Aromatic Hydroxylation Resulting from Attack of Lignin by a Brown-Rot Fungus

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A valuable means for studying the aromatic substitution in lignins is the identification of methoxylated aromatic carboxylic acids formed on oxidative degradation of methoxylated samples. In connection with investigations of the effects of brown-rot fungi on the chemical structure of lignin it was of interest to determine whether any significant differences were to be found between aromatic substitution patterns of sound and fungus-altered lignin.

Lignin isolated from wood of sweetgum (Liquidambar styraciflua L.) decayed by the brown-rot fungus Lenzites trabea Pers. ex Fries ("enzymatically liberated lignin") has thus been compared with Björkman lignin (milled wood lignin) of sound sweetgum wood. Following methylation, the lignins were oxidized with permanganate at pH 11–12 and then with hydrogen peroxide at pH 9–10.25 and the resulting acids methylated with diazomethane. The mixtures of methyl esters were examined by gas chromatography-mass spectrometry.

The gas chromatograms indicated close similarity between the two lignins in the products and in the approximate relative proportions of products. The major aromatic acids included veratic acid (I), tri-O-methylgallic acid (II), isohemipinic acid (III), metahemipinic acid (IV), and 3,4,5-trimethoxyphenolic acid (V). These acids are also major products formed on similar treatment of Björkman lignin of birch (Betula verrucosa Ehrh.).

In addition to the mutual chromatographic peaks, there were two significant peaks from the enzymatically liberated lignin that were not observed in the case of the ester mixture derived from Björkman lignin. The compounds responsible for

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these peaks have been identified by gas chromatography-mass spectrometry as the methyl esters of 2,3,4-trimethoxybenzoic acid (VII) and 2,3,4,5-tetramethoxybenzoic acid (VII). These clearly arose from structures which had been hydroxylated as a result of fungal action. The hydroxylations occurred ortho to the side chains in the phenylpropane units of the lignin. It is not discernible from our data whether normal units (structures VIIa and b, R₂ = Me) or methoxy-deficient units \( \text{VIIa} \) (structures VIIa and b, \( R_2 = H \)) or both were hydroxylated. Neither is the hydroxylation mechanism known. Evidently the origin of structures giving rise to 2,3,4,5-tetramethoxybenzoic acid (VII) involved hydroxylation at C-2 or C-6 of lignin units of type VIIa. Similarly, the origin of structures giving rise to 2,3,4-trimethoxybenzoic acid (VII) involved hydroxylation at C-2 of VIIb. Hydroxylation at C-6 of VIIb did not occur; this would have led to 2,4,5-trimethoxybenzoic acid (IX), which was not detected in the mixture of esters derived from either lignin. Investigations are underway to determine the nature of the hydroxylation reaction and to identify the structures giving rise to VI and VII.

**Experimental. Reference compounds.** For compounds I–VI, see Ref. 2. 2,3,4,5-Tetramethoxybenzoic acid (VII), m.p. 85–86° (Ref. 7, m.p. 87–88°); asaronic acid (IX), m.p. 143–143.5° (Ref. 8, m.p. 142°). The respective methyl esters were prepared by methylation with diazomethane in methanol-ether.

**Lignins.** The enzymatically liberated lignin was a fraction (Fraction 3) prepared in connection with an earlier study. Björkman lignin was prepared from sound sweetgum sapwood. **Methylation** of the lignins was accomplished with diazomethane in dimethylformamide in the manner used for ethylation with diazomethane. Oxidation of the methylated lignin was done as described for ethylated lignins. Gas chromatography. Chromatograph: Perkin-Elmer Model 880. Column dimensions: column A) 100 x 0.3 cm o.d., column B) 180 x 0.3 cm o.d. stainless steel tubing. Solid support: A) Chromosorb G, acid-washed and treated with dimethyl dichlorosilane, 250–150 mesh; B) Gas Chrom Z, 250–100 mesh. Stationary phase: A) General Electric SE-30, 5% by weight of solid support; B) combination of SE-30, 1% by weight of solid support, and General Electric SE-60, 2% by weight of solid support. Temperatures: Injection 300°, Detector 230°. Column: A) 170° constant, or 160–250°, 5°/min; B) 180° constant. Carrier gas: \( N_2 \), 25 ml/min. Detector: Differential flame ionization. Compounds I–VII were analyzed with column A. The absence of compound IX could not be established with column A, but was possible with column B, which permitted authentic IX to be well separated from compounds obtained on methylation-oxidation of the lignins.

**Identification of methyl esters.** Compounds I–VII, as methyl esters, were identified by comparison of retention times and mass spectra with those of synthesized materials. Mass spectra were taken with an LKB 9000 gas chromatograph-mass spectrometer unit.

**Retention times of methyl esters.** (Retention times are expressed relative to that of the methyl ester of III). Column A (constant temperature of 180°) I–VI; \( \text{VII} \) 0.64; IX 0.65. Column B (constant temperature of 180°) I 0.26; II 0.40; III 1.00; IV 1.59; V 1.59; VI 0.34; VII 0.46; IX 0.73.

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High Pressure Synthesis of Nb$_2$O$_5$F with U$_5$O$_8$-type Structure
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Structure determinations of niobium oxide fluorides have revealed the existence of six-coordinated metal atoms in Nb$_2$O$_5$F and mixtures of six- and four-coordinated niobium atoms in the structures of Nb$_2$O$_5$F, Nb$_2$O$_5$F$_3$, Nb$_4$O$_{17}$F$_x$, Nb$_4$O$_{16}$F$_y$, and Nb$_5$O$_{14}$F$_z$. All these structures are related to the ReO$_3$ type of structure. Nb$_2$O$_5$F shows crystallographic shear in one dimension, while the others are of the Wadsley block type with shear in two dimensions.

Recently, Holmberg has determined the structure of Nb$_2$O$_5$F with the niobium atoms in six- as well as in seven-coordination, and there is no obvious relation to the ReO$_3$ structure.

Several compounds of intermediate compositions have also been found to exist in the system TaO$_2$F-Ta$_2$O$_5$. The compound Ta$_2$O$_4$F was reported to occur in two different forms, one of the LiNb$_2$O$_6$F structure type, the other of a type related to U$_2$O$_5$. Both of these have six- as well as seven-coordinated metal atoms. These structures are packed more densely than those of the corresponding niobium oxide fluoride structures that are based on the ReO$_3$-type building units.

The starting materials used were H-Nb$_2$O$_5$ and Nb$_2$O$_6$F; the latter was prepared according to Ref. 8. A sample of Nb$_2$O$_5$F was obtained by heating a mixture of Nb$_2$O$_5$F and H-Nb$_2$O$_5$ (mole ratio 1:1) at 850°C in a sealed nickel capsule.

The pressure experiments were performed in a girdle apparatus at 25 kb in the temperature range 850–1100°C. Mixtures of H-Nb$_2$O$_5$ and Nb$_2$O$_5$F in the mole ratio 1:1 as well as samples of Nb$_2$O$_5$F were exposed to the pressure for 2–14 h. Crystalline materials were always obtained and X-ray powder analysis at atmospheric pressure and room temperature indicated the presence of a new phase. A reaction time in excess of 3 h at 900°C resulted in a slight reduction of the products as evidenced by dark coloration.

Weissenberg photographs of a single crystal, selected from a white nonreduced sample prepared at 900°C and 20 kb, were taken with CuK$_\alpha$ radiation with the a axis chosen parallel to the rotation axis (a = 3.9 Å). The Laue symmetry was mmm. On the Weissenberg photographs 0kl and 1kl and the powder photograph (v. infra) the following reflections were absent:

$$hkl$$ for $$k + l = 2n + 1$$

Thus probable space groups are $A222$, $A2mm$, $Amm2$, and $Ammm$.

The cell constants were derived from a powder photograph, taken with a Hagg-Guinier camera, using CuK$_\alpha$ radiation. All lines were indexed on the basis of an orthorhombic cell, with the following edge lengths:

$$a = 3.927 \pm 1 \text{ Å}; \quad b = 10.514 \pm 1 \text{ Å}; \quad c = 6.475 \pm 1 \text{ Å}; \quad V = 237.4 \text{ Å}^3$$

The indexed powder pattern is given in Table 1. The density of the sample determined from the loss of weight in benzene was 5.23 g cm$^{-3}$, which corresponds to two formula units of Nb$_2$O$_5$F per unit cell ($d_{calc}=5.08 \text{ g cm}^{-3}$).

The composition of the starting materials as well as the observed density, suggests the formula Nb$_2$O$_5$F. The unit cell dimen-