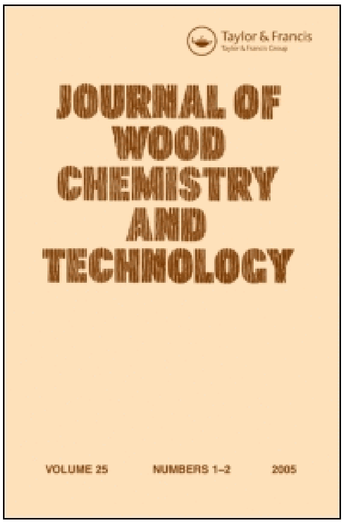

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A ^{14}C BALANCE ON NITROBENZENE OXIDIZED KENAF LIGNIN

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ABSTRACT

Kenaf, containing unlabeled or ^{14}C-labeled lignin was oxidized by nitrobenzene. Reaction products were separated, the ^{14}C-content measured, and the products identified by mass spectroscopy (MS) and high pressure liquid chromatography (HPLC). Principal products and their approximate percentage of "original-lignin-^{14}C-content" were: syringaldehyde 20%, oxalic acid 20%, high-molecular-weight species 16%, vanillin 8.2%, benzoic acid and 4-hydroxy-azobenzene 10.7%, neutral species 3% or less, syringic acid 2.4%, vanillic acid 1.5%, and malic acid (trace). Numerous aromatic products at about the 1% concentration level remain unidentified.

INTRODUCTION

In a previous paper we reported that nitrobenzene oxidation completely solubilizes lignin from wheat, kenaf, oak, and some pine samples.[1] Many reaction products have been identified and quantitated from the nitrobenzene oxidation of conifer wood.[2-4] Reported yields of reaction products from nitrobenzene oxidation of lignin varied from 25% of lignin (as measured by Klason analysis)[5] to 49% of the milled wood lignin starting material,[6] but no complete accounting of the lignin has been reported. A material balance is difficult to develop from weights of products because oxidized species have fewer phenolic units and more total weight than the unoxidized lignin. ^{14}C-Lignin and ^{14}C-product balance overcomes this problem because only labeled carbon needs to be balanced. Aliphatic acids isolated from nitrobenzene-oxidized conifer wood have not been clearly identified as to their source, i.e. whether they came from lignin or other plant components. This is especially important because up to 80% of a plant material is often solubilized by nitrobenzene oxidation in 2N NaOH. Aromatic aldehydes resulting from nitrobenzene oxidation of lignin were often the only products reported because their structures and yield were related to the structure of lignin in the plant. The combination of syringaldehyde and vanillin as oxidation products, often in a 3:1 ratio, indicates that the starting material was a syringyl-guaiacyl lignin. Vanillin alone indicates a guaiacyl lignin. Syringaldehyde, vanillin, and p-hydroxybenzaldehyde together points to a syringyl-guaiacyl-p-hydroxyphenyl lignin typical of grasses.

We chose a syringyl-guaiacyl lignin labeled with ^{14}C from kenaf (Hibiscus cannabinus, L.) in order to determine the nature and carbon balance of nitrobenzene-oxidized lignin products. The products from oxidation of kenaf lignin should be typical of those from syringyl-guaiacyl lignins in other plants. Syringyl-guaiacyl lignins are the most frequently encountered in the plant kingdom.

RESULTS AND DISCUSSION

Each separation step (Figure 1) was tested for loss of ^{14}C by decomposition or volatilization. Table 1 shows that little or no

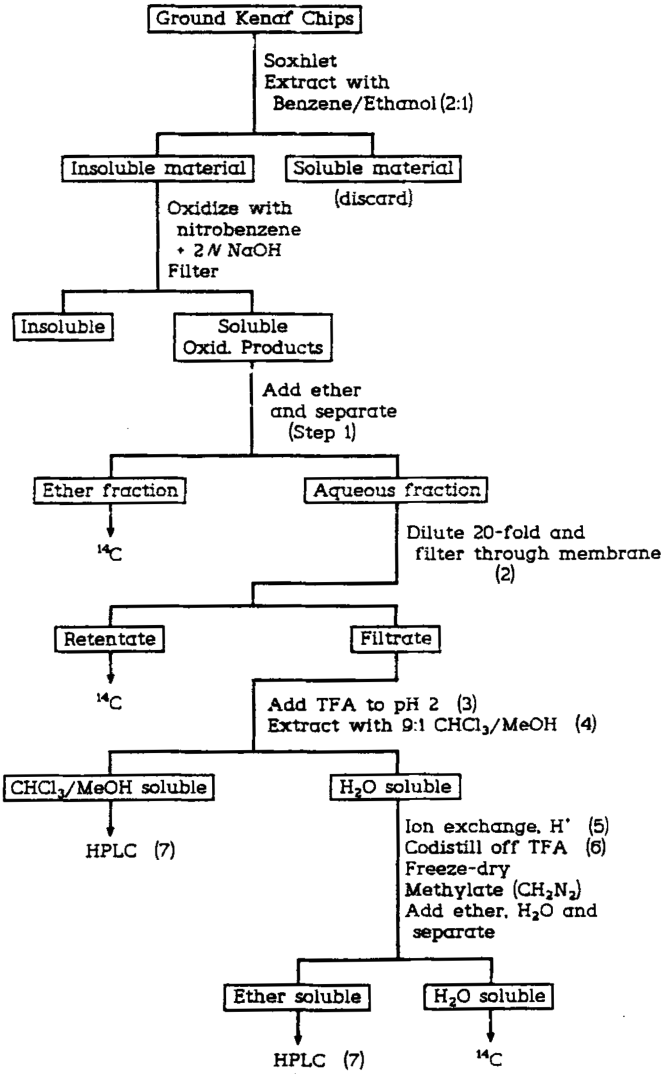

Figure 1. Separation of Nitrobenzene Oxidation Products. Numbered steps have been tested for ^{14}C loss and results are reported in Table 1.

TABLE 1

^{14}C Losses in Separation

Separation Step[1]	Percentage Lost	DPM x 10^5 Before	DPM x 10^5 After
(1) Ether Extraction of Basic Solution	0.1, 0.9, 3.4		
(2) Membrane Separation		7.84	8.02 (102%)
(3) Acidification, N_2 Bubbling	0.3		
(4) $CHCl_3$:CH_3OH Evaporation	N.D.		
(5) Ion Exchange Column		1.25	1.25
(6) TFA Distillation	N.D.		
(7) HPLC Separations	0 to 10		

[1] Numbers of separation steps are illustrated in Figure 1.

N.D. = None detected, DPM = disintegration of ^{14}C per min, TFA = trifluoroacetic acid, HPLC = high pressure liquid chromatography.

loss occurs in the steps tested. Recoveries of ^{14}C were 90% or better of starting ^{14}C. Based on gas analysis after acidification and N_2 bubbling, it could be concluded that no CO_2 was formed from lignin during oxidation, confirming Brink's findings.[3] In each fraction the ^{14}C content was normalized to the total ^{14}C recovered in that fractionation step. Neutral compounds soluble in ether were always 3.4% or less. High-molecular-weight but soluble reaction products constituted 16.4% of the total reaction products.

$CHCl_3/CH_3OH$-soluble products made up 51.1% of the low-molecular-weight species. These were further separated by HPLC into syringaldehyde (39%), vanillin (16%), syringic acid (4.6%), vanillic acid (2.8%), benzoic acid containing some 4-hydroxyazobenzene (13.8%), and a fraction which contained only 4-hydroxyazobenzene (7.2%). The ratio of syringaldehyde to vanillin was similar to those found by thin-layer chromatography and ^{14}C detection in an earlier report.[7] The benzoic acid content was higher than reported for nitrobenzene oxidation of conifer wood,[3] but kenaf lignin is significantly different from conifer lignin. The contamination of the benzoic acid fraction with some 4-hydroxyazobenzene questions whether the ^{14}C is in only one or the other component. The 4-hydroxyazobenzene could be labeled by sidechain displacement of p-hydroxyphenyl units, and the 4-hydroxyazobenzene fraction which appears to contain no contaminants by GC or HPLC tests contains 7.2% of the ^{14}C in the $CHCl_3/MeOH$ extract. Therefore we must conclude that 4-hydroxyazobenzene is ^{14}C-active but the benzoic acid is not definitely shown to be a product of lignin degradation.

The reaction products that remained unextracted by 90:10 $CHCl_3:CH_3OH$ from trifluoroacetic acid-water solution were not easily separated by HPLC. The products were, therefore, methylated with diazomethane in ether-ethanol. Methyl formate, methyl acetate, and a few other volatile esters may have been partially lost when the methylated reaction product was concentrated for HPLC. Methylation with diazomethane often generates more than one product from a single starting material. As a result of the derivatization reaction and the complexity of the starting material, not all components could be isolated by HPLC. Some methylated reaction products remained water soluble and were identified as oxalic acid half esters and salts (38% of recovered ^{14}C after methylation).

The remaining 62% in the ether-soluble phase was separated into more than 15 components by HPLC. Oxalic acid esters were present at 10% of ^{14}C recovered after methylation. Syringic and malic acids and 3,4-dimethyoxybenzaldehyde were identified in the various fractions. Most fractions contained unidentified aromatic compounds. Their characterization as aromatics was based on PMR resonances in the region of 6.5 to 7.5 ppm downfield from TMS, IR absorbances in the region of 3000 to 3100 cm^{-1}, and mass spectra of individual GC constituents. The last procedure revealed mass peaks typical of methoxy-substituted aromatics with unsaturated side-chains, (e.g. ions of m/e 181, 167, 165, 151, 135, and 131).

The data indicated that a high-molecular-weight fraction of lignin was solubilized by nitrobenzene oxidation. Oxalic acid, although found previously in oxidation products from conifers,[3] was identified as a major reaction product of lignin breakdown in syringyl-guaiacyl lignins but not from other plant components p-Hydroxyazobenzene and benzoic acid products need further research to clarify their relationship to lignin degradation. This more complete picture of the carbon balance from nitrobenzene oxidation of lignin helps us to understand this important reaction better.

EXPERIMENTAL

Kenaf lignin was labeled with ^{14}C by feeding L-[U-^{14}C] phenylalanine to the plant through a few exposed roots.[7] The kenaf plants used in this study were harvested 60 days after labeling with ^{14}C. After removing soluble extracts and protein according to previous methods,[7] the dry, ground kenaf was oxidized at 160°C with nitrobenzene in 2N NaOH.[1] Unlabeled kenaf was grown, recovered, and oxidized in the same manner as labeled plants. The separation scheme followed after the reaction is pictured in Figure 1.

An Amicon YM-2 membrane was used for separation of low-molecular-weight species (nominal <1000).

The 90:10 $CHCl_3$:CH_3OH soluble fraction (Figure 1) was taken to dryness, and a portion taken up in .01N NaOH for HPLC separation. Separation was accomplished on a 9.4 mm x 25 cm Zorbax SAX column eluted with .02 M KH_2PO_4. UV absorbance at 210 nm and ^{14}C content, determined with a Radiomatic Model HP FLO-ONE system, were monitored as the sample eluted. Kenaf grown and treated exactly like the ^{14}C-labeled plant but containing no ^{14}C-label was also oxidized and separated. By correlating UV absorbance curves of labeled and unlabeled HPLC eluent, the peaks containing lignin degradation products could be isolated from the unlabeled material. Relative amounts of each compound were determined from the ^{14}C-content curve obtained from the ^{14}C detector. Each compound was identified by GC-MS and by comparison of its HPLC retention time to that of an authentic sample.

Preparative HPLC was performed on the ether-soluble methylated products (7, Figure 1) that were not extracted in 90:10 $CHCl_3$:CH_3OH. A Dynamax 21.2 mm x 25 cm C-18 column was used with a solvent gradient of 50 to 100% methanol in water.

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