Pyrolysis-gas chromatography-mass spectrometry of isolated, synthetic and degraded lignins

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Abstract—Curie-point pyrolysis-gas chromatography-mass spectrometry was applied to study the chemical structure of sound and fungus degraded, industrial and synthetic lignins. Pyrolysis products reflected in some detail the structural units present in the lignin polymer. Thus, sound spruce lignin yielded *trans*-isoeugenol, coniferaldehyde and *trans*-coniferyl alcohol as major pyrolysis products. Biodegraded lignin yielded oxidized units, including vanillin, acetoguaiacone, methyl vanillate, propioguiacone, vanilloyl methyl ketone and vanillic acid as major products. Kraft lignin also showed evidence of oxidation, although not as much as the biodegraded lignin. Major products from this industrial lignin were guaiacol, methylguaiacol, vinylguaiacol and homovanillic acid. Results indicated that synthetic lignin duplicates fairly well the structure of natural lignin. However, coniferylaldehyde and *trans*-coniferyl alcohol were the dominant products only from the synthetic lignin, indicating the presence of large amounts of coniferyl alcohol and coniferylaldehyde end groups.

Key words: pyrolysis, lignins, biodegradation, kraft lignin, DHP lignin, spruce lignin

INTRODUCTION

Lignins are phenolic polymers which occur as major components of vascular plants and hence can be considered as markers of land-derived organic matter in lacustrine and marine sediments.

Lignins are biosynthesized by an oxidative polymerization of three substituted cinnamyl alcohols: *p*-coumaryl-, coniferyl- and sinapyl alcohols (4-hydroxy-, 4-hydroxy-3-methoxy- and 4-hydroxy-3,5-dimethoxycinnamyl alcohol, respectively). The proportions of the three precursor alcohols differ between angiosperm and gymnosperm lignins (Adler, 1977).

Although a wide range of micro-organisms, especially fungi and bacteria, can decompose lignins to some extent, only certain higher fungi (Basidiomycetes) have been shown to decompose them extensively (Kirk and Fenn, 1982).

In soils, and probably also in sediments when present, lignins and/or their degradation products are thought to play an important role in the formation of humic substances (Saiz-Jimenez and de Leeuw, 1984).

Besides the natural input of lignin in soils and sediments via plant debris, an anthropogenic origin of lignin has to be considered. In fact, industrial lignins, produced as side-products in chemical pulping, are often discharged into the environment. As industrial lignins appear to be resistant to microbial decomposition in a variety of neutral and acidic,

anoxic environments (Zeikus *et al.*, 1982), their accumulation in polluted areas has to be taken into account.

The chemical nature of the lignins has been studied by often tedious chemical degradation techniques such as nitrobenzene, permanganate, cupric oxide oxidation or acidolysis. However, the structure of lignins can be analysed rapidly by analytical flash pyrolytic methods (Martin *et al.*, 1979b; Obst, 1983; Schenck *et al.*, 1983). In this paper, pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) for sound, fungus degraded and industrially modified lignins is reported. Also, an artificial lignin synthesized *in vitro* from coniferyl alcohol using a peroxidase is included for comparative purposes. Because complex polymeric mixtures present in sediments and soils, coals, etc. are often studied by analytical pyrolytic techniques (Saiz-Jimenez *et al.*, 1979; Martin *et al.*, 1979a; van de Meent *et al.*, 1980; Schenck *et al.*, 1983) the understanding of the structures of pyrolysis products generated from different lignins will provide useful information about the origin of the lignin-related components most frequently identified in the various types of humic and other macromolecular materials. Moreover, a better understanding of the lignin structure after fungus mediated transformation can be obtained, and the structure of artificial lignin can be compared with that of natural lignins.

EXPERIMENTAL

Spruce milled wood lignin and lignin degraded by *Coriolus versicolor* (L. ex Fr.) Quel. were kindly

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provided by Dr T. Kent Kirk. Characteristics of the wood used, the extraction procedures and the isolation and the incubation methods are reported by Kirk and Chang (1974). Moreover, chemical characterization studies of both lignins have been reported (Kirk and Chang, 1975).

Indulin AT, a pine kraft lignin with an ash content less than 1%, was kindly provided by Dr J. Marton. Details of its chemical structure are described elsewhere (Marton, 1964; Marton and Marton, 1964).

The synthetic lignin, guaiacyl DHP (dehydrogenative polymerisate), was kindly supplied by Dr O. Faix. Its synthesis is described by Schweers and Faix (1973).

Pyrolysis-GC

The samples were suspended in methanol. One droplet of the suspension (~10–20 µg of sample) was applied to a ferromagnetic wire with a Curie temperature of 510°C.

The pyrolysis gas chromatographic analyses were carried out using a pyrolysis unit similar to the one described by Meuzelaar *et al.* (1975), modified for use at high temperatures (van de Meent *et al.*, 1980). Chromatographic separations were achieved with a capillary glass WCOT column (28 m × 0.5 mm i.d.) coated with CP sil 5 (1.3 µm film thickness) using a Packard Becker 419 gas chromatograph. Helium was used as the carrier gas.

Pyrolysis-GC-MS

Pyrolysis was performed as described above. The 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 a rate of 5°C min⁻¹. Helium was used as the carrier gas. The chromatograph (Varian model 3200) was coupled to a Varian Mat 44 mass spectrometer operated in the EI mode at 80 eV with a cycle time of 2 s.

RESULTS AND DISCUSSION

Pyrograms of the sound spruce lignin, the biodegraded spruce lignin, the industrial lignin and the synthetic lignin are shown in Figs 1–4.

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

The pyrogram of spruce lignin (Fig. 1) shows several major peaks, identified as 4-methylguaiacol (6), 4-vinylguaiacol (9), vanillin (12), *trans*-isoeugenol (15), coniferaldehyde (27) and *trans*-coniferyl alcohol (29). Coniferylaldehyde and coniferyl alcohol were not reported in previous Py-GC-MS studies (Martin *et al.*, 1979b; Philp *et al.*, 1982) of spruce lignin, but were recently identified from pine lignin by Obst (1983). Other prominent pyrolysis products identified in the spruce lignin pyrogram were guaiacol (4), eugenol (10), homovanillin (14),

acetoguaiacone (16), propioguaiacone (22) and homovanillic acid (25). All of these pyrolysis products seem to reflect structural units present in the lignin polymer.

It is noteworthy the presence of methyl vanillate (19) among the pyrolysis products of lignins. Obst (personal communication) treated loblolly pine MWL with 1 N NaOH at 100°C, which would cleave all carboxylic esters. The pyrogram of the saponified MWL showed about the same amount of methyl vanillate as the untreated MWL. Therefore, it appears that methyl vanillate is an artefact produced during pyrolysis and does not represent methyl esters in lignin. Apparently vanillic acid is methylated during pyrolysis.

The distribution pattern of the pyrolysis products encountered in the fungus degraded lignin (Fig. 2) is very different from the pattern observed in the sound lignin (Fig. 1). Major components in the biodegraded lignin are guaiacol (4), acetoguaiacone (16), methyl vanillate (19) and vanilloyl methyl ketone (23). Other prominent peaks were identified as 4-vinylguaiacol (9), vanillin (12), propioguaiacone (22) and vanillic acid (24). The total absence of coniferaldehyde and *trans*-coniferyl alcohol, the relatively low intensity of 4-methylguaiacol, 4-vinylguaiacol and *trans*-isoeugenol and the presence of vanilloyl methyl ketone and vanillic acid among the pyrolysis products of the fungal degraded lignin is good evidence for oxidation of the C₃-alkyl chain at the C_α and C_β positions, and for cleavage in the side-chain, mainly between C_α and C_β. These conclusions based upon the pyrolysis data are in good agreement with those of Chen *et al.* (1982, 1983) who studied the fungal degradation of spruce lignins by other methods.

The pyrogram of kraft lignin (Fig. 3) shows significant differences from that of natural conifer lignin. Major peaks were identified as guaiacol (4), 4-methylguaiacol (6), 4-vinylguaiacol (9), *trans*-isoeugenol (15), and homovanillic acid (25). Other important pyrolysis products were identified as 4-ethylguaiacol (8), eugenol (10), vanillin (12), acetoguaiacone (16) and *trans*-coniferyl alcohol (29). The differences between the sound lignin and the kraft lignin are especially evident in the relatively high abundance of guaiacol, 4-methylguaiacol, 4-vinylguaiacol and homovanillic acid in the latter.

During delignification of wood in the kraft process, the lignin component is solubilized via degradation and ionization to free the fiber for the manufacture of paper. The lignin alteration is characterized by degradation of the side-chain, including a partial loss of C_γ atoms, β-guaiacyl ether bond cleavage, limited demethylation, and formation of stilbene structures from phenylcoumarans (Marton, 1964; Adler *et al.*, 1964; Lundquist *et al.*, 1977). These changes in the lignin structure are corroborated to some extent by the prominence of homovanillic acid together with the low intensities of coniferaldehyde and *trans*-coniferyl alcohol, in the pyrolysis mixture.

Fig. 1. Pyrogram of spruce milled wood lignin.

Fig. 2. Pyrogram of spruce milled wood lignin degraded by *Coriolus versicolor*.

Among the very volatile pyrolysis products of kraft lignin we encountered a number of organic sulphur compounds such as H_2S , SO_2 , CH_3SH , $\text{C}_2\text{H}_5\text{SH}$ and/or $\text{CH}_3\text{S-CH}_3$, $\text{CH}_3\text{-S-S-CH}_3$ and $\text{CH}_3\text{-S-S-S-CH}_3$. These pyrolysis products are very probably generated from the sulphur-containing alkyl side-chain moieties present in the industrial lignin. This

observation is in agreement with other work on kraft lignins (Marton, 1964).

The presence of characteristic sulphur-containing compounds, together with lignin pyrolysis products, can discriminate an anthropogenic origin of industrial lignins in recent sediments from a natural contribution of lignin or biodegraded lignins. Further,

Fig. 3. Pyrogram of kraft lignin.

Fig. 4. Pyrogram of synthetic lignin.

characteristic lignin pyrolysis products most frequently identified in soil and aquatic humic substances and coals can be considered as polymeric biomarkers, indicating a plant origin.

Pyrograms of sound lignin (Fig. 1) and guaiacyl DHP (Fig. 4) are similar in that both have the same type of pyrolysis products. However, because the

ratios of these products are different, it must be concluded that DHP and sound lignins are not identical, but merely similar. Compared to sound lignin the relative amounts of guaiacol (4), coniferaldehyde (27), and *trans*-coniferyl alcohol (29) are increased in DHP. Model DHP lignin as synthesized by the action of peroxidase on coniferyl alcohol

Table 1. Pyrolysis products from lignins

Peak No.	R ₁	R ₂	R ₃	Remarks
1	OH			
2	OH	CH ₃		
3	OH		CH ₃	
4	OH	OCH ₃		
5				Positional isomer of 6
6	OH	OCH ₃	CH ₃	
7	OH	OH		
8	OH	OCH ₃	CH ₂ -CH ₃	
9	OH	OCH ₃	CH ₂ =CH ₂	
10	OH	OCH ₃	CH ₂ -CH=CH ₂	
11	OH	OCH ₃	CH ₂ -CH ₂ -CH ₃	
12	OH	OCH ₃	CHO	
13	OH	OCH ₃	CH=CH-CH ₃	<i>cis</i>
14	OH	OCH ₃	CH ₂ -CHO	
15	OH	OCH ₃	CH=CH-CH ₃	<i>trans</i>
16	OH	OCH ₃	CO-CH ₃	
17				
18	Cl	Cl	OCH ₃	R ₄ =OCH ₃
19	OH	OCH ₃	COO-CH ₃	
20	OH	OCH ₃	CH ₂ -CO-CH ₃	
21	OH	OCH ₃	C ₃ H ₅ O	
22	OH	OCH ₃	CO-CH ₂ -CH ₃	
23	OH	OCH ₃	CO-CO-CH ₃	
24	OH	OCH ₃	COOH	
25	OH	OCH ₃	CH ₂ -COOH	
26	OH	OCH ₃	CH=CH-CH ₂ OH	<i>cis</i>
27	OH	OCH ₃	CH=CH-CHO	
28	OH	OCH ₃	CH ₂ -CH ₂ -COOH	
29	OH	OCH ₃	CH=CH-CH ₂ OH	<i>trans</i>
30				dialkyl phthalate

appears to be similar to certain conifer lignins. However, Kirk *et al.* (1975) have shown that in the synthetic lignin the relative amounts of coniferyl alcohol end groups are more abundant and that the natural lignin has a higher degree of cross-linking. These phenomena are supported by our pyrolysis data.

The dominant presence of coniferaldehyde and *trans*-coniferyl alcohol in the pyrogram of the DHP lignin point to a polymeric structure in which the original substrate (*trans*-coniferyl alcohol) is linked less firmly than in the natural lignin. In summary, we think that these synthetic lignins are very valuable models for the chemical study of many aspects of natural lignins.

CONCLUSIONS

(1) Pyrolysis-gas chromatography and pyrolysis-gas chromatography-mass spectrometry are power-

ful tools to rapidly chemically characterize isolated and synthetic lignins and lignin-derived polymers.

(2) Lignins and lignin-derived products can, therefore, be considered as polymeric biomarkers, less prone to external influences than extractable biomarker molecules.

(3) Biodegradation of lignin by white-rot fungi results in severe side-chain oxidation. These recognizably oxidized polymers are present among the building blocks of humic substances (Saiz-Jimenez and de Leeuw, 1984).

(4) An anthropogenic origin of lignin products in recent sediments, e.g. kraft lignin from papermills can be recognized and can be discriminated from a natural contribution of lignins or biodegraded lignins, based on pyrolysis data.

Acknowledgements—The authors wish to express their gratitude to Dr T. Kent Kirk and Dr J. R. Obst for critically reading the manuscript.

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