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Correction to "The Determination of Cytochrome *c* Reductase Activity"*

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The cyanide concentration, as given on p. 4, line 26 from above, is erroneous. The concentration actually used was 7×10^{-3} mM.

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Catechol Moieties in Enzymatically Liberated Lignin

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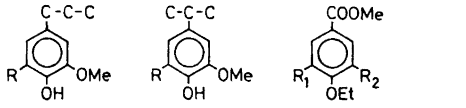
The methoxyl content of lignin in wood decreases markedly during removal of carbohydrates by certain Basidiomycetes known as "brown-rot" fungi.[1–3] Little specifically is known, however, about the effects of these fungi on the lignin;[3] the structures of the methoxyl-deficient units in the lignin apparently have not been studied. We have examined such lignin, which is known as "enzymatically liberated lignin",[4] for the possible occurrence of catechol (*o*-diphenol) moieties, which could arise *via* a net demethylation of guaiacylpropane (*1*) and syringylpropane (*2*) units as well as of the corresponding 2-arylpropane structures (*3*, *4*) present [5,6] in lesser



1 R=H
2 R=OMe
3 R=H
4 R=OMe
5 R_1=H, R_2=OMe
6 R_1=H, R_2=OEt
7 R_1=R_2=OMe
8 R_1=OMe, R_2=OEt

amounts in lignin. We used an approach suggested by the work of Hayashi and Namura,[7] who reported that permanganate oxidation of an ethylated lignosulfonate yielded, among other products, small amounts of 3,4-diethoxybenzoic acid, which must have been derived from catechol units present in the lignosulfonate. The detailed method that we used in the present study was adapted from a technique developed recently in this laboratory in connection with the oxidative degradation of methylated lignins.[8–10]

The enzymatically liberated lignin had been prepared from sapwood of sweetgum (*Liquidambar styraciflua* L.) by the use of *Lenzites trabea* Pers. ex Fries in an earlier study.[11] The whole lignin had been separated by preparative gel chromatography

into four fractions of decreasing molecular weights. The second lowest molecular weight fraction, "fraction 3", was used in the present investigation, and had a molecular weight (M_n) of 1100.[11] It contained 14.9 % methoxyl, whereas Björkman lignin[12] prepared from sound sweetgum had a methoxyl content of 21.4 %. Samples of fraction 3 were ethylated with diethyl sulfate and the resulting material oxidized, first with permanganate at pH 12 at 80°, then with 5 % H_2O_2 at pH 9–10 at 50°; the acids thus produced were methylated with diazomethane.

Gas chromatography of the mixture of methyl esters revealed that four compounds were particularly prominent (Fig. 1); these were identified as the methyl esters of 3-methoxy-4-ethoxybenzoic acid *5*, 3,4-diethoxybenzoic acid *6*, 3,5-dimethoxy-4-ethoxybenzoic acid *7*, and 3,4-diethoxy-5-methoxybenzoic acid *8*. Quantitative estimations (Table 1) were made by the use of an internal standard, trimethylgallic acid methyl ester *9*.

The methyl esters *5* and *7* obviously originated from (phenolic) guaiacyl (*1,3*) and syringyl (*2,4*) units, respectively. As was expected, when ethylated Björkman lignin of sweetgum was oxidatively degraded as above, methyl esters *5* and *7* were the major products formed. The presence of compounds *6* and *8*, which were produced in substantial amounts (Table 1) from the enzymatically liberated lignin, indicates that catechol groups were formed in the lignin by a net demethylation of (phenolic) guaiacyl and syringyl units by *L. trabea*. Comparison of the amounts of compounds *6* and *8* with the amounts of compounds *5* and *7*, respectively, (Table 1) indicates that approxi-

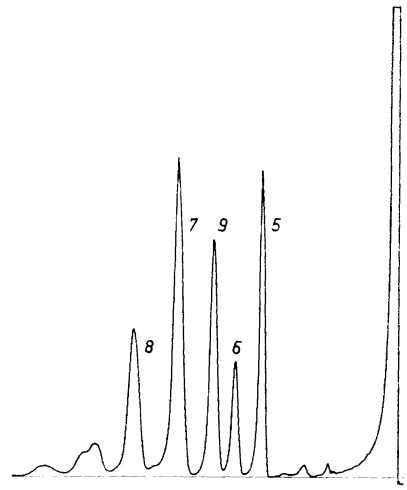


Fig. 1. Gas chromatographic separation of methyl esters of acids produced by oxidatively degrading an ethylated enzymatically liberated lignin of sweetgum (stationary phase: "OV-1").

Table 1. Yields of methyl esters (mg per 100 mg of non-ethylated lignin).

Lignin	Methyl ester			
	5	*6*	*7*	*8*
Björkman lignin of sweetgum	3.7	0 [a]	5.3	0 [a]
Enzymatically liberated lignin of sweetgum	2.3	1.0	3.9	1.8
Björkman lignin of spruce	9.2	0 [a]	—	—
Enzymatically liberated lignin of spruce	6.1	2.0	—	—

[a] Small peaks in the chromatograms (corresponding to less than 0.1 mg), having the same retention time as *6* or *8*, were observed but the compounds responsible for the peaks have not been identified.

mately 30 % of the (phenolic) guaiacyl and syringyl units had undergone net demethylation.

It was of interest to examine an enzymatically liberated lignin produced by a different fungus. Consequently, an enzymatically liberated lignin, with a methoxyl content of 11.6 %, was prepared from sitka spruce wood (*Picea sitchensis* Carr.) that had been attacked by the brown-rot fungus *Poria monticola* Murr. Oxidative degradation of this lignin as above yielded methyl ester *6* in a substantial amount, in addition to methyl ester *5*; approximately 25 % of the (phenolic) guaiacyl units in the spruce lignin had undergone a net demethylation (Table 1). Similar treatment of Björkman lignin (methoxyl = 15.7 %) of Norway spruce (*Picea abies* Karst.) yielded methyl ester *5* as the major product (Table 1). Thus demethylation is not a process unique to the sweetgum-*L.trabea* combination, but is characteristic of the action of at least two different brown-rot fungi on lignins from two different woods.

Syringyl moieties are present only in small amounts in spruce lignins,[10,13] so that the methyl esters *7* and *8* were not expected to be found in significant quantities in the mixture of degradation products; no attempt was made to establish their presence.

It is concluded from these studies that brown-rot fungi effect a net demethylation of the (phenolic) guaiacyl and syringyl units in lignin, which results in the formation of catechol moieties. Investigations are underway to determine whether demethylation also occurs in guaiacyl and syringyl units in which the phenolic hydroxyl group became etherified during lignification.

Gas chromatography. Chromatograph: Perkin-Elmer Model 880. *Column dimensions:* 200 × 0.3 cm o.d. stainless steel tubing. *Solid support:* Chromosorb G, acid washed and treated with dimethyldichlorosilane, 80–100 mesh. *Stationary phase:* Silicone elastomer SE-30, General Electric, or OV-1, Applied Sciences Laboratories, (5 % by weight of solid support). These two stationary phases gave practically identical separations. *Temperatures:* Injection: 300°. Detector: 230°. Column: 187°. *Carrier gas:* N_2, 25 ml/min. *Detector:* Differential flame ionization detector. The instrument was used with two packed columns.

Identification of methyl esters. The mass spectra of components *5*, *6*, *7*, and *8* were obtained using an LKB 9000 gas chromatograph-mass spectrometer unit, and were shown to be identical with mass spectra of the synthesized known compounds.

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