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Lignin Structure. XI. A Quantitative Study of the Alcoholysis of Lignin[1]

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The following changes occur on re-ethanolysis of ethanol–spruce lignins, isolated in 57% yield: a maximum of 42 to 57% of the guaiacylpropane units become ethoxylated by a rapid reaction which seems to be independent of the liberation of phenolic groups. New phenolic hydroxyl and α-carbonyl groups are generated by a slower cleavage reaction in nearly 1-to-1 molar ratio. The concurrent decrease in molecular weight indicates that the liberation of the phenolic group usually results in fission of the molecule but that some cyclic structures may be present. Both unconjugated phenols and phenols with a carbonyl in the α-position are formed by the cleavage. The α-carbonyl groups formed are mainly responsible for the change in the ultraviolet spectrum during ethanolysis. The rate of the phenolic liberation decreases more rapidly than would be expected if only a single structure were reacting. Only about 60% of the para-phenoxy groups in ethanol lignin are converted to free phenols by acid-catalyzed ethanolysis. An extensive decrease in oxygen content (0.3 to 0.6 oxygen atom per guaiacyl propane unit) takes place and is apparently due to independent intramolecular dehydration reactions. Most of these observations correspond closely to similar changes occurring on ethanolysis of guaiacylglycerol β-aryl ethers.

Alcoholysis is one of the important degradative reactions which can be used to dissolve lignin from the tissues of woody plants. Although the reaction can be considered formally analogous to the alcoholysis or hydrolysis of proteins or polysaccharides, there are marked differences between the degradation of lignin by this reaction and the degradation of other polymers. For example, the final products even from a softwood lignin, which apparently is derived almost exclusively from a single monomeric skeleton, are always a complex mixture of oligomers. Furthermore, that small fraction of lignin which has been degraded to propylguaiacyl monomers has had the functional groups on the aliphatic side chains extensively rearranged.

Four monomeric ethanolysis products from spruce lignin have been isolated and identified by Hibbert.[2] These are probably degradation and rearrangement products derived from a single structure, such as a derivative of α-oxyconiferyl alcohol[3] or guaiacylglycerol,[4–8] which exists as a part of the lignin macromolecule in wood. The reactions occurring in that part of lignin which resists degradation to the monomeric state are, however, less clear. The introduction of ethoxyl groups,[9] the formation of phenolic hydroxyl[10] and C-methyl groups,[11] the increase in structures capable of yielding benzene tetra- and pentacarboxylic acids by direct oxidation[12] or *m*-hemipinic acid by oxidation of the methylated product,[13] depolymerization reactions[14] and changes in ultraviolet absorption spectrum[15] have been observed qualitatively. Polymerization reactions also have been claimed to occur.[14]

This article reports the results of a study on the re-ethanolysis of ether-insoluble ethanol lignins, isolated under mild conditions[16,17] from spruce wood. The formation of monomeric products by re-ethanolysis from analogous preparations has been shown to be almost negligible (less than 3%).[14] Therefore, these results describe reactions which occur on that portion of lignin which remains almost exclusively as a complex mixture of low polymers.

It is quite possible that several independent reactions occur on re-ethanolysis of ethanol lignin. The interpretation of analytical data is therefore not as obvious as in the case of simple homogeneous materials. For example, if two functional groups are formed or destroyed in a simple molar ratio (1:1 or 2:1) after some period of time, it is not justified to conclude that the changes are caused by a single reaction. However, there is little likelihood that two independent reactions would have exactly the same rate and therefore if such a ratio is constant throughout the reaction it is very probable that the two molecular changes are caused by the same reaction. Therefore by following changes in analysis over a period of time, it is possible to distinguish which changes are independent and which are probably interrelated. As will be shown in detail, changes in light absorption and in number-average molecular weight may similarly be interrelated with changes in functional group content. For correlations of this type, it is obviously necessary to recover quantitatively and analyze carefully a number of samples allowed to react for varying periods of time. The method used in this investigation seems suitable for application to the reactions of many substrates, the homogeneity of which is doubtful.

It seemed advantageous to isolate fractions of the whole substrate as soon after solution as practicable in order to follow the ethanolysis through as large a fraction of the whole reaction as possible. Therefore samples SI, SII and SIII were isolated

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(2) E. West, A. S. McInnes and H. Hibbert, THIS JOURNAL, **65**, 1187 (1943).

(3) H. E. Fisher, M. Kulka and H. Hibbert, *ibid.*, **66**, 598 (1944).

(4) K. Freudenberg, *Forts. Chem. Org. Naturstoffe*, 8, Wien (1939).

(5) C. Gustafsson, K. Sarkanen, S. Kahila and E. Niskasaari, *Paper and Timber, Finland*, **33**, 74 (1951).

(6) E. Adler and B. O. Lindgren, *Svensk Papperstidn.*, **55**, 563 (1952).

(7) E. Adler and S. Yllner, *Acta Chem. Scand.*, **7**, 570 (1953).

(8) E. Adler and S. Yllner, *Svensk Papperstidn.*, **57**, 78 (1954).

(9) B. Holmberg and S. Runius, *Svensk Kemisk Tidskrift*, **37**, 189 (1925).

(10) E. Hagglund and H. Richtzenhain, *TAPPI*, **35**, 281 (1952); C. Schuerch, THIS JOURNAL, **72**, 3838 (1950).

(11) W. S. MacGregor, T. H. Evans and H. Hibbert, *ibid.*, **66**, 41 (1944).

(12) D. E. Read and C. B. Purves, *ibid.*, **74**, 120 (1952).

(13) H. Richtzenhain, *Svensk Papperstidn.*, **53**, 644 (1950).

(14) W. B. Hewson and H. Hibbert, THIS JOURNAL, **65**, 1173 (1943).

(15) R. F. Patterson and H. Hibbert, *ibid.*, **65**, 1869 (1943).

(16) C. Schuerch, *ibid.*, **74**, 5061 (1952).

(17) H. G. Arlt, K. Sarkanen and C. Schuerch, *ibid.*, **78**, 1904 (1956).

as ether-insoluble fractions of ethanol lignins in that order from a single batch of spruce wood meal by successive, mild ethanolyses. In all essential aspects, the characteristics of SII were intermediate between SI and SIII. The study on this sample was therefore less comprehensive than that of the other two fractions. Sample NI is a part of SI, separated from it by countercurrent distribution in 10% yield. It differs from the rest of sample SI by the fact that its phenolate is extracted from aqueous alkali by chloroform–ethanol. It is called NI to indicate its apparently "neutral" character. The "neutral" parts of SII and SIII were negligibly small and were not investigated separately.

Experimental

Preparation of Ethanol-Spruce Lignins.—The ethanol lignins used in this study were those prepared as described previously[17] by a three-step ethanolysis of Norway spruce woodmeal in anhydrous chloroform–ethanol (volume ratio 4/1) using 0.2 *N* hydrogen chloride as catalyst. The ether-insoluble powders SI, SII and SIII were extracted in that order and amounted to 28, 14 and 15% of the original Klason lignin. An ether-insoluble fraction poor in phenolic groups, NI, was separated in about 10% yield from SI, or 2.8% of the original Klason lignin, by countercurrent distribution between chloroform–alcohol and alkali as previously.[16]

became clear. The solution was dried with sodium sulfate and evaporated in water-pump vacuum under nitrogen atmosphere. Ten ml. of dioxane (freshly distilled over sodium) was added to the residue and evaporated again. The residue was dissolved in 8 ml. of dioxane and transferred for freeze drying into a test-tube (diam. 6/8", length 3.5") with a small indentation in the middle. The contents were thoroughly frozen in an acetone–Dry Ice-bath, placed inside the evaporation flask of a freeze-drying apparatus and the solvent sublimed for 21 hr. under oil-pump vacuum. The final drying was done *in vacuo* over phosphorus pentoxide at 40° for three days.

Because of the tendency of hydrogen chloride to evaporate from chloroform–ethanol, the ethanolysis stock solution was frequently rechecked by titration and the acid concentration corrected by adding a proper amount of a more concentrated ethanolysis solution.

Analysis of Samples.—The methods used for the determination of methoxyl, ethoxyl, phenolic hydroxyl content and ultraviolet absorption spectra have been given previously.[17] Carbon and hydrogen analyses were carried out by Dr. K. Ritter, Basel, Switzerland. The number average molecular weight determinations were made by Dr. Sonja K. Gross in this Laboratory using a cryoscopic determination in ethylene carbonate.[18] A standard Beckmann freezing point apparatus was used. Lignin concentrations were 2%, and molecular weights were found to be nearly concentration independent.

The analytical data are given in Table I. It may be noted that dissolving sample SI in cold ethanolysis solution and recovering it immediately caused a slight change in composition. Samples SII and SIII, on similar treatment, remained unchanged. It seems probable that, in the preparation of SI, the removal of carbohydrates was incomplete and therefore the data on SI_0 were used in subsequent calculations.

TABLE I

ANALYTICAL DATA ON ETHANOL LIGNIN SAMPLES

Sample	Time of re-ethanolysis, hr.	Carbon, %	Hydrogen, %	Methoxyl, %	Ethoxyl, %	Phenolic hydroxyl, %	Cryoscopic mol. wt.	Absorptivity,[a] l.(mole MeO^{-1}) cm.$^{-1}$ At 305 mμ	At 255 mμ	Formulas
SI	0	62.86	6.45	13.80	10.80	2.75	..	..	..	$C_9H_{8.2}O_{2.57}(OMe)_{0.93}(OEt)_{0.50}$
SI_0[b]	0	62.81	6.73	14.70	10.20	2.75	908	2560	3180	$C_9H_{8.7}O_{2.52}(OMe)_{0.99}(OEt)_{0.48}$
SI_1	4.1	...	..	14.55	11.75	3.38	800	3240	3490	
SI_2	11.2	...	..	14.45	11.70	4.06	743	3720	3760	
SI_3	17.6	64.99	6.80	14.75	12.30	4.31	787	4000	3930	$C_9H_{8.2}O_{2.07}(OMe)_{0.98}(OEt)_{0.56}$
SI_4	42.0	...	..	14.80	11.70	4.79	654	4620	4440	
SI_5	59.8	...	..	14.50	11.80	4.99	585	4910	4780	
SI_6	83.9	...	..	14.50	11.30	4.91	626	4900	4870	
SI_7	115.7	66.60	6.34	14.60	11.05	5.05	..	5340	5590	$C_9H_{7.3}O_{1.91}(OMe)_{0.93}(OEt)_{0.48}$
NI_0	0	64.0	6.66	14.46	10.4	1.03	955	..	..	$C_9H_{8.4}O_{2.31}(OMe)_{0.95}(OEt)_{0.47}$
NI_1	19.0	...	..	14.82	12.0	3.54	..	..	..	
NI_2	25.3	...	..	14.82	11.9	3.90	..	..	..	
NI_3	50.0	...	..	14.90	11.7	4.57	..	..	..	
NI_4	115.5	...	..	14.90	11.7	4.57	..	..	..	
SII	0	63.40	6.46	14.55	10.2	3.02	..	..	..	
SII_1	7.2	...	..	14.62	11.0	3.94	858	..	..	
SII_2	13.0	...	..	14.60	10.4	4.51	571	..	..	
SIII	0	62.57	6.23	14.55	8.69	3.11	1320	2980	3990	$C_9H_{8.0}O_{2.66}(OMe)_{0.97}(OEt)_{0.40}$
$SIII_1$	7.2	...	..	14.95	9.05	3.56	1127	3520	4470	
$SIII_2$	13.0	...	..	14.55	10.30	3.53	959	..	..	
$SIII_3$	46.7	64.58	6.30	15.20	8.85	4.28	828	4110	4850	$C_9H_{7.7}O_{2.24}(OMe)_{0.97}(OEt)_{0.39}$
$SIII_4$	91.7	...	..	14.68	8.95	..	734	4560	5440	
$SIII_5$	141.0	64.15	5.88	14.60	8.30	4.41	705	4610	5730	$C_9H_{7.1}O_{2.41}(OMe)_{0.94}(OEt)_{0.36}$

[a] Solvent: SI and SII series, 95% ethanol, containing 2% of dioxane; SIII series, dioxane. [b] Sample SI_0 is SI dissolved in cold re-ethanolysis solution and immediately recovered similarly to the reacted samples. SI_1, SI_2, etc., are samples of SI recovered after various periods of re-ethanolysis.

Re-ethanolysis Experiments.—Lignin samples (1 g.) were dissolved in 100 ml. of ethanolysis solution (0.38 *N* hydrogen chloride in chloroform–ethanol 4:1) and heated in sealed round-bottomed flasks for different periods of time in a constant-temperature bath adjusted to 65 ± 0.02°. The reaction was stopped by cooling the flask in crushed ice. The flask was opened and the contents shaken with a saturated sodium bicarbonate solution to neutral reaction and finally with distilled water. If some cloudiness appeared during the extraction, ethanol was added until the solution

Results

Changes in Ethoxyl Content.—The changes in

(18) S. K. Gross and C. Schuerch, *Anal. Chem.*, **28**, 277 (1956); S. K. Gross, K. Sarkanen and C. Schuerch, manuscript in preparation.

ethoxyl and phenolic hydroxyl content of samples NI, SI and SIII occurring during re-ethanolysis are shown in Fig. 1. First, it is obvious that the ethoxylation reaction has been completed in sample SIII before it is isolated from the woodmeal and is nearly complete in the fractions SI and NI isolated earlier. A rapid rate previously has been reported for the ethoxylation of black spruce "native lignin."[19]

The ethoxyl content reaches a maximum value of about 0.55 ethoxyl group per monomer unit in lignin SI after a few hours of re-ethanolysis and thereafter decreases slightly. The ethoxyl content of fraction SIII may also decrease very slowly on re-ethanolysis from an initial value of 0.41 unit per monomer.

While the ethoxyl content is thus remaining almost unchanged, the phenolic hydroxyl content is increasing substantially. It is clear, therefore, that these changes are not connected and the cleavage reaction at this stage of the process should properly not be called ethanolysis at all. The assumption that phenolic liberation and ethoxyl introduction are related during[20] the original isolation from wood therefore appears unlikely.

Liberation of Phenolic Groups.—Just as the ethoxyl content reaches a nearly constant value which is much less than one ethoxyl group per propylguaiacyl unit, the phenolic content also reaches a constant value of 0.55 to 0.65 phenolic group per monomer (Fig. 1). Since every propylguaiacyl unit contains either a phenoxy derivative such as a phenolic ether or a free phenolic hydroxyl group, there are present in the completely reacted samples 0.35–0.45 "unreactive" phenolic ether group per monomer unit that cannot be cleaved by the ethanolysis reaction.

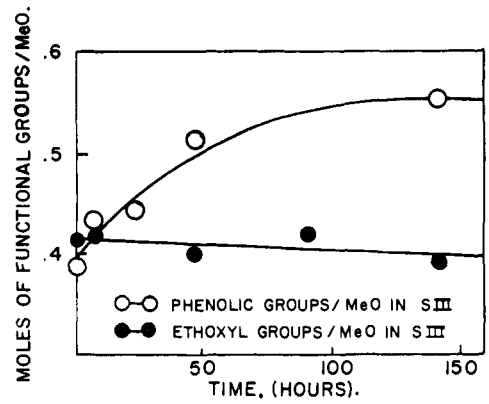
Some information regarding the structure of the "reactive" phenoxy derivatives in ethanol lignin can be obtained by a study of the rate of their ethanolysis. In most acid-catalyzed reactions the rate is known to be first order with respect to the substrate.[21] The rate constant k can therefore be computed using the equation

$$\ln (A_0/A) = kt$$

where A_0 and A are the amounts of the reactant at the start of the reaction and after time t, respectively. For the liberation of phenolic groups, A can be defined as the moles of "reactive" phenolic ether groups per mole methoxyl. These are not directly determinable. However, if no side-reactions occur, the number of "reactive" phenolic ether groups at the beginning of the reaction equals the total number of phenolic hydroxyl groups liberated after infinite time of reaction. The latter quantity can be estimated from the experimental data with a reasonable accuracy and the above equation thus assumes the form

$$\ln \frac{(\Delta C_6H_5OH)_\infty}{(\Delta C_6H_5OH)_\infty - (\Delta C_6H_5OH)_t} = kt$$

where ΔC_6H_5OH denotes the amount of phenolic groups liberated in moles per mole methoxyl, t is time in hours and subscripts ∞ and t refer to infinite time and time t, respectively.



Change in phenolic hydroxyl and ethoxyl contents in the re-ethanolysis of sample SIII.

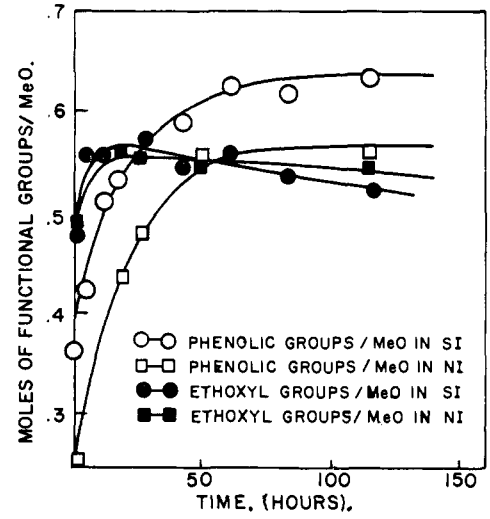


Fig. 1.—Change in phenolic hydroxyl and ethoxyl contents in the re-ethanolysis of samples SI and NI.

If the formation of phenolic groups in ethanolysis were due to the cleavage of one single reactive phenolic ether structure, the logarithmic plot of the data against time in Fig. 2 should form a single straight line, regardless of the ethanolic lignin preparation. This condition is obviously not fulfilled. The rate of liberation of phenolic groups in sample SIII is clearly less than the initial rate in sample SI or NI and the data on sample SI suggest that its first-order rate constant becomes less as the reaction proceeds.

The observed kinetic behavior would be expected if phenolic hydroxyl groups were liberated from two (or more) "reactive" phenolic ether structures. As the reaction proceeded the constant decrease in the ratio of rapidly reacting phenolic ether groups to those reacting slowly would result in a decrease in the over-all rate, as was observed in case of sample SI. Since sample SIII, when isolated, had been subjected to ethanolysis for a longer period of time than SI, it would be expected to contain proportionately less of the rapidly reacting groups and would have a lower rate as is observed.

(19) E. Adler and J. Gierer, *Acta Chem. Scand.*, **9**, 84 (1955).

(20) C. Schuerch, THIS JOURNAL, **73**, 2385 (1951).

(21) K. J. Laidler, "Chemical Kinetics," McGraw-Hill Book Co., Inc., New York, N. Y., 1950, p. 282.

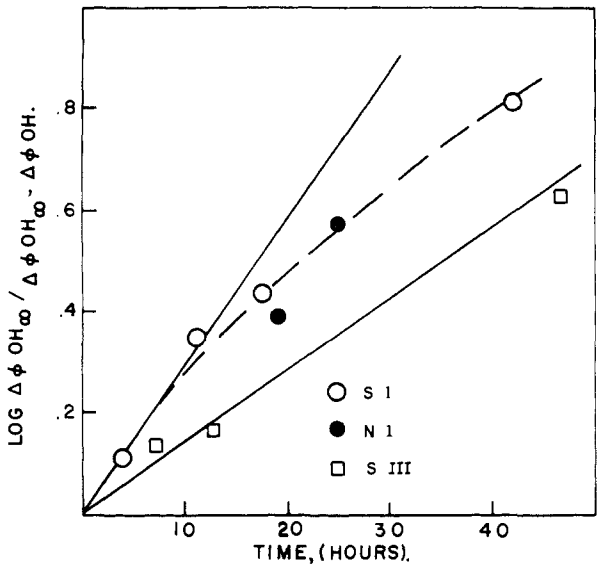


Fig. 2.—First-order rate diagram for the liberation of phenolic groups in re-ethanolysis.

The present results suggest that ethanol lignin (and perhaps spruce lignin in its native state) contains at least three different phenolic ether linkages between the monomeric units, one of which resists degradation by ethanolysis, while the others are cleaved at different rates. It is, therefore, not correct to assume that carbon-to-carbon linkages between monomer units are the only structural feature which prevents lignin from being degraded to monomers on ethanolysis (*cf.* ref. 14).

Nature of the Phenolic Groups Formed.—In order to determine what kind of phenolic groups were liberated during re-ethanolysis, each individual product from re-ethanolysis of SI was dissolved in neutral and alkaline solution, and the difference in the spectra of the two solutions was determined by the method of Goldschmid.[22] The height of the two maxima of the difference spectra of these products at various stages of reaction are given in Table II. The increase in the peak at

TABLE II

MAXIMA IN THE DIFFERENCE SPECTRA BETWEEN NEUTRAL AND ALKALINE SOLUTIONS OF REACTED SI SAMPLES

Time of reacn., hr.	Absorptivity, l. g.$^{-1}$ cm.$^{-1}$[a] At 300 mμ	At 350 mμ
0	0.45	1.80
4.1	1.27	4.01
13.0	0.96	6.49
17.5	..	7.55
59.8	0.40	11.60

[a] Because of the low solubility of samples 30% of ethanol was used in *p*H 12 and 6 buffer solutions.

350 mμ during re-ethanolysis can be interpreted[23] to mean that phenols with a carbonyl group in the α-position of the side chain are formed throughout the re-ethanolysis. The increase in the 300 mμ peak during the first 5-hour period of the same reaction indicates the formation of unconjugated phenols early in the reaction. The disappearance of the peak later may be due either to the formation of more α-carbonyl groups, from a previously unconjugated side chain, or may simply be due to the formation of more conjugated phenols, since the values of their difference spectra are generally negative in the 300 mμ region.[23]

Changes in the Ultraviolet Spectra.—Ultraviolet spectral changes occurring during ethanolysis were determined by subtracting the spectra of neutral solutions of the original lignin samples SI or SIII from the neutral solutions of the same products after re-ethanolysis for various periods of time. In both series the spectral change was greatest at 255 and at 305 mμ (Fig. 3a). Since there is little

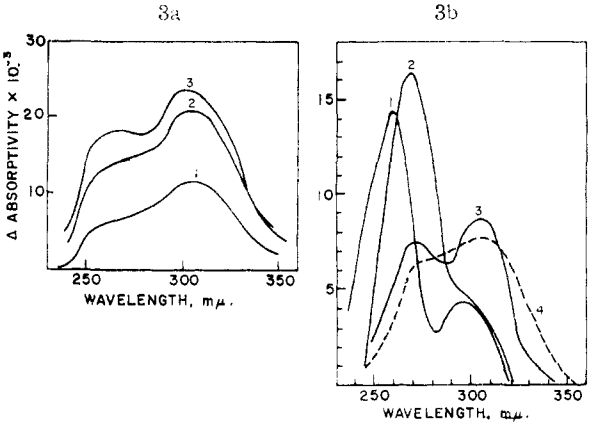
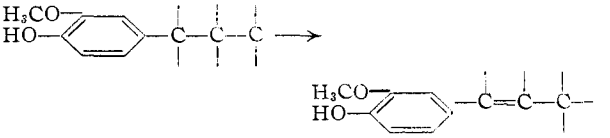


Fig. 3a.—Change in ultraviolet spectrum in the re-ethanolysis of sample SI; time of reaction: curve 1, 11.2 hr.; curve 2, 43.0 hr.; curve 3, 85.9 hr.

Fig. 3b.—Spectral changes caused by the introduction of a conjugated ethylenic bond (curves 1 and 2) or a conjugated carbonyl group (curves 3 and 4) to guaiacyl derivatives.

difference between the molar absorptivity of a phenol and its simple derivatives at these wave lengths, the change in spectrum must indicate the formation of another functional group than the phenolic hydroxyl. It must be unsaturated and conjugated with the aromatic ring because guaiacylpropane derivatives show absorption at wave lengths higher than 305 mμ only when such groups are present. In order to establish the nature of this group, comparison was made to similar spectral changes in known model compounds.

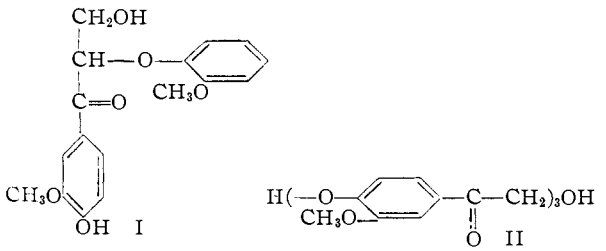
By subtracting the reported absorption spectrum of *p*-propylguaiacol from that of isoeugenol and of dihydro-dehydrodiisoeugenol from that of dehydrodiisoeugenol,[24] typical spectral changes were obtained for the transformation



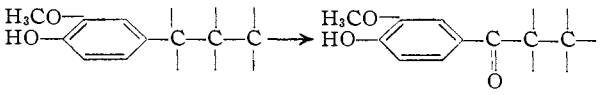
These spectral changes are shown as curves 1 and 2, respectively, in Fig. 3b. Similarly, by subtracting the reported spectrum of guaiacylglycerol-β-guaiacyl ether from that of ketone I[25] and of dihydroeugenol from that of II (on a methoxy basis), spec-

(22) O. Goldschmid, *Anal. Chem.*, **26**, 1421 (1954).
(23) G. Aulin-Erdtman, *Svensk Papperstidn.*, **56**, 287 (1953).
(24) G. Aulin-Erdtman, *ibid.*, **55**, 745 (1952).
(25) E. Adler, B. O. Lindgren and U. Saeden, *ibid.*, **55**, 245 (1952).

tral changes were obtained for the following trans-



formation (curves 4 and 3, respectively, in Fig. 3b).



The spectral change for the formation of a conjugated ethylenic bond in this series is characterized by a strong main peak at 260–270 mμ and a shoulder or low maximum at 300 mμ. The spectral change corresponding to the formation of an α-carbonyl, however, contains a main peak at 305 mμ and a somewhat lower maximum in the 270 mμ region (Fig. 3b) and is thus very similar to that produced during ethanolysis (Fig. 3). The absorptivity change at 305 mμ is therefore almost certainly due to the formation of an α-carbonyl group during ethanolysis.

Furthermore, when the change in absorptivity at 305 mμ (ΔA_{305}) is plotted as a function of change in phenolic content, a single linear correlation between the two changes is found for the products from both SI and SIII (Fig. 4). This appears to be strong evidence that a phenolic hydroxyl and an α-carbonyl group are produced in the same reaction.

It can be shown by the following reasoning that these two functional groups must also be produced in nearly equivalent amounts rather than in some non-stoichiometric ratio. The maxima of curves 3 and 4 (Fig. 3b) at 305 mμ are 8.7×10^3 and 7.8×10^3 l. mole^{-1}cm.$^{-1}$, respectively. The average of these two values, 8.3×10^3, gives the approximate change in absorptivity at 305 mμ, caused by the introduction of one mole of α-carbonyl in a guaiacylpropane derivative. On the other hand, the liberation of one mole of phenolic group in the ethanolysis of lignin corresponds to a change in absorptivity of 9.17×10^3 l. (mole $CH_3O)^{-1}$cm.$^{-1}$ (Fig. 4). By comparing this figure with the average value given above, it can be concluded that for every liberated phenolic hydroxyl group approximately 1.1 α-carbonyl groups are formed. The 10% variation between these numbers is well within the range of uncertainty introduced by ignoring those functional groups in the model substances which contribute less to ultraviolet absorption.

The formation of an α-carbonyl very largely explains the ultraviolet spectral changes during re-ethanolysis, but the rate of formation of the 255 mμ peak differs somewhat from that at 305 mμ. Furthermore, the 255 mμ peak continues to grow as long as ethanolysis continues while the change at 305 mμ is very slight in those later periods of ethanolysis during which there is little or no phenolic liberation. The 255 mμ peak must therefore be formed in part by another unexplained reaction.

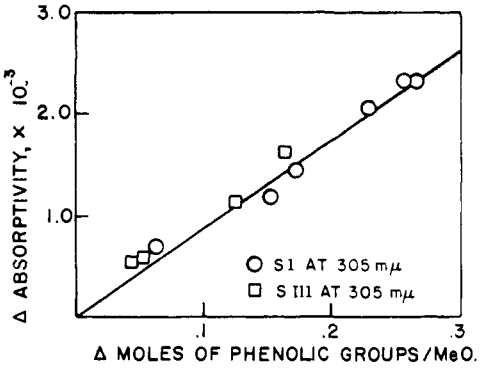


Fig. 4.—The relationship between the change in absorptivity at 305 mμ and the change in phenolic hydroxyl content in the re-ethanolysis of samples SI and SIII.

Decrease in Oxygen Content of Lignin.—The molecular formulas given in Table I show that quantitatively the most important reaction occurring during re-ethanolysis is one which results in a loss of oxygen. Sample SI over a period of 116 hr. loses about 0.6 atom of oxygen per guaiacylpropane unit and SIII loses about 0.3 oxygen atom. For comparison the total number of phenolic hydroxyl groups formed are 0.28 and 0.16 per guaiacylpropane unit. This reaction may be partially related to the increase in absorptivity at 255 mμ, but it is not possible to make any linear correlation such as was possible with phenolic hydroxyl liberation and change in absorptivity at 305 mμ.

Change in Molecular Weight (With Sonja K. Gross).—In the past, repolymerization reactions have been postulated to occur during lignin ethanolysis.[14] However, under our conditions of high dilution in a good solvent system, the number average molecular weight at first decreased and then, like the phenolic hydroxyl content, remained constant (Fig. 5). It seemed possible, therefore, that the fission of phenolic ether linkages might be the only reaction altering the lignin molecular weight.

Now in linear or branched polymers, it is obvious that a cleavage reaction will be accompanied by a decrease in number average molecular weight corresponding to the formation of two molecules from one. This relationship can be expressed by the equation

$$M_n = \frac{G_o \bar{M}_{no}}{G(1 + \Delta F_s \bar{M}_{no})} \qquad \text{(III)}$$

where G represents the content of some stable functional group (in the case of lignin, methoxyl expressed as per cent.), and ΔF_s is the *increase* in the number of moles of *product* functional group per gram (in the case of lignin ethanolysis, phenolic hydroxyls).

If during a cleavage reaction the molecular weight decreases less than this equation predicts, the molecular structure cannot be linear but must contain cycles of some size which are opened by the reaction, or side reactions must be occurring.

Theoretical number average molecular weights of re-ethanolized samples were calculated from the

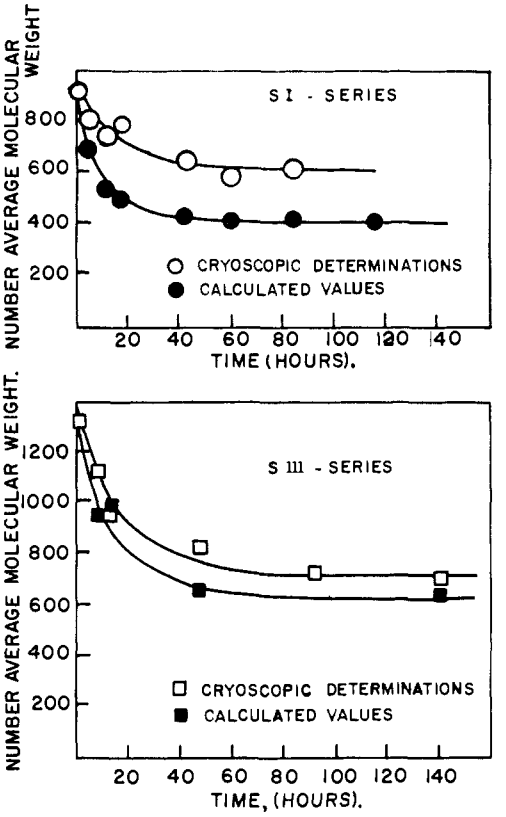


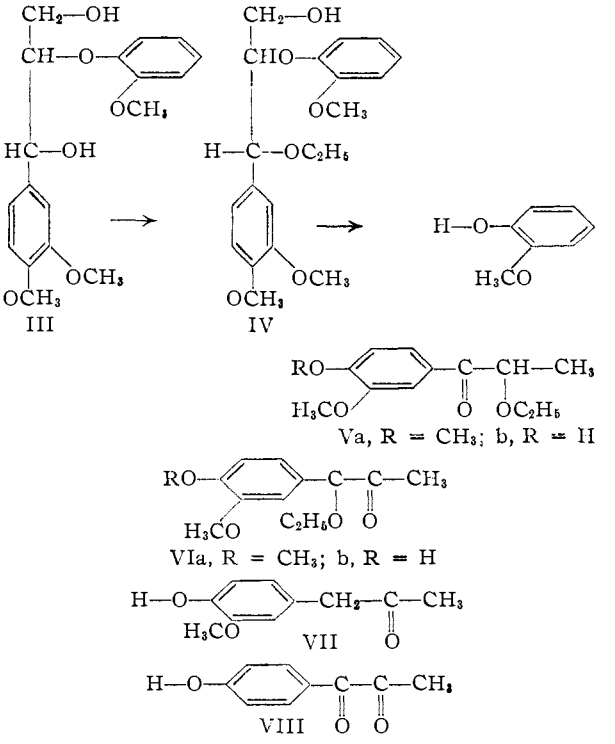
Fig. 5.—Cryoscopically determined number-average molecular weights compared with a hypothetical curve calculated from the change in phenolic group content.

experimental molecular weights of the starting materials SI and SIII and from the corresponding increase in phenolic content using the above equation and thus assuming that each liberation of a phenolic group resulted in the formation of two molecules from one. The calculated values were consistently lower than the experimental values (Fig. 5) although the changes followed each other closely. There may therefore be some cyclic structures initially present or new bonds formed during degradation. In any case, this method of gaining evidence of cyclic structures in lignin should be applied further as our knowledge of lignin reactions develops and molecular weight determinations of lignin are improved.

Discussion

The behavior of ethanol–spruce lignins on re-ethanolysis is rather closely analogous to that of guaiacylglycerol-β-aryl ethers. Compounds of this general structure have been proposed as the structural elements of lignin responsible for the formation of the ethanolysis monomers,[5-8] and they now appear to be satisfactory models for at least two of the reactions occurring on the amorphous portion of lignin.

Veratrylglycerol-β-guaiacyl ether (III) reacts with ethanolic hydrogen chloride first to form an ethyl ether, presumably IV, and later to form small amounts of guaiacol and the two phenylpropane derivatives V_a and VI_a and an amorphous product containing carbonyl groups.[6]



Veratrylglycerol reacts similarly.[8] From the ethanolysis products of guaiacylglycerol (Vb, R = H), VII and VIII have been isolated and probably VIb also is formed.[8] The careful work of Gardner[26] shows that ethanolysis of oxyconiferyl alcohol, a compound which may well exist as a reaction intermediate from guaiacylglycerol, produces all four lignin ethanolysis monomers V_b, VI_b, VII and VIII. Furthermore, the two compounds (Vb and VIII) with α-carbonyl groups amount to 83% of the monomeric products of ethanolysis.

This research shows that lignin re-ethanolysis gives results similar in many respects to the above. The ethoxyl content of lignin rises sharply during the initial phase of the ethanolysis (compare references 22 and 23). The ethoxylation of lignin is accompanied by a slower reaction in which a phenolic hydroxyl and an α-carbonyl group are formed in nearly equimolar proportions. The ethoxyl content of the lignin decreases slightly during re-ethanolysis as might be expected if a conversion like that giving rise to VII and VIII were occurring.

It is reasonable to assume that the same reactions here shown to be occurring on about one-fifth of the units in SI, SII and SIII on re-ethanolysis must have occurred throughout the extraction process as well, that a very substantial fraction of the phenolic groups liberated must have been liberated by the same reaction and that the liberation of monomers by ethanolysis followed a similar course. The best present model for these reactions of ethoxylation and cleavage seems to be the guaiacylglycerol-β-aryl ether system.

The fact that the liberation of phenolic groups was not kinetically homogeneous does not necessarily mean that the reacting structures were origi-

(26) J. A. F. Gardner, *Can. J. Chem.*, **32**, 532 (1954).

nally different, for a change in the side chain structure of a monomer unit could affect the rate of liberation of its phenolic group.

While it has been implied that the amorphous ethanol lignins are analogous to the reversion products produced on ethanolysis of III,[6] our results indicate that they are nearer to being incompletely reacted fragments of the original lignin since we obtained no direct evidence of an increase in molecular weight during ethanolysis.

There is, however, an unexplained loss of oxygen (0.6 atom per C_9 unit in SI) in the re-ethanolysis. The proposed cleavage of IV produces no over-all change in oxygen content, and therefore it seems likely that the oxygen loss may be occurring in those monomer units which do not react to produce a free phenolic hydroxyl group. If this is the case, these units lose nearly one atom of oxygen per C_9 unit. Since the formation of a conjugated ethylenic bond appears to be excluded by the ultraviolet spectra and the molecular weight data seem to exclude repolymerization, we postulate an intramolecular condensation reaction to account for this oxygen loss.

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The Constitution of Corn Starch Dextrin[1]

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Commercial corn starch dextrins have been fractionated and subjected to periodate oxidation studies and one of them has been examined by methylation. Periodate oxidation shows that the amount of glucose stable to oxidation is higher (5%) in the dextrin than in the parent starch. The hydrolyzate of the methylated dextrin has been shown to contain: 2,3,4,6-tetra-*O*- (16.5%); 2,3,6-tri-*O*- (57.3%), 2,3,4-tri-*O*- (2.6%), 2,4,6-tri-*O*- (1.2%), 2,3-di-*O*- (6.3%), 2,6-di-*O*-(10.0%), 3,6-di-*O*- (3.2%), 2-*O*- (1.5%), 3-*O*- (0.8%) and 6-*O*-methyl-D-glucose (0.5%). From the periodate and methylation data it is deduced that dextrinization is accompanied by considerable transglycosidation and the development of a highly branched structure.

In spite of the industrial importance of starch dextrins, prepared from native starches by roasting usually in the presence of acidic reagents, little is known about the detailed structural changes that the starch undergoes during the dextrinization process. In the early work[2,3] it was suggested that dextrins are formed by cleavage of the starch chains with concomitant anhydro-ring formation to give smaller molecules terminated by non-reducing units of the levo-glucosan type. Dextrinization of amylose and amylopectin also has been investigated by adsorption chromatography[4] whereby it was shown that amylopectin undergoes dextrinization more readily than amylose.[4,5] Methylation studies[5] enabled the deduction to be made that the dextrinization process caused breakdown of the starch molecules to smaller fragments which were much more highly branched than the original starch. This was recognized from the increased percentage of 2,3,4,6-tetra-*O*-methyl-D-glucose obtained from the methylated dextrin as compared with the yield from the methylated derivative of the parent starch.

This paper deals with periodate oxidation studies on a number of acid converted corn starch dextrins and methylation studies on one of them.

Four commercial corn starch dextrins were fractionated from aqueous solution with ethanol and the various fractions (see Table II) were examined by a procedure[6] involving periodate oxidation, reduction and hydrolysis. This procedure not only provides the usual information on the periodate consumption and formic acid production, but it also determines the percentage of glucose residues in the dextrin that are immune to periodate cleavage. Since the native starches contain less than 1% of glucose residues that are immune to periodate oxidation, it was tentatively concluded that the relatively high percentage of glucose residues in the dextrin that survived periodate oxidation was an indication of the structural rearrangements that occur during the dextrinization process.

On the basis of these periodate oxidation studies (see Tables I and II), the dextrin fraction chosen for further investigation by the methylation technique was one which was found[6] to contain the largest percentage of glucose units that were immune to periodate oxidation.

TABLE I

PERIODATE OXIDATION OF DEXTRIN

Reacn. time, hr.	Moles anhydrohexose per mole formic acid produced	Moles of periodate consumed per mole of anhydrohexose	Reacn. time, hr.	Moles anhydrohexose per mole formic acid produced	Moles of periodate consumed per mole of anhydrohexose
0	22.6	0.19	144	4.7	0.99
17	10.7	.45	241	4.7	0.99
45	5.5	.49	480	4.5	1.00
91	4.8	.87	700	4.3	1.00

The dextrin fraction, purified through its acetate, was exhaustively methylated with methyl sulfate and alkali in the usual way after which it was hydrolyzed. The composition of the mixture of methylated sugars so formed, as revealed by column[7] partition chromatography using buta-

(1) Paper No. 3696, Scientific Journal Series, Minnesota Agricultural Experiment Station.

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