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## Pyrolysis-GC-MS Characterization of Forage Materials

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Pyrolysis-GC-MS pyrograms from a series of alfalfa preparations, a grass, an angiosperm wood, a cellulose, and an arabinoxylan were obtained under pyrolytic conditions optimal for aromatic components of plant cell walls. Approximately 130 pyrolytic fragments have been identified by a combination of mass spectral interpretation, comparison with literature data, and, where possible, confirmation with authentic compounds. Several new fragments not previously noted in pyrograms have been conclusively assigned including both guaiacyl- and syringylpropyne and a range of alcohols. Diagnostic peaks from arabinose and xylose components of forages were found, along with markers for protein. Pyrolysis-GC-MS was particularly valuable for screening and differentiating *p*-coumaric acid and ferulic acid derivatives in tissues.

### INTRODUCTION

Analytical pyrolysis coupled with gas chromatographic (GC) separation of the components and mass spectral (MS) detection is an emerging technique which is proving useful in characterizing plant materials. Pyrolysis-GC-MS, along with pyrolysis-mass spectrometry (in which the sample is pyrolyzed and the unseparated volatile components are introduced directly into the mass spectrometer to yield a composite spectrum of all products) has been recently reviewed by Boon (1989).

Lignins may be pyrolyzed reproducibly to produce a mixture of relatively simple phenols which result from cleavage of ether and certain C-C linkages. Most of these phenols retain their substitution patterns from the lignin polymer, and it is thus possible to identify components from *p*-hydroxyphenyl, guaiacyl, and syringyl moieties. An intractable tar fraction and a gas fraction that are also produced are usually not analyzed in this context. Much of the pyrolysis-GC-MS work to date has been on woods and isolated wood lignins (Meier and Faix, 1991; Faix et al., 1987, 1990; Pouwels and Boon, 1987, 1990; Salo et al., 1989; Boon et al., 1987; Evans and Milne, 1987; Genuit et al., 1987; Pouwels et al., 1987; Saiz-Jimenez et al., 1987; Obst and Landucci, 1986; Fullerton and Franich, 1983; Obst, 1983; Martin et al., 1979). Grass and legume lignins differ from wood lignins. Grasses contain higher amounts of *p*-hydroxyphenyl units resulting from inclusion of *p*-coumaryl alcohol along with coniferyl and sinapyl alcohols in the monomer pool for free-radical-induced polymerization (Sarkanen and Ludwig, 1971). Both legumes and grasses contain a substantial fraction of ferulic and *p*-coumaric acids (Harris and Hartley, 1980; Hartley and Jones, 1976; Higuchi et al., 1967; L-Basyouni et al., 1964), both esterified and etherified to lignins, as well as esterified to carbohydrate components (Scalbert et al., 1985). On pyrolysis of grasses, Meier and Faix (1991) and Martin et al. (1979) examined pyrolysis-GC-MS of bamboo, and Boon (1989) reviewed work with Hartley on barley straw, corn stems, and agropyron. Hartley and Haverkamp (1984) have correlated phenolic acids in grasses with total ion (nonseparated) pyrolysis-MS. To our knowledge, the only paper on pyrolysis-GC-MS studies involving legumes

is a study correlating pyrolysis product production with measures of crude protein, fiber, lignin, and in vitro digestibility (Reeves, 1990).

Carbohydrate pyrolysis products are considerably more difficult to unequivocally identify by mass spectrometry because of their facile fragmentation (under electron impact conditions) to nondiagnostic low molecular weight ions. Here we have relied heavily on a combination of studies reporting pyrolysis products from wood holocellulose, cellulose, xylans, pectins, and other oligo- and polysaccharides (Boon, 1989; Pouwels et al., 1987, 1989; Aries et al., 1988; Evans and Milne, 1987; Helleur, 1987; Helleur et al., 1987; Van der Kaaden et al., 1984).

Our objective was to develop a rapid qualitative method for comparing plant cell walls with particular emphasis on phenolic components but with a view to glean information on important carbohydrate components and proteins as well. This paper is intended to provide a ready reference compilation of the variety of plant pyrolysis components—a compilation resulting from comparison with previous literature information along with the identification of several new compounds authenticated in this study. Interpreted pyrolysis chromatograms (pyrograms) of a series of alfalfa samples representing different fractionation stages, a grass (bromegrass), an angiosperm (hardwood) lignin, and two carbohydrate preparations (cellulose and an arabinoxylan) are presented. Alfalfa and bromegrass were chosen because of their importance as forage crops. The arabinoxylan was chosen because it represents a major type of hemicellulose in forages (Wilkie, 1983; Bailey, 1973) and because of the current interest in the esterification of phenolic acids to it (Hatfield et al., 1991). In particular, ferulic acid has been found esterified to C<sub>5</sub> of arabinose in arabinoxylans in a number of species (Kato et al., 1983; Harris et al., 1980; Smart and O'Brien, 1979; Harris and Hartley, 1976; Hartley, 1973). The willow wood lignin was included to provide a comparison to both grasses and legumes which, having both guaiacyl and syringyl components (Sarkanen and Ludwig, 1971), most closely resemble angiosperm lignins.

### EXPERIMENTAL PROCEDURES

**Samples.** Alfalfa (*Medicago sativa* L.) and bromegrass (*Bromus inermis* L.) samples were collected from plants grown in a greenhouse. High-pressure sodium lamps supplemented sunlight to give a constant 14 h/10 h day/night regime. Alfalfa stems

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were from early bud stage and included the lower nodes and internodes. Alfalfa samples for each of the fractions pyrolyzed for Figure 1 were not taken from the same batch of plant material. Bromegrass samples were taken at full bloom stage, using whole stems with sheaths removed.

Alfalfa stems were prepared for pyrolysis by freeze-drying freshly harvested stems. These were ground through a 1-mm screen in a Udy mill and used without fractionation. Alfalfa and bromegrass stem cell walls were isolated according to the procedure of Hatfield (1990). Briefly, stem tissue was homogenized in cold phosphate buffer (10 mM  $\text{NaH}_2\text{PO}_4$  plus 50 mM NaCl, pH 7.0), collected over double Teflon mesh filters (52  $\mu\text{m}$ ), and washed extensively with cold 50 mM NaCl (ca. 200 mL). The residue was further washed with acetone and chloroform/methanol (2:1) before air-drying. The cell wall residue was transferred to a jar mill with 80% ethanol and milled for 4–5 h to give particles of ca. 500  $\mu\text{m}$ . The milled walls were washed with 80% ethanol, followed by acetone, and air-dried. An amylase treatment was administered to remove any possible residual starch contamination.

The alfalfa enzyme lignin was prepared from the cell wall material by vibratory ball milling and treating with crude cellulases following literature procedures (Minor, 1986; Obst and Kirk, 1988). Briefly, Cellulysin Cellulase (1.5 g, Calbiochem, 10.4 units/mg) was dissolved in 30 mL of acetate buffer (pH 4.6) and insoluble material removed by centrifugation. The centrifugate was diluted to 300 mL with acetate buffer, and 1 mL of toluene and 5 mL of Tween 80 were added. Vibratory ball milled solvent-extracted alfalfa stem material (23 g) was suspended in 125 mL of this cellulase solution, which was stirred at 48 °C for 7 days. Soluble material was decanted following centrifugation, and fresh enzyme solution added for a further 7-day treatment period. The product was centrifuged and the residue washed three times with water before lyophilizing. The product, obtained in 17% yield, contained ca. 17% total sugars and 6% uronic acids.

The willow milled wood lignin (MWL) was prepared from extracted willow (*Salix alba*) by ball milling and extracting with dioxane/water as described by Björkman (1956). The MWL yield was ca. 5.9%, which contained ca. 16% total sugars and 4% uronic acids.

**Pyrolysis-GC-MS.** The sample, 200–500  $\mu\text{g}$ , was pyrolyzed in a quartz tube in a Pyroprobe 120 (Chemical Data Systems) at 700 °C (>50 °C/ms) for 10 s using helium as the carrier gas with a mean linear velocity of 14.3 cm/min. The sample was carried onto a 0.25 mm  $\times$  60 m DB-1 (J&W Scientific) column fitted in an HP 5890 GC set in the splitless mode. The temperature was held at 50 °C for 2 min to trap and focus the volatile components, then programmed to a final temperature of 275 °C at 4 °C/min, and held at that temperature to give a total run time of just over 60 min. Eluting compounds were detected with a Hewlett-Packard 5970 mass selective detector controlled by an HP UNIX data-station, and total ion chromatograms (TICs) reconstructed. Compounds of interest eluted between 15 and 60 min (Figure 1).

**Authentic Compounds.** Some compounds were required for authentication of assignments or to make new assignments not previously reported in the literature. The compounds included 14, 19, 22, 23, 26 (a known impurity in commercial 52), 27, 30, 31, 34, 36, 39, 41, 43, 45, 48, 51, 52, 58, 59, 61, 65, 67–70, 72, 74, 75, 77, 78, 80–87, 90, 93, 97, 99, 102, 104, 107–111, 113, 114, 116, 117, 122. Additionally, the following compounds are well authenticated from pyrolysis of simple compounds: 42 from arabinose, 54 from xylose, 83 and 95 from cellobiose. The styrenes 53, 64, and 94 were obtained very cleanly from decarboxylation, by pyrolysis, of the parent cinnamic acids. Compounds 123 and 124 were obtained by extracting dried ground alfalfa with acetone and pyrolyzing the extract.

All compounds were obtained commercially (Aldrich Chemical Co.) or were derived from commercially available materials as noted below.

Primary alcohols 65, 80, and 108 were obtained in high yield by reduction of aldehydes 67, 75, and 102 using sodium borohydride in 50:50 ethanol/water.

Secondary alcohols 69, 84, and 110 were similarly obtained from ketones 77, 85, and 111.

Methyl esters 78, 90, and 116 were obtained by methylation

of acids 86, 93, and 117 with methanolic HCl, prepared from methanol and acetyl chloride (Fieser and Fieser, 1967).

Methyl ethers 19, 22, and 23 were prepared by diazomethane methylation of the corresponding cresols 27, 30, and 31.

Propiovanillone (97) was prepared by Friedel-Crafts acylation of propionic acid onto guaiacol (Ralph, 1982).

*trans*-Coniferyl alcohol (113) was prepared according to standard methods (Kirk, 1988).

Dihydroconiferyl alcohol (104) was obtained by 1,4-reduction of methyl ferulate using lithium aluminum hydride in tetrahydrofuran at room temperature.

Compound 109 (*trans*-2,6-dimethoxy-4-propenylphenol) was prepared by refluxing commercial (Aldrich) 4-allyl-2,6-dimethoxyphenol (99) with  $\text{RhCl}_3$  in ethanol (Andrieux et al., 1977).

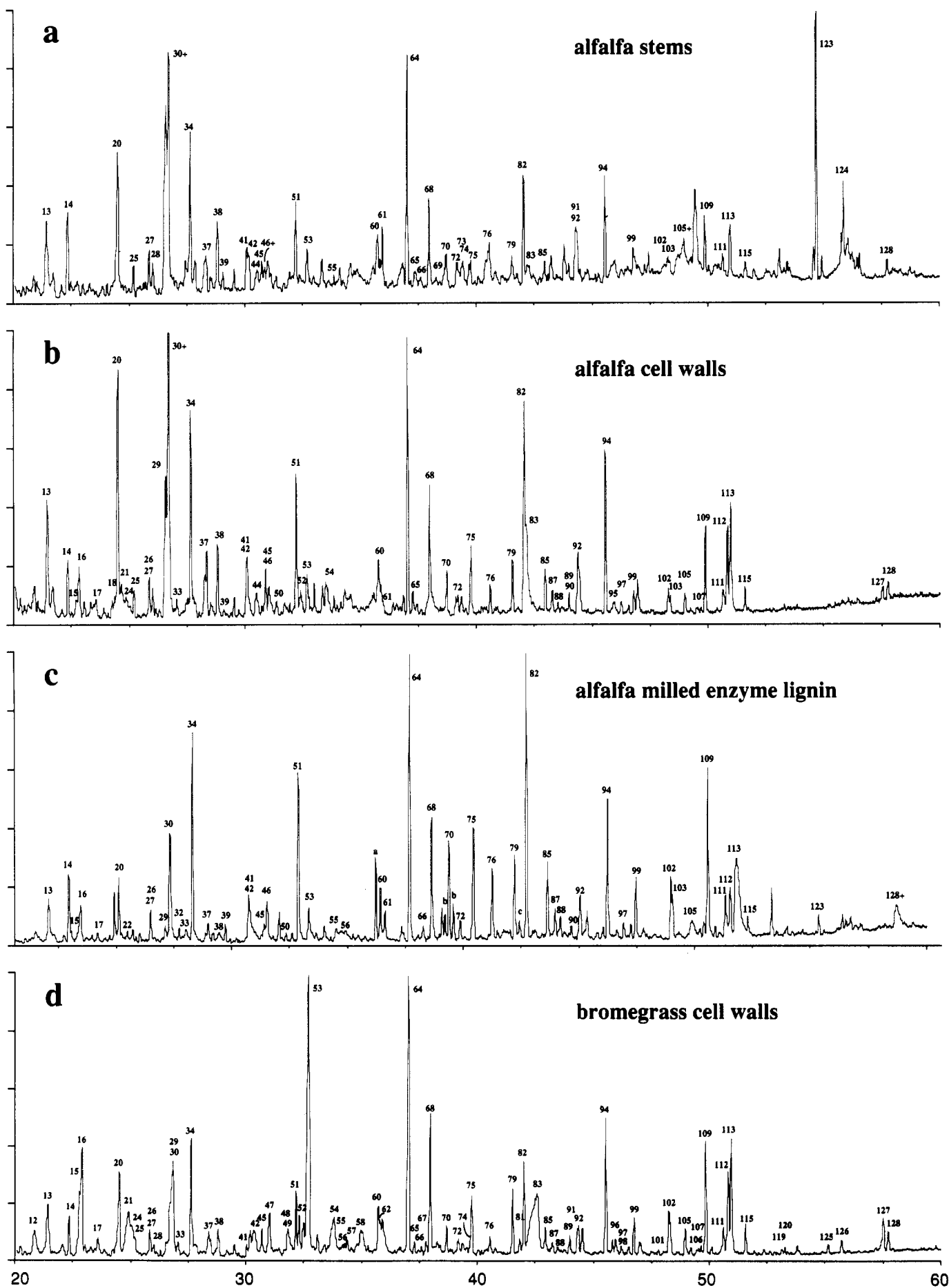
Acetylenic compound 87 was prepared as follows: *trans*-isoeugenol (82) was acetylated with acetic anhydride/pyridine to give the 4-acetoxy compound. To this compound in  $\text{CHCl}_3$  was added 1 equiv of  $\text{Br}_2$  to give the  $\alpha,\beta$ -dibromide as a mixture of diastereomers. This dibromide was doubly dehydrohalogenated by refluxing with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in toluene for 24 h to give the acetate of 87:  $^1\text{H}$  NMR (acetone- $d_6$ )  $\delta$  2.01 (s,  $\gamma\text{-CH}_3$ ), 2.22 (s, acetate methyl), 3.81 (s, methoxyl);  $^{13}\text{C}$  NMR (acetone- $d_6$ )  $\delta$  3.8 (q,  $\text{C}_7$ ), 20.3 (q, acetate methyl), 56.2 (q, methoxyl), 79.7 (s,  $\text{C}_6$ ), 86.1 (s,  $\text{C}_a$ ), 116.1 (d,  $\text{C}_2$ ), 123.6 (d,  $\text{C}_6$ ), 124.5 (d,  $\text{C}_6$ ), 132.6 (s,  $\text{C}_1$ ), 140.6 (s,  $\text{C}_4$ ), 152.0 (s,  $\text{C}_3$ ), 168.7 (s, acetate carbonyl). Finally, the acetate was saponified with dioxane/1 M aqueous NaOH (50:50) to give compound 87, which was purified by preparative TLC using 10% ethyl acetate in petroleum ether as eluant:  $^1\text{H}$  NMR (acetone- $d_6$ )  $\delta$  1.96 (s,  $\gamma\text{-CH}_3$ ), 3.82 (s, methoxyl), 6.72–6.90 (m, aromatics), 7.77 (s, phenolic OH);  $^{13}\text{C}$  NMR (acetone- $d_6$ )  $\delta$  3.8 (q,  $\text{C}_7$ ), 56.2 (q, methoxyl), 80.6 (s,  $\text{C}_6$ ), 83.6 (s,  $\text{C}_a$ ). The unoptimized yield from isoeugenol 82 was ca. 50%.

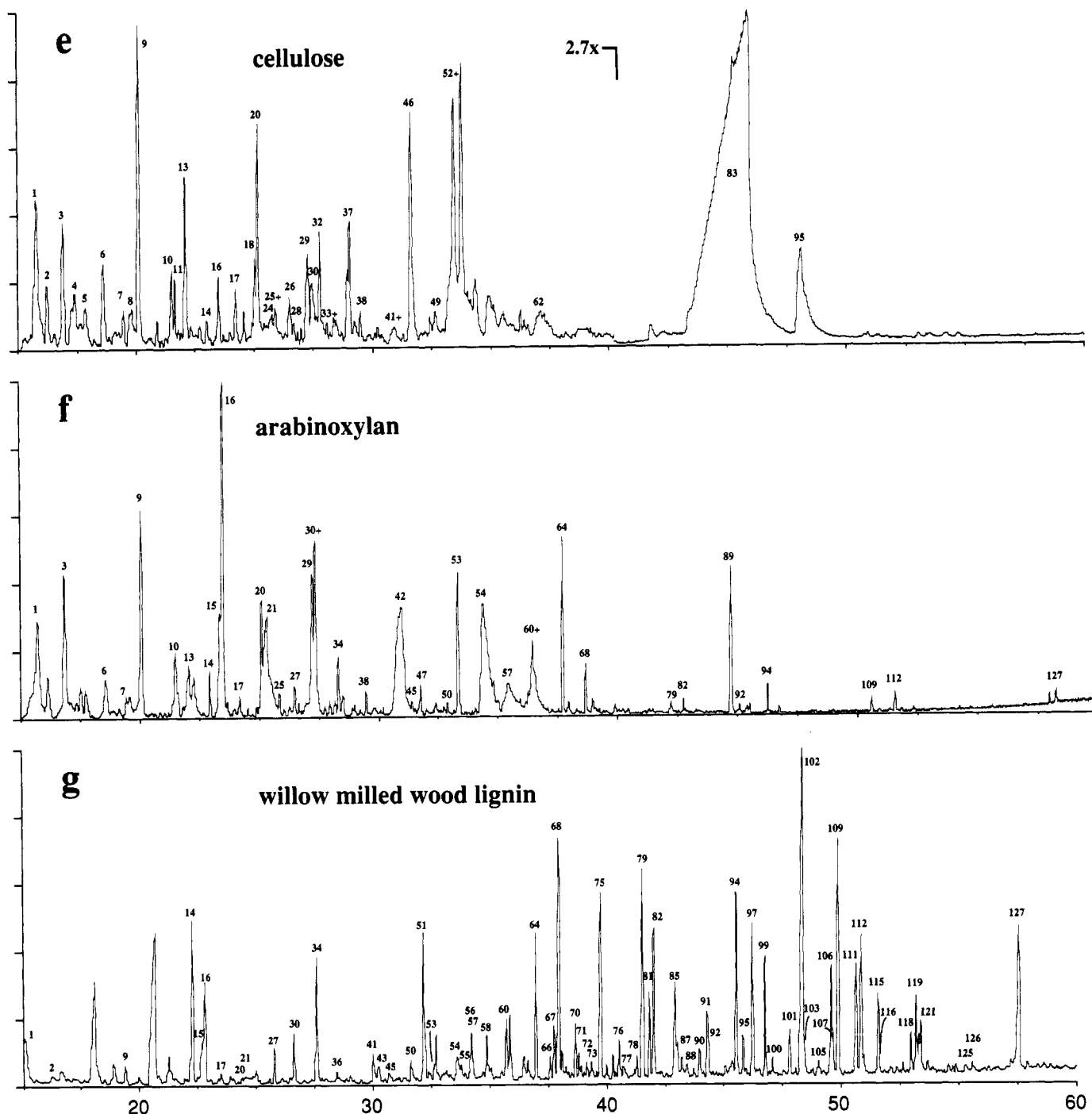
Compound 107, the syringyl analogue of 87, was synthesized from 109 in an analogous manner to the synthesis of 87 from 82:  $^1\text{H}$  NMR (acetone- $d_6$ )  $\delta$  1.97 (s,  $\gamma\text{-CH}_3$ ), 3.80 (s, methoxyls), 6.63 (s, 2,6-aromatics), 7.39 (phenolic OH);  $^{13}\text{C}$  NMR (acetone- $d_6$ )  $\delta$  3.9 (q,  $\text{C}_7$ ), 56.6 (q, methoxyls), 80.8 (s,  $\text{C}_6$ ), 83.7 (s,  $\text{C}_a$ ), 109.9 (d,  $\text{C}_{2,6}$ ), 133.4 (s,  $\text{C}_1$ ), 137.4 (s,  $\text{C}_4$ ), 148.5 (s,  $\text{C}_{3,5}$ ).

Attempts to prepare the allene 88 analogously from eugenol (70) were unsuccessful, leading to polymeric tars and other unidentified compounds.

## RESULTS AND DISCUSSION

**General.** Low molecular weight monomers derived from pyrolysis of lignin chromatograph cleanly and are often easily identified by their diagnostic mass spectra (Figure 2). Many of these compounds have been identified previously in pyrograms of wood and plant materials (see references under Introduction). However, frequently there are isomers (e.g., eugenol (70), *cis*-isoeugenol (76), and *trans*-isoeugenol (82)) which are not distinguishable from each other by their mass spectra alone. The GC retention time data from authentic compounds are required, or additional data from GC-FTIR or LC-NMR, techniques which are more sensitive to isomeric differences, are required. Also, there are a number of minor compounds throughout the total ion chromatogram that have lignin-fragment-like spectra but are beyond reasonable scope for interpretation at this level. Still other compounds have resisted interpretation to date, and researchers have speculated as to their structure. A particular case is the pair of peaks 87 and 88 present in the legume, grass, and wood samples (Figure 2). These clearly derive from lignin fragmentation. Meier and Faix (1991) have postulated the allene structure 88 to fit these mass spectra but, since 1-(4-hydroxy-3-methoxyphenyl)allene (88) has no geometric isomers (no *cis* and *trans* forms, for example), this can, at best, identify only one of the two peaks. Furthermore, arylallenes dimerize readily at room temperature (Müller, 1977), and there is some question as to whether monomers would result during pyrolysis. As





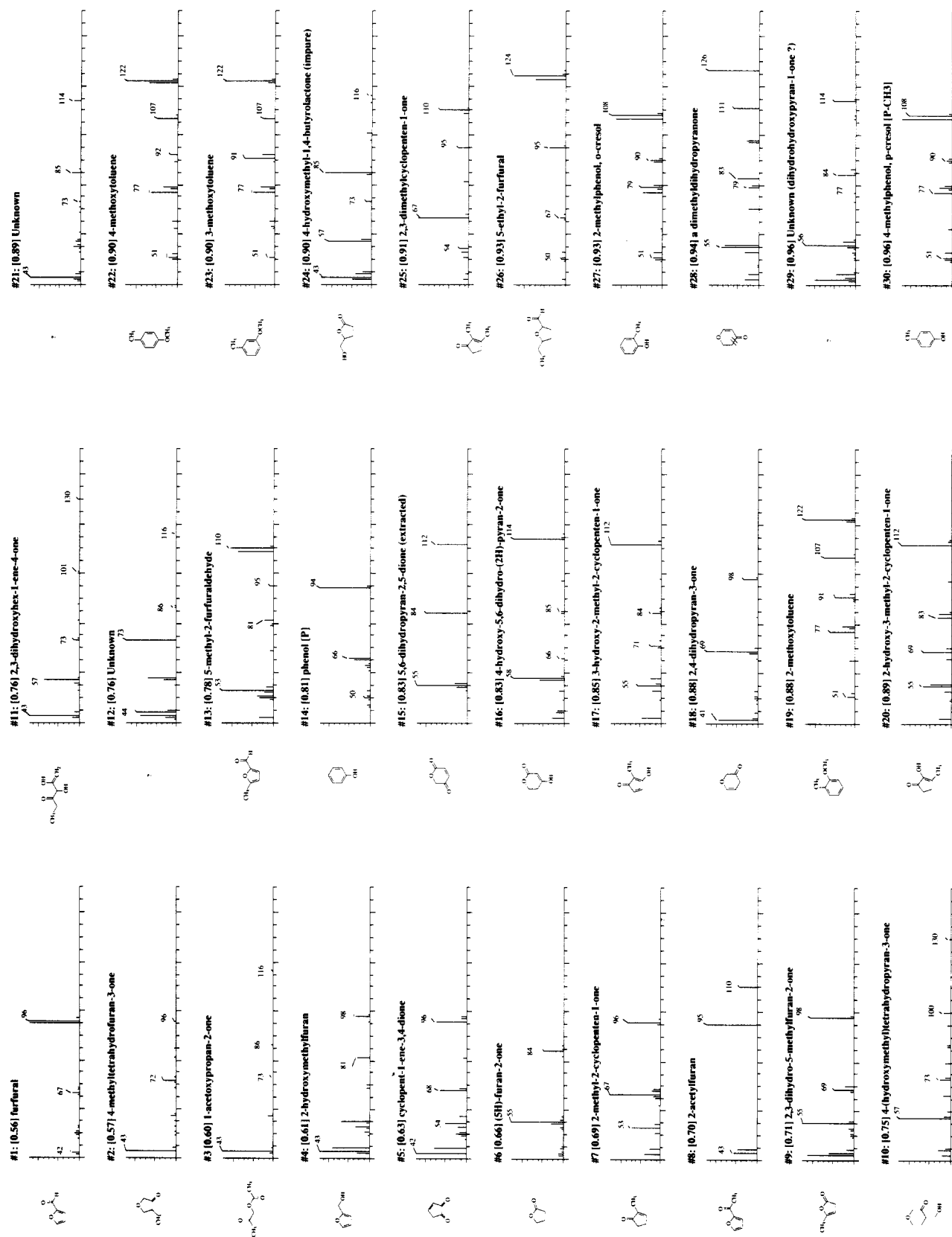
**Figure 1.** Pyrograms (total ion chromatograms) of (a) alfalfa stems, (b) alfalfa cell walls, (c) alfalfa milled enzyme lignin, (d) bromegrass cell walls, (e) avicell cellulose, (f) arabinoxylan, and (g) willow milled wood lignin. Relative intensity vs time (minutes). A "+" on as peak label signifies overlapping peaks (i.e., compounds other than the labeled compound are present in the peak).

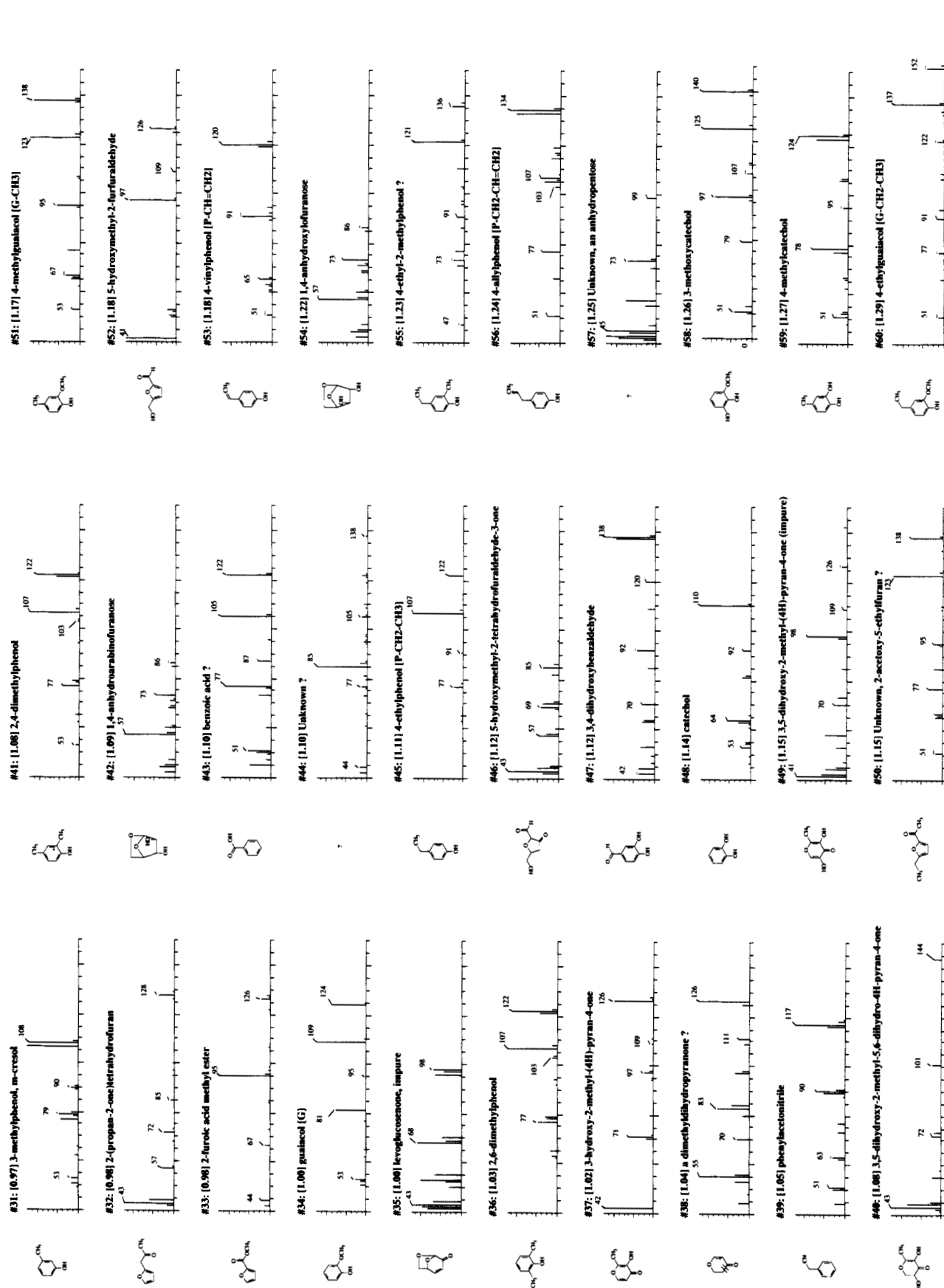
noted under Experimental Procedures, we synthesized authentic compound 87 (and confirmed its structure by the more diagnostic proton and  $^{13}\text{C}$  NMR spectra). Isolated compound 87 survives pyrolysis (i.e., simply volatilizes), and it was therefore possible to prove its identity with peak 87 rather than 88 by spiking the sample with authentic 87 prior to pyrolysis (Figure 3). Additionally, it was clear that compound 107 was a syringyl homologue of the guaiacyl compound 87. We were not able to find a second syringyl peak but again synthesized compound 107, the acetylene isomer, to obtain retention data confirming the 107 assignment as the acetylene. Attempts to prepare the allene 88 from eugenol by an analogous manner failed, producing only intractable tars

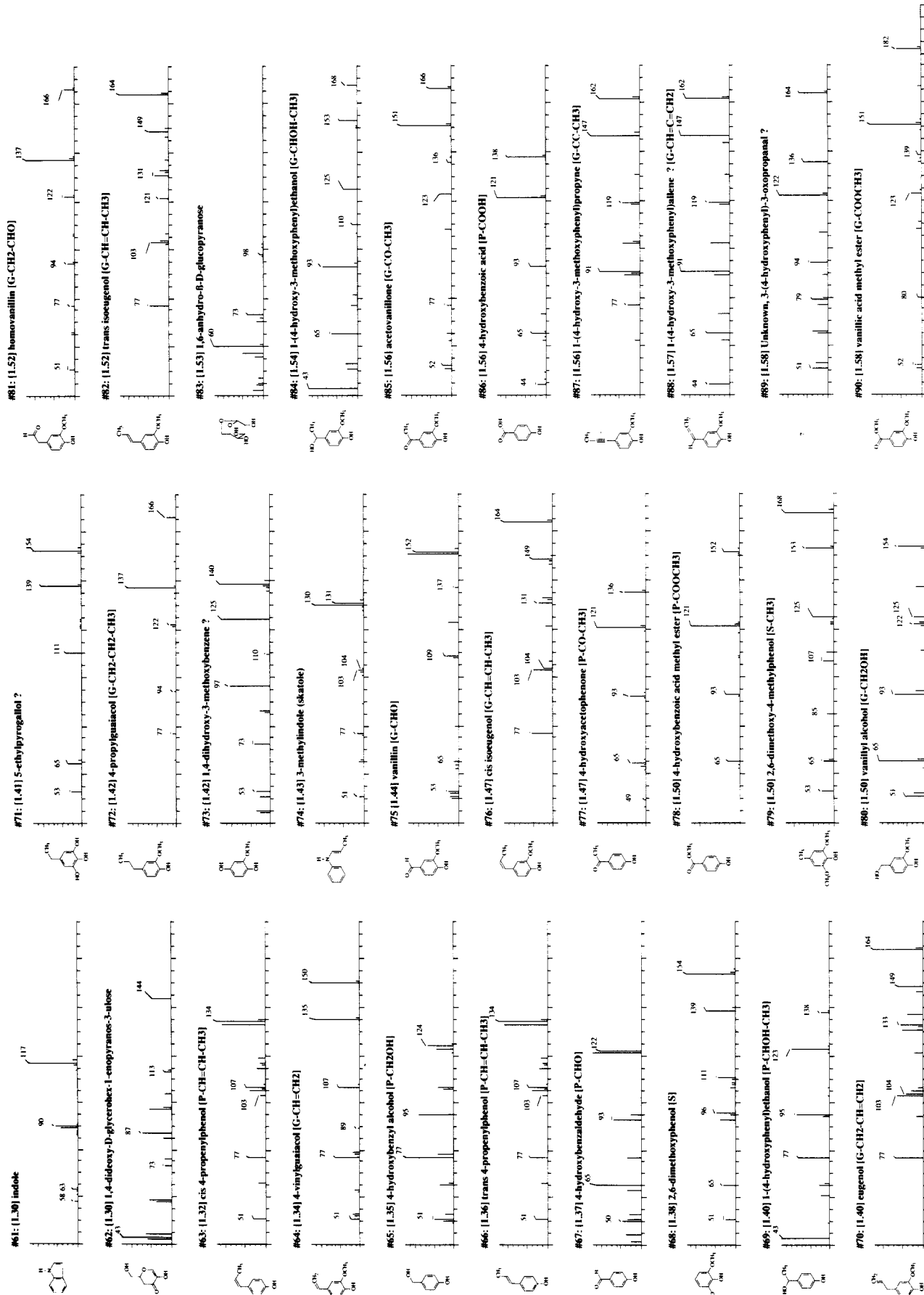
and unidentified products. Thus, the actual identity of peak 88 remains uncertain.

We have also often seen evidence for the smaller alcohols 65, 80, and 108 and 69, 84, and 110 in our pyrograms, but these have never been reported by others. We confirm the presence of these structures in our pyrograms by comparison with authentic compounds, prepared in high yield from the corresponding keto compounds (see Experimental Procedures). Compounds 65 and 69 are sufficiently abundant to be labeled in some of the TICs in Figure 1, but all of the alcohols are more easily found by obtaining selected-ion chromatograms.

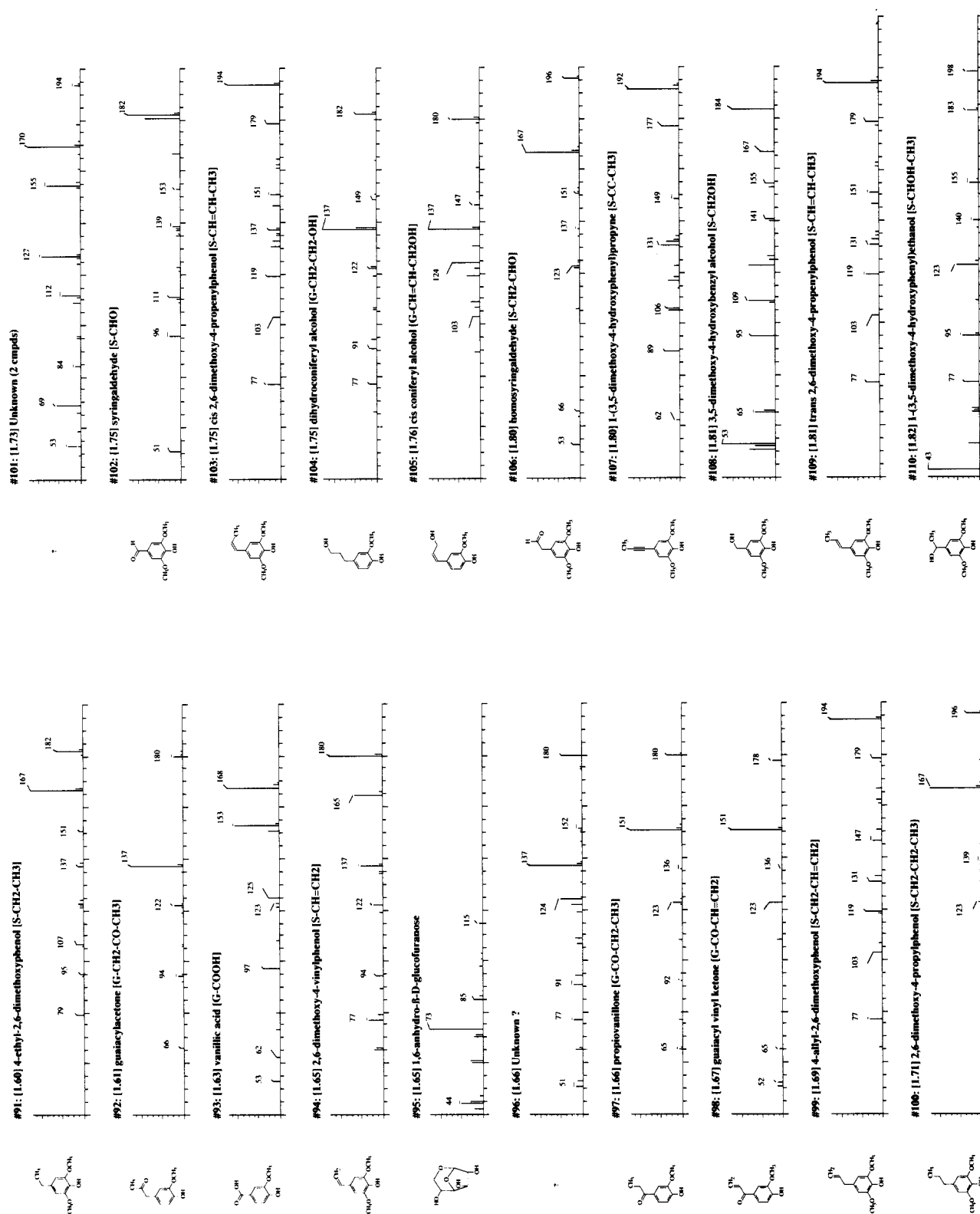
Although lignin pyrolysis peaks dominate the pyrograms for most lignified plant materials, we have also been











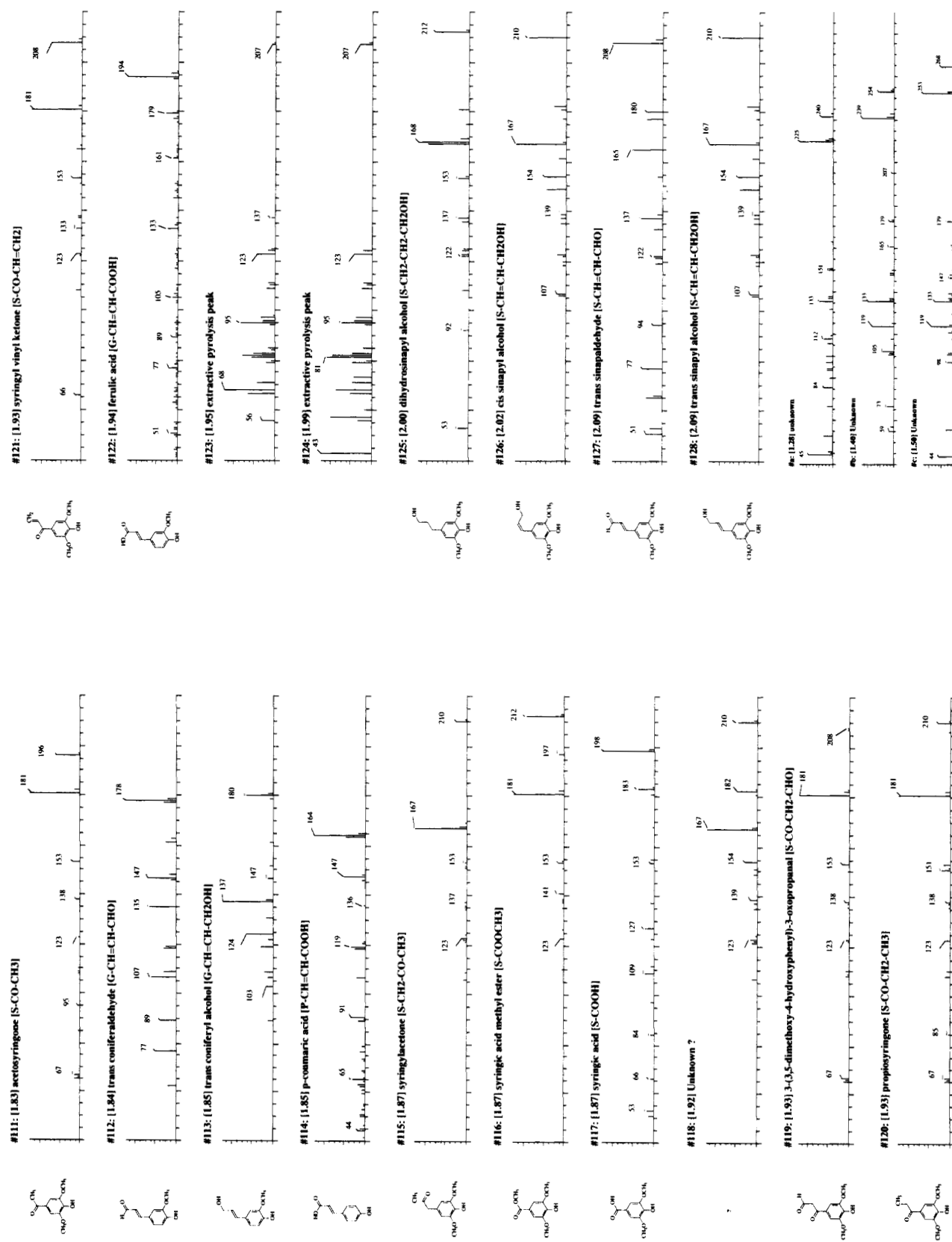
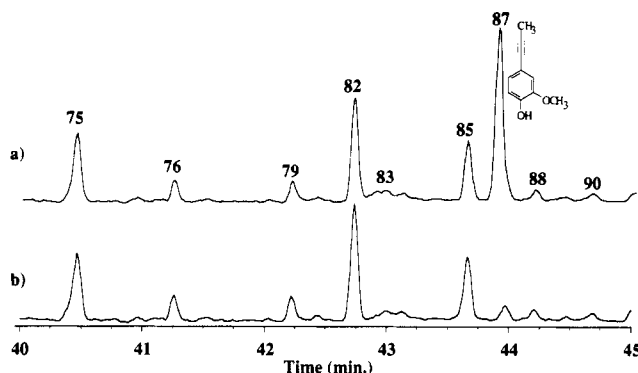


Figure 2. Mass spectra for pyrolysis components 1–128 and artifacts a–c. The number in square brackets after the peak number is the relative retention time with respect to guaiacol (34).



**Figure 3.** Partial pyrogram of alfalfa enzyme lignin: (a) sample spiked with authentic guaiacylpropyne (87); (b) normal sample.

interested in determining the extent to which pyrolysis-GC-MS can be used to detect protein contamination (either from plant or microbial sources) and to detect certain carbohydrate fractions. Under the pyrolysis conditions chosen (700 °C), the pyrolytic efficiency of carbohydrate polymers to give diagnostic volatile products is low, especially compared to lignin, but it is possible to detect anhydroarabinose (42), anhydroxylose (54), and the anhydroglucoses (83, 95) in the pyrograms along with many smaller fragments. The protein-derived peaks phenylacetonitrile (39), indole (61), and 3-methylindole (skatole) (74) make good protein markers (Boon et al., 1984; Tsuge and Matsubara, 1985). Toluene, also a diagnostic marker from phenylalanine, elutes at ca. 13.9 min, relative retention time 0.49 (with respect to guaiacol (34)). Proteins may also contribute to the phenol (14), 4-methylphenyl (30), and 4-vinylphenol (53) peaks which arise from tyrosine residues (Tsuge and Matsubara, 1985).

Before a more detailed look at the pyrograms in Figure 1, it is instructive for future researchers in this area to stress one aspect of variability that is clearly overlooked by most others using pyrolysis-GC-MS, ourselves included. It is notable that some pyrograms contain coniferyl alcohol (113) and sinapyl alcohol (128) as major peaks, while they are entirely absent from other spectra, although the corresponding aldehydes may be present. For example, Figure 1g shows absolutely no coniferyl alcohol (113), although it is present in prior runs on the same material. Additionally acids 86, 93, and 117 are sometimes reported but were not initially seen in this study. Also, carbohydrate-derived peaks may be quite broad. Freshly silylating the GC insert has a tremendous impact on the detection of these peaks. Under normal GC conditions (i.e., by direct injection of compounds rather than via pyrolysis) coniferyl alcohol is easily detected and chromatographs nicely if the insert is freshly silylated but can be completely undetectable when it is "dirty". The acids similarly will stick to an insert with active glass sites and are not detected. Even *p*-coumaric acid (114) and ferulic acid (122) can be chromatographed, without derivatization, on this column, with a silylated insert. The reason to belabor this point is because, although in normal chromatography it is usual to maintain a "clean" insert, under extended pyrolyses substantial amounts of low molecular weight aliphatic acids are produced and the insert does not remain inactive for long.

**Comparison of Alfalfa Samples.** The three alfalfa samples illustrate the differences between using crude native plant material and an enriched lignin sample. The alfalfa stems (Figure 1a) were simply ground and subjected to the standard pyrolysis-GC-MS conditions without pre-extraction. Immediately noticeable is that lignin and phenolic acid derived fragments dominate the spectrum.

Thus, even though the lignin represents only about 12.6% by weight, its breakdown to simple volatile products with relatively high pyrolytic efficiency is striking. Compound 16 (4-hydroxy-5,6-dihydro-2*H*-pyran-2-one) is an indicator for xylans, although it also derives from cellulose (Pouwels et al., 1989). Peaks 42 and 54 arise from arabinose and xylose, respectively. The peaks around 123/124 are particularly indicative of extractive components, including chlorophyll, and are the predominant peaks in the pyrogram of acetone-extracted leaf components (not shown). Phenylacetonitrile (39), indole (61), and 3-methylindole (74) are markers for proteins and clearly must derive from natural protein components within the plant stems (mature alfalfa stems contain 8–12% protein on a dry weight basis; Buxton et al., 1985).

The cell wall sample (Figure 1b) was prepared as described under Experimental Procedures. The protein markers 39, 61, and 74 are all substantially less abundant due to the protein removal that accompanies the isolation procedure, and the characteristic extractive peaks 123 and 124 are completely absent. As can be seen, the ratios of all major lignin-derived peaks and peak 64, which derives primarily from pyrolysis of ferulic acid derivatives, are very similar between the cell wall sample (Figure 1b) and the crude stem sample (Figure 1a).

The enzyme lignin sample (Figure 1c) prepared by digesting the cell wall material with cellulases, has reduced amounts of carbohydrate-derived products, peak 20 (2-hydroxy-3-methyl-2-cyclopenten-1-one) being a good indicator, and represents the cleanest lignin pyrogram. The anhydroglucose peak 83 is almost entirely absent. This sample does, however, still contain ca. 17% neutral sugars and 6% uronic acids. In particular, peak 42, (1,4-anhydroarabinofuranose) is only marginally depleted, which may be a reflection of the involvement of arabinose in esterification of ferulic acid. As described below, the pyrolysis of arabinose residues to give 42 is about 6 times more efficient than that of xylose residues to give 54, the corresponding anhydroxylose. In this sample, we find the return of protein marker peaks 39 and 61 presumably now due to contamination from the enzyme used in the carbohydrate degradation. The appearance of a homologous series of peaks, labeled a–c, is also presumably due to contamination during the enzyme lignin preparation. At this time, we can only speculate on the identity of these peaks.

**Bromegrass Cell Walls.** The most striking feature of the bromegrass cell wall pyrogram, as has been observed from bamboo (Martin et al., 1979; Meier and Faix, 1991), is the appearance of 4-vinylphenol (53), which derives primarily from *p*-coumaric acid (114) (4-hydroxycinnamic acid) residues in an analogous manner to the way in which 4-vinylguaiacol (64) is derived from ferulic acid (122) residues. It is well-known that grasses contain significant amounts of *p*-coumaric acid (as well as some *p*-hydroxyphenyllignin; Sarkanen and Ludwig, 1971), whereas legumes contain only minor amounts. Peak 53 is, however, visible in the alfalfa cell wall pyrogram. Since these acids represent about 0.8% (Jung and Casler, 1990) vs 20% lignin, it is clear that their pyrolytic efficiency to generate styrenes 53 and 64 is high. It should be noted that styrene 64 can result from pyrolysis of normal "core" lignin since it is a significant peak in the willow pyrogram (Figure 1g); significant levels of phenolic acids have not been reported in woods. From the preponderance of both syringyl and guaiacyl compounds it is clear that the lignin from legumes and grasses is more like typical angiosperm woods such as willow and unlike typical gymnosperm woods which

contain at most only traces of syringyl components (Obst and Landucci, 1986; Fullerton and Franich, 1983).

**Pyrolysis of Carbohydrate Fractions.** The pyrolysis of cellulose has been well documented by Pouwels et al. (1989). Under our conditions of pyrolysis at 700 °C, the major volatile products were 83 and 95, the anhydroglucoses. These were grossly overloaded in this ion chromatogram (Figure 1e) to see detail in the earlier part of the pyrogram. Most of the assignments were made from papers cited in the introduction, along with our own authentic compounds listed under Experimental Procedures.

Arabinoxylans, key components of forage cell walls, were isolated from Sigma xylan as described under Experimental Procedures. Of the fractions isolated, 1:6 (Figure 1f) and 1:12 (not shown) arabinose/xylose fractions were pyrolyzed. The two levels of arabinose substitution helped distinguish arabinose-derived from xylose-derived peaks. The most surprising feature is that many of the key peaks in the ion chromatogram arise from lignins and phenolic acids. Clearly, the pyrolysis-GC-MS technique is very sensitive to even trace lignin impurities. What is particularly interesting is the styrene peaks 53 and 64. While these can arise from pyrolysis of lignin containing both *p*-hydroxyphenyl and guaiacyl units (see 64 in the MWL sample, Figure 1g), their high intensity with respect to the guaiacol (34) peak, a standard marker for all lignins, suggests that they derive from the phenolic acids *p*-coumaric and ferulic acid. Since it is unlikely that esters could have survived the xylan purification steps involving base extraction, we assume these represent phenolic acids that are etherified, presumably to the residual lignin. Clearly (Figure 1f) this lignin is not representative of plant lignins and is either modified or fractionated as evidenced by the huge 89 peak, but again, this xylan is clearly derived from a grass because of the presence of syringyl and guaiacyl components as well as ferulic and *p*-coumaric acid derivatives. (In fact, the Sigma xylan is from oats.)

Of the carbohydrate-derived peaks, 42 is derived from arabinose and 54 from xylose, each being the 1,4-anhydrofuranoses. The similar areas of these peaks indicate that the pyrolytic efficiency of arabinose to its anhydro sugar vs xylose to its anhydro sugar is about 6 times as high. In the arabinoxylan sample with 1:12 arabinose/xylose, peak 42 is ca. half the area of 54, indicating the linearity. It is this efficiency of arabinose pyrolysis that makes peak 42 visible in pyrolyses of crude plant cell walls, even though this sugar is not a major component of the cell wall. Compounds 21 and 57 are clearly carbohydrate derived but remain unknowns at present. Their spectra are given in Figure 2. Of the early peaks, compound 16 is the most diagnostic for pentosans, being less intense in the cellulose spectrum. Compound 16 has a substantial abundance in the plant spectra of Figure 1 but not, for unknown reasons, in the crude alfalfa stems.

## CONCLUSIONS

Pyrolysis-GC-MS provides a rapid way of gaining valuable information from submilligram samples of plant material. It is particularly effective for lignins and phenolic acid components that pyrolyze to simple diagnostic volatile compounds with reasonable efficiency. As the aromatic nuclei largely retain their substitution patterns, the syringyl/guaiacyl (and to a lesser extent *p*-hydroxyphenyl) nature of the lignin is clearly revealed. The technique is particularly valuable for screening and differentiating *p*-coumaric acid and ferulic acid derivatives in tissues. The information about carbohydrate content is consid-

erably lower, but a few diagnostic peaks from xylose-, arabinose-, and glucose-containing polymers are useful. Finally, the ability to detect protein contamination, from plant or microbial sources, in cell wall preparations will be valuable in future studies on cell wall residues remaining after ruminant digestion.

## ACKNOWLEDGMENT

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