

Lignin pyrolysis products: Their structures and their significance as biomarkers

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Abstract—Pyrolysis in combination with gas chromatography and mass spectrometry was used to characterize softwood, hardwood and grass lignins as well as the corresponding synthetic dehydro-polymers. The method permitted differentiation of the three types of lignins. Softwood lignins yielded exclusively guaiacyl derivatives, coniferaldehyde and coniferyl alcohol being major compounds. Hardwood lignins gave rise to guaiacyl and syringyl derivatives, among which syringaldehyde, coniferyl alcohol and sinapyl alcohol were the most prominent. Grass lignins, represented by bamboo lignin, yielded *p*-vinylphenol as major compound. In addition, other guaiacyl and syringyl pyrolysis products were identified. The results indicate that guaiacyl and syringyl compounds are unique pyrolysis products of lignins and woods. Because of the relatively high resistance of lignins these pyrolysis products can be considered as characteristic biomarkers for terrestrial plant input.

Key words: milled wood lignins, dehydrogenative polymers, gymnosperm lignins, angiosperm lignins, grass lignins

INTRODUCTION

Lignins form an essential component of the woody stems of arborescent gymnosperms and angiosperms in which their amounts range from 15% to 36%. Lignins are not, however, restricted to arborescent plants, but are found as integral cell wall constituents in all vascular plants including the herbaceous varieties.

Investigations on the chemistry of lignin have shown it to be a substance of great structural complexity. Its structure is based on molecular units of the phenylpropane type. The complexity of the lignin molecule arises in part from the manner in which the C₆–C₃ units are linked to each other (and in part from the fact that these units are not chemically identified).

Lignins are polymeric products arising from an enzyme-initiated dehydrogenative polymerization of three primary precursors: *trans*-coniferyl, *trans*-sinapyl and *trans*-*p*-coumaryl alcohols.

The proportions of the three precursor alcohols differ between angiosperm and gymnosperm lignins. Gymnosperm lignin is made up by *trans*-coniferyl alcohol units, angiosperm lignin is constituted from *trans*-coniferyl and *trans*-sinapyl alcohol units and grass lignin contains all three alcohols as building blocks.

Living, dying and dead plants containing lignins are incorporated into soils and sediments, undergoing biochemical and chemical degradation reactions. Aerobic degradation of lignocellulose is slow since

the presence of the lignin moiety severely decreases cellulose decomposition and because lignin is not the best microbial energy source. The absence of significant wood decomposition under anaerobic conditions is consequence of the requirement of oxygen for microbial degradation (Zeikus *et al.*, 1982). Therefore, in soils lignins are slowly biodegraded by certain fungi and bacteria, and partly degraded lignins and lignin phenols may contribute to the formation of humus and are present in humic fractions (Saiz-Jimenez and de Leeuw, 1985), while in anaerobic wetlands and sediments of marine and freshwater environments, lignocellulosic materials are better preserved.

Lignin derivatives have been identified in soil organic matter fractions (Saiz-Jimenez and de Leeuw, 1984a, 1985), buried woods (Hedges *et al.*, 1985), fossil woods (Sigleo, 1978; Hatcher *et al.*, 1982) and kerogen (Habermehl and Hundrieser, 1983). Because lignin components are relatively stable upon diagenesis, they can be considered as specific biomarkers for terrestrial plant input.

In previous work, Saiz-Jimenez and de Leeuw (1984b) demonstrated that isolated, synthetic, biodegraded and kraft lignins can be analysed by flash pyrolysis. Further, pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS) data have shown a high correlation between pyrolysis products and the lignin units from which they arise (Martin *et al.*, 1979; Obst, 1983). Therefore, it is possible to discriminate between gymnosperm and angiosperm lignins as original contributors to sediments and soils.

In this paper, Py–GC–MS of three types of isolated and synthetic lignins is reported. This study of soft-

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wood, hardwood and grass lignins and the characterization of their pyrolysis products will improve our present knowledge of the chemical structure of this biopolymer and will provide useful information when comparing sound, biodegraded, fossil or buried woods.

EXPERIMENTAL

Samples

Two spruce milled wood lignin samples (MWL) were provided by Dr T. Kent Kirk (MWL-1) and by Dr O. Faix (MWL-2). Beech, aspen and bamboo MWL samples were supplied by Dr O. Faix. MWL samples were isolated according to Björkman (1956).

Dehydrogenation polymers (DHPs) were synthesized using *trans*-coniferyl, *trans*-sinapyl and *trans*-coumaryl alcohols in fixed proportions to reproduce the three types of lignins. They were kindly supplied by Dr O. Faix. Further

information on these samples is reported by Kirk and Chang (1984), Faix and Besold (1978), Martin *et al.* (1979) and Saiz-Jimenez and de Leeuw (1984b).

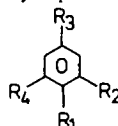
Pyrolysis-GC and pyrolysis-GC-MS

Details of the methods have been described in a previous paper (Saiz-Jimenez and de Leeuw, 1984b). Briefly, pyrolysis was performed with ferromagnetic wires having a Curie temperature of 510°C (pyrolysis time 10 sec) and pyrolysis products were separated on a capillary glass WCOT column (28 m × 0.5 mm i.d.) coated with CP sil 5 (1.25 µm film thickness) held at 50°C for 3 min and subsequently programmed to 250°C at a rate of 3°C min⁻¹. He was used as a carrier gas.

RESULTS

Pyrograms of the MWL and DHP samples are shown in Figs 1-8. In this study we concentrated on

Table 1. Pyrolysis products from lignins



Peak No.	R ₁	R ₂	R ₃	R ₄	Remarks
1	OH				
2	OH	CH ₃			
3	OH		CH ₃		
4	OH	OCH ₃			
5	OH		CH ₂ -CH ₃		
6					Positional isomer of 7
7	OH	OCH ₃	CH ₃		
8	OH		CH=CH ₂		
9	OH		CH ₂ -CH=CH ₂		
10	OH	OCH ₃	CH ₂ -CH ₃		
11	OH		CH=CH-CH ₃		<i>cis</i>
12	OH	OCH ₃	CH=CH ₂		
13	OH	OCH ₃		OCH ₃	
14	OH	OCH ₃	CH ₂ -CH=CH ₂		
15	OH		CHO		
16	OH	OCH ₃	CH ₂ -CH ₂ -CH ₃		
17	OH	OCH ₃	CHO		
18	OH	OCH ₃	CH=CH-CH ₃		<i>cis</i>
19	OH	OCH ₃	CH ₃	OCH ₃	
20	OH	OCH ₃	CH ₂ CHO		
21	OH	OCH ₃	CH=CH-CH ₃		<i>trans</i>
22	OH	OCH ₃	CO-CH ₃		
23	OH	OCH ₃	COO-CH ₃		
24	OH	OCH ₃	CH ₂ -CH ₃	OCH ₃	
25	OH	OCH ₃	CH ₂ -CO-CH ₃		
26	OH	OCH ₃	CH=CH ₂	OCH ₃	
27	OH	OCH ₃	CO-CH=CH ₂		
28	OH	OCH ₃	COOH		
29	OH	OCH ₃	CO-CH ₂ -CH ₃		
30	OH	OCH ₃	CH ₂ -COOH		
31	OH	OCH ₃	CH ₂ -CH=CH ₂	OCH ₃	
32	OH	OCH ₃	CH ₂ -CH ₂ -CH ₃	OCH ₃	
33	OH	OCH ₃	CHO	OCH ₃	
34	OH	OCH ₃	CH=CH-CH ₃	OCH ₃	<i>cis</i>
35	OH	OCH ₃	CH=CH-CH ₂ OH		<i>cis</i>
36	OH	OCH ₃	CH ₂ -CHO	OCH ₃	
37	OH	OCH ₃	CH=CH-CH ₃	OCH ₃	<i>trans</i>
38	OH	OCH ₃	CO-CH ₃	OCH ₃	
39	OH	OCH ₃	CH=CH-CHO		
40	OH	OCH ₃	CH=CH-CH ₂ OH		<i>trans</i>
41	OH	OCH ₃	CH ₂ -CO-CH ₃	OCH ₃	
42	OH	OCH ₃	COO-CH ₃	OCH ₃	
43	OH	OCH ₃	CO-CH=CH ₂	OCH ₃	
44	OH	OCH ₃	CO-CO-CH ₃	OCH ₃	
45	OH	OCH ₃	CH ₂ -CH ₂ -CH ₂ OH	OCH ₃	
46	OH	OCH ₃	CH=CH-CHO	OCH ₃	
47	OH	OCH ₃	CH=CH-CH ₂ OH	OCH ₃	<i>trans</i>

Note that the numbering of the ring atoms is different from the numbering used in Fig. 9.

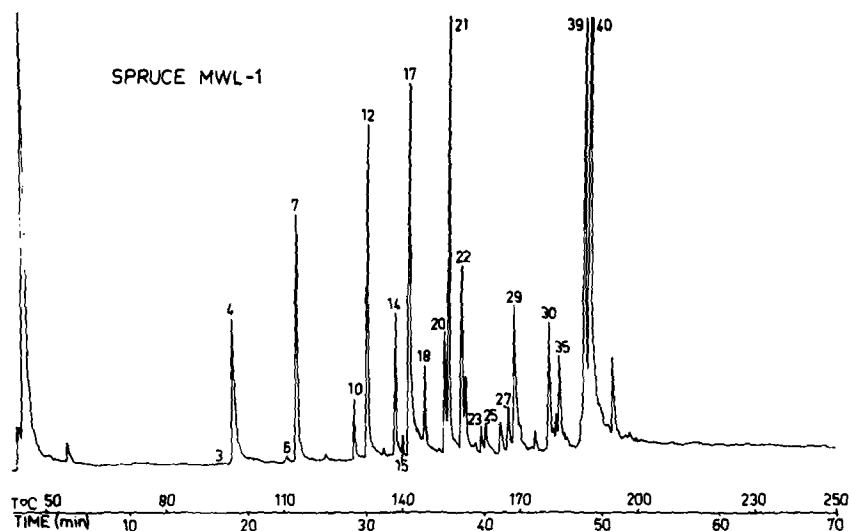


Fig. 1. Pyrogram of a *Picea engelmannii* milled wood lignin.

the phenolic pyrolysis products because they are highly representative of each lignin sample. Other products, like furan derivatives, which sometimes are present in the samples, and low molecular weight aromatic hydrocarbons such as benzene, toluene, styrene, alkylbenzenes, etc., are not discussed since they are minor pyrolysis products.

The structural elucidation of the pyrolysis products (Table 1) is based on comparison of both retention time and mass spectral data with those of standards and with literature data.

Milled wood lignins

Figure 1 shows the pyrogram of a spruce lignin (MWL-1). Major peaks are identified as 4-methylguaiacol (7), 4-vinylguaiacol (12), vanillin (17), *trans*-isoeugenol (21), coniferaldehyde (39) and *trans*-coniferyl alcohol (40). Other prominent pyrolysis

products encountered are guaiacol (4), eugenol (14), homovanillin (20), acetoguaiacone (22), propioguaiacone (29) and homovanillic acid (30).

The other spruce lignin (MWL-2) analysed (Fig. 2) shows a high similarity with the MWL-1 sample. The presence of a few additional furan derivatives and the decrease of the peaks corresponding to coniferaldehyde (39) and *trans*-coniferyl alcohol (40) are noted. Moreover, vanillic acid (28) is only present in this sample.

Differences between the two spruce samples analysed are expected to some extent because interspecies variation and variations within a single plant (e.g. normal and compression wood) lignins have been reported (Sarkanen and Hergert, 1971). Further, differences may also be ascribed to slight modifications in the analytical isolation procedures, although basically the same procedure was used in both laboratories.

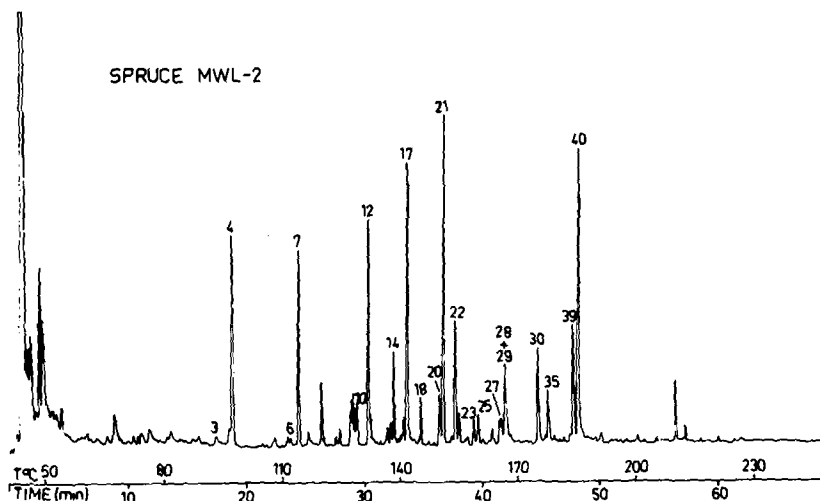


Fig. 2. Pyrograms of a *Picea abies* milled wood lignin.

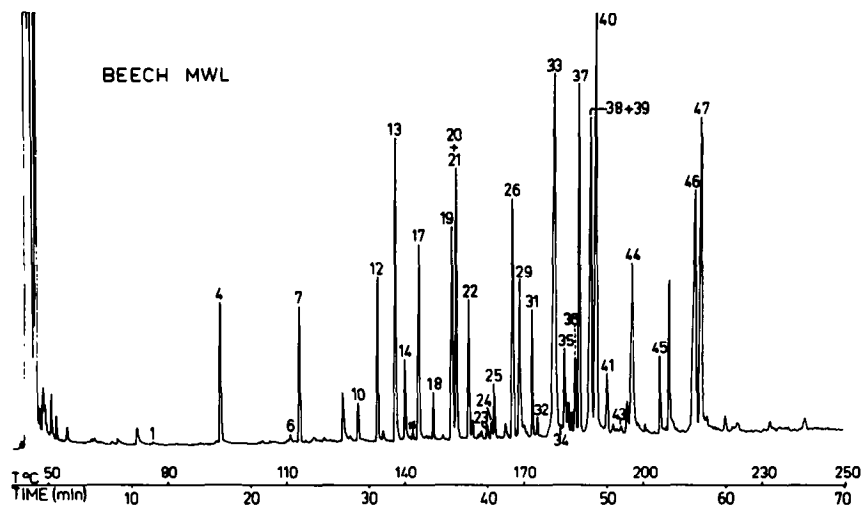


Fig. 3. Pyrogram of *Fagus silvatica* milled wood lignin. Contribution of acetoguaiacone (20) and acetosyringone (38) to the peaks are lesser than their corresponding coeluted compounds. A minor amount of dialkyl phthalate contributes to peak 46. The contribution of the identified compound to peak 44 is minor as compared with a syringyl derivative of MW 210 and unknown structure.

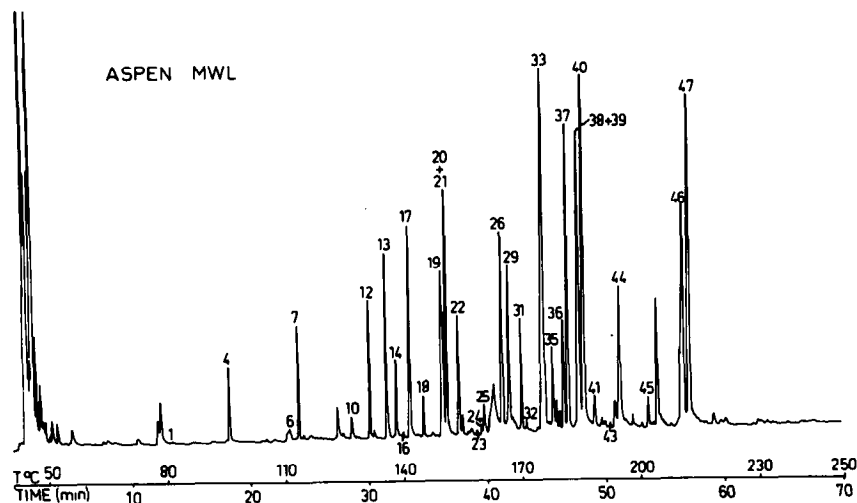


Fig. 4. Pyrogram of a *Populus* sp. milled wood lignin. Captions as in Fig. 3.

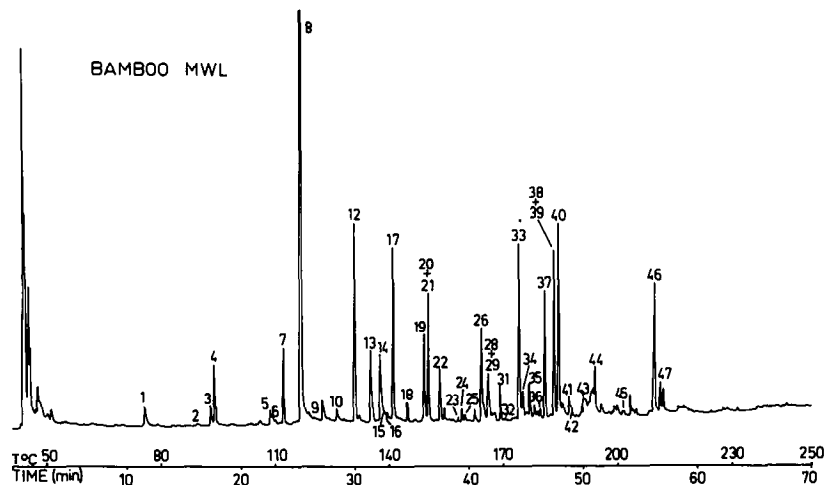


Fig. 5. Pyrogram of a *Bambusa* sp. milled wood lignin. Captions as in Fig. 3.

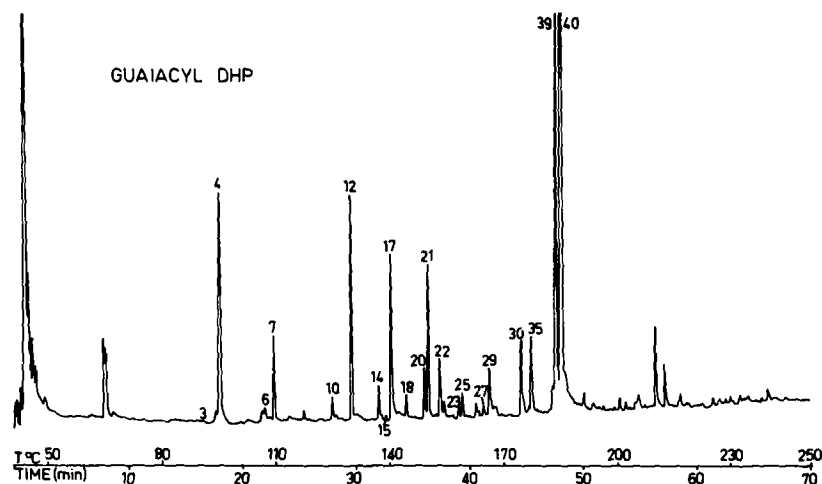


Fig. 6. Pyrogram of a guaiacyl dehydropolymer.

The pyrogram of beech MWL (Fig. 3) shows major peaks for 2,6-dimethoxyphenol (13), *trans*-isoeugenol (21), 4-vinyl-2,6-dimethoxyphenol (26), syringaldehyde (33), *trans*-4-propenyl-2,6-dimethoxyphenol (37), coniferaldehyde (39), *trans*-coniferyl alcohol (40), sinapaldehyde (46) and sinapyl alcohol (47). Prominent pyrolysis products are also guaiacol (4), 4-methylguaiacol (7), 4-vinylguaiacol (12), vanillin (17), 4-methyl-2,6-dimethoxyphenol (19), acetoguaiacone (22), propioguaiacone (29) and 4-allyl-2,6-dimethoxyphenol (31).

The pyrogram of an aspen MWL (Fig. 4) is quite similar to those of beech lignin and only a few differences are found in peak intensities.

The pyrogram of bamboo MWL is shown in Fig. 5. In addition to typical guaiacyl and syringyl pyrolysis products *p*-hydroxyphenyl compounds, as known to occur in grass lignins, are observed. The major pyrolysis product identified is *p*-vinylphenol (8). Other important compounds are 4-vinylguaiacol (12), vanillin (17), *trans*-isoeugenol (21), syringalde-

hyde (33), *trans*-4-propenyl-2,6-dimethoxyphenol (37), coniferaldehyde (39), *trans*-coniferyl alcohol (40) and sinapaldehyde (46).

Dehydrogenation polymers

Guaiacyl DHP (Fig. 6) is similar to spruce MWLs in that both have the same type of pyrolysis products. However, the ratios of these products are different because compared to natural lignins the relative amounts of guaiacol (4), coniferaldehyde (39) and *trans*-coniferyl alcohol (40) are increased. This indicates differences between the natural and synthetic lignins probably in the proportion of linkages.

The pyrogram of the guaiacyl-syringyl DHP is displayed in Fig. 7. Major compounds are identified as 2,6-dimethoxyphenol (13), syringaldehyde (33), coniferaldehyde (39), *trans*-coniferyl alcohol (40), sinapaldehyde (46) and sinapyl alcohol (47). Other important compounds are 4-vinylguaiacol (12), vanillin (17) 4-methyl-2,6-dimethoxyphenol (19) *trans*-isoeugenol (21), 4-vinyl-2,6-dimethoxyphenol (26),

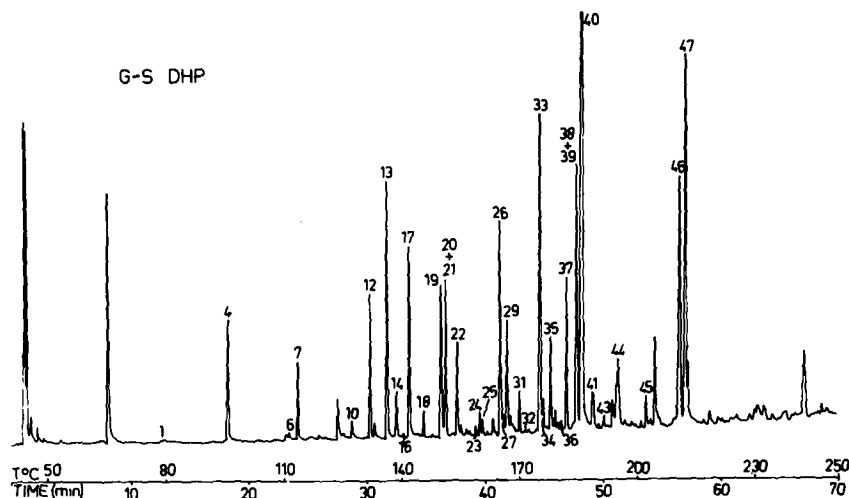


Fig. 7. Pyrogram of a guaiacyl-syringyl dehydropolymer. Captions as in Fig. 3.

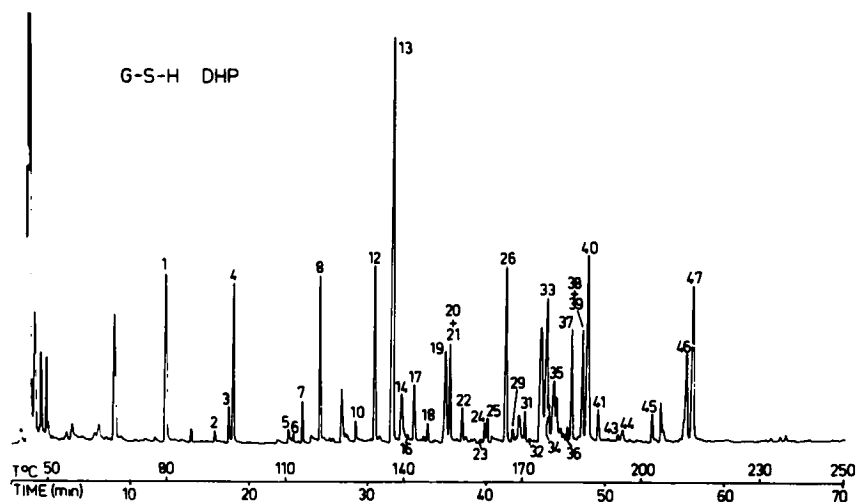


Fig. 8. Pyrogram of a guaiacyl-syringyl-*p*-hydroxyphenyl dehydropolymer. Captions as in Fig. 3.

and *trans*-4-propenyl-2,6-dimethoxyphenol (37). As for the guaiacyl DHP, the guaiacyl-syringyl DHP contains the same pyrolysis products as the natural lignin, but peak intensities are different, especially for *trans*-coniferyl alcohol.

The guaiacyl-syringyl-*p*-hydroxyphenyl DHP pyrogram (Fig. 8) shows a very different pattern with regard to bamboo MWL. The major phenolic compound identified is 2,6-dimethoxyphenol (13). Other prominent compounds are phenol (1), guaiacol (4), *p*-vinylphenol (8), 4-vinylguaiacol (12), 4-vinyl-2,6-dimethoxyphenol (26), syringaldehyde (33), *trans*-coniferyl alcohol (40) and syringyl alcohol (47). In this case the synthetic lignin does not reproduce well the main linkages present in the bamboo MWL, as indicated by the very different relative amounts of pyrolysis products.

To further substantiate the possible pyrolysis mechanisms of *p*-coumaril alcohol precursors, a *p*-hydroxyphenyl DHP was also studied. Among the phenolic compounds *p*-cresol and *trans*-*p*-propenylphenol are abundant pyrolysis products. Other important compounds are phenol, *p*-ethylphenol, *p*-vinylphenol, *p*-propylphenol, *cis*-*p*-propenylphenol and *p*-coumarylalcohol. Most of them are also encountered in guaiacyl-syringyl-*p*-hydroxyphenyl DHP. The *p*-coumaryl alcohol peak is lower than any of the identified compounds in the pyrolysate of *p*-hydroxyphenyl DHP, which might explain the difficulty of identifying this compound in bamboo MWL and its synthetic counterpart.

DISCUSSION

Pyrolysis products vs lignin units

Lignins are thought to be composites of two relatively well defined structural moieties: the so-called "bulk-polymer" characterized by a large number of free C₃-side chains and the "end-wise polymer", in which the β-O-4 linkage is dominant (Sarkanen,

1971). The identification of coniferaldehyde and coniferyl alcohol as major pyrolysis products in soft and hardwood lignins and their synthetic counterparts indicate that their presence is related with definite and relatively abundant structures. From all possible lignin substructures, two types, those with free end groups and those with arylglycerol-β-ether linkages appear to be the most likely candidates to explain the origin of these products. In fact, from units with free end groups the coniferyl derivatives will originate through cleavage of a C-O bond. Most probably they are released from their association with the lignin molecule through the cleavage of a β-O-4 linkage (Fig. 9). In the case of ether structures, the cleavage of two or more C-O bonds is involved. C-O bonds are the relatively weakest linkages present among the structural units of lignins, which could explain the abundance of the encountered major coniferyl derivatives. Both types of the substructures mentioned might account for about 50% of the natural lignins and perhaps more in the synthetic ones.

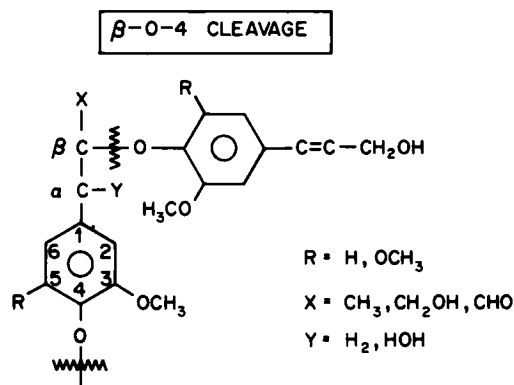


Fig. 9. Possible major types of lignin substructures thought to release coniferaldehyde/sinapaldehyde and coniferyl alcohol/sinapyl alcohol upon pyrolysis. Note that the numbering of the ring atoms in this figure is different from the numbering used throughout the text and in Table 1.

The occurrence of sinapaldehyde and sinapyl alcohol also points to the presence of free end groups in syringyl substructures. In this case β -O-4 bonds are not so abundant because the dual O-substitution at positions 3 and 5 (Fig. 9) forms a barrier against β -O couplings. Therefore, the β - β coupling might be more important among syringyl units (Sarkanen, 1971).

The relatively lower intensities of sinapyl alcohol as compared to coniferyl alcohol in the aspen and beech MWL sample might be due to this more pronounced β - β type of linkage, since these lignins are built up from almost equal amounts of coniferyl and sinapyl alcohols.

It is noteworthy that no hydroxyphenyl or syringyl compounds were encountered in the pyrolysates of softwood lignins, since Leopold and Malmström (1952) reported *p*-hydroxybenzaldehyde and syringaldehyde as minor components in the nitrobenzene oxidation of conifers.

Pyrolysis of bamboo MWL produces a rather unique suite of compounds (vinylphenol, propenylphenols, *p*-hydroxybenzaldehyde, etc.) that were not encountered in the softwood and hardwood lignins studied. Higuchi *et al.* (1967) reported that the larger proportion of *p*-hydroxybenzaldehyde produced in the alkaline nitrobenzene oxidation of grass lignins is formed from *p*-coumaric acid esterified with the lignin, and not from a *p*-hydroxyphenyl moiety in the lignin polymer itself. Further, these authors conclude that the main polymeric systems of the grass lignins are not very different from hardwood lignins. So, it is expected that the *p*-coumaric acid esterified with the lignin, upon pyrolysis, will yield the decarboxylated pyrolysis product *p*-vinylphenol.

Milled wood lignins vs dehydrogenation polymers

Milled wood lignins are considered to be, in many respects, almost identical with the original lignin, although they represent only 30–50% of the total lignin components, and generally contain small amounts of associated carbohydrates. The major differences between natural and synthetic lignins is the increase of coniferyl alcohol and aldehyde in the DHP samples, which indicates that an important moiety of the dehydropolymers is composed of the so-called "bulk polymer". In this connection, Haider *et al.* (1985) reported that ^{13}C labelling at the C- γ position of the side chain in the coniferyl alcohol precursor causes an enhancement of the signal corresponding to C- γ of coniferyl alcohol units with a free chain, which was the most intense signal in the DHP spectrum. The same result was obtained with C- α and C- β when labelled at the corresponding positions.

Spectroscopic and chemical studies point out that DHPs contain essentially the same structural units as MWLs (Kirk *et al.*, 1975). Previous analytical pyrolysis (Saiz-Jimenez and de Leeuw, 1984b) demonstrated that spruce and synthetic lignins are similar in

that both generate the same type of pyrolysis products. However, because the ratios of these products were different, it was concluded that MWL and DHP were not identical, but merely similar. Further, in this study it is shown that differences between hardwood lignins and their synthetic counterpart are also evident. However, the much larger differences observed between the bamboo MWL and the guaiacyl-syringyl-*p*-hydroxyphenyl DHP indicate that the complexity of the natural lignin is not reflected in the synthetic one, probably due to the fact that the ester linkage is not present in the synthetic dehydropolymers.

Present pyrolysis data vs literature data

The results presented in this paper agree relatively well with those of Obst (1983) who studied loblolly pine and white oak MWL by Py-GC-MS. However, the different pyrolysis system (Pyroprobe) and temperature (800°C) probably result in a higher fragmentation of subunits (e.g. demethylation, as indicated by the identification of catechols). Catechol was only found upon Curie-point pyrolysis in biodegraded and kraft lignins (Saiz-Jimenez and de Leeuw, 1984b), where the action of respectively fungi and chemicals cause a demethylation. In the investigation reported here no catechols were encountered.

Martin *et al.* (1979) studied the same MWLs as those reported here. Also, in this case the pyrolysis system (Pyroprobe) and temperature (700°C) were different. The distribution patterns of pyrolysis products were somewhat different from those reported here. The largest difference concerned their identification of vinylphenols in the spruce MWL (equivalent to our MWL-2). A detailed study of the two spruce and the beech and aspen MWL samples failed to recognize vinylphenols among the pyrolysis products. Therefore, it is possible that the pyrolysis conditions used by the authors caused fragmentation of guaiacyl units to give rise to vinylphenols. However, Obst (1983) using an even higher temperature also did not report vinylphenols in pine and oak MWL.

From these data it must be concluded that comparison of results, even obtained for the same samples using different analytical systems and pyrolysis temperature is difficult.

Philp *et al.* (1982) studied lignins using Curie-point pyrolysis with an end temperature of 610°C. In pine lignin, among other compounds, 1,4-dimethoxybenzene and 1,2,3-trimethoxybenzene were identified and in eucalyptus lignin the major compound was identified as 3,4-dimethoxyphenol. Neither of these compounds could be identified in softwood and hardwood lignins by Obst (1983) or in this study, as expected, because there are no parent structures present in lignins which can give rise to them upon pyrolysis. As suggested by Obst (1983) identifications might have been wrong owing to misinterpretation of library spectra. This could occur because many of the

lignin pyrolysis products are not present in most library files and the best match does not always give the right structure. Reinterpretation of the data is necessary before a comparison can be made.

Sigleo (1978) reported that wood polymers survive 200 million years of silicification and diagenesis in the Petrified Forest National Park (Arizona) while Habermehl and Hundrieser (1983) found typical lignin oxidation products in Messel kerogen, 50 million years old. Because the guaiacyl and syringyl derivatives are unique to lignins and woods, and obviously can be preserved for millions of years, lignins can be considered as macromolecular biomarkers. Unequivocal evidence for the presence of lignin and/or wood in fossil materials can be obtained by studying the pyrolysis products. Moreover, Py-GC-MS analysis can clearly distinguish the three types of lignins.

The presence of *p*-vinylphenol as major compound in the pyrolysates of lignocellulosic materials, in addition to guaiacyl and syringyl derivatives, points to grass lignin. Guaiacyl and syringyl derivatives, in absence of *p*-hydroxyphenyl compounds, direct to hardwoods, while the presence of guaiacyl units alone indicate that the lignin was biosynthesized by conifers. To further prove these statements and to obtain more information about the fate of buried wood components such as carbohydrate and lignin structures, a study of spruce, alder and oak woods deposited in coastal sediments is in progress.

Note added in proof

Unidentified peak before 10 in Figs 3, 4, 5, 7 and 8 is methoxydihydroxybenzene. Also peak before 46 is *as*-sinapyl alcohol.

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