. The first is that the softwood spectrum closely resembles the G component of the hardwood spectrum. Consistent chemical shifts are evidenced by the nonexistence of a hardwood/softwood distinction for the high-resolution G-unit chemical shifts (Lüdemann and Nimz 1973). With respect to resonance intensities, structural studies suggest that the abundances of G structural units are mostly unaffected by the

incorporation of S units. This conclusion is based upon the abundances of structural units that involve C-5 and therefore must contain a G unit. On a per-G-unit basis, the respective numbers of 5-0-4 (diphenylether) and β-5 (phenylcoumaran) structures found in hardwood (Nimz 1974) are comparable to those in softwood (Adler 1977). Some minor intensity differences between the softwood spectrum and the G component of the hardwood spectrum are expected owing to the lower abundance of the exceptional 5-5 (biphenyl) structure in hardwood (Nimz 1974; Adler 1977). The second assumption is the existence of a G-resonance window from about 146 to 148 ppm in the hardwood spectrum. The proximity of the chemical shift of C-3 and C-5 in unalkylated S units (148 ppm) is presumed to be inconsequential in view of the low abundance for this structure (vide supra).

The relative total intensities of the S and G components of the hardwood spectrum are determined in the following manner. A hardwood spectrum is paired with a softwood spectrum and the latter is linearly scaled by a factor that renders it less intense than the G component of the hardwood spectrum. This is indicated by positive intensity in the G-resonance window in the difference spectrum (hardwood minus softwood). The softwood spectrum is then scaled upward, and difference spectra generated, until some point in the difference spectrum, presumably in the G-resonance window, has just begun to go negative (illustrated in Fig. 4). When this occurs, the total intensity and the spectrum of the S component are given by the difference spectrum (exclusive of tannin resonances); the G-component total intensity is equal to the total intensity of the softwood spectrum. Before S/G is calculated, the S and G intensities are normalized to an equal number of unprotonated aromatic carbons in their respective structures.

Decompositions of the hardwood spectra in Figure 3 with the cedar softwood spectrum (Fig. 2b) gave the S-component (difference) spectra shown in Figure 5. Each spectrum consists of two peaks, except the cherry (Fig. 5c), which also contains the previously identified tannin resonances. The chemical shift of the upfield resonance in the S spectrum, which is assigned to C-1 and C-4, is 137 ppm; the position of the downfield peak (C-3 and C-5) was unaffected by the decomposition. The intensities of the two resonances provide evidence for the validity of the decomposition. Since each resonance is assigned to two carbons in the S unit structure, equal intensities are indicated. In the S spectra, the intensity of the peak at 137 ppm ranges from 0.85 to 0.96 times the intensity of the peak at 153.5 ppm.

With regard to the abundance of unalkylated S units in hardwood, it is noted that only the elm S spectrum (Fig. 5g) exhibits significant intensity at 148 ppm. A simulation of the unalkylated component of the S spectrum (Fig. 6a) (cf. Haw and Schultz 1985) was constructed from the poplar S spectrum by isolating a segment of the spectrum containing only the peak at 153.5 ppm, shifting the segment 5 ppm upfield, and adding it to the remaining upfield resonance. After scaling the total intensity of the unalkylated spectrum to a certain percentage of the elm S spectrum intensity, it was subtracted from the original elm spectrum and the decomposition was repeated. The resulting S spectra are compared in Figure 6b. The intensity at 148 ppm is completely removed when the subtracted unalkylated intensity is equal to about 6% of the total S-unit intensity. More significant, the intensity of the G component was not changed from the value obtained in the original decomposition. Accordingly, S/G was also unaffected.

The values of S/G for the hardwood/softwood pairs are given in Table 1. In each case the use of the cedar spectrum as the softwood spectrum resulted in the greatest S/G; spruce and pine gave comparable values. Most were within 0.05 ratio units of their respective averages, and this interval was adopted as the uncertainty in the average value of S/G. Averages ranged from 0.45, for elm, to 1.20 for poplar. Published methoxyl/elemental results (Sarkanen and Hergert 1971) for five of the hardwoods and nitrobenzene oxidation results for the samples used in this study are included in Table 1. The solid-state ^{13}C NMR S/G values are in close agreement with the methoxyl/elemental results; the orders of S/G values, excluding the basswood entries, that were obtained by the two methods are the same. Hence the solid-state ^{13}C NMR method provides confirmation of the methoxyl/elemental method for determining S/G. Nitrobenzene oxidation yielded syringaldehyde/vanillin (S/V) ratios that were approximately three times greater than the corresponding NMR S/G values. Interestingly, Sarkanen and Hergert (1971) suggested that the S/V ratios were, as a rule of thumb, three times greater than the ratios of the corresponding units in hardwoods (see Introduction).

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Table 1

Hardwood Values of S/G Obtained by Solid-State ^{13}C NMR and Other Methods

Hardwood	Spruce	-Softwood- Cedar	Pine	Arithmetic Mean[a]	Methoxyl/ Elemental[b]	Nitrobenzene Oxidation[c]
Elm	0.42	0.49	0.44	0.45	0.32	1.3
Basswood	0.67	0.77	0.63	0.70	0.92	2.0, 2.1
Oak	0.65	0.76	0.68	0.70	0.72	2.2, 2.4
Maple	0.79	0.88	0.81	0.85	0.79	3.0
Cherry[d]	0.98	1.05	0.99	1.00	--	3.9, 4.2
Birch	0.98	1.05	0.99	1.00	0.92	2.9, 3.2
Poplar	1.16	1.23	1.16	1.20	--	3.6

[a]Rounded to nearest pentad, uncertainty is ± 0.05.
[b]Sarkanen and Hergert (1971). For lignin thioglycolic acids.
[c]Syringaldehyde-vanillin molar ratio. Method in Obst and Landucci (1985). Duplicate analyses were performed on some samples.
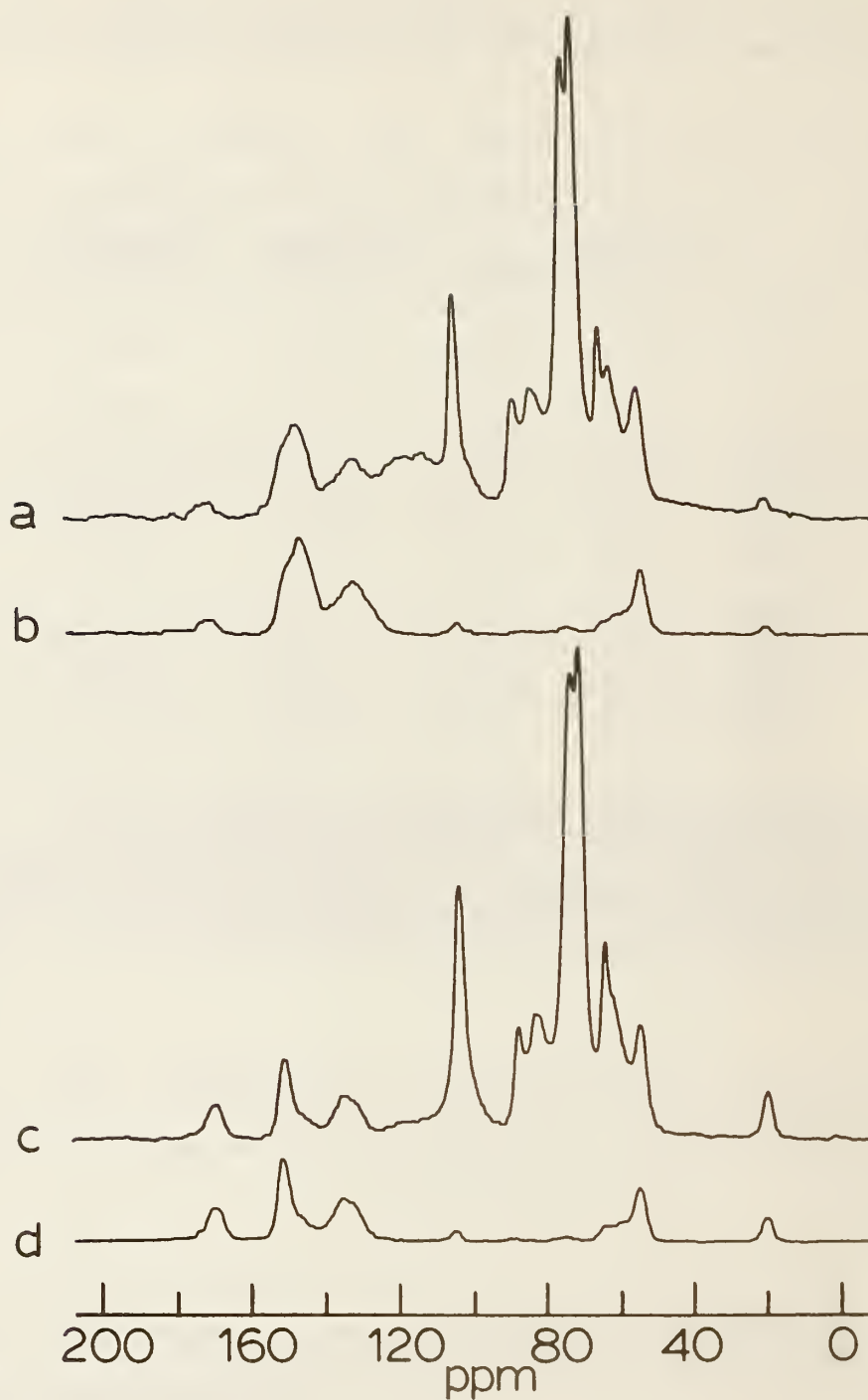[d]Tannin resonances in this spectrum increase the uncertainty of S/G.

Figure 1. CP/MAS ^{13}C NMR spectra of (a) cedar, a representative softwood; (b) cedar with delayed decoupling; (c) poplar, a representative hardwood; and (d) poplar with delayed decoupling.
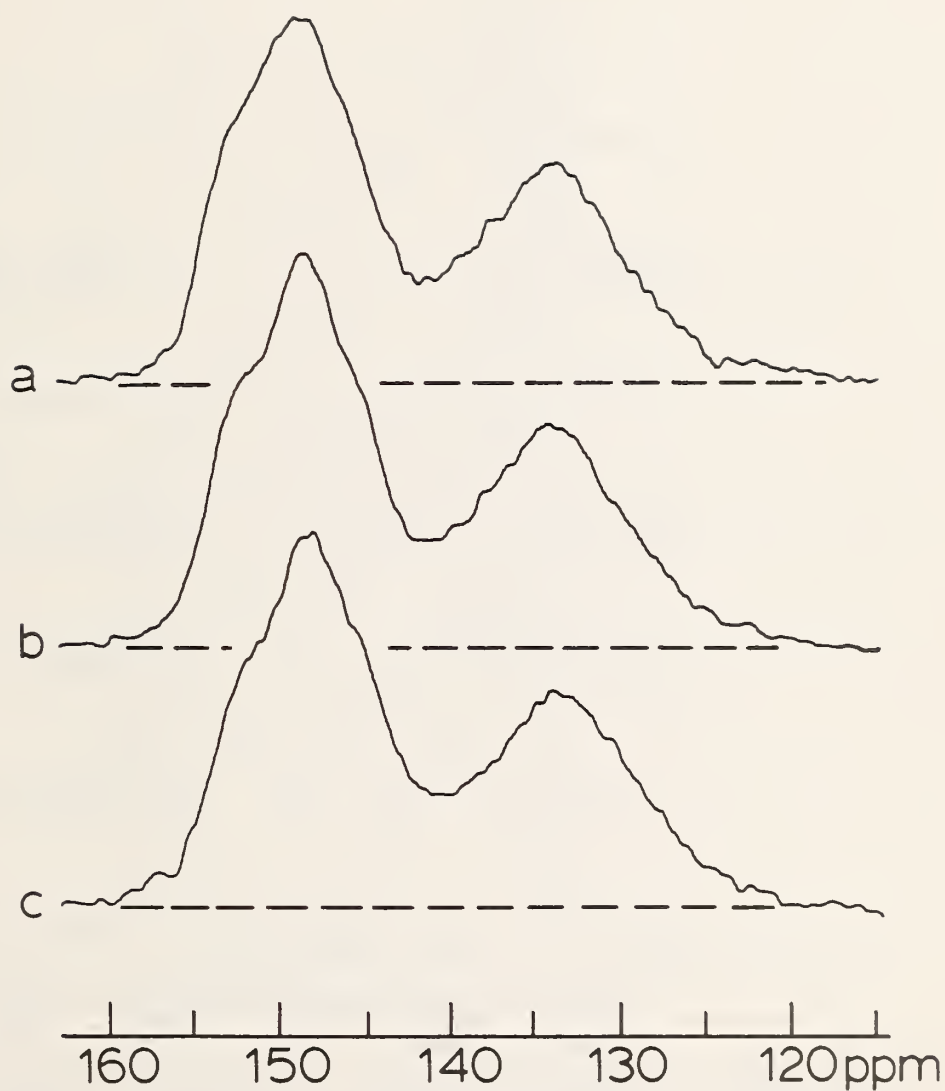
Figure 2. The unprotonated aromatic region of the delay-decoupled
CP/MAS ^{13}C NMR spectra of selected softwoods: (a)
spruce, (b) cedar and (c) pine. Total spectral
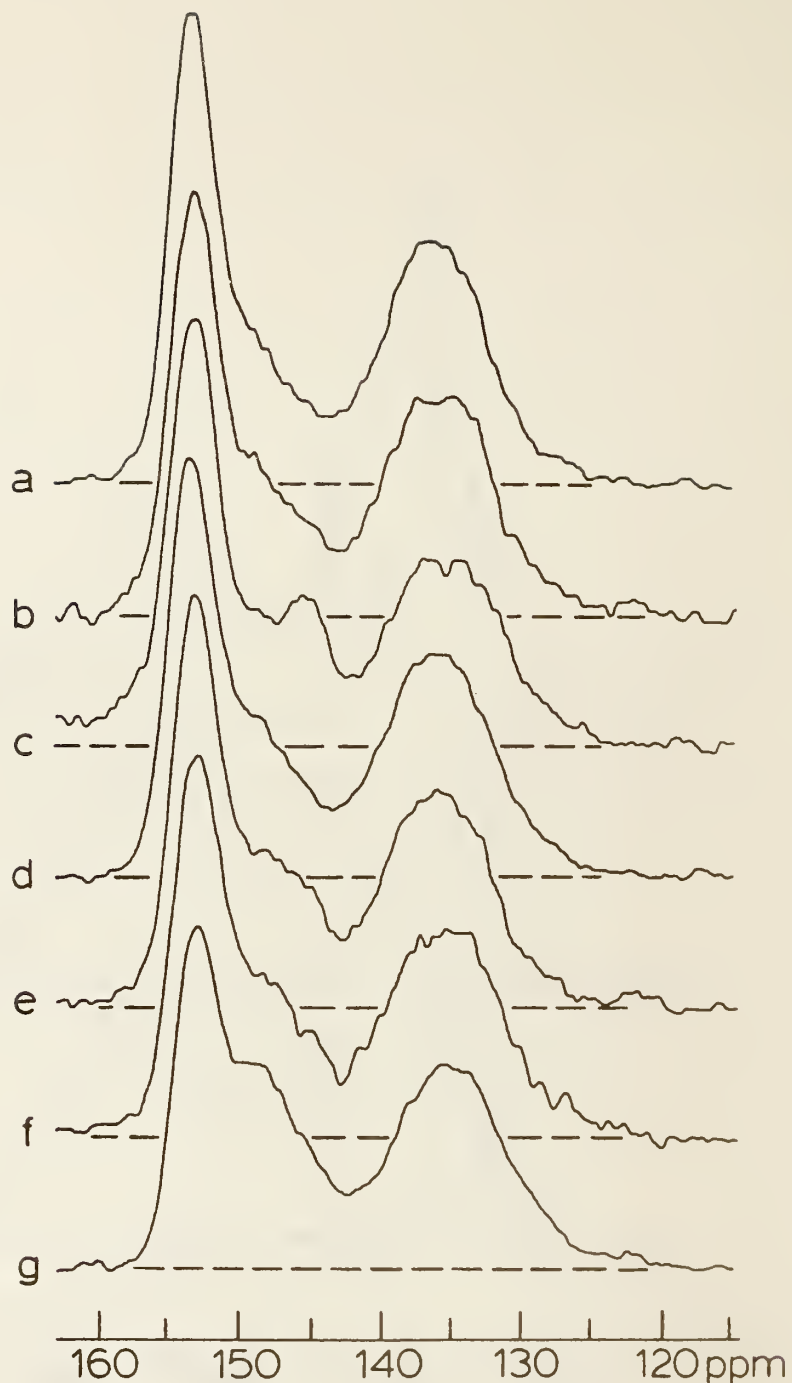intensities are equivalent.

Figure 3. The unprotonated aromatic region of the delay-decoupled
CP/MAS ^{13}C NMR spectra of selected hardwoods: (a)
poplar, (b) birch, (c) cherry, (d) maple, (e) oak, (f)
basswood and (g) elm. The spectra are arranged from
top to bottom in order of decreasing S/G. The change
in the intensity of the sharp S-unit peak at 153.5 ppm
relative to its upfield wing, due to G units, can be
seen. Total spectral intensities are normalized to an
equivalent total of monomeric units by utilizing the
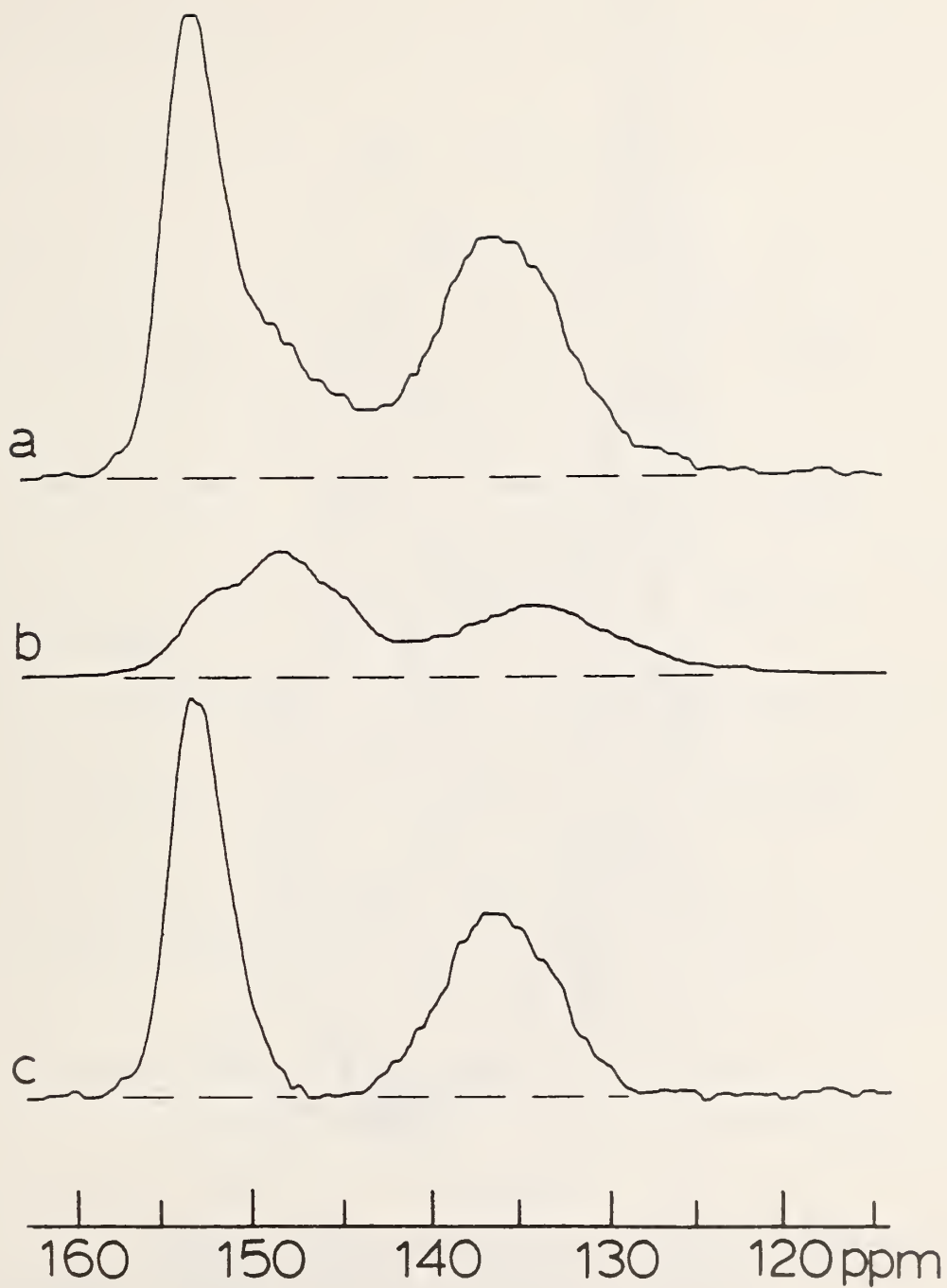average S/G values in Table 1.

Figure 4. Illustration of decomposition method for obtaining the individual intensities of the S and G components. The properly scaled softwood (cedar) spectrum (b) is subtracted from the hardwood (poplar) spectrum (a) to give its S component (c). (See text for details.)
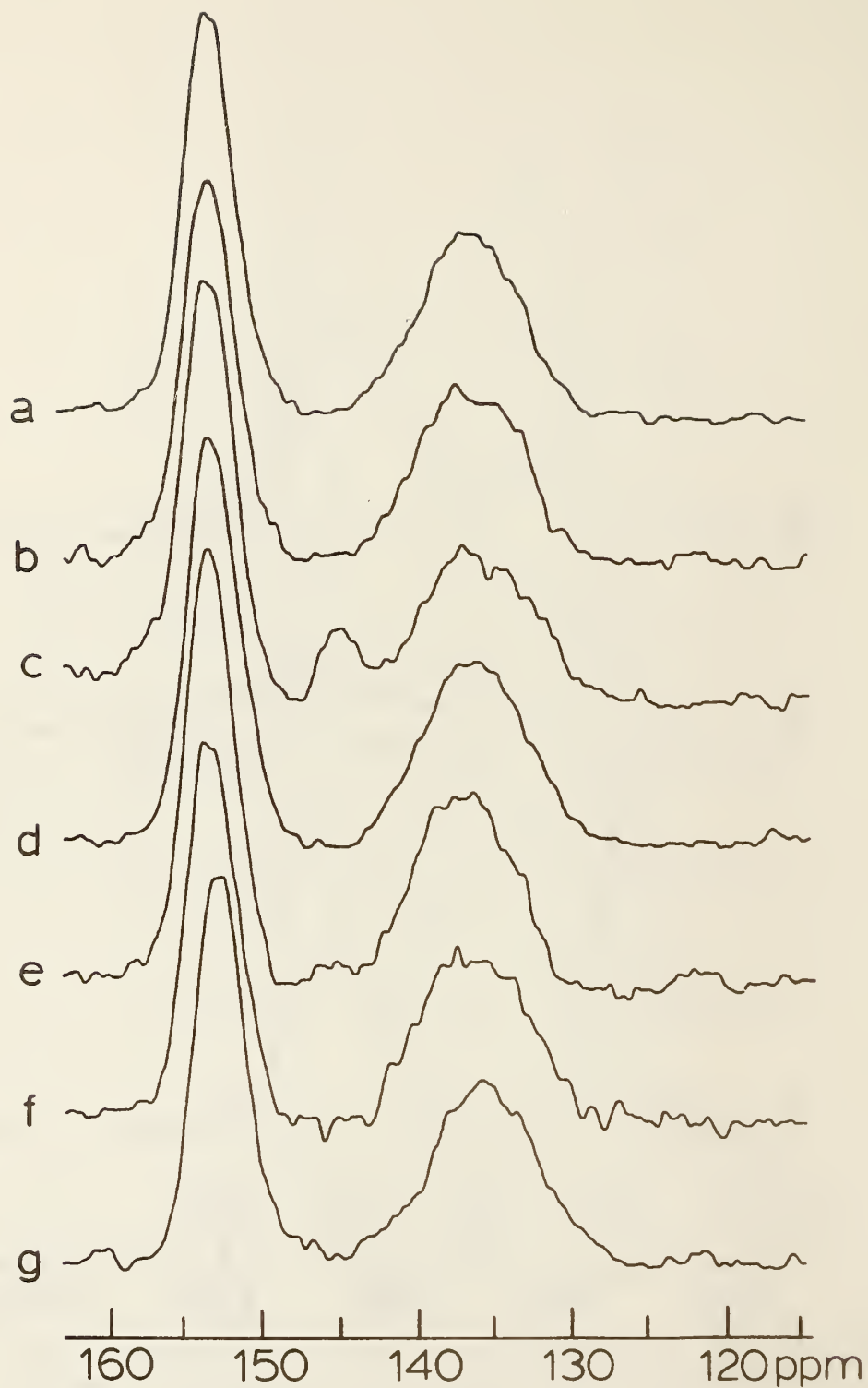
Figure 5. The S-component spectrum of the following hardwoods:
(a) poplar, (b) birch, (c) cherry, (d) maple, (e) oak,
(f) basswood and (g) elm. (See text for details.)
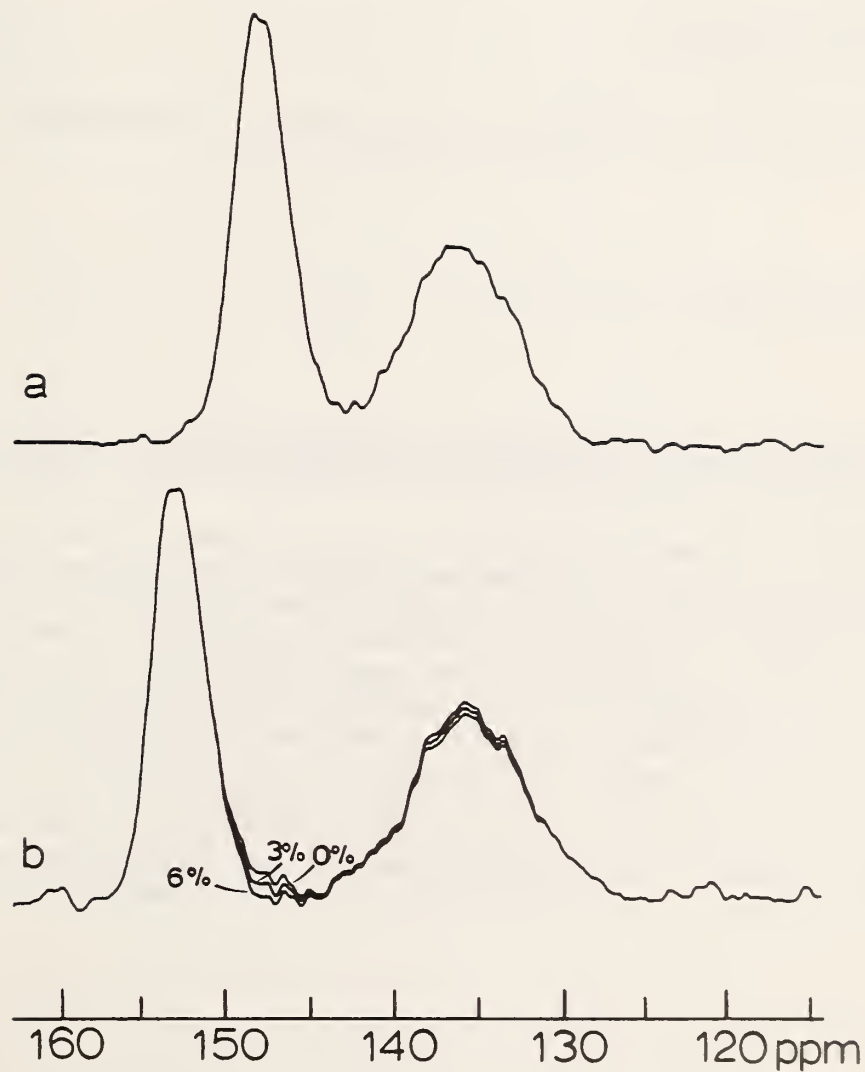Total spectral intensities are equivalent.

Figure 6. The simulated unalkylated S spectrum is shown in (a).
Scaled versions of (a) representing the indicated
abundances were subtracted from Fig. 3g and its
decomposition with the cedar softwood spectrum was
repeated.   The resulting S component spectra are
compared in (b).

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11. ABSTRACT *(A 200-word or less factual summary of most significant information. If document includes a significant bibliography or literature survey, mention it here)*

The unprotonated aromatic regions of the solid-state ^{13}C NMR spectra of several hardwoods and softwoods are examined. Spectra are acquired with cross polarization, magic-angle spinning, and delayed proton decoupling. The hardwood spectra are decomposed into syringyl and guaiacyl components with the aid of a softwood spectrum, which is assumed to be the same as the guaiacyl component of the hardwood spectrum. The molar ratio of syringylpropanoid units to guaiacylpropanoid units (S/G) in hardwood is determined from the intensities of their respective component spectra. Radios for elm (0.45), basswood (0.70), oak (0.70), maple (0.85), cherry (1.00), birch (1.00), and poplar (1.20) are reported. These results were comparable to literature values that were obtained by a combination of methoxyl and elemental analyses. Nitrobenzene oxidation gave syringaldehyde/vanillin ratios that were approximately three times greater than the corresponding NMR S/G ratios.

12. KEY WORDS *(Six to twelve entries; alphabetical order; capitalize only proper names; and separate key words by semicolons)*

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