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The Chemistry of Several Novel Bioconversion Lignins

Wolfgang G. Glasser,* Charlotte A. Barnett, Peter C. Muller, and Kyosti V. Sarkanen

Fourteen lignin-rich residues from six different bioconversion technology schemes currently under investigation for converting lignocellulosic resources into chemicals were analyzed with regard to their chemical structures. Process schemes included (a) acid hydrolysis with sulfuric acid, (b) acid hydrolysis with hydrofluoric acid, (c) steam explosion followed by solvent or alkali extraction, (d) organosolv pulping, and (e) digestion with cellulase or (f) digestion with cellulase-producing organisms. The quantitative analysis regime tested for elemental composition, functionality, interunit linkages, molecular mass, and glass transition temperature. The results were computed on the basis of the composition of average lignin-building, phenylpropane (C_9) structures. They suggest (a) that bioconversion lignins are subject to, in part, severe hydrolytic depolymerization of their alkyl aryl ether bonds, (b) that the degree of depolymerization differs significantly with the process, (c) that significant secondary condensation occurs only during acid hydrolysis, and, to a minor extent, during steam explosion and organosolv pulping, (d) that, except for phenolic hydroxyl groups, only minor variations in functionality exist, (e) that molecular mass ranges between 500 and 20 000 (g M^{-1}) and was lowest with the organosolv and steam explosion lignins, (f) that glass transition temperatures vary between 95 and 160 °C, and (g) that the isolation of homogeneous, solvent- or alkali-soluble, lignin-rich fractions from the residues of the HF acid hydrolysis and the cellulolytic enzyme process options is extremely difficult.

The conversion of renewable lignocellulosic resources into chemicals, liquid fuels, and feed supplements has gained considerable attention in recent years (Abelson and Hammond, 1976; St. Pierre and Brown, 1980; Sarkanen and Tillman, 1979; Paul, 1979; Bungay, 1981; Worthy, 1981; Myerly et al., 1981). The bulk of the raw materials under consideration for this "bioconversion technology" consists of woody biomass, which contains 20–30% lignin by weight (35–40% by energy content) and 60–80% polysaccharides (Falkehag, 1975). A large number of proposed bioconversion schemes start with a resource treatment aimed at separating (physically or chemically) lignin from polysaccharides (Bungay, 1981). This has been reported to result in greater chemical and biological conversion efficiency (Marchessault and St. Pierre, 1980). If ethanol is the intended end product, this pretreatment generates as much as 1 lb of lignin/lb of ethanol (Muller and Glasser, 1983). The utility and marketability of this coproduced lignin as a prepolymer for engineered materials depend significantly on its particular chemical structure and physical properties (Falkehag, 1975; Narain, 1981). These are related to pretreatment and isolation conditions (Glasser et al., 1981, 1983a,b). The market value of coproduced lignins will significantly influence the economic feasibility of bioconversion technologies in the future (Glasser, 1981; Bungay, 1982; Abelson, 1982; Muller and Glasser, 1983; Kringstad, 1980).

Bioconversion processes that aim at separating lignin from carbohydrates in their initial process step include acid hydrolysis ("wood saccharification") with hydrochloric (Papadopoulus et al., 1981), sulfuric (Church and Wooldridge, 1981; Rugg et al., 1981) or hydrofluoric acid (Rogovin and Pogosov, 1958; Defaye et al., 1983; Selke et al., 1982), treatment with steam under conditions known as "autohydrolysis" (Lora and Wayman, 1978) or "steam explosion" (Marchessault and St. Pierre, 1980), delignification with aqueous organic solvents known as "organosolv pulping" (Kleinert, 1974), and mechanically pretreating (two-roll mill) lignocellulose followed by enzymatic/microbiological saccharification of the carbohydrates (Man-

Department of Forest Products, Virginia Polytechnic Institute and State University, Blacksburg, Virginia (W.G.G., C.A.B., and P.C.M.), and College of Forest Resources, University of Washington, Seattle, Washington.

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dels et al., 1981; Emert and Katzen, 1980; Esterbauer et al., 1981; Blanch and Wilke, 1982). These bioconversion process options have recently been reviewed in the literature. Several options have been explored on pilot plant level. The "NYU process" is based on sulfuric acid catalyzed saccharification in a twin-screw extruder at high temperatures (220–245 °C) and short retention times (<1 min) (Rugg et al., 1981). The HF wood hydrolysis process operates in either aqueous (Selke et al., 1982) or nonaqueous medium (Defaye et al., 1981, 1983) at ambient or elevated temperatures. The "Iotech process" employs wood chip impregnation with water at high pressure and temperature followed by sudden, explosive decompression (Marchessault and St. Pierre, 1980; Bungay, 1982). Delignification with aqueous mixtures of alcohols (methanol, ethanol, butanol) (Kleinert, 1967) and catalysts is the basis on which the Penn/GE and other "organosolv" processes (Katzen et al., 1980; Hansen and April, 1981; Nakano et al., 1981) operate. "Two-roll milling" followed by incubation with cellulolytic enzymes isolated from *Trichoderma viridae* and other organisms is employed by the "Natick process" (Marchessault and St. Pierre, 1980; Mandels, 1982), and if the mechanical pretreatment is followed by "simultaneous saccharification–fermentation", this process is known as the "GULF/EMERT/ARKANSAS process" (Emert and Katzen, 1980).

Coproduced lignin fractions from several of these process schemes have been characterized in part. Where acid hydrolysis lignins were severely condensed, intractable polyaromatic moieties (Goldstein et al., 1981; Concin et al., 1981), autohydrolysis, and steam explosion lignins were found to constitute phenolic prepolymers of much more desirable chemical and viscoelastic properties (Sarkanen et al., 1981; Marchessault et al., 1982; Fengel et al., 1981; Lange et al., 1981; Meier et al., 1981; Chua and Wayman, 1979). Organosolv lignins resemble steam-treated lignins (Glasser et al., 1983a,b), and enzymatically liberated lignins are regarded as the preferred lignin preparation representative of the total lignin in wood (Chang et al., 1975).

It is the objective of the present study to compare the chemical structures of lignins coproduced with six different bioconversion technology schemes (two acid hydrolysis processes, steam explosion, organosolv pulping, and two enzymatic saccharification options) and to describe the composition of lignins in terms of functionality, interunit linkages, and macromolecular properties such as molecular mass and thermal transition. The bioconversion lignins will be compared to milled wood and kraft lignins.

EXPERIMENTAL SECTION

Materials. *Acid Hydrolysis Lignins.* Lignin residues from the acid hydrolysis of aspen and white pine wood chips with dilute sulfuric acid (about 2%) at temperatures in excess of 220 °C were obtained from a pilot plant in operation at New York University (Rugg et al., 1981). The pilot plant consists of a twin-screw extruder that operates continuously with an overall retention time of <10–60 s. Solid residues, separated from the aqueous hydrolysate by centrifugation, were shipped in moist form in plastic containers. A part of these residues were fractionated further by alkali and by dioxane.

Two additional lignin residues from the acid hydrolysis of benzene–ethanol (2:1 v/v) extracted birch wood meal with anhydrous hydrogen fluoride were obtained from Grenoble, France (Defaye et al., 1983). These samples had been treated with ca. 4 mL of HF/g of wood at below ambient temperature (rising to ambient during the reaction) for 8 min and for 2 h. The reaction products were washed first with diethyl ether and then with water, and the lignin residues were collected by centrifugation. The residues were obtained as nearly ash-free, dry, solid powders. They were fractionated further by alkali and by dioxane.

Steam Explosion Lignins. Barley straw, poplar, and aspen steam explosion lignins were obtained from a pilot plant operated by Iotech Corp. of Ottawa, Ontario, Canada (SERI, 1981). These were obtained by extracting steam-exploded biomass samples with aqueous ethanol or aqueous alkali. All lignin preparations were obtained as dry brown powders.

Organosolv Lignins. Acetone-extracted cottonwood chips were pulped at 160 °C in 50% aqueous ethanol containing 0.25 M ammonium sulfide for 6 h ("cottonwood A" preparation). Two other cottonwood organosolv lignins, "cottonwood B" and "cottonwood C" preparation, were obtained by two-stage pulping at 175 °C in 50% aqueous ethanol containing 0.008 M sodium bisulfate for 1 h (stage 1, cottonwood B), and by subsequently pulping at 175 °C in 50% aqueous ethanol containing 0.02 M $NaHSO_4$ for 1 h (stage 2, cottonwood C). The pulped chips were washed with an equal amount of aqueous ethanol as was used for pulping, and the combined pulping and wash liquors were concentrated to ca. $^1/_5$ of their original volume in a rotation evaporator. Cottonwood A was obtained by acidification with 1 N HCl to pH 4, collection by centrifugation and decantation, and freeze-drying. Cottonwoods B and C were collected by centrifugation following the addition of Na_2SO_4 and mild heating to assist in coalescing the fine lignin particles. The air-dried solid lignin samples were prepared in the laboratories of the College of Forest Resources of the University of Washington [cf. Sarkanen et al. (1981) for more details of organosolv pulping]. They were shipped as dry brown powders. Two additional organosolv lignin preparations, one from aspen and one from pine, were obtained from Biological Energy Corp. of Valley Forge, PA, and they were analyzed as dry brown powders, also. Neither yield nor conditions of separation of these samples are known.

Cellulase Enzyme Lignin. Lignin-rich residue from the enzymatic saccharification of two-roll-milled newsprint was obtained from the U.S. Army Laboratories in Natick, MA. This residue was from a pilot plant operation involving mechanical milling followed by treatment with a cellulase enzyme preparation isolated from *Trichoderma reesei* (Mandels, 1982). The dry residues were shipped and subjected to Soxhlet extraction with azeotropic mixtures of aqueous dioxane, leaching with 1 N aqueous NaOH, and preparation of milled wood lignin by the conventional procedure of Bjorkman (1956). Dry samples were obtained by freeze-drying.

Simultaneous Saccharification and Fermentation (SSF) Lignin. Lignin-rich residues from the simultaneous saccharification and fermentation pilot plant operation at the University of Arkansas Research Laboratories (Emert and Katzen, 1980) were obtained in dry form, also. These samples were prepared by mechanical two-roll milling of newsprint followed by degradation with cellulase enzymes and ethanol-producing microoganisms and filtration. The residues were obtained as dry solid powders, which were subjected to Soxhlet extraction with an acetone–water azeotrope and the normal milled wood lignin preparation procedure (Bjorkman, 1956). Dry, solid lignin preparations were obtained by freeze-drying.

Kraft Lignin. The kraft lignin preparation was a commercial Indulin-ATR sample obtained from Westvaco, North Charleston, SC. This sample was purified by dis-

solution in alkali followed by acid precipitation, filtration, and washing.

Milled Wood Lignins. Milled wood lignin (MWL) preparations from spruce and red alder sapwoods were obtained from Wiley-milled and extracted wood meal that was milled with steel balls for 2 weeks (Glasser and Barnett, 1979). The lignin was subsequently solvent extracted and purified following the procedure of Bjorkman (1956).

Methods. *Alkali Extraction.* Approximately 5 g of lignin were added to 100 mL of 1 N NaOH solution in a 500-mL erlenmeyer flask. The mixture was purged with nitrogen and sealed to prevent oxidation. The suspension was kept at a constant temperature (60 °C) with agitation for 48 h. The suspension was then filtered or centrifuged, and the lignin dissolved in the supernatant was precipitated by acidification. The precipitate was centrifuged and washed to a neutral pH. A powdered lignin was obtained by freeze-drying.

Dioxane Extraction. Approximately 50 g of lignin or lignin-rich biomass sample was precisely weighed and Soxhlet extracted with azeotropic aqueous dioxane for 48 h. The thimble and the liquid extract were removed and analyzed separately. The thimble was air-dried and its contents were weighed to determine weight loss, and the dioxane solution was freeze-dried.

Elemental Analysis. Elemental analysis was conducted by Galbraith Laboratories in Knoxville, TN.

Functionality. Methoxyl contents were determined by titration according to Tappi Standard T209su72. Total hydroxyl contents were determined by reaction with acetic anhydride in pyridine and titration of the unreacted acetic acid with aqueous NaOH, by H NMR spectroscopy, and by *O*-acetyl determination (Mansson and Samuelson, 1981). Phenolic OH groups were determined by H NMR spectroscopy, by UV spectroscopy, and by treating a diethyl sulfate derivatized lignin preparation with hydrogen iodide followed by gas chromatographic separation of the ethyl and methyl iodide. Total carbonyl contents were determined by nitrogen determination of the pentafluoro phenylhydrazone derivative (Sano and Glasser, 1980).

Carbohydrates. The carbohydrate content was determined by the method of Borchardt and Piper (1970), which involves acid hydrolysis, $NaBH_4$ reduction, acetylation, and gas chromatographic separation of the alditol acetates.

H NMR Spectroscopy. Acetylated lignin samples were prepared by reaction with acetic anhydride and pyridine, followed by isolation by precipitation into ether or water. The H NMR spectra were recorded in deuteriochloroform solutions in the usual way.

Analytical Degradation. Lignin preparations were analyzed after analytical degradation with alkaline permanganate solutions in accordance with the procedure of Sano and Glasser (1980). This method is based on sequential ethylation (Et_2SO_4), oxidative depolymerization, methylation (Me_2SO_4), and oxidation with potassium permangante and H_2O_2 (Larsson and Miksche, 1969; Morohoshi and Glasser, 1979a). Monomeric and dimeric degradation products were separated quantitatively by gas chromatography as methyl esters. In addition, some of these mixtures were subjected to gas chromatography/mass spectrometry for the positive identification of degradation products. These analysis techniques, and their quantitative interpretation, have, in part, been the subject of earlier publications (Glasser and Glasser, 1981; Glasser et al., 1981).

Molecular Mass Determinations. Number- and weight-average molecular weights were determined by vapor pressure osmometry and high-pressure size exclusion chromatography. A series of four μ-Spherogel (Altex Corp.) size exclusion chromatography columns were employed, having gel sizes of 500, 1000, 10000, and 100000 Å, and they were calibrated with polystyrene standards. Lignins were chromatographed in a 0.1 N solution of lithium bromide in dimethyl formamide. Similar molecular weight distribution experiments have been reported previously (Connors et al., 1980b; Sarkanen et al., 1982; Concin et al., 1981; Faix et al., 1981).

Glass Transition Temperatures. Glass transition temperatures of lignin were determined by differential scanning calorimetry (DSC) on a Perkin-Elmer Model 4 instrument.

RESULTS AND DISCUSSION

Sample Preparation. Lignin preparations were obtained as bioconversion coproducts from pilot plant operations, where possible. These samples were investigated in view of their potential for generating a uniform fraction suitable for use as the phenolic prepolymer. The results of isolation and fractionation efforts are summarized in Table I. Yields of crude extracts ranged between less than 1 and about 20% on total biomass. On the basis of UV absorptivity determinations of these fractions, lignin contents of the crude extracts were between <20 and >90%. On the basis of these data it is estimated that reasonably uniform lignin coproduct fractions can be generated in yields ranging between <10 and about 80% of total lignin. Where the mechanically pretreated, enzymatically and microbiologically liberated residues exhibited greatest resistance to releasing uniform, lignin-rich fractions, steam explosion and hydrolysis and organosolv pulping produced such fractions in high yield. Wood saccharification residues by the NYU process proved to be easily fractionated into high yields of alkali- and dioxane-soluble components. Hydrofluoric acid residues, by contrast, were resistant to fractionation by alkali or dioxane.

Since mechanically pretreated, enzyme-liberated bioconversion and HF hydrolysis residues yielded only insignificant quantities of uniform lignin fractions by solvent extraction, these samples were disregarded from further analytical characterization.

Analysis Techniques. The chemical structures of lignins are most appropriately described in terms of the composition of average phenylpropane (C_9) units (Glasser and Glasser, 1981). This description may concern functionality features as well as interunit linkages by type and concentration. This information can be obtained by conventional chemical analysis techniques, quantitative analytical degradation, and/or spectroscopic methods. Analytical degradation with alkaline permanganate solutions and hydrogen peroxide (Larsson and Miksche, 1969) has been found to be capable of generating highly quantitative information, especially on the content of alkyl aryl ethers, on the content of interunit linkages between aliphatic and aromatic carbons, and on the content of interunit linkages between aromatic rings (Morohoshi and Glasser, 1979a,b; Glasser et al., 1981). This method was modified to allow alkyl aryl ether content determination in one reaction sequence by beginning the degradation analysis by diethyl sulfate derivatization followed by methylation of the depolymerized reaction product (Sano and Glasser, 1980; Glasser et al., 1983a,b). This method is illustrated in Figure 1. A typical gas chromatogram of a degradation product mixture from a hardwood (guaiacyl–syringyl) lignin is illustrated in Figure 2. Where the initial six prominent peaks, region A (benzoic acid derivatives), represent orginally phenolic and alkyl aryl ether linked

Table I. Isolation and Fractionation Data

	yield by extraction		extract characteristics		
	% of wood	% of conversion residue	UV absorptivity coeff (a_{280})	lignin content[a]	extractable lignin, % of total lignin
MWL					
loblolly pine	16[b]		20.8	87	45-50
red alder	26[c]		10.0	45	40-50
kraft lignin (pine)	NA		22.6	94	~75
acid (H_2SO_4) hydrolysis lignin	NA				
aspen: dioxane		57	24.8	100	~50
1 N NaOH		82	23.4	97	80-90
white pine: dioxane		52	26.3	100	~50
1 N NaOH		73	19.1	80	70-80
acid (HF) hydrolysis lignin	NA				
8 min: birch, dioxane		6	6.4	27	~5
1 N NaOH		7	11.7	49	~5
120 min: birch, dioxane		7	5.0	21	~5
1 N NaOH		16	7.5	31	5-10
steam explosion lignin					
barley straw	NA		19.8	86	70-80
poplar	NA		19.2	83	70-80
aspen	NA				
organosolv lignin					
cottonwood A	12.5		20.2	88	50
cottonwood B	10.3		21.7	94	45
cottonwood C	6.6		19.7	86	25
aspen	NA				
pine	NA				
enzyme lignin (NATICK)			8.1	35	
dioxane		27	4.2	18	~10
1 N NaOH		9	14.4	63	~10
MWL[b]		19	26.0	90 (est.)	60-70
SSF lignin (GULF)			7.3	41 (53)	
dioxane		19			
1 N NaOH		6			
MWL[b]		7	17.9	90 (est.)	~25

[a] Based on lignin absorptivities of 24.0 and 23.0 g L^{-1} cm^{-1} for softwood and hardwood, respectively. [b] By extraction into 90% aqueous acetone. [c] By extraction into 90% aqueous dioxane.

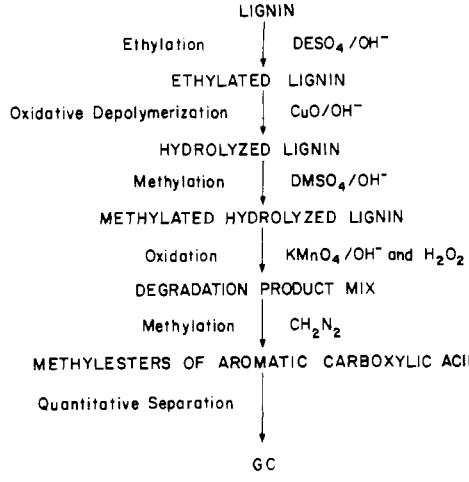

Figure 1. Reaction scheme of the degradative lignin analysis technique by permanganate oxidation.

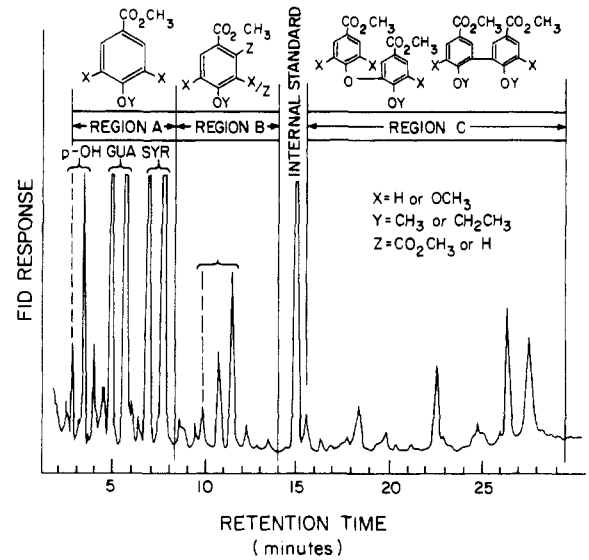

Figure 2. Typical gas chromatogram of a permanganate oxidation product mixture of (hardwood) lignin.

uncondensed *p*-hydroxylphenyl, guaiacyl, and syringyl moieties, the second region (region B) represents phthalic and isophthalic acid derivatives of various compositions. The third and final region (region C) is constituted by various dimeric biphenyl and diaryl ether components. The method of interpreting primary analysis results from functional group determination and by analytical degradation by permanganate oxidation has been described in detail previously (Glasser and Glasser, 1981; Glasser et al., 1981).

Chemical Lignin Structures. Results from elemental and functional group analysis, as well as carbohydrate contents, are listed in Table II. Carbon contents ranged between 57 and 68%, corresponding to fuel values of approximately 10 000–11 700 Btu/lb. All bioconversion lignins analyzed were remarkably free from carbohydrate contamination. Little variation was found for prominent functional groups, such as total hydroxyl and carbonyl groups. Phenolic character varied with degree of depolymerization.

Permanganate oxidation degradation results are listed in Table III. The results summarize data from duplicated degradations (triplicated in case of loblolly pine MWL) in

Table II. Elemental Composition, Functionality, and Carbohydrate Content

lignin preparation	elemental and functional group composition, %						carbohydrate content, %		
	C	H	OCH_3	total OH	phenolic OH[a]	total carbonyl[b]	total	hexoses	pentoses
MWL									
loblolly pine	61.56	5.90	13.97	10.54	2.75	3.01	3.1	1.9	1.1
red alder	57.19	5.83	18.19	10.09	3.27	2.12	8.0	0.6	7.4
kraft lignin (pine)	64.33	5.88	13.70	11.86	5.52	1.76	0.7	0.5	0.2
acid (H_2SO_4) hydrolysis lignin									
aspen	64.36	5.80	22.82	8.76	5.40	2.62			
pine	60.00	6.00	9.30	6.29	4.07	2.84			
steam explosion lignin									
barley straw	63.79	5.92	10.30	8.52	5.38	4.74	1.8	0.4	1.3
poplar	62.28	5.65	15.02	9.82	4.30	2.92	3.4	0.9	2.5
aspen	61.90	5.34	18.23	9.59	4.25	2.13	0.6	0.3	0.3
organosolv lignin									
cottonwood A	57.22	5.50	17.03	10.13	2.71	2.19			
cottonwood B	59.14	5.35	19.81	10.11	3.43	1.53	0.2	0.1	0.1
cottonwood C	59.82	5.78	20.17	9.61	2.22				
aspen	66.41	5.89	20.07	8.11	5.40	2.19	0.8	0.4	0.4
pine	68.15	6.08	15.09	8.17	6.32	2.33			

[a] By H NMR spectroscopy and by Et_2SO_4 ethylation, HI hydrolysis, and GC analysis of EtI [according to Glasser et al. (1980)]. [b] By N determination of pentafluorophenylhydrazone derivatives [according to Sano and Glasser (1980)].

Table III. Primary and Secondary Permanganate Oxidation Results

lignin preparation	yield of monomers and dimers[a]	distribution of degradation products[b]			yield, mmol/C_9[c]		molar ratio of BA/PA[d]	hydrolysis factor[e]
		A	B	C	A	B		
MWL								
loblolly pine: A	29.3	54	17	30	471	99	4.8	0.28
B	30.3	50	25	24	418	113	3.7	0.31
C	29.6	64	13	24	493	120	4.1	0.32
red alder: A	42.5	77	6	17	683	53	13	0.22
B	49.8	75	6	18	690	45	16	0.22
kraft lignin (pine): A	18.5	64	14	18	333	187	2.0	0.81
B	19.1	68	16	16	352	186	2.2	0.85
acid (H_2SO_4) hydrolysis lignin[g]								
aspen: A	13.4	30	43	25	141	538	0.32	0.77
B	13.4	39	32	24	178	458	0.46	0.81
white pine: A	13.4	36	25	34	135	363	0.76	0.88
B	9.8	44	21	27	131	296	0.67	0.94
steam explosion lignin								
barley straw: A	9.1	74	11	15	298	161	2.2	0.84
B	7.4	71	13	16	248	162	1.8	0.78
poplar: A	40.5	70	10	20	481	79	8.1	0.78
B	30.4	68	8	23	440	102	5.6	0.78
aspen: A	38.6	69	9	21	549	92	6.8	0.52
B	30.4	43	9	48	340	71	5.2	0.51
organosolv lignin								
cottonwood A: A	23.46	82	7	11	403	96	4.7	0.27
B	17.41	64	8	28	307	95	3.7	0.30
cottonwood B: A	21.9	77	7	17	459	100	6.4	0.35
B	16.5	78	5	17	351	73	8.7	0.25
cottonwood C: A	46.5	56	6	38	480	52	11	0.45
high mol wt fraction[f]	39.4	83	6	11	764	53	15	0.21
low mol wt fraction[f,g]	54.4	48	12	40	484	257	1.9	0.53
aspen: A	27.3	40	14	35	301	192	1.8	0.90
B	12.8	56	15	20	286	210	1.6	0.83
pine: A	29.6	55	19	24	371	176	2.4	0.87
B	27.8	54	21	24	347	214	1.8	0.87

[a] As separated by gas chromatography, in mg/100 mg of lignin. [b] As per gas chromatogram, Figure 2, distinguishing between monomeric monocarboxylic acids (region A), monomeric di- and multicarboxylic acids (region B), and dimeric acids (region C); in % by weight. [c] Yield of guaiacyl and syringyl derived benzoic acids (A) and of guaiacyl and syringyl derived phthalic and isophthalic acids (B), in mmol/C_9 after yield correction according to the method of Larsson and Miksche (1971). [d] Molar ratio of monocarboxylic benzoic acids (BA), including *p*-alkoxybenzoic acid, to guaiacyl and syringyl derived phthalic and isophthalic acids (PA) (after yield correction). [e] Molar ratio of phenolic to total [phenolic and alkyl aryl ether linked, guaiacyl-derived (softwood), and guaiacyl and syringyl derived (hardwood)] monomeric monobasic benzoic acids (after yield correction). [f] Solvent fractionated by diethyl ether. [g] Degradation product mixture consisted of 57% *p*-ethoxybenzoic acid, which was deducted from the data listed.

terms of the total yield of gas chromatographically identified monomers and dimers, the weight distribution of degradation products in regions A–C (cf. Figure 2) representing monomeric monocarboxylic acids (region A), monomeric di- and multicarboxylic acids (phthalic and isophthalic acids) (region B), and dimeric acids (region C). Primary degradation product yields are corrected in accordance with the method proposed by Larsson and

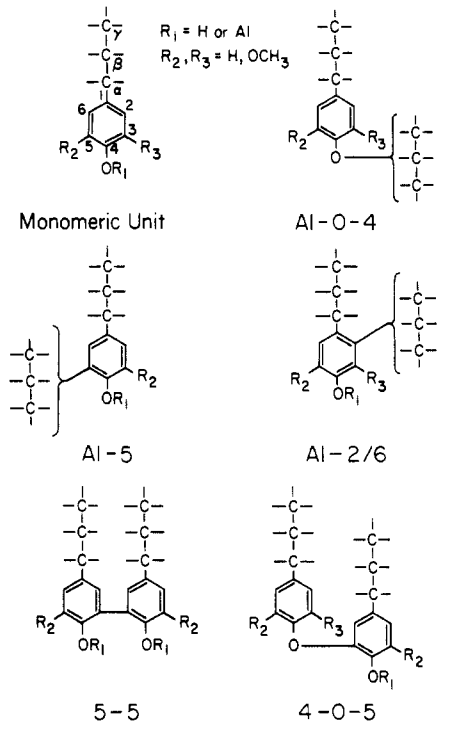

Figure 3. Prominent interunit linkages in lignin.

Miksche (1971) and revised by Morohoshi and Glasser (1979b), and the corrected product yields are also expressed in millimoles per average phenylpropane unit (mM/C_9) in Table III. The molar ratio of benzoic acid to phthalic and isophthalic acid derivatives, Table III, gives an expression for the degree of condensation between aliphatic and aromatic carbons. A hydrolysis factor is used for describing quantitatively the degree of alkyl aryl ether linking in a lignin preparation. This factor is computed as the molar ratio of phenolic to total (phenolic and alkyl aryl ether linked) guaicyl-derived (softwood), and guaiacyl plus syringyl derived (hardwood), monomeric monobasic benzoic acids. This factor has been used previously for describing the alkyl aryl ether nature of lignins (Glasser et. al., 1983a,b). Degradation products contained in regions A–C are illustrated in the gas chromatogram of Figure 2.

The permanganate oxidation degradation product mixture of the aspen acid (H_2SO_4) hydrolysis lignin preparation was subjected to gas chromatography/mass spectrometry. The mass spectra of several prominent monomeric degradation products are summarized in Table IV with their characteristic fragments. Where the methyl esters of mono-, di-, and trimethoxybenzoic acid exhibit major peaks at the mass of the molecule ions, at $(M - C_2H_4)^+$, or at $(M - C_2H_4 - OCH_3)^+$, most di- and tribasic acids of region B, Figure 2, have significantly reduced molecule peak intensities and become less well recognizable by GC/MS. Dimethyl 2,4-dimethoxy-3-ethoxyphthalate (M^+ at m/e 298) is a surprising exception in this category. Many of the components identified by GC/MS have previously been synthesized, and their mass spectra have been recorded.

The composition of average phenylpropane, C_9 units, in terms of functionality and interunit linking is summarized for all lignin preparations in Table V. Common interunit linkage configurations are illustrated in Figure 3. These include connections between aliphatic (side-chain) carbon atoms and C_5 on the aromatic ring resulting in the formation of isophthalic acids (i.e., isohemipinic acid) by permanganate oxidation. Connections between aliphatic carbons and positions 2 or 6 on the aromatic ring lead to

Table IV. Mass Spectroscopic Identification of Several Monomeric Permanganate Oxidation Products of the Aspen Acid (H_2SO_4) Hydrolysis Lignin by GC/MS (Retention Times in Accordance with Figure 2)

retention time, min	molecule peak		prominent fragments, m/e (rel intensity, ion)	methyl benzoate derivatives
	m/e	intensity, %		
2.6	166		MS patterns suggest incomplete separation and presence of other substances, by comparison with model compounds	$R_2 = R_3 = R_5 = R_6 = H$; $R_4 = OCH_3$
3.2	180			$R_2 = R_3 = R_5 = R_6 = H$; $R_4 = OCH_2CH_3$
4.8	196	82	181 [12, $(M - CH_3)^+$]; 165 [100, $(M - OCH_3)^+$]; 137 [20, $(M - COOCH_3)^+$]	$R_2 = R_5 = R_6 = H$; $R_3 = R_4 = OCH_3$
5.6	210	90	195 [10, $(M - CH_3)^+$]; 182 [90, $(M - C_2H_4)^+$]; 179 [20, $(M - OCH_3)^+$]; 151 [100, $(M - C_2H_4 - OCH_3)^+$]	$R_2 = R_5 = R_6 = H$; $R_3 = OCH_3$; $R_4 = OCH_2CH_3$
6.8	226	100	211 [70, $(M - CH_3)^+$]; 195 [70, $(M - OCH_3)^+$]; 155 (25)	$R_2 = R_6 = H$; $R_3 = R_4 = R_5 = OCH_3$
7.8	240	73	212 [100, $(M - C_2H_4)^+$]; 209 [13, $(M - OCH_3)^+$]; 197 [20, $(M - C_2H_4 - CH_3)$]; 181 [98, $(M - C_2H_4 - OCH_3)^+$]	$R_2 = R_6 = H$; $R_3 = R_5 = OCH_3$; $R_4 = OCH_2CH_3$
9.6	275/273	23/62	247/245 [34/100, $(M - C_2H_4)^+$]; 232/230 [5/18, $(M - C_2H_4 - CH_3)^+$]; 216/214 [40/100, $(M - C_2H_4 - OCH_3)^+$]	
10.1	254	42	223 [82, $(M - OCH_3)^+$]; 221 [100, $(M - OCH_3 - 2H)^+$]	$R_2 = R_6 = H$; $R_3 = R_4 = OCH_3$; $R_5 = COOCH_3$
10.3	268	24	253 [13, $(M - CH_3)^+$]; 240 [10, $(M - C_2H_4)^+$]; 237 [14, $(M - OCH_3)^+$]; 222 [27, $(M - OCH_3 - CH_3)^+$]; 220 [43, $(M - OCH_3 - CH_3 - 2H)^+$]; 208 [75, $(M - 2OCH_3 + H)^+$]; 180 [100, $(M - 2OCH_3 - C_2H_4 + 2H)^+$]	$R_2 = R_6 = H$; $R_3 = OCH_3$; $R_4 = OCH_2CH_3$; $R_5 = COOCH_3$
10.8	284	6	253 [15, $(M - OCH_3)^+$]; 221 [12, $(M - 2OCH_3 - H)^+$]; 73 (100)	$R_6 = H$; $R_3 = R_4 = R_5 = OCH_3$; $R_2 = COOCH_3$
11.5	298	100	267 [85, $(M - OCH_3)^+$]; 236 [50, $(M - 2OCH_3)^+$]; 206 [60, $(M - 2OCH_3 - C_2H_4)^+$]	$R_6 = H$; $R_3 = R_5 = OCH_3$; $R_4 = OCH_2CH_3$; $R_1 = COOCH_3$
12.1	342	5	311 [48, $(M - OCH_3)$]; 280 [88, $(M - 2OCH_3)^+$]; 208 (100)	$R_2 = R_6 = COOCH_3$; $R_3 = R_4 = OCH_3$ (?)

Table V. Composition of Average Phenylpropane Units in Terms of Functionality and Interunit Linkages

lignin preparation	composition of av phenylpropane unit (C_9 basis) total H	aromatic H	total OH	phenolic OH	OCH_3	total CO	ratio of Syr/Gua	approximate linkage distribution,[a] per 100 C_9 units Al-5	Al-2/6	1/5-5, 4-O-5,	Al-O-4
MWL											
loblolly pine	8.52	2.46	1.07	0.30	0.89	0.22		7-12	3-5	30-35	50-55
red alder	8.24	2.15	1.09	0.39	1.36	0.16	1.2	2-5	2-4	17-22	55-65
kraft lignin (pine)	8.10	2.51	1.32	0.59	0.81	0.14		10-15	7-10	40-45	5-10
acid (H_2SO_4) hydrolysis lignin											
aspen	7.19	1.77	1.12	0.61	1.28	0.18	0.5	5-10	22-30	20-25	2-5
pine	9.63	2.58	1.24	0.46	0.60	0.20		7-12	22-30	30-35	2-5
steam explosion lignin											
barley straw	8.42	1.98	1.12	0.61	0.64	0.33	1.5	5-8	8-12	25-35	3-8
poplar	7.75	2.01	1.11	0.48	0.97	0.21	1.5	2-6	4-6	15-20	8-12
aspen	7.00	2.10	1.14	0.49	1.13	0.15	1.8	2-5	6-8	17-22	28-32
organosolv lignin											
cottonwood A	7.76	1.75	1.41	0.34	1.22	0.18	1.5	3-7	3-7	15-23	40-50
cottonwood B	7.29	1.96	1.31	0.42	1.25	0.11	1.3	2-6	3-7	17-22	40-50
cottonwood C	7.95	2.27	1.17	0.27	1.31		1.7	2-6	3-5	17-22	35-40
aspen	7.31	1.62	1.09	0.59	1.03	0.15	1.0	2-6	15-20	20-25	3-8
pine	8.08	2.36	1.15	0.64	0.64	0.14		7-12	10-15	32-37	3-8

[a] Designation of linkage types is explained in Figure 3.

phthalic acid derivatives by permanganate oxidation. Biphenyl and diaryl ether connections of the types 5–5, 1–5, and 4–O–5 give rise to the most common dimers in the permanganate oxidation product mixture. Alkyl aryl ether linkages cleavable by cupric sulfate in alkaline solution at 170 °C are signified by the presence of *p*-methoxybenzoic acids of any type. The diversity of interunit linkages present in lignin has recently been reviewed in relation to some quantum mechanical calculations of phenoxy radical precursors (Glasser, 1980).

The data of Table V suggest insignificant differences in total OH contents between various lignins. Phenolic OH contents vary between 1/4 C_9 units (MWL hardwood) and 0.6 phenolic OH group/C_9 with some organosolv and acid hydrolysis lignins. Methoxyl contents and syringyl to guaiacyl ratios cover the range from 0.6 to 1.4 and 0.5 to 1.8 per C_9, respectively. Moderate increases in phenolic hydroxyl contents, slight methoxyl losses, and partially elevated carbon contents were found also with organosolv lignins by Fengel et al. (1981) and by Meier et al. (1981). These authors also report the detection of ethoxy groups in concentrations between 1.3 (Meier et al., 1981) and 4.8% (Fengel et al., 1981). In the present study, organosolv lignins were found to contain small amounts (0.5–1.5%) of hydrolyzable (HI) ethoxy groups as well.

Significant differences are also detected for interunit linkage distributions. Where midly isolated softwood and hardwood lignins (MWL) contain 10–20 and 5–15 interunit linkages between side-chain and aromatic ring carbons, respectively, lignins subjected to severe acid hydrolysis conditions exhibit significantly increased numbers of side-chain to aromatic ring connections. Some lignins contain as much as 30–40 alkyl aryl linked structures in 100 C_9 units. Small differences were noticed for biphenyl and diaryl ether concentrations, which were between 17 and 22 for most hardwood lignins and 30 and 40 per 100 C_9 units for most softwood lignins. Hyrolyzable alkyl aryl ether contents ranged between 5 or less to more than 55 per 100 C_9 units, and they varied with the method of isolation. Mildly isolated MWL and some steam explosion lignins were particularly rich in polyether bonds. Significant differences were noticed among lignins generated by steam explosion and organosolv delignification. Some of the lignins were obviously severely hydrolytically depolymerized, whereas others retained a high alkyl aryl ether content. These differences in interunit linking must be expected to greatly influence thermodynamic properties of lignins and thus utility for polymer preparations.

Table VI. Molecular Mass and Glass Transition Temperature Data

	molar mass, $\times 10^{-3}$ g/mol M_n by VPO	M_n by GPC	M_w by GPC	T_g by DSC, °C
MWL				
loblolly pine	4.0	1.3	11.4	160[b]
red alder	2.7	1.2	7.7	110-130[c]
kraft lignin (pine)	1.5	1.3	4.3	169
acid (H_2SO_4) hydrolysis lignin				
aspen	NS[a]	0.66	10.1	95
pine	NS	0.8	40.0	96
steam explosion lignin				
barley straw	0.7	0.4	1.1	125
poplar	0.6	0.9	3.0	113
aspen	1.2	0.8	2.3	139
organosolv lignin				
cottonwood A		0.6	4.9	
cottonwood B		0.7	4.3	
cottonwood C		0.9	8.0	
aspen	1.2	0.6	2.1	97
pine	0.8	0.5	1.4	91

[a] Not sufficiently soluble. [b] Sample was Virginia pine. [c] Range includes red oak, chestnut (heartwood), and black cherry.

Some selected polymer characteristics, such as molecular mass and mass distribution and glass transition temperature, are summarized in Table VI. Number-average molecular weights (M_n) seem to range between 500 and 4000, and weight-average molecular weights (M_w) are between 1000 and 40000. These values are in agreement with reports by Meier et al. (1981) for orgnosolv lignins. Glass transition temperatures range between 95 and 160 °C.

Lignin–Carbohydrate Separation Process in View of Lignin Structure. Bioconversion processes generate lignin coproduct fractions of widely different chemical structures. Where wood saccharification at high temperatures and low retention times produces residues that are still soluble, at least in part, in aqueous dioxane and alkali

and that therefore allow the preparation of lignin fractions comparable to kraft lignin, enzymatically liberated lignin-rich residues cannot be fractionated in a similar fashion. Steam explosion and organosolv delignification processes are best qualified for producing homogeneous lignin fractions of suitably pure character. Acid hydrolysis and some organosolv lignins contain few alkyl aryl ethers and many interunit linkages based on combinations between aliphatic and aromatic carbons. Steam explosion lignins are depolymerized along their alkyl aryl ether linkages to the extent of from <5 to >75%. Steam explosion and organosolv delignification can apparently be performed under conditions that are more or less harmful to the original lignin structure. Tailoring the lignin to attain particular chemical and physical properties by judicious choice of pretreatment and isolation conditions thus becomes a distinct possibility.

Lignins with many linkages between aliphatic and aromatic carbons and low overall methoxy contents are likely to have suffered secondary condensation reactions with furfural. This seems to be true with the acid hydrolysis lignins and with two of the organosolv lignins. Such reactions have previously been observed with lignins obtained from autohydrolysis experiments (Chua and Wayman, 1979).

Most of the lignins examined were obtained from hardwood resources; two were derived from pine wood, and one was obtained from an agricultural residue (barley straw). It is generally accepted that softwood is more difficult to delignify than hardwood, and it is therefore not surprising that the two pine lignin preparations, from acid hydrolysis and organosolv delignification, were greatly depolymerized and severely condensed as compared to most hardwood lignin preparations. The straw lignin sample had a low carbon-to-carbon linkage content, a high phenolic hydroxyl content, a high syringyl-to-guaiacyl ratio, and a low overall methoxyl content.

Prospective Coproduct Utilization. It appears that two factors primarily determine the value of a lignin for prepolymer utilization, and these are viscoelasticity and phenolic functionality (Falkehag, 1975; Marton et al., 1966). Viscoelasticity is related to such factors as molecular mass, cross-link density, glass transition temperature, solubility, flow properties (viscosity), and chemical structure as determined by functionality and interunit linkage type. Lignin preparations high in alkyl aryl ether content can be expected to impart greater flexibility and toughness and less rigidity to materials derived from them than lignins with high contents of C–C bonding.

On this basis some steam explosion and organosolv lignins seem to hold promise for incorporation into polymeric materials without imparting excessive rigidity. Steam explosion and organosolv delignification constitute versatile processes in terms of their ability to coproduce lignins with widely different chemical structures and viscoelastic properties. Acid hydrolysis with sulfuric acid at high temperatures must be expected to produce alkali-soluble lignin fractions that are inferior to kraft lignin in terms of utilization features.

Depolymerization of lignins to mixtures of phenols continues to be regarded as a possible outlet for low-value lignin-rich residues (Goldstein, 1975). Yield estimates of degradation products are usually made on the basis of some hydrolytic, pyrolytic, hydrogenolytic, or oxidative depolymerization technique. A selection of data from the recent literature is compiled in Table VII, where these data are compared to the yields of monomeric degradation products from analytical permanganate oxidation. The results show that oxidative (Meier and Schweers, 1979), pyrolytic (Kaminsky et al., 1980), hydrogenolytic (Meier and Schweers, 1981; Connors et al., 1980a) and hydrolytic (Goldstein et al., 1981; Papadopoulos et al., 1981) depolymerization of bioconversion lignins failed to produce the monomeric degradation product yields in excess of 20% (with one exception of nitrobenzene oxidation to 27% yield) and that yields were below 10% in most cases. Permanganate oxidation produced higher yields of monomeric aromatic degradation products in all cases, and it stands to reason that results from this degradation technique constitute an upper limit of potential monomeric phenol yields from lignin preparations. The low overall yields suggest that this type of lignin utilization does not hold much promise for adding value as two-product process scheme to bioconversion technologies.

Table VII. Yields of Identifiable Monomeric Degradation Products from Hydrolysis (HL), Pyrolysis (PY), Hydrogenation (HG), and Oxidation with Alkali/O_2 (OA) or Nitrobenzene (ON) As Compared to Permanganate/H_2O_2 Oxidation (PO), from Several Lignin Preparations (in Milligrams of Degradation Products per 100 mg of Lignin)

	literature data	method	present study (PO)[i]
native lignin			
softwood			20.8-22.8
hardwood	11.6-27.0[a]	HL/ON	35.3-40.3
alkali lignin			
softwood	4.2-5.9[b]	OA/HG	11.8-13.0
hardwood	3.6-5.3[c]	OA/HG	
acid (H_2SO_4) lignin			
softwood			6.4-8.2
hardwood	trace[d]	HL	9.5-9.8
acid (HCl) lignin			
softwood			
hardwood	8.5-11.1[e]	HL/ON	
organosolv lignin			
softwood	5.3-7.3[f]	HG/HL	20.9-21.9
hardwood	8.0-14.1[g]	HG/OA	9.1-20.9
steam explosion lignin			
softwood			
hardwood	3.9-14.4[h]	ON	15.8-32.4
straw			5.3-6.7

[a] Low value, sweetgum wood by alkaline hydrolysis at 250 °C (Goldstein et al., 1981); high value, sweetgum wood by nitrobenzene oxidation (after elimination of calibration factor) (Papadopoulus et al., 1981). [b] Low value, pine lignin by alkali/O_2 oxidation (Meier and Schweers, 1979); high value, pine lignin by hydrogenolysis (Meier and Schweers, 1981). [c] Same as footnote *b*, except beech lignin. [d] Klason lignin by alkaline hydrolysis at 250 °C (Goldstein et al., 1981). [e] All values, sweetgum wood by alkaline hydrolysis at 250 °C (Goldstein et al., 1981); higher yields were obtained by nitrobenzene oxidation of incompletely hydrolyzed sweetgum wood samples (Papadopoulus et al., 1981). [f] Values from spruce and pine lignin by hydrogenolysis (low values) and alkali/O_2 oxidation (high value) by Meier and Schweers (1979, 1981). [g] Values from beech, birch, and white oak lignin by hydrogenolysis (low values), alkali/O_2 oxidation (Meier and Schweers, 1979, 1981), and fluidized bed oxidation (high value) (Kaminsky et al., 1980). [h] Values from aspen autohydrolysis lignin treated for 5 and 120 min to generate the high- and low-yield value, respectively, by alkaline nitrobenzene oxidation (Chua and Wayman, 1979). [i] Values from Table III by multiplying total yield (in mg/100 mg of lignin) with percent of products in region A.

CONCLUSIONS

(1) Uniform solvent or alkali-soluble lignin fractions of high purity can be obtained in 50–80% yield of total lignin from the acid (H_2SO_4) hydrolysis, steam explosion, and

organosolv delignification process. Enzymatic liberation of lignin-rich residues from mechanically pretreated newsprint does not produce uniform lignin preparations in high yields. (2) Significant differences exist between the chemical structures of various lignins from different bioconversion processes in relation to process type, to process condition, and to lignocellulosic resource species. These differences concern primarily phenolic hydroxyl content, interunit linkages, and molecular parameters (molecular mass and T_g). Acid hydrolysis lignins and some organosolv lignins were found to be significantly condensed by secondary dehydrating condensation reactions. These seemed to coincide with hydrolytic depolymerization of alkyl aryl ethers as well as phenolic OH groups. Hardwood lignins varied in terms of poly(alkyl aryl ether) nature. (3) Molecular weights were found to be between <1000 and >40 000. Organosolv and steam explosion lignins exhibited typically narrow molecular mass distributions, with the average polymer consisting of a molecular size corresponding to a degree of polymerization of 4 or 5. (4) Glass transition temperatures ranged between 95 and 160 °C. (5) Steam treatment and organic solvent delignification in conjunction with agricultural residues (straw) or hardwoods seem to constitute process configurations that hold high promise of coproducing lignin preparations attractive for use as prepolymers for the manufacture of engineered materials.

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Registry No. MWL, 8068-00-6; kraft lignin, 8068-05-1; hydrolytic lignin, 8072-93-3; explosion lignin, 86747-42-4; organosolv lignin, 67479-30-5; enzyme lignin, 67479-30-5; lignin, 9005-53-2.

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Partial Characterization of Condensate Derived from Volatiles-Sublimate of Homogenized Leaf-Cured Burley Tobacco during Storage

Roger A. Andersen

Approximately 100 mg dry weight of an orange-red water-soluble condensate was deposited as a viscous oil on the interior glass walls of the sealed storage chamber housing 1 kg of homogenized leaf-cured tobacco during a 20-weak storage period at 16% moisture and 30 °C. The condensate presumably emanated from the tobacco by volatilization and sublimation. Partial characterization of the substance indicated that it contained a mixture of organic compounds. One or more of these compounds was amphoteric. Elemental composition of the material was C 47.0%, H 5.5%, N 17.1%, and O 20.5%. Chemical, HPLC, GC, and GC–MS analyses showed that nicotinic acid (3.4%), nicotine (6.3%), and *N*-nitrosonornicotine (0.07–1.4%) were constituents accounting for about 10% of the dry weight. Total volatile bases as nitrogen equivalent were 6.6 mequiv/g.

An experimental homogenized leaf-cured (HLC) tobacco procedure for the rapid curing of burley tobacco is currently under investigation. The process involves homogenization of green leaves, incubation of the homogenate, and dehydration of the material (Tso et al., 1975; Yoder et al., 1976). During a recent investigation conducted to determine chemical changes that occur during prolonged storage ("aging") of HLC and conventionally air-cured burley tobacco under controlled environmental conditions, it was noted that quantities of an orange-red substance condensed on the upper walls of the storage chambers housing the HLC tobacco (Andersen et al., 1982). No measurable (weighable) amount of this kind of condensate was observed with the air-cured tobacco, however. Chemical changes in the HLC tobacco accompanied this loss in volatile or sublimed components with resultant decreases in concentrations of total alkaloids as nicotine, nitrate N, nitrite N, ammonia N, total volatile nitrogenous base N, and petroleum ether extractables in addition to a concomitant increase in *N*-nitrosonornicotine (NNN) levels. NNN was shown to have carcinogenic activity (Hecht et al., 1978). The purpose of this investigation is to characterize the condensed material that collected on storage chamber walls during the aging of HLC tobacco.

MATERIALS AND METHODS

Growth, Curing, and Storage (Aging) of Tobacco. Burley tobacco (*Nicotiana tabacum* L. cv. Ky 14) plants were grown, harvested, homogenized leaf-cured, and prepared for sampling as previously described for tobacco that was not treated for the removal of protein (Andersen et al., 1982).

Agricultural Research Service, U.S. Department of Agriculture, and Department of Agronomy, University of Kentucky, Lexington, Kentucky 40546-0091.

A 1-kg sample of tobacco that had been stored temporarily at a 3–4% moisture content was moisturized in a high-humidity chamber to 16% moisture on a "wet weight" basis and then packed into an 8-L glass desiccator that served as the storage container. The container was placed in an unlighted controlled-environment chamber maintained at 30 ± 0.5 °C. The desiccator cover had a gas-inlet tube that was closed except for a brief opening to equilibrate air pressure followed by removal of the cover for single 1-min periods at weekly intervals to allow exchange of storage-container gases with ambient air. The short periods of gaseous exchange did not cause an appreciable change in the tobacco moisture content.

Isolation of Condensate. After 20 weeks of storage, three-fourths of the orange-red condensate that collected as a viscous oil on the upper walls and cover of the storage chamber (desiccator) was transferred to a 1-mL glass vial. The vial contents were dried over P_2O_5 for 96 h. The transferred dried material weighed 75 mg and portions of it were used for the analyses.

Elemental and Chemical Analyses of Aged Tobacco and Condensate. Elemental analyses for carbon, hydrogen, nitrogen, oxygen, chlorine, and sulfur were performed on an 18-mg sample of the condensate material by Gailbraith Laboratories, Inc., Knoxville, TN. A Beckman Acta III recording spectrophotometer was used to scan the absorbance of a 1 mg/mL aqueous solution of the condensate material from 400 to 190 nm.

Total volatile nitrogeneous bases as nitrogen equivalence (TVB-N) were determined on a 20-mg sample of the condensate material by titration with standard sulfamic acid after the following pretreatment sequence: initially, bases in the sample were steam distilled from an alkaline solution (pH 12.0) into dilute hydrochloric acid, the resultant solution that contained excess hydrochloric acid was concentrated and a Kjeldahl digestion of the residue with