inantly in the molecular weight range of about 500 to 10,000. However after 3 hr. when the delignification has been nearly completed, the molecular weights are found to range on up to around 100,000. Longer reaction time to a total of 10 hr. brings about a general shift in the cumulative distribution curve into a substantially lower range of molecular weights while still further treatment again shifts the curve but this time into a higher range of molecular weights.

These results, together with others previously obtained here and in other laboratories, are believed to provide additional evidence in support of the following concepts: (a) that lignins are branched chain polymers existing in woody tissue at least mostly as relatively large molecules, probably combined with each other and/or with carbohydrates to make three dimensional networks; (b) that the bonds between the lignin structural units are of at least two types, one relatively easily hydrolyzable in acidic aqueous solution and the other not; (c) that for removal of lignin from wood into acidic aqueous solution, hydrolysis of some linkages between structural units must occur to set free segments or "ζ-lignins" from the presumed proto lignin polymer and also these segments must have already been sulfonated or must become sulfonated to the degree necessary to permit them to dissolve in water; (d) that lower molecular weight lignins are first removed during delignification because the distribution of hydrolyzable and of hydrolyzed linkages may be approximately random, and the smaller ζ-lignins diffuse out from the wood residue into solution more rapidly than the larger fragments which are removed later with the result that the average molecular weight increases as delignification proceeds; (e) that acidic hydrolysis of the ζ-lignin sulfonates continues even after they have become dissolved in the aqueous medium so that although the average molecular weight observed for dissolved lignin sulfonates reaches a maximum at about the time that the delignification is completed, thereafter the molecular weight decreases as hydrolysis proceeds, (f) that the average molecular weight of the lignin sulfonates finally reaches a minimum value at which presumably all of the hydrolyzable linkages have been hydrolyzed to provide an "ω-lignin"; and (g) that an increase may then become evident in the average molecular weights of the lignins as a result of progress of irreversible condensation reactions, perhaps of the phenol-carbonyl type, which have presumably continued throughout the delignification process and can finally bring about condensation of the lignin sulfonates to a water-insoluble state.

Additional research is in progress toward the development of a quantitative formulation of these relationships.

SEATTLE, WASHINGTON

[CONTRIBUTION FROM PULP MILLS RESEARCH, DEPARTMENTS OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF WASHINGTON]

Lignin. IX. Molecular Weights of Lignin Sulfonates as Influenced by Certain Acidic Conditions

BY VINCENT F. FELICETTA AND JOSEPH L. MCCARTHY

RECEIVED SEPTEMBER 17, 1956

Lignin has been removed incrementally by treating hemlock wood in five steps with sodium bisulfite–sulfurous acid solutions of progressively increasing acidity. Low molecular weight lignins were removed first, and the later removed lignins were found generally to be of progressively increasing molecular weight. The lignin sulfonates in each increment were hydrolyzed in bisulfite–sulfurous acid solutions and a substantial decrease in molecular weights occurred. Generally similar results were obtained when purified non-dialyzable lignin sulfonates were hydrolyzed in acidic bisulfite–sulfurous acid solutions and also in hydrochloric acid solutions. Hydrolysis proceeds only to a limiting degree and condensation seems to occur simultaneously but more slowly. The new end groups may be carbonyl and phenolic hydroxyl.

Introduction

Many studies have been made of the removal of lignin from woody tissue,[1] and evidence has recently been submitted[2] indicating that as lignin is removed from gymnosperm woods using bisulfite–sulfurous acid solutions, relatively low molecular weight lignin sulfonates are first obtained in solution. Then, as the time of treatment is extended, the average molecular weight of the dissolved lignin sulfonates increases to a maximum, decreases to a minimum and finally begins to rise again. These changes are attributed to the effects of the following three processes which are thought to proceed simultaneously but at different rates: hydrolysis of hydrolyzable bonds in the lignin polymer, polycondensation of some lignin molecules with others and diffusion of soluble lignin sulfonates from the woody tissue into solution.

However, the usual delignification procedure which was used in our prior study comprised the heating of wood with aqueous bisulfite–sulfurous acid solutions in a closed autoclave or bomb. Under these batch conditions, the lignin sulfonates dissolved early in the period of reaction remain exposed to an acidic environment at elevated temperature for the remaining reaction time. In this situation hydrolysis and polycondensation reactions may be important. Thus the now-reported investigation was carried out using an "incremental" delignification method designed to minimize progress of these reactions during delignification. Experiments also have been conducted to establish

(1) F. E. Brauns, "The Chemistry of Lignin," Academic Press, Inc., New York, N. Y., 1952.

(2) E. Nokihara, M. J. Tuttle, V. F. Felicetta and J. L. McCarthy, THIS JOURNAL, **79**, 4495 (1957).

a basis for a study of the hydrolysis and polycondensation reactions in homogeneous solution.

Experimental

Incremental Delignification of Hemlock Wood.—Hemlock wood meal was prepared by passing hemlock wood shavings through a hammer mill. The resulting wood meal was exhaustively extracted with 2:1 ethanol–benzene followed by hot water and then air-dried and sieved to remove fines leaving a wood meal of mesh size 35 to 65. Analysis of this wood meal gave a methoxyl content of 4.62% OCH_3 on air-dried wood of 9.25% H_2O. Air-dried wood meal (12.8 g.) was used for the first delignification step, and each succeeding delignification was conducted on the wood residue from the preceding step. Delignification was carried out in sealed glass bombs at 130 ± 0.5° for 15 hr. except for the first step where an additional 2 hr. was used to slowly bring the temperature to 130°. The treating solution was prepared by passage of sulfur dioxide into an aqueous sodium hydroxide solution until the desired composition was attained as shown in Table I. The bombs were tumbled at about 25 r.p.m. in a stirred thermostated oil-bath.

TABLE I

INCREMENTAL DELIGNIFICATION[a] OF HEMLOCK WOOD

Characteristic	Treatment[b] or incremental sample number I	II	III	IV	V
Wood residue					
Weight, %	...	82.1	72.9	61.1	51.5
S, %	1.08	1.15	1.09	0.67	0.25
OCH_3, %	3.76	3.85	2.81	1.40	0.40
Delignfcn., % OCH_3 basis	(26.7)	36.3	55.6	81.3	95.5
Solution product					
*p*H	4.8	4.8	3.4	2.5	2.1
D_A 10^7 × cm.^{2} sec.$^{-1}$	25.5	20.1	14.9	9.5	12.1
Mol. wt.	3400	6600	14,500	30,000	20,500
Solution after hydrolysis[a]					
D_A, 10^7 × cm.^{2} sec.$^{-1}$	30.9	22.5	19.5	17.2	15.8
Mol. wt.	1900	4800	7200	10500	13000

[a] Conditions for the delignification and hydrolysis reactions are given in the Experimental part. [b] Treatments to secure samples I, II, III, IV and V were conducted using aqueous solutions containing the following proportions of "free" and "combined" SO_2: 2.5, 2.5; 2.5, 2.5; 2.6, 2.4; 2.75, 2.25; and 3.25 and 1.75 g. of SO_2/liter, respectively.

After each delignification step the wood residue was separated from the solution product by filtration, washed thoroughly with water, air-dried and weighed and then subjected to the next delignification step. A portion of each wood residue was set aside for sulfur and methoxyl and moisture analyses which were carried out by previously described methods.[3] Solutions obtained from each step were analyzed for methoxyl and characterized by measurement of diffusion coefficient, D_A.[4] Molecular weights were estimated from the diffusion coefficients[5] and are average values, M_A, related to the molecular weights of the individual species, M_i, by the approximate expression

$$M_A = [\Sigma(w_i)(M_i)^{0.5a}]^{2/a} \cong [\Sigma(w_i)(M_i)^{1.5}]^{0.67}$$

where w_i = weight fraction of i-th species, and since $a \cong 3$.

Hydrolyses of the lignin sulfonates were conducted at 130° for 15 hr. in glass bombs. For sample I the hydrolyzing medium was adjusted to 2.25% "combined" SO_2 and 2.89% "free" SO_2 whereas for samples II, III, IV and V the composition was 1.75% "combined" SO_2 and 3.25% "free" SO_2. Diffusion coefficients of the lignin sulfonates were also measured after hydrolysis.

Sulfonation of Lignin Sulfonates.—The non-dialyzable lignin sulfonates used in this investigation were secured by continuous exhaustive dialysis of an industrial sulfite spent liquor made from a mixture of about 85% western hemlock and 15% white fir woods. Determinations of total sulfur, methoxyl, strong and weak acids, and diffusion coefficient on the sodium salt of the non-dialyzable lignin sulfonates were conducted. The treating solution was prepared by passing sulfur dioxide into cold water and then adding solid sodium bisulfite to attain the desired composition. Thirty cc. of the non-dialyzable sodium lignin sulfonate solution was combined with 45 cc. of the concentrated resulfonation solution to give a final combined "SO_2" content of 0.75% and free "SO_2" content of 4.7% and sodium lignin sulfonate concentration of 37.4 g./l. Reactions were conducted in sealed glass tubes with rotation at 25 r.p.m. in an oil-bath at 130 ± 0.5° for varying periods of time as shown in Table II. A separate sample was used for each reaction period.

The products from the reaction were prepared for analysis by the following sequence of steps: (a) filtration to remove free sulfur, (b) de-ashing and steam-distilling to remove sulfur dioxide and convert sulfates to sulfuric acid, (c) preparation of barium salts by slow addition of barium carbonate with stirring, (d) centrifuging to remove barium sulfate and excess barium carbonate and finally, (e) exhaustive dialysis through a Visking cellophane membrane tubing against distilled water.

Measurements for total solids and diffusion coefficient were made on each product before and after dialysis. Determinations of sulfur, methoxyl and strong and weak acids were conducted on the solutions after dialysis. Determination of strong and weak acids was conducted by titrating the free acids with standard 0.1 *N* carbonate-free NaOH and following the progress of neutralization with a Leeds and Northrup glass electrode *p*H electrometer.

Hydrolysis of Lignin Sulfonates.—Acidic hydrolysis was conducted on two non-dialyzable lignin sulfonate preparations, A and B, obtained by exhaustive dialysis of gymnosperm spent sulfite liquors obtained from two different industrial sources. Reactions were carried out in sealed glass tubes in 0.01 *N* HCl solutions using several conditions of time, temperature and sodium lignin sulfonate concentra-

TABLE II

HOMOGENEOUS SULFONATION OF LIGNIN SULFONATES

Characteristic	Sulfonation time in hours or sample number 0	1	2	4	8	24	48
Solution product							
D_A × 10^7, cm.^{2}/sec.	7.5	9.2	12.5	13.5	13.9	16.5	15.2
Mol. wt.	44,000	25,000	19,500	17,000	16,000	12,000	14,000
Soln. after dialysis							
Wt. loss, %	~0	14	9	15	10	18	15
S, %	6.25	5.53	5.81	5.95	6.30	6.99	8.16
OCH_3, %	11.56	10.3	10.4	10.2	10.3	9.7	10.1
Strong acids, meq./g.	2.02	1.50	1.50	1.54	1.83	1.85	2.06
Weak acids, meq./g.	0.13	0.36	0.41	0.54	0.33	0.79	0.61
D_A, 10^7 × cm.^{2} sec.$^{-1}$	7.5	.....	9.4	9.5	9.9	11.1	14.0
Mol. wt.	44,000	.....	31,000	30,000	28,000	23,000	16,000

(3) Q. P. Peniston and J. L. McCarthy, THIS JOURNAL, **70**, 1324 (1948).

(4) V. F. Felicetta, A. E. Markham, Q. P. Peniston and J. L. McCarthy, *ibid.*, **71**, 2879 (1949).

(5) (a) J. Moacanin, V. F. Felicetta, W. Haller and J. L. McCarthy, *ibid.*, **77**, 3470 (1955), and (b) V. F. Felicetta, A. Ahola and J. L. McCarthy, *ibid.*, **78**, 1899 (1956).

tion as shown in Table III. Diffusion coefficients of the hydrolyzed mixture were determined and molecular weights were estimated from the diffusion coefficients.

TABLE III

HOMOGENEOUS HYDROLYSIS OF LIGNIN SULFONATES

Hydrolysis time, hr.	$D_A \times 10^7$, cm.²/sec.	Mol. wt.	Hydrolysis time, hr.	$D_A \times 10^7$, cm.²/sec.	Mol. wt.
(A: 40 g./l.; 131°)[a]			(A: 0.4 g./l.; 147°)		
0	7.4	45,000	0.17	9.6	30,000
1	9.9	28,000	.33	11.7	21,000
2	10.6	25,000	.67	14.2	15,500
4	11.7	21,000	1.50	14.7	14,500
8	13.4	17,000	3.00	15.4	13,500
(A: 40 g./l.; 146°)			(B: 40 g./l.; 146°)		
1	12.1	20,500	0.0	7.7	42,000
2	13.4	17,500	.25	8.8	34,000
4	13.5	17,000	.5	10.1	27,000
8	13.5	17,000	1.0	12.4	20,000
			2.0	13.3	17,000
(A: 4 g./l.; 145°)			4.0	13.9	16,500
0.17	14.3	26,500	8.0	14.0	16,000
0.5	14.4	15,500			
1.0	15.4	13,500			
2.0	15.4	13,500			
8.0	15.4	13,500			

[a] Hydrolysis reactions were conducted in 0.01 *N* HCl aqueous solutions at the indicated temperatures and sodium lignin sulfonate concentrations with preparation A or B. For Fig. 2, the samples are designated as, for example, A-40–131, which signifies preparation A treated at 40 g./liter and at 131°, etc.

Discussion

Hemlock wood has been delignified by successive treatments with five aqueous sodium bisulfite–sulfurous acid solutions of increasing acidity, *i.e.*, at *p*H 4.8, 4.8, 3.40, 2.51 and 2.09, respectively. After each treatment the solution was removed and the wood residue retreated with a fresh increment of bisulfite–sulfurous acid solution, and this procedure is somewhat similar to one followed by Freudenberg, Lautsch and Piazolo.[6]

Results of analyses of the several wood residues and solutions (Table I) show that about one-third of the lignin, as indicated by methoxyl groupings, can be removed from wood under weakly acidic conditions as previously observed by Abrahamson Lindgren and Hagglund.[7] Increasingly acidic solutions are required to remove the remaining lignin. The lignin sulfonates obtained in the first increment are found to be of relatively low average molecular weight, about 3000 as estimated by a diffusion method[4] calibrated by light scattering observations.[5a] The lignin sulfonates obtained in later increments proved to be of increasingly high average molecular weight up to about 30,000 for IV, but yet only about 20,000 for V.

Since it was thought that the disorderly molecular weight of V might have arisen as a result of progress of more extensive hydrolysis of lignin molecules in this increment compared with the others, further heating of each of the lignin sulfonate solutions in a more acidic environment was carried out. The average molecular weights of the lignin sulfonates were again estimated. They were now found to have decreased substantially as represented in Fig. 1 and, moreover, to increase progressively from the first to the last extracted increment.

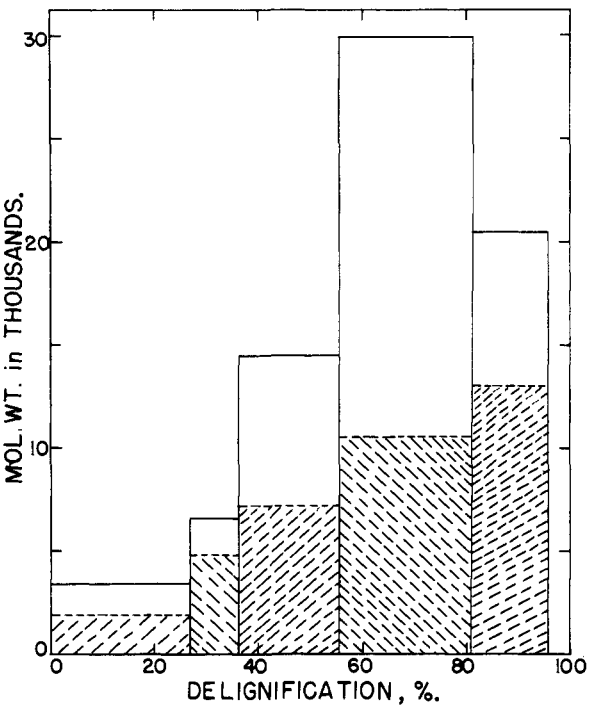

Fig. 1.—Estimated molecular weight of lignin sulfonates from incremental delignification and after hydrolysis (cross-hatched).

The weighted average molecular weight for the five lignin sulfonate preparations after hydrolysis, *i.e.*, for nearly the total lignin of the wood, is about 6000. After making allowances for the influences of the differences in types of molecular weight averages and also for the proportions of the total lignin examined, this value is believed to be in approximate agreement with a limiting value of about 3500 found by Gralen[8] and with the value of 3900 given by Loughborough and Stamm[9] and with quite a number of other average molecular weights of lignin preparations which have been reported in the literature.[1] However the molecular weights of these lignins are considerably higher than the values of around 1000 found in this Laboratory for the lignin sulfonates from maple wood,[2] and this difference may prove to be a characteristic distinction between gymnosperm and angiosperm lignins.

To observe the hydrolysis of lignin sulfonates in homogeneous aqueous solution in the substantial absence of sugars and other non-lignin substances, a non-dialyzable lignin sulfonate preparation was dissolved in sodium bisulfite–sulfurous acid solution adjusted to about *p*H 2 and then heated at 130° for various periods of time up to 48 hr. Average

(6) K. Freudenberg, W. Lautsch and P. Piazolo, *Cellulosechem.*, **22**, 97 (1944).

(7) B. Abrahamson, B. Lindgren and E. Hagglund, *Svensk Papperstidn.*, **51**, 471 (1948).

(8) N. Gralen, *J. Colloid Sci.*, **1**, 453 (1946).

(9) D. L. Loughborough and A. J. Stamm, *J. Phys. Chem.*, **40**, 1113 (1936); **45**, 1137 (1941).

molecular weights were estimated and were found to decrease progressively and to approach a limiting value of about 12,000 (Table II and Fig. 2) Lignins which have been hydrolyzed to this apparently limiting degree may be conveniently designated as "ω-lignins."

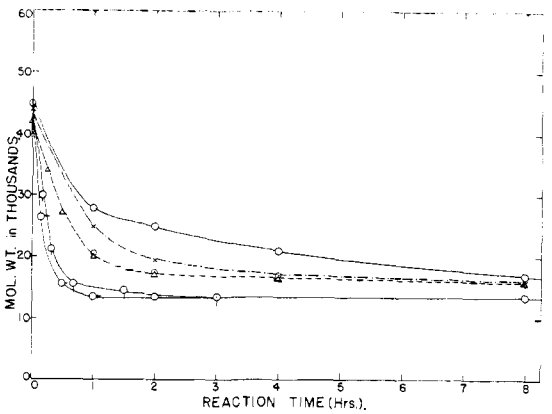

Fig. 2.—Estimated molecular weights of non-dialyzable lignin sulfonates after acidic treatments: ×, homogeneous sulfonation; O, A, 40–131; ◊, A, 40–146; ○, A, 4–145; ♀, 0.4–147; and △, B, 40–146. See Table III, footnote *a*, for interpretation of sample code.

For preliminary investigation of the gross changes which occur in lignin sulfonates during hydrolysis, each treated lignin sulfonate was exhaustively dialyzed and the non-dialyzable product was analyzed (Table II). The sulfur to methoxyl ratio increases from the initial value of 0.52 to a final value of 0.78 after 48 hr. Judging from the values found for "strong acid" groups, this increase occurs mostly as the result of increased sulfonation. The "weak acid" groups increase three- to sixfold, apparently because of formation of carboxylic or phenolic hydroxyl groups. From the estimated weight of the lignin sulfonates which passed through the membrane during dialysis, together with the average diffusion coefficients of the treated lignin sulfonates before and after dialysis, it is evident that the average molecular weights of these sulfonates which passed through the dialysis membrane must be small.

Preliminary hydrolysis experiments were also conducted under several conditions with aqueous solutions of hydrochloric acid, and average molecular weights and some other characteristics of the products were estimated (Table III and Fig. 3). With increasing reaction time, a decrease in average molecular weight is again found to occur and a limiting value is approached. For non-dialyzable lignin sulfonate preparations from two different sources, hydrolyses conducted at relatively high concentration (40 g./liter) gave rise to ω-lignin sulfonates with molecular weights in the range of 17,000 while hydrolyses at lower concentrations (4 and 0.4 g./liter) yielded products of substantially lower molecular weight, presumably because polycondensation reactions were minimized by dilution. An approximate fit to the present results can be obtained by assuming that the rate of hydrolysis is proportional to the concentration of unhydrolyzed hydrolyzable bonds. On this basis, values for reaction rate constants can be computed from the present data and correlated. However, for a serious treatment of the kinetics of the hydrolysis and condensation reactions of the lignin polymer, molecular weight distributions need to be known after various times of reactions under several different conditions. In this Laboratory such data are now being assembled and studied to provide a basis for a detailed statistical kinetic formulation.

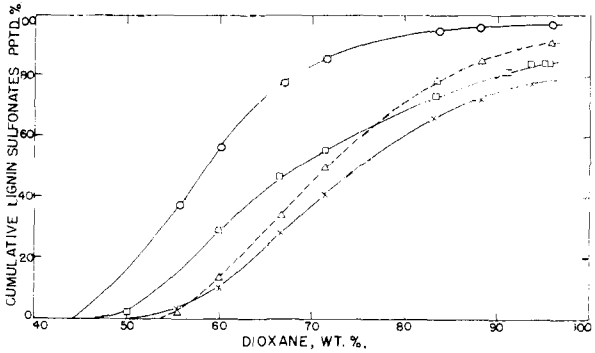

Fig. 3.—Precipitability of barium lignin sulfonate preparations in dioxane–water mixtures: whole sulfite spent liquor, □; dialyzable lignin sulfonate, ×; non-dialyzable lignin sulfonate, O; hydrolyzed lignin sulfonate (sample B, 40–146, 8 hr.), △.

The ultraviolet spectra of non-dialyzable lignin sulfonate preparation A and of its 8-hr. hydrolysis product (expt. A, 40–146) were ascertained. The spectra were very similar except that for the hydrolyzed sample the absorption minimum at about 2600 Å. was less pronounced and its absorptivity at 2800 Å. had increased by about 15%. For preparation B hydrolysis for 4 and 8 hr. (expt. B, 40–146) brought about increases of 70 and 86%, respectively, in the reducing substances calculated as glucose compared with the unhydrolyzed material. These changes may indicate formation of carbonyl end-groups as a result of the hydrolysis reaction, and the nature of the end-groups formed is now under study.

A non-dialyzable ω-lignin sulfonate (expt. B, 40–146, 8 hr.) was converted to a barium salt, dioxane was added in various proportions to aqueous solutions of the salt and the percentages of lignin precipitated were determined. Precipitation occurs over quite a wide range of dioxane concentrations (Fig. 3) which suggests that the preparation is rather polydisperse. Similar measurements were carried out with a non-dialyzable lignin sulfonate preparation which had not been hydrolyzed, and its precipitation at lower ranges of dioxane concentration may be a manifestation of the lower solubility of higher molecular weight lignin sulfonates in a less polar solvent medium. This interpretation is supported by the observation that precipitation of the relatively low molecular weight lignin sulfonates which had passed through a cellophane dialysis membrane took place only at the higher dioxane concentrations while the total lignin sulfonates in whole sulfite spent liquor were precipitated at intermediate concentrations of dioxane.

SEATTLE, WASHINGTON