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Degradation of Insoluble Lignin by Chloride Monoxide

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Moistened spruce cuoxam and periodate lignins, treated with Cl_2O in CCl_4, were ring chlorinated, as deduced from infrared spectra, and became soluble in NaOH solution, Me_2SO, dioxane, and THF. The extent of solubility depended upon the quantity of Cl_2O applied. Molecular weights (by sedimentation equilibrium) of the dissolved lignins were a function of the fraction dissolved. GPC on Styragel of the soluble chlorolignins showed broad, skewed distributions, each having a single maximum at the low molecular weight end. Similar fractions chromatographed on Sephadex G-50 exhibited bimodal chromatograms. The high molecular weight peak was demonstrated to be an artifact, and the distribution given by Styragel chromatography is probably correct. It is concluded that the original cuoxam lignin, and hence lignin in wood, was a three-dimensional, cross-linked, infinite network polymer gel.

Of all the polymers of nature, the plant polymer lignin, composed of phenylpropane building blocks arranged in complex patterns, has alone continued to resist substantial efforts to unravel the details of its molecular architecture and to elucidate the chemical processes by which it can be separated from its accompanying polysaccharides. Research on the delignification of plant tissue—a process of prime commercial importance—usually focusses either on the organic chemistry of the degradative reactions, or on the properties of the polymeric degradation products.

Among the latter investigations, McNaughton et al. (1967) have found that in delignification at 150–180 °C by means of a solution of sodium hydroxide and sodium sulfide (i.e., kraft pulping), the lignin dissolved early in the process has a low molecular weight. As the extraction proceeds, the molecular weight of the dissolved material becomes progressively higher. Similar behavior has been observed during the acidolysis of sprucewood in methanol (Kosikova and Skamla, 1968), in the preparation of milled-wood (Björkman) lignin from pinewood (Bogomolov et al., 1974), and in other reactions (Yean and Goring, 1964; Rezanowich et al., 1963; Albrecht and Nicholls, 1976).

When a new delignifying agent, chlorine monoxide (Cl_2O; the anhydride of HOCl), was brought to light (Bolker and Liebergott, 1972), we undertook, as reported here, to determine whether the soluble products of its attack on lignin would exhibit the same pattern. The significance of this aspect of our investigation is that increasing molecular weight with increasing solubility of lignin is predicted by our previous work (Bolker and Brenner, 1970). It led to the conclusion that the relationship could be explained if lignin in wood is an infinite network of cross-linked chains and is solubilized by the cleavage of cross-links.

There have been other, substantially different, explanations proposed for the increase in molecular weight of the dissolved lignin as the delignification reaction proceeds. The most common explanation (Alekseev et al., 1969; 1971; Lacan and Matasovic, 1966; Alekseev and Reznikov, 1970; Chupka et al., 1970), which might be called the "condensation theory", is that lignin in wood consists of finite macromolecules with reactive sites that "condense", i.e., form intermolecular bonds, when the lignin is extracted. Since "condensation" occurs under both acidic and alkaline conditions, the concept, and its terminology as applied to lignin, probably arose by analogy with the chemistry of phenol–formaldehyde resins. Whatever its origin, the theory implies that isolated, soluble lignins, when further exposed to extracting reagents and conditions, would either increase in molecular weight (if all the reactive sites had not been used up during the original isolation), or would not change (if they had). Adler et al. (1968) found, however, that there is a marked decrease in the molecular weight of Björkman (milled-wood) lignin upon mild hydrolysis. A decrease in the molecular weight

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of soluble sulfite lignin upon a second cooking (Felicetta and McCarthy, 1957) is further evidence against condensation in an acid medium.

A second explanatory proposal is that the lignin in the middle lamella is finite and of higher molecular weight—and hence of greater size—than the lignin in the secondary wall. This model requires that in order for the lignin to enter solution, it must pass through pores in the cell wall into the lumen thence into the solvent (Ahlgren et al., 1971). These pores have been shown to increase in size by removal of some of the carbohydrate material during delignification. The cell wall thus acts as a sieve of gradually increasing hole size, effectively sorting the lignin molecules so that the average molecular weight of the lignin increases as the extraction continues. The model may explain the behavior of lignin in wood chips, but for milled wood, ground to fragments much smaller than the size of an individual cell, it is not adequate. In milled wood, there is no longer any need for the middle lamella lignin fragments to diffuse through the secondary wall, it being exposed to the solution directly. There is a report, however, by Bogomolov et al. (1974), of the molecular weight of Björkman lignin increasing with increasing yield. Unfortunately, contamination of milled wood lignins by carbohydrates, whether covalently bound, trapped in "snake cages" (Pew and Weyna, 1962), or loose, may confuse the issue.

In the present investigation we have eliminated the possible influence of diffusion by using periodate (Wald et al., 1947; Ritchie and Purves, 1947) and cuoxam (Freudenberg, 1955) lignins. Although these lignins are generally not employed for organic structural studies—and for good reason (Lai and Sarkanen, 1971)—they have been unjustly neglected in research on the polymer chemistry of lignin. They are prepared by removing the carbohydrate from around the lignin—and hence are "naked"—and whatever their shortcomings in terms of precise structural chemistry, they possess the virtues of having retained the morphological structure of the cells from which they came and of being insoluble until attacked by degradative reagents.

Their insolubility is one indication that they have the architecture of three-dimensional cross-linked polymers. The literature contains other evidence (Bolker, 1974), some of it quantitative (Bolker and Brenner, 1970), which supports this conclusion. It may also be argued on an a priori basis from the published formulations (Freudenberg, 1965; Ludwig et al., 1964; Adler, 1968) of the structure of lignin, for they all contain some trifunctionally linked branch-points.

Some evidence, particularly the broad molecular weight distributions of soluble lignins, has suggested that the lignin in wood might be a finite polmer of very high molecular weight, and highly branched (Yean and Goring, 1964; Adler et al., 1968), in the pattern designated by Flory (1953) as $(AB_{f-1})_n$. However, our knowledge of the detailed structure of lignin (Lai and Sarkanen, 1971) does not support this hypothesis, nor does that knowledge accord with the requirement that a finite, branched molecule must have more end-groups than branch-points.

Rather, formulations of lignin structure are consistent with the concept that its architecture is that of a three-dimensional, infinite network, with a gel phase (insoluble without degradation) and a sol phase (finite molecules, soluble in appropriate solvents).

The experimental evidence that lignins give fragments of higher and higher molecular weight the more they are degraded has thus far been quantitatively analyzed for only a limited number of degradative reactions (Szabo and Goring 1968; Bolker and Brenner, 1970). A primary purpose of the present research was to bring forward evidence obtained by employing a reagent of a rather different type. At the same time, we wished to evaluate the use of gel permeation chromatography on Styragel and of gel filtration on Sephadex for research of this nature.

In the course of the gel filtration experiments, we, like others (James et al., 1968; Wayman and Obiaga, 1974; Obiaga and Wayman, 1973), observed that isolated lignins frequently exhibit bimodal distribution curves. New evidence, presented in this paper, suggests that the bimodality is an artifact of the gel filtration method, and that the distribution curves have only one maximum.

EXPERIMENTAL SECTION

Infrared spectra were obtained on a Unicam SP 200 Grating Infrared Spectrometer from KBr pellets. Molecular weight determinations were done by the short column sedimentation equilibrium technique in a Spinco Model E ultracentrifuge. The partial specific volume of a lignin, $\bar{V}$, was assumed to be 0.7, and the refractive index increment, $-dn/dc$, was measured as −0.0868. All such determinations were made in Me_2SO taken from the same bottle, Fisher analyzed, density 1.099.

Microanalyses (C, H, O, Cl, OCH_3) were performed by Schwartzkopf Microanalytical Laboratories. Solubility in Me_2SO was judged by the absorptivity at 280 nm, using an extinction coefficient of 75.6 $cm^{-1}/\%$ by weight (approximately 6.9 g^{-1} cm^{-1} L). This value was derived from the absorbance of cuoxam lignin treated with 5 mol of Cl_2O per mol of phenylpropane units, 100% soluble in Me_2SO.

Spruce Periodate Lignin. Spruce periodate lignin was prepared essentially by the method of Ritchie and Purves (1947). Spruce (*Picea mariana*) wood meal, 40 mesh, was exhaustively extracted with alcohol–benzene (1:2) and hot water. The wood meal was then stirred for 20 h at room temperature (~25 °C) in a 4.5% solution of trisodium paraperiodate acidified with acetic acid. The wood residue was collected on a large sintered glass funnel (filter paper was found to disintegrate under these conditions) and washed continuously with distilled water until the filtrate liberated no iodine from an acidified potassium iodide solution. After being washed once more with distilled water, the residue was extracted for 3 h under reflux in boiling distilled water and subsequently recovered and given a final wash with distilled water. The periodate oxidation–extraction cycle was repeated six times more, then the isolated lignin was extracted at room temperature with methanol and benzene, and dried under reduced pressure. Microanalysis, corrected for ash content, was C, 58.6%; H, 5.65%; OCH_3, 12.8%; Cl, 1.34%. Infrared spectrum (KBr pellet) λ_{max} 1750, 1600, 1510 (doublet), 1470, 1430, 1270, 1230, 1140, 1085, 1035, 860, 815 cm^{-1} (no cellulose bands detectable).

Examination under a microscope showed that the periodate lignin retained the general morphology of the wood, with a clearly visible parallel cellular structure. Observed in polarized light with a cross polarized objective, the lignin showed only faint traces of light, also indicating the essentially complete removal of cellulose.

Cuoxam Lignin. Spruce cuoxam lignin was provided by Dr. J. J. Renard (Renard et al., 1975). Its elemental analysis was C, 61.47%; H, 6.43%; OCH_3, 15.20%. Infrared spectrum (KBr pellet) λ_{max} 1600, 1510 (doublet), 1470, 1430, 1270, 1230, 1140, 1085, 1035, 860, 815 cm^{-1} (no cellulose bands detectable).

Chlorine Monoxide. The procedure was derived from that of Cady (1957). Mercuric oxide (Anachemia, Reagent

Grade) was heated in an oven at 120 °C for a minimum of 2 days before use, then stored in a desiccator over P_2O_5. The carbon tetrachloride used in these experiments was first dried over P_2O_5 and then distilled. The first 20% and the last 30% of the distillate were discarded. Then chlorine gas was dissolved in dry CCl_4 at 0 °C to a concentration exceeding 50 g/L, as monitored by iodometric titration. The solution was brought to room temperature, an excess of HgO was added, and the suspension was stirred vigorously for half an hour. It was then filtered through a sintered glasss funnel, and the solid material was retained for recovery of HgO. The concentrations of Cl_2 and Cl_2O were measured by iodometric titration. Any sample containing a measurable amount of Cl_2 was discarded. The concentration of Cl_2O was reduced to the required value by dilution with dry CCl_4.

Mercuric oxide was regenerated by stirring the filtered sludge with aqueous NaOH, filtering, washing with cold water, and drying at 100 °C.

Treatment of Lignin with Cl_2O. Large portions of both periodate and cuoxam lignins were dried at room temperature under vacuum for over 20 h, then brought to 10% by weight of water with a fine spray of distilled H_2O and shaken in a sealed flask for 3 days. The moisture was always readily absorbed into the lignin. Except when otherwise stated, the treatments of cuoxam lignin proceeded as follows: Two hundred milligrams of lignin ($\sim$0.001 mol on a C_9 basis, 10% H_2O) was suspended in the required volume of 5×10^{-2} M chlorine monoxide in CCl_4 solution at room temperature (23 °C) and stirred for 3 h. The mixture was filtered, and the solid residue was washed with fresh dry CCl_4 and dried at reduced pressure.

Treatment of the periodate lignin was similar except that the lignin was suspended in CCl_4 before exposure to the Cl_2O.

Chromatography on Sephadex. Chromatograms were obtained on an apparatus consisting of a graduated 1-cm diameter glass column connected by a siphon to a reservoir and with the outlet connected through Teflon tubing to a Uvicord II ultraviolet absorptiometer equipped with a filter which passed light at 282 nm. The transmittance of the eluent was recorded on a chart recorder as a function of time. The output was converted into absorbance by the equation $A = \log (100/T)$, and the time was multiplied by the flow rate determined for each sample to give the elution volume.

Most of the chromatography was done on Sephadex G-50 (fine) gel with Me_2SO as the eluent. The bed volume was 27 mL with a height of 40 cm and the flow rate was 0.09–0.10 mL/min under 60 cm of hydrostatic pressure. Samples of Blue Dextran 2000 (Pharmacia, Uppsala) and vanillin were passed through the column periodically to verify V_0 and to check the packing of the column.

Samples for chromatography were prepared by dissolving as much as possible of 0.05 g of treated lignin in 2.0 mL of Me_2SO. The insoluble fraction was removed by centrifugation. Aliquots of 0.2 mL (0.05 mL when $W_s >$ 0.8) of the clarified solution were layered on the gel and eluted until the ultraviolet absorption returned to its initial value.

Chromatography on Styragel. Chromatograms were obtained on a Waters Associates Gel Permeation Chromatograph equipped with 120-cm columns of THF–swollen Styragel, with separation up to a $\sim 10^7$ g/mol. Superimposed upon the output of the detector, a differential scanning refractometer, was a series of spikes, each indicating the draining of a 5-mL siphon, thereby marking the elution volume. Samples, approximately 0.1% solutions in tetrahydrofuran, were injected via a 2-mL sample loop into a continuous flow of distilled THF.

Table I. Solubilities of Spruce Periodate Lignin Treated with Chlorine Monoxide

Cl_2O/-lignin,[a] mole ratio	Fraction sol. in CCl_4, % of starting material	Residue insol. in CCl_4, % of starting material	Soluble in dioxane–water (9:1), % of residue insol. in CCl_4
0.0	0.0	100	0
0.5	2.2	123	16
1.0	3.0	129	38
1.5	3.7	140	74
2.0	8.3	140	86
3.0	12.4	140	98
4.0	*b*	54	100

[a] One mole of lignin was taken as the formula weight of a C_9 unit. [b] Not determined.

RESULTS

Morphological Structure. Microscopic examination revealed that both cuoxam and periodate lignins, particularly the latter, retained a morphological resemblance to the original wood. The cellular structure was clearly evident, and the lignin was only slightly less difficult than sawdust itself to grind in KBr for infrared spectroscopic examination. On treatment with Cl_2O, however, the structure broke down. A sample treated with 4 molar equivalents and examined under the microscope was seen to have a greatly reduced average particle size. Grinding this sample to produce a satisfactory KBr pellet was relatively easy.

Solubility. When dry periodate lignin in carbon tetrachloride was treated with 4 mol of Cl_2O/C_9 unit, the CCl_4-insoluble residue had 75% of the weight of the original lignin and was only partially soluble in dioxane–water (9:1). When periodate lignin containing 10% of moisture was similarly treated, the solid residue represented 54% of the weight of the starting material, and was completely soluble in dioxane–water. With respect, then, to solubilization of the lignin, the presence of some water appears to be necessary during the reaction. Whether the role of water is chemical (e.g., to hydrolyze the Cl_2O to HOCl) or physical (e.g., to swell the lignin and permit the reagents to enter its structure) remains to be determined. Whatever its role, water (10%) was present in the lignins used in all subsequent experiments, as indeed it always is in pulps undergoing technological bleaching.

In the presence of moisture, treatment with 3 mol of Cl_2O made the chlorinated residue completely soluble in dioxane–water (Table I). Treatment with 4 mol led to overdegradation in that the solid residue represented only 54% of the starting material, with the rest, a mixture of oligomers, presumably having been washed away in the carbon tetrachloride. The presumption is unconfirmed, however, because in this one experiment, the carbon tetrachloride soluble fraction was not collected and weighed. In all the other experiments represented in Table I, the yields of CCl_4-soluble material were relatively insignificant and did not exceed the 12.4% measured when the lignin had been reacted with 3 mol of Cl_2O/C_9 unit.

Table I also shows how the weight of the lignin increased with the extent of treatment, up to 3 mol/C_9, clearly, as the elemental analysis confirms (Table II), due to the addition of chlorine. In the same way, the solubility of the residue in dioxane–water increased with the extent of treatment. The residue was also soluble in 1% NaOH, 1% Na_2CO_3, and in tetrahydrofuran (THF).

The effect of reaction with Cl_2O on the solubility of cuoxam lignin in Me_2SO is included in Table III. Similar

Table II. Elemental Analyses of Cl_2O-Treated Lignins

Starting material	Treatment, mol of Cl_2O/mol of C_9	Analysis, % C	H	Cl	OCH_3	Empirical formula, based on C_9
Periodate lignin	0	58.6	5.65	1.3	12.8	$C_9H_{8.9}O_{3.66}(OCH_3)_{0.82}$[a]
	0.5	47.5	4.26	18.7	8.1	$C_9H_{8.4}O_{3.87}Cl_{1.28}(OCH_3)_{0.64}$
	1.0	43.5	3.73	23.3	6.5	$C_9H_{8.1}O_{4.3}Cl_{1.73}(OCH_3)_{0.55}$
	1.5	40.3	2.97	26.9	5.5	$C_9H_{6.8}O_{4.8}Cl_{2.15}(OCH_3)_{0.50}$
	2.0	38.3	2.97	28.4	4.4	$C_9H_{7.5}O_{5.2}Cl_{2.37}(OCH_3)_{0.42}$
	3.0	35.3	2.78	30.8	3.7	$C_9H_{7.7}O_{5.8}Cl_{2.77}(OCH_3)_{0.38}$
Cuoxam lignin	0.0	61.5	6.43	0.02	15.2	$C_9H_{9.6}O_{2.94}(OCH_3)_{0.95}$
	1.0	48.9	4.52	14.1	8.3	$C_9H_{8.7}O_{4.16}Cl_{0.94}(OCH_3)_{0.63}$
	3.0	39.1	3.48	26.5	4.3	$C_9H_{8.7}O_{5.16}Cl_{2.16}(OCH_3)_{0.40}$
	4.0	37.1	3.02	29.3	4.0	$C_9H_{8.0}O_{5.44}Cl_{2.51}(OCH_3)_{0.38}$

[a] Neglecting Cl.

Table III. Solubilities in Me_2SO and Molecular Weights of Cl_2O-Treated Cuoxam Lignin

Treatment, mol of Cl_2O/mol of C_9	Fraction soluble in Me_2SO, $\bar{w}_s$	Wt av mol wt, $\bar{M}_w$	Wt av deg. of polymerization, $\bar{x}_w$
1	0.32	6100	25
2	0.34	3800	18
3	0.95	18900	65
4	1.0	420000	1400
5	1.0	47600	192

trends of solubility in 0.1 N NaOH and in THF were observed.

Like Table I, Table III shows that 3 mol of Cl_2O/phenylpropane unit was required to make the cuoxam lignin completely soluble. In fact, iodometric titration of the spent Cl_2O/CCl_4 filtrate from the 3:1 reaction revealed a residual oxidizing capacity equivalent to 10% of the original solution. Thus, although 3 mol of Cl_2O was applied, only 2.7 mol was consumed.

Elemental Analysis. The analyses and empirical formulae (Table II) of the treated lignins to some extent reflect the difficulty of drying isolated lignin derivatives and keeping them dry. Nevertheless, there is a clear trend of incorporation into the lignin of substantial amounts of chlorine and oxygen, and loss of methoxyl groups, as the degradation proceeds. The empirical formulae based on C_9 are included here as a matter of form. Their significance, however, may be limited, since possible cleavage of the C_9 backbone might yield volatile or CCl_4-soluble fragments, either of which would be lost during the workup. Indeed, such losses may explain why the formula of the residue from the treatment of periodate lignin with 0.5 mol of Cl_2O, which, in substitution reactions (eq 1) supplies one atom of chlorine per phenylpropane unit (Renard and Bolker, 1976), shows 1.28 atoms of chlorine. Despite their obvious deficiencies, the formulae do give formula weights which permit the estimation of degrees of polymerization from polymer molecular weights.

$$C_6H_5OR + 0.5Cl_2O \rightarrow C_6H_4ClOR + 0.5H_2O \quad (1)$$

Infrared Spectra. The infrared spectra of undegraded cuoxam and periodate lignins contain the very distinct, well-known absorption band (actually a doublet) at 1510 cm^{-1} that arises from the aromatic rings in their structure. On treatment of both lignins with Cl_2O, this band decreases in intensity, and, after sufficient treatment, disappears entirely. At intermediate stages of treatment, it is markedly less intense in the THF-soluble fraction than in the insoluble fraction.

Simultaneous with the loss in intensity of the 1510 cm^{-1} band is a gain at 1730 cm^{-1}, clearly a C=O stretch. The infrared spectrum of the vapor above the reaction mixture indicates the presence of CO_2 at higher than atmospheric concentration. The evolution of CO_2 is hardly surprising as Rashback and Yorston reported as early as 1931 that it was a product of bleaching with calcium hypochlorite. It does emphasize, however, that considerable care should be taken with the interpretation of elemental analyses "on a C_9 basis". No firm conclusions can yet be made concerning the actual reactions taking place.

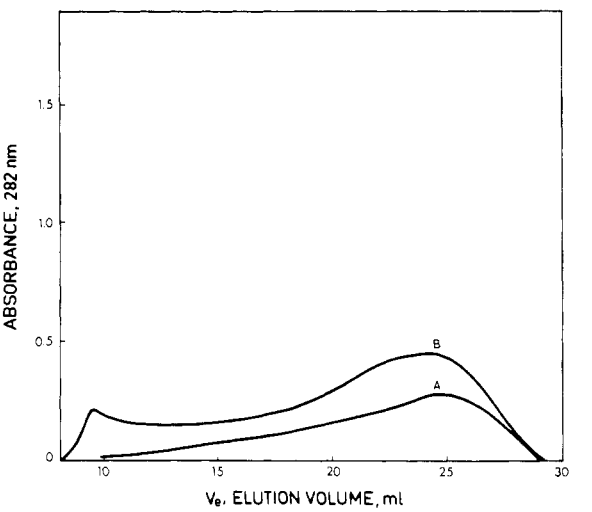

Figure 1. Elution curves (Me_2SO) of Cl_2O-treated periodate lignins on Sephadex G-50. Mole ratios of Cl_2O to lignin C_9 unit: A, 1:1; B, 2:1.

Molecular Weights and Distribution. As measured by the ultracentrifuge, the weight-average molecular weight, $\bar{M}_w$, of the soluble portion of the Cl_2O-treated cuoxam lignin increased with the weight-fraction of sol, W_s, until the entire sample was soluble (Table III). The maximum was 4×10^5 at 100% solubility, achieved on treatment with 4 mol of Cl_2O/mol of C_9 unit. Treatment with 5 mol led to a reduction of molecular weight, presumably because an excess of Cl_2O breaks many more bonds than necessary to cause only solubilization. When the lignin was treated with a massive excess of Cl_2O (10:1), further decrease in molecular weight was evident, but appeared to be limited in extent. This lower limit of molecular weight represents polymeric fragments resistant to further degradation by Cl_2O and merits future investigation in that they may constitute, in this reaction, the primary chains which form the basis of the infinite network.

The probable loss of the sol phase (Braun's, "native" lignin) during the original isolation of the insoluble lignin should have had no significant effect on the molecular weights. Even if it represented as much as 5% of the total lignin sample, the effect on the cumulative molecular weight would have been very small, less than 5%, to yield a value of $\bar{X}_w$ slightly large for the corresponding W_s. Qualitatively, the results would remain the same.

Table IV. Frequency of the First Absorption Band below 1520 cm^{-1} in the Infrared Spectra of Substituted Benzenes[a]

Trisubstituted benzenes				Disubstituted benzenes		
X	Y	Z	$\bar{\nu}$, cm^{-1}	X	Z	$\bar{\nu}$, cm^{-1}
COOH	OH	OH	1518	CH_3	CH_2Cl	1518
OH	Cl	OH	1515	OH	$C(CH_3)_3$	1512
OCH_3	OCH_3	CH_2COOH	1510	OH	OH	1510
OH	OC_2H_5	COOH	1510	OC_2H_5	OC_2H_5	1510
OH	OCH_3	$CH=CHCOOC_2H_5$	1510			
CH_3	Cl	C	1480	OH	Cl	1495
Cl	Cl	CCl_3	1470	CH_3	Cl	1490
CH_2Cl	Cl	Cl	1470	CH_2Cl	Cl	1490
OH	Cl	Cl	1470	COOH	Cl	1490
Cl	Cl	COOH	1470	Cl	Cl	1475
Cl	Cl	CH_3	1465			

[a] All $\bar{\nu}$ values were read to within ±4 cm^{-1} from the Sadtler spectra.

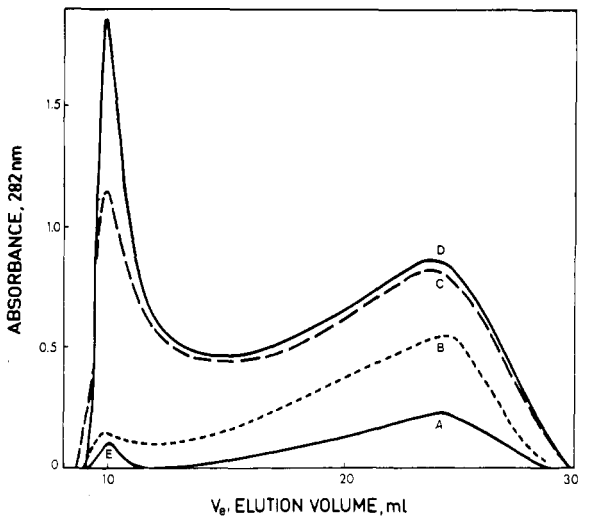

Figure 2. Elution curves (Me_2SO) of Cl_2O-treated cuoxam lignins on Sephadex G-50. Mole ratios of Cl_2O to lignin C_9 unit: A, 1:1; B, 3:1; C, 4.5:1; D, 5:1. Curve E was obtained by chromatography of Blue Dextran 2000, and its peak thus represents the exclusion limit of the column.

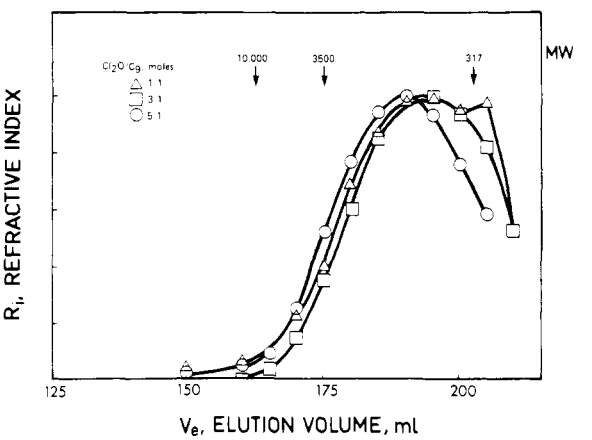

Figure 3. Elution curves (THF) of Cl_2O-treated periodate lignins on Styragel. The molecular weight calibrations are for polystyrene and are of limited significance with respect to the chlorinated lignins.

In obtaining the molecular weight distribution curves by chromatography on Sephadex (Figures 1 and 2), the sample sizes were selected so that the area under the curves would be proportional to the solubility. The increase in $\bar{M}_w$ with increasing extent of solubilzation was manifested by the growth of a "high" peak (Figure 2) coinciding with the exclusion limit as indicated by Blue Dextran 2000.

Attempts to obtain gel permeation chromatograms of Cl_2O-treated periodate lignin on Styragel using THF as the eluent led to quite different results than on the Sephadex–Me_2SO system. Qualitatively all the samples appeared the same (Figure 3). All contained a maximum at low molecular weight, with some high molecular weight tail. There was no evidence in any sample of the sort of high molecular weight band apparent in the Sephadex chromatograms, and no significant variation was observed in the position of the maximum.

DISCUSSION

Nature of the Reactions. It is not expected that an investigation of this type will shed much light on the nature of the reactions of Cl_2O with specific functional groupings on the lignin molecule. Such information will come from hitherto unpublished research (Lee, 1973) on the reactions of model compounds. The present work does reveal, however, that lignin takes up a good deal of chlorine in its reaction with Cl_2O (Table II). Model compound studies (Lee, 1973) suggest that most of this chlorine is taken up by aromatic substitution. However, it was observed that the absorption band at 1510 cm^{-1} was lost on treatment of the lignin.

Since the band represents an aromatic C=C linkage in compounds that are 1,4-disubstituted, 1,3,4-trisubstituted, and occasionally 1,3,4,5-tetrasubstituted (no other substitution pattern gives significant bands in this region), and since there was earlier evidence that chlorine *dioxide* cleaves the rings of aromatic model compounds (Husband et al., 1955; Dence et al., 1962; Sarkanen et al., 1962), it seemed possible that the disappearance of the band represented a loss of aromaticity. A search of the literature (Sadtler Spectra) revealed, however, that, as seen in Table IV, substitution of Cl into either the 1 or 4 position would also result in the disappearance of the 1500 cm^{-1} band. Furthermore, Brownlee et al. (1969) have found that in chlorine-containing para-disubstituted benzenes, the corresponding band lies at a much lower frequency (1475–1490 cm^{-1}) than that observed in undegraded lignin. Thus the decrease in intensity of the 1510 cm^{-1} band in an aromatic material treated with a chlorinating agent is not necessarily evidence for loss of aromaticity, but more likely for substitution by chlorine.

Interpretation of Chromatograms. The chlorinated, soluble lignins exhibited two types of molecular weight distribution patterns: a bimodal distribution when

chromatographed on Sephadex (Figures 1 and 2) and a broad distribution with a single maximum at low molecular weight when chromatographed on Styragel (Figure 3). Any interpretation of the chromatograms must reconcile these two rather different results.

Certainly, the use of gel permeation chromatography over the past two decades has established (Brown et al., 1967; Alekseev et al. (1969a); Babikova et al., 1974; Smirnova et al., 1974) that soluble lignins isolated under the mildest of extraction conditions from a wide variety of plants are polydisperse, polymeric materials (Goring, 1971).

As to the bimodal curves obtained by chromatography on Sephadex, examples are numerous (e.g., Brown et al., 1967; James et al., 1968; Obiaga and Wayman, 1974). The example provided by James et al. (1968) is particularly relevant to the present argument, because it shows that the shapes of chromatograms of the same lignosulfonate vary with gel pore size. Their results suggest that for a material like lignin (rather than a polypeptide), it may be wrong to interpret a narrow band on a Sephadex chromatogram as a narrow distribution of molecular weight, or a bimodal curve as representing a truly bimodal distribution.

Another possible source of interpretive error is to be seen in chromatograms of milled-wood lignin preparations from spruce and hemlock groundwood pulps as presented by Obiaga and Wayman (1974). The overwhelming preponderance of the material falls into the "high" molecular weight category, even on a Sephadex G-100 column (separation of Dextrans to mol wt 10000, peptides to mol wt 30000). However, the preparation of these lignins included purification by washing and reprecipitation. In the course of this procedure a substantial portion of the lower molecular weight lignin could have been removed, and the chromatograms were probably not representative of the lignin made soluble by the milling process.

If we consider that lignin in wood is a three-dimensional cross-linked polymer and is solubilized by the cleavage of its cross-links in a reversal of the post-gel phenomena (Szabo and Goring, 1968; Bolker and Brenner, 1970), then it is possible to calculate from theory (Flory, 1953) that the most abundant fraction in the molecular weight distribution of the solubilized material will be that of lowest molecular weight. The abundance of fractions then decreases with increasing molecular weight. This was the very pattern found some years ago by Gupta et al. (1960) on examining a number of fractions of soda lignin by ultracentrifugal sedimentation. It is also the pattern of molecular weight distribution that we have found on chromatographing chlorolignins on Styragel (Figure 3).

If one assumes that ultracentrifugal sedimentation and gel permeation chromatography on Styragel give the correct molecular weight distribution—with a single maximum at its low molecular weight end—one can, starting with this distribution and simply decreasing the exclusion limits, generate distribution patterns that contain all the essential features of the bimodal lignin Sephadex chromatograms which appear in the literature. The exclusion limit of the particular Sephadex employed has the effect of displacing the high molecular weight tail to form a peak at the exclusion volume. This peak represents the accumulation of all molecules of hydrodynamic volume greater than that acceptable to the chromatographic medium. Although the peak does not represent a real maximum, the area under it is extremely sensitive to changes in molecular weight distribution, such as those which occur as the degradation of lignin proceeds. The peak at the exclusion volume can therefore be an important tool for investigating variations in the molecular weight of lignins. In this sense, the Sephadex chromatograms are more useful than the Styragel chromatograms. Since the latter have tails extending as far as mol wt 10^7, differences between isolated polymers are more difficult to discern.

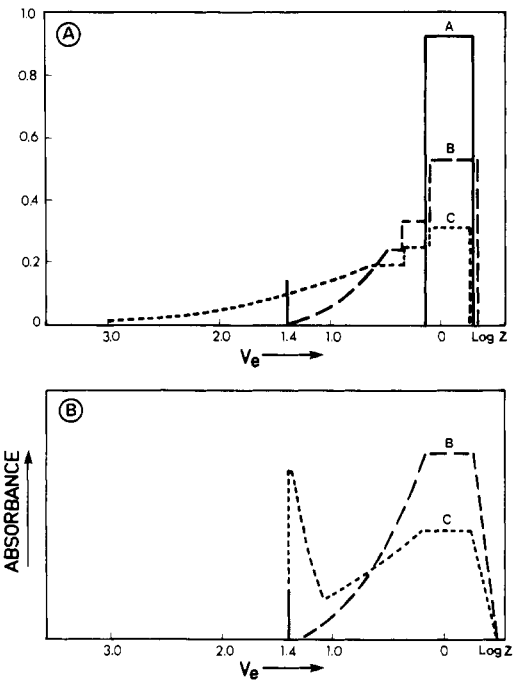

Figure 4. Calculated chromatograms of the sol fractions of cross-linked polymer gels: (A) no exclusion limit; (B) exclusion limit, log z = 1.4, where z = total number of primary chains in the polymer. A, γ = 0; B, γ = 0.5; C, γ = 1.0. γ is the cross-linking index, such that γ = 1.0 represents the point of incipient gelation where $\bar{x}_w$ is infinite. The curves represent samples of equal weight. Spreading in the vicinity of z = 1 is expected to be significant.

The coincidence of the high molecular weight peak with the exclusion volume, V_0, of the Sephadex gel is easily tested (Figure 2) by passing Blue Dextran 2000, a commercially prepared dye, mol wt $\sim 2 \times 10^6$, through the column. Unfortunately, such verification of the exclusion limit has rarely been published in papers on lignin chemistry.

In mathematical terms, it is possible to demonstrate the effect of a low exclusion limit by calculating a family of idealized gel chromatograms (Figure 4A), assuming a series of sols derived by the breakdown of a network of primary chains. The distribution is governed (Flory, 1953) by:

$$W_z = z^{(z-1)}(\gamma e^{-\gamma})^z/\gamma z! \qquad (1a)$$

in which W_z is the weight fraction of primary chains having y units, z is the total number of primary chains in the polymer, and γ is the cross-linking index.

To simplify the calculations, it was assumed that the relation between z and the elution volume, V_e, was of the form $\log z = -nV_e + \log z_0$, where n and z_0 are constants, implying exact linearity of the calibration curve over the entire range. The implication of linearity is not realistic, but it gives an acceptable approximation that serves to make the point. In real systems, the elution profile of a monodisperse compound is approximately Gaussian, and a polymer chromatogram is the sum of a large number of individual peaks, yielding a smooth curve. For the calculation of the curves in Figure 4A it is assumed that an x-mer is eluted in a rectangular peak, the base width being the elution volume corresponding to (V_e at $z - {}^1/_2$) - (V_e

at $z + {}^1/_2$), which is proportional to ln $[(z + {}^1/_2)/(z - {}^1/_2)]$. Since the area of any one of these rectangles is W_z, the height, h, equals $W_z/\ln [(z + {}^1/_2)/(z - {}^1/_2)]$.

Except at low values of z, $z = 1$ and perhaps $z = 2$, where there is not a significant number of overlapping peaks on each side, the rectangular approximation holds well. One can therefore plot h vs. log z and obtain curves resembling the detector response vs. elution volume for any value of γ. This has been done (Figure 4A) for three values of γ, including $\gamma = 1.0$ (the gel point).

The curves in Figure 4A were calculated without regard for peak spreading due to processes independent of the macromolecular size. Even so, they illustrate why chromatograms obtained on gels with a large separation range may be deceptively similar. However, if one imposes an exclusion limit on the system by arbitrarily slicing through the curves at a given value of z (log $z = 1.4$ has been chosen, Figure 4A), some radical changes emerge. In order to satisfy the requirement that the total area under the curves remains the same, one is forced to introduce a high molecular weight peak at the exclusion limit (Figure 4B).

It should be noted that yet another factor can affect the chromatograms of lignins at the high molecular weight end. It arises from the approximately logarithmic relationship between the elution volume, V_e, and the molecular weight, M, in the central portion of a column calibration curve. When a UV detector is used, the total area under the absorbance vs. elution volume curve for a particular sample represents a weight fraction of 1.0 of the dissolved sample. Although the detector response is proportional to concentration, the distance on the V_e scale is proportional to log M_2–log M_1, rather than M_2–M_1. Thus, the same weight fractions of material might lie between $M_1 = 10$ and $M_2 = 20$ (log $M_2/M_1 = 0.302$) on the one hand, and $M_1 = 1010$ and $M_2 = 1020$ (log $M_2/M_1 = 0.005$) on the other. Since the latter two points lie 60 times closer together, the detector response in the range $M = 1010$–1020 would have to be 60 times greater than in the range $M = 10$–20. In other words, at the high molecular weight end of a gel permeation chromatogram, there is a "stacking" of the detector response which could lead to the recording of a peak in the distribution where none exists. This, then, is another cause for enhanced peaks in the high molecular weight range of a "bimodal" distribution curve, and its effect is additive to that of the exclusion.

The polydispersity ($\bar{M}_w/\bar{M}_n$) of soluble lignins, if they are derived by breaking a three-dimensional network, probably owes more to a high $\bar{M}_w$, which, at nearly 100% extraction could approach infinity, than to a low $\bar{M}_n$, which must always be finite (Flory, 1953). There are polymers (carbohydrate) in wood which may have a high $\bar{M}_w$ and be much less polydisperse than the type of condensation polymer degraded lignin is expected to be. If such polymer molecules are grafted or otherwise directly joined to lignin units, in a lignin–carbohydrate complex (LCC), the high molecular weight peak could, in fact, represent a true maximum in the molecular weight distribution suggested by chromatography on Sephadex of this complex dissolved in dimethyl sulfoxide or aqueous alkali. However, since the LCC are insoluble in THF, in which (see Experimental Section) the chlorolignins were soluble, the corresponding peak would not appear in the Styragel–THF chromatograms. This hypothesis is consistent with the observation reported here, except that it does not explain why the high molecular weight peaks on the Sephadex chromatograms should grow with increasing solubilization.

It is highly improbable, moreover, that, as has been suggested, the high molecular weight peaks contain a significant amount of carbohydrate macromolecule with lignin covalently bound to them. Indeed, the magnitude of the "high" molecular weight peaks in some published chromatograms is so great as to indicate a carbohydrate fraction far larger than conceivable for the lignin preparation.

The LCC explanation is certainly inapplicable to the present results. Even if the starting lignins had not been demonstrated to be essentially cellulose-free (and, hence, presumably free of other carbohydrates as well) both by microscopic examination of the samples between crossed polarizers and by their spectral analysis, then the UV detection employed in the chromatography would provide the evidence. Since the UV detector was scanning at 282 nm, it would respond only to the attached lignin fragments, and the high molecular weight peak represents up to 25% of the integrated absorption. Brown et al. (1968) reported finding a high molecular weight peak of similar size in chromatograms of biologically isolated carbohydrate-free lignin.

Architecture of Lignin in Wood. Bimodal chromatograms of lignins isolated by the action of brown rot fungi (Brown et al., 1967) were originally interpreted by dividing the curves into three portions, one dialyzable, one of low molecular weight but not dialyzable, and one of high molecular weight. Since the "high" peak increased with the extent of the rot, and the rot is believed to attack from the lumen, this peak was attributed to middle lamella lignin, rendered diffusible when the fungi removed material from the secondary wall. According to this reasoning, the bimodal chromatograms of extracted lignins constitute a reasonable reflection of the state of affairs within the wood substance itself where lignins are of finite molecular weight, low in the secondary wall and high in the middle lamella. Although this interpretation is an extension of the conventional wisdom, it is no longer acceptable unless the high molecular weight peak is proven not to be an artifact.

Similarly, without proof that the peak is genuine, one would have to reject the interpretation (Wayman and Obiaga, 1974; Obiaga and Wayman, 1973, 1974) of bimodal curves which suggests that dissolved lignin comprises essentially two fractions, the one of low molecular weight representing the "modules" of which the high-weight fraction is composed.

Rather, given the evidence in this paper, the bimodal chromatograms represent a distortion of the true distribution. Changes in the relative height of the excluded peak represent changes in the skewing of a unimodal distribution and not the accumulation or depletion of a single, narrow fraction.

This particular type of unimodal distribution is consistent with the dissolved lignin being the product of the degradation of a highly branched $(AB_{f-1})_n$ polymer. However, for the reasons given in the Introduction, this possibility may be dismissed, leaving the possibility of lignin as an infinite network.

In degrading the lignin network, one reagent (e.g., sulfite pulping liquor and other acidic media) might predominantly attack and cleave chemical bonds of one kind (probably benzyl ether linkages) within the lignin. For such reagents the average basic chain length ($\bar{D}\bar{P}$) was calculated (Bolker and Brenner, 1970) as 18 (Figure 5). Another reagent might attack and cleave (as yet unknown) bonds of another kind. Such, indeed appears to be the case when lignin is cleaved by chlorine monoxide, for the experimental results obtained so far appear to fit the curve calculated for an average basic chain length ($\bar{D}\bar{P}$) of 7 (Figure 5). This different value likely means that Cl_2O

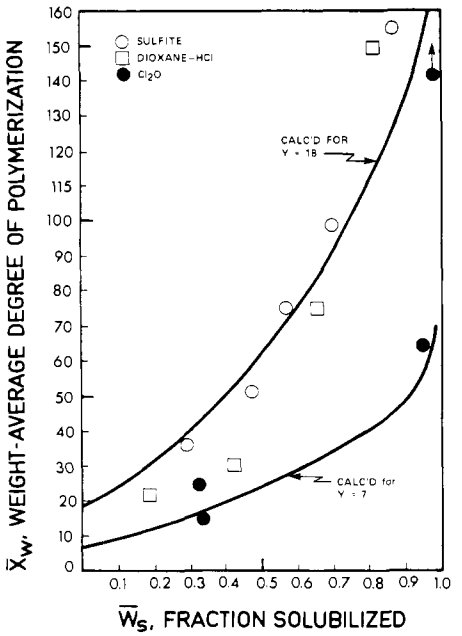

Figure 5. Experimental points from lignin degradation experiments and curves calculated from the theory of cross-linking. The upper curve is redrawn from Bolker and Brenner (1970), which also gives the source of the sulfite and dioxane lignin data. The solid circles are the results from the present work. The curves are calculated from the equation (Bolker and Brenner, 1970):

$$\bar{x}'_w = \frac{y\left\{1 - w_s\left(\frac{1 - w_s^{1/y}}{1 - w_s}\right)[1 + 2(1 - w_s^{1/y})]\right\}}{1 - (y - 1)\left\{w_s\left(\frac{1 - w_s^{1/y}}{1 - w_s}\right)[1 + 2(1 - w_s^{1/y})]\right\}}$$

where $\bar{x}_w'$ is the weight-average degree of polymerization of the lignin in solution, $\bar{w}_s$ is its yield, and y is the weight-average degree of polymerization of the basic chains.

cleaves bonds that are different from those cleaved by sulfite and other acidic reagents. In molecular terms, the net qualitative effects are similar: the release into solution of straight chains, branched chains, and microgel particles. The more of the lignin that is solubilized, the higher the molecular weight of the solubilized fragments. The smallest sizes of the fragments, however, depend on the reaction which produced them.

Taken together, the results presented so far in the present paper (Table III, Figures 2 and 5) are all in accord with the conclusion that periodate and cuoxam lignins, on treatment with Cl_2O, degrade in a pattern that suggests that these lignins are initially a three-dimensional, cross-linked, infinite network of gelled primary chains. Since, in addition, these lignins retain the morphological appearance of the cells from which they are derived and are completely insoluble without a chemical or physical treatment that results in fragmentation, it is a reasonable deduction that lignin in wood, which follows the same pattern, is a similar network.

An observation which would seem to stand in the way of interpreting the present results as according with the concept of lignin as a gel is the topochemical effect (Goring, 1971; Proctor et al., 1967). This effect is manifested in the difference between the rates of delignification of the secondary wall and the middle lamella by kraft and acid–sulphite liquors. In the early stages of delignification there is more rapid removal of most of the lignin from the secondary wall than from the middle lamella. The origin of this effect is not yet certain, but it can be explained if the lignin in the two morphological regions consists of gels differing only in ρ, the degree of cross-linking (larger in the middle lamella). In the course of degradation, the secondary wall would reach ρ_c, the critical value for degelation, well in advance of the middle lamella, and would be delignified first. Such a scheme, would require a slight alteration of the theoretical curves for X_w vs. W_s (Figure 5) to allow a molecular weight maximum or inflexion when the secondary wall lignin is just dissolved (somewhat above $W_s = 0.70$).

Control of Molecular Weight by Diffusion. Ahlgren et al. (1971) have suggested that in the delignification of wood, the cell walls exert a "sieving" action, and the size of lignin molecules extracted at any time during the process will depend on the size of the pores in the wall at that time. The size of the pores, in turn, seems to be correlated with the removal of hemicellulose from the secondary wall (Wood et al., 1972; Kerr and Goring, 1975). Nevertheless, the increase of molecular weight with increasing yield of extracted lignin cannot depend upon the size of the pores in the cell wall, since the experiments with Cl_2O were not conducted with whole wood, but with naked lignin, and the effects of polysaccharides were eliminated. At best, the presence of hemicellulose in the cell walls may affect the rate of delignification, but not its mechanism. It is indeed pertinent to note here that a previous account of the sulfonation of naked periodate lignin (Goring and Rezanowich, 1958) reported that its rate of solubilization was about the same as the rate of delignification of commercial wood chips, thus suggesting that diffusion processes had no gross effect on the rate of solution.

More recently, it has also been found (Kerr and Goring, 1976) that when pressure-refined black spruce fibers, with exposed middle lamella lignin on their outside surfaces, were pulped by the kraft process, the delignification of the exposed lignin occurred at the same rate as if it had been enclosed between cells. Furthermore, the curve of molecular weight vs. percent delignification of the lignin from the pressure-refined fibers coincided, within experimental error, with the curve for the lignin from black spruce wood chips. Accordingly it was concluded that the primary and secondary walls did *not* act as physical barriers inhibiting the diffusion of dissolved lignin, but that the topochemical effect "must" be caused by intrinsic properties of the individual morphological regions. We would interpret "intrinsic properties" as meaning the degree of cross-linking and the basic chain length.

CONCLUDING REMARKS

As long ago as 1952, Schuerch proposed for lignin-in-wood the architecture of a three-dimensional network polymer because he could not otherwise explain its insolubility in good solvents. Since then, Gupta et al. (1960) have pointed out that such an architecture would account for the molecular weight distribution of isolated lignins. Later, starting with the assumption that wood lignin is cross-linked gel, and using different aspects of the theories of synthetic gel formation (Flory, 1953), Szabo and Goring (1968) and Bolker and Brenner (1970) were able to duplicate by calculation the molecular weight/yield relationship of kraft, sulfite, and dioxane lignins. These latter results have led to the very important, but not yet generally accepted, conclusion that one need not invoke the "condensation" of lignin in order to explain the increase of molecular weight that occurs with increasing yield.

The present paper provides another example of the molecular weight/yield relationship. Although this re-

lationship is quantitatively different from those previously observed, it still represents additional evidence that the degradation of lignin follows the pattern of the degradation of a cross-linked polymer gel.

The starting materials and techniques employed in this investigation have provided evidence whereby experimental information already in the literature could be reevaluated. Although originally given other interpretations, this experimental evidence appears to be compatible with the conclusions of the present paper.

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