(Skraup[2] recorded 122°). The same product was obtained when the reaction was repeated using ethyl hydrogen DL-galactarate and a reaction time of 3 min. The compound reduced hot Fehling solution.

Anal. Calcd. for $C_{14}H_{18}O_{10}$: C, 48.57; H, 5.21; CH_3CO, 37.3. Found: C, 48.66; H, 5.46; CH_3CO, 37.3.[13]

4-Acetoxy-6-ethoxycarbonyl-α-pyrone.—When the reaction mixture (from ethyl DL-galactarate lactone) described in the preceding experiment was heated at 100° for 90 min., the solution became a very dark brown. On pouring the mixture onto ice and water a brown solid was obtained which, having been washed and dried, was boiled with hexane. The dark color remained in the insoluble residue from which the solution was decanted. Long needles of 4-acetoxy-6-ethoxycarbonyl-α-pyrone separated from the cooled solution, yield 2.5 g., m.p. 68°. Pure material was obtained on one recrystallization from hexane, m.p. 69°. The substance was insoluble in water and was soluble in alcohol, ether, hexane and chloroform.

When ethyl hydrogen DL-galactarate (5 g.) was used as the starting compound, the same product was obtained, yield 1.5 g., m.p. 68°.

Anal. Calcd. for $C_{10}H_{10}O_6$: C, 53.20; H, 4.43. Found: C, 53.42; H, 4.48.

The compound decomposed slowly at room temperature, losing acetic acid, but it could be stored at 0°. It reduced warm Fehling solution; an alcoholic solution of it gave no color with ferric chloride. The compound dissolved in cold 10% aqueous sodium hydroxide to give a yellow solution. When hydrochloric acid was added dropwise until the solution was slightly acid, the yellow color became paler, and the resulting solution gave a deep-red coloration with ferric chloride, decolorized bromine water and reduced alkaline potassium permanganate.

Diethyl Tetra-*O*-acetyl-galactarate.—Diethyl galactarate (5 g.) was heated with acetic anhydride (15 ml.) and sodium acetate (1.5 g.) for 90 min. The solid obtained on decomposing the reaction mixture with ice and water was insoluble in hexane, and was recrystallized from methanol as needles of diethyl tetra-*O*-acetyl-galactarate, m.p. 195° (Skraup[2] recorded 189°).

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(13) The authors are indebted to Mr. A. Chaney of this Laboratory for the acetyl analysis.

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

Lignin. VII. Distribution in Molecular Weight of Certain Lignin Sulfonates[1a]

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Several lignin sulfonate preparations, representative of nearly all of the lignin in gymnosperm woods, have been fractionated and estimates have been made of the average molecular weights of the lignin sulfonates in the fractions. It is found that the molecular weights of the lignin sulfonates range from a few hundred to about one hundred thousand, average around ten or twenty thousand, and are distributed somewhat differently in the several preparations studied. An ordered change in the ultraviolet absorpton spectrum and presumably in phenolic hydroxyl content occurs with increase in the molecular weight. In fractions of the lowest molecular weight, the presence of several individual lignin sulfonates, or of closely related groups of lignin sulfonates, has been observed by use of an electrophoresis technique.

Introduction

Several investigations of the molecular weight of lignins have been carried out but the results so far reported[2-4] have in most cases been obtained using lignin preparations which seem to have been representative of only a part of the total lignin available from the wood, or to have been incompletely purified or inadequately fractionated.

Thus, one of the objectives of research conducted in this Laboratory over the last several years has been to develop methods by which molecular weights of lignins could be estimated under such conditions that the above-mentioned difficulties could be minimized or avoided. Investigations have been carried out with the sulfonate derivatives of lignin in part because (a) nearly all of the lignin in xylem tissue can be converted to these derivatives, (b) the conversion takes place under mildly acidic conditions whereby the possibilities of alkaline rearrangements are avoided although acidic condensation reactions may proceed to a limited degree,[5] and (c) almost the total lignin sulfonates are soluble and are thereby amenable to investigation by well established procedures. It has been found[2,6] that an aqueous solution of total lignin sulfonates and other wood components is separated by dialysis into dialyzable material consisting of some lignin sulfonates, sugars and other substances of low molecular weight, and also into non-dialyzable material consisting of higher molecular weight lignin sulfonates in a good state of purity. These non-dialyzable lignin sulfonates were fractionated[7] and the fractions proved to be of approximately constant composition and to manifest progressively different diffusion coefficients[8] as expected for a polymeric series of lignins. Recently, the molecular weights of some carefully purified and fractionated non-dialyzable lignin sulfonates have been determined by a light scattering method.[9] The diffusion coefficients of the same samples were also measured. The relationship found between the diffusion coefficients and the molecular weights, along with other evidence,

(1) (a) Presented in part at American Chemical Society Lignin Symposium in New York in September, 1950, and at the Pacific Northwest Regional Meeting in Eugene, Oregon, in June, 1955. (b) American Association of University Women and Fullbright Research Fellow. Urheilukatu 4, Helsinki-Toolo, Finland.

(2) (a) F. Brayns, "The Chemistry of Lignin," Academic Press, Inc., New York, N. Y., 1952; (b) N. Gralen, *J. Colloid Sci.*, **1**, 453 (1946).

(3) (a) E. Olleman and D. M. Ritter, THIS JOURNAL, **69**, 665 (1947); (b) E. Olleman, D. Pennington and D. M. Ritter, *J. Colloid Sci.*, **3**, 185 (1948).

(4) B. Ivarsson, *Svensk Papperstidn*, **54**, 1 (1951).

(5) V. F. Felicetta and J. L. McCarthy, unpublished research.

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

(7) A. E. Markham, Q. P. Peniston and J. L. McCarthy, *ibid.*, **71**, 3599 (1949).

(8) V. F. Felicetta, A. E. Markham, Q. P. Peniston and J. L. McCarthy, *ibid.*, **71**, 2839 (1949).

(9) J. Moacanin, V. F. Felicetta, W. Haller and J. L. McCarthy, *ibid.*, **77**, 3470 (1955).

gives support to the view that lignin molecules exist in solution as non-rigid and branched-chain polymers which are solvated and more or less randomly coiled.

While these prior studies have given considerable knowledge of the molecular weights of the non-dialyzable lignin sulfonates, little information is available concerning the dialyzable lignin sulfonates which may comprise nearly half of the total lignins. Thus, the presently reported experiments have been carried out to learn something of the distribution of molecular weights in the total lignin sulfonates available from the wood, including those of low as well as of high molecular weight. This has been done for several different lignin sulfonate preparations and some optical and electrophoretic properties have been evaluated for a number of the fractions obtained.

Experimental

Sample Preparation.—Five lignin sulfonate preparations designated as A, B, C, D and E were fractionated. A was a calcium sulfite spent liquor from a mixture of gymnosperm woods (about 85% Western Hemlock and 15% White Fir) which had been industrially fermented and spray dried to give a powder found by previously described procedures[6-8] to have the following analytical characteristics: 6.85% H_2O, 7.97% OCH_3, 8.96% reducing substances as glucose, 6.64% S, 0.53% sulfate as SO_3, 0.16% "free SO_2," 1.55% "loosely combined SO_2," 17.19% sulfated ash, 6.74% CaO, 0.105% N by Kjeldahl, 0.073% Fe, *p*H 4.23 in water at 110 g./liter, 2.31 meq. strong acids/g., 0.48 meq. weak acids/g., 17.2 cm.^{2}/sec. $\times 10^{-7}$ diffusion coefficient at 25.2°, 8.9 liters/g. cm. absorptivity at 2800 Å. This material was dissolved in distilled water (10 g. in 50 cc.), deashed using a column of cation exchange resin, and then extracted five times with equal volumes of ether. Then barium carbonate was added to the aqueous solution, the suspended barium sulfate and excess carbonate removed by centrifugation, and the solution was concentrated at reduced pressure to 56 ml.

B was secured from an authentic Western Hemlock (*Tsuga heterophylla*) tree cut by the authors, then debarked and deknotted. Bole wood was converted by use of a table saw to wood meal (10 to 35 mesh) which was air dried and placed in a large glass column and extracted exhaustively by continuous percolation with a solution fifty volume per cent. each in ethanol and benzene whereby 1.7 weight per cent. of the air dry wood was removed as extractives. The wood meal was washed with 95% ethanol to displace benzene and then extracted batchwise and nearly exhaustively with water at 80° whereby 1.1% of the air-dried wood meal was extracted. This extracted wood meal was air-dried and analyzed with the following results on an oven-dry basis: 6.14% H_2O and 5.24% OCH_3. Delignification to obtain B was carried out by sealing in a glass bomb 40 g. of air-dried extracted wood meal together with 360 ml. of an aqueous sulfurous acid–sodium bisulfite solution containing 50 g. SO_2/liter and 9.67 g. Na_2O/liter (4% "free SO_2" and 1% "combined SO_2") heating for six hours at 137–139°, then cooling and opening the bombs. After separation of the cellulosic residue, which amounted to 46 weight per cent. of the dry wood meal, the solution was concentrated under reduced pressure, deashed using Dowex-50 cation exchange resin, extracted five times with ether, treated with barium carbonate as described above for A, and finally analyzed with the following results: 57.8 g. total solids/liter, 11.8 g. reducing substances as glucose/liter, 17.2 g. sulfated ash/liter, with 5.43% OCH_3 in dry total solids.

C, D and E were obtained from the same extracted wood meal as was B and by use of a delignification solution of the same composition. Each sample was prepared in two glass bombs, each of which contained 20 g. of the wood meal and 180 ml. of solution. Delignification reactions to obtain C, D and E were carried out at 135° for 6.5 hours, 90° for 135 hours and 135° for 20 hours, respectively. The cellulosic residues were recovered by filtration, thoroughly washed with water, dried and analyzed to obtain for C, D and E the following values for percentage yield, and methoxyl in the residue: 44% and 0.03% OCH_3, 46% and 0.06% OCH_3, and 35% and 0.02% OCH_3, respectively. The solutions and washings for each of C, D and E were combined, concentrated under reduced pressure, deashed using a cation exchange resin, extracted five times with equal volumes of ether, neutralized to *p*H 5.5 with 1 *N* NaOH solution and then vacuum evaporated again to a volume appropriate for fractionation. Diffusion coefficients found for C, D and E are 18.5, 18.7 and 23.0 cm.^{2}/sec. $\times 10^{-7}$, respectively.

Fractionation Procedures.—For A, 13 ml. of the above-described solution (equivalent to 2.32 g. of the original solids) was added dropwise and with vigorous stirring to 345 ml. of dry ethanol in which was suspended 12.5 g. of a highly purified wood cellulose previously dry-disintegrated in a Koerner mill. This suspension was poured into a glass column (65 cm. high, 2.4 cm. i.d.) which had been tightly packed to 31 cm. height with 40 g. of the disintegrated cellulose and washed with ten column volumes of water followed by five column volumes of 95 volume per cent. ethanol. The cellulose on which the barium lignin sulfonates had been precipitated was also tightly packed. Through this column various ethanol–water mixtures of 250–500 ml. each were passed at about 15 ml./(sq. cm.)(min.) under a 2-meter liquid head, and column effluents were collected incrementally. Liquid associated with the initial suspension was drained from the column until the liquid level fell to the top of the packing; this collected liquid or the solids therein dissolved was called fraction 1. Then a 500-ml. volume of 95% ethanol was added to the top of the column, permitted to flow through the column until the liquid level again fell to the top of the packing; this collected liquid was called fraction 2. Similarly other volumes of progressively decreasing ethanol concentration were passed through the column and fractions were collected and numbered successively. The volume and ethanol concentration of each fraction was determined. The absorbance of 2800 Å. light was measured for each fraction as collected or after appropriate dilution and results are given in Table I.

B was treated similarly except with the following differences. Sixty ml. of B containing 29.5 g. of solids was precipitated onto 140 g. of Johns-Manville Co. Hyflo-Super-Cel suspended in 750 ml. of dry ethanol. The slurry was added to a 6.6-cm. diameter glass column containing filter aid in 95% ethanol to a height of 5.3-cm. with the result that the bed height became 19.4 cm., and then additional filter aid in 95% ethanol was added to bring the final total bed height to 24.3 cm. Alcohol–water solutions, starting at 95% ethanol concentration and decreasing by 2% concentration per day, were passed through the column at about 0.02 ml./(sq. cm.)(min.) and one fraction of about 800-ml. volume was collected per day. All A and B fractions were freed of ethanol by evaporation under reduced pressure to about 30 ml. and the barium salts were then converted to sodium salts by ion exchange on Amberlite IR 100 columns and made to 100 ml. with water.

C, D and E fractionations were carried out using a 50-ml. aliquot of each sample which contained about 10 g. of sodium lignin sulfonates and sufficient sodium chloride to provide a 0.1 *N* NaCl solution. To each sample 500 ml. of absolute ethanol was added dropwise and with stirring to yield a system 90% ethanol by volume. The suspension was vigorously stirred in a 25° water-bath for 15 minutes, centrifuged at about 2500 r.p.m. for 10 minutes, and then the clear supernatant solution was decanted off and designated as fraction 1. The precipitated and settled solids were redissolved in water, more sodium chloride was added to provide a 0.1 *N* NaCl solution, and then absolute ethanol was again added as described above but in amount sufficient to bring the ethanol concentration to 85% by volume. This suspension was centrifuged and the clear liquid decanted to yield fraction 2. This procedure was repeated at progressively lower final ethanol concentrations to provide additional fractions. After being freed of ethanol and concentrated by evaporation under reduced pressure, the C, D and E fractions as well as those from A and B were saturated with toluene and stored in a refrigerator until examined further at which time the toluene was removed.

Characterization Methods.—Absorption spectra for the components in the several fractions, as sodium salts in aqueous solution at a concentration of about 50 mg. total solids/liter, were determined from 2500 to 3100 Å. using a

TABLE I

SOLUTION FRACTIONATION OF BARIUM LIGNIN SULFONATES A

Fraction	Ethanol concn.,[a] vol. %	Cum. u.v.[b,c,d] collected, %	D,[e] 10^7 cm.^{2}/sec.	Mol. wt.
1	95	0.3	..	.....
2	95	1.7	52	400
3	92	5.8	52	400
4	89	7.6	48	500
5	84	9.1	42	750
6	82	11.6	40	850
7	79	14.5	39	940
8	77	16.1	34	1,400
9	77	17.5	34	1,400
10	72	24.4	30	2,100
11	67	27.9	28	2,500
12	67	30.8	27	2,900
13	62	46.6	20	7,000
14	59	53.6	17	10,000
15	58	57.9	16	11,000
16	58	61.2	15	12,000
17	58	63.5	14	13,000
18	52	76.3	10	25,000
19	47	81.4	10	25,000
20	47	82.4	10	25,000
21	41	87.8	8.9	31,000
22	36	92.2	7.0	48,000
23	36	94.5	6.8	50,000
24	30	96.2	6.6	53,000
25–26	25	97.5	5.4	76,000
27	6	99.1	5.3	78,000
28–29	0	101.0	3.7	150,000

[a] The ethanol solution which had passed through the column was collected in increments of about 250 or 500 ml. volumes, and concentration of ethanol therein was determined with results as shown. [b] Cumulative ultraviolet absorption for 2800 Å. is reported as percentage of the total original ultraviolet in sample A. [c] The shift in the ultraviolet absorption peak between *p*H 5 to 12 amounted to 145, 75, 25, 15, 10 and 5 Å. for fractions 4, 7, 11, 14, 17 and 21, respectively. [d] Cumulative percentages of reducing substances, based on the total originally present, amounted to 6, 37, 44, 47, 50, 55, 64, 68, 70 and 71 for fractions 1, 2, 3, 4, 5, 10, 15, 20, 22, 28–29, respectively. [e] Diffusion coefficients reported here were measured in 0.02 *M* NaCl solution at 25.2°.

Beckman model DU spectrophotometer. A fractions were examined in unbuffered water solution and, in some cases, in 0.1 *N* NaOH solution. B fractions were examined at *p*H 5 (0.2 *N* acetate buffer) and also at *p*H 12 (0.05 *M* phosphate buffer). C, D and E fractions were examined in unbuffered water solution only.

Reducing substances calculated as glucose were determined on all fractions from A, on the first nine fractions from B, but not on the C, D or E fractions. Reducing substances attributable to sugars are found in the first seven or eight A and B fractions. Recovery of reducing substances from A was incomplete probably because of microörganism action, but sugar components were not investigated further in view of other work done in this Laboratory.[10]

Diffusion coefficients were determined by a previously described solution-to-gel method[8] in which diffusion proceeded at 25.2° into an agar gel from an aqueous solution containing sodium lignin sulfonates at about 200 mg./liter and also 0.02 *M* KCl. The components diffused into a gel made using purified agar at 0.6% in 0.02 *M* KCl and held in a cell made from silica microscope slides through which 2800 Å. light was passed for observation of absorption at various distances from the boundary. Graphs on "probability paper" of this distance *versus* the ratio of position concentration to boundary concentration proved to be nearly straight lines for most of the fractions investigated. For fractions with diffusion coefficients less than 9×10^{-7} cm.^{2}/sec., a specially purified agar was used at 0.37% concentration in 0.02 *M* KCl to avoid hindrance to the diffusion of large lignin sulfonate molecules. The substantial absence of a hindrance effect in each determination was established by the agreement found between the absorbance actually measured in the liquid phase from which diffusion was occurring with the absorbance estimated for the liquid phase by extrapolation to the boundary of the absorbances measured in the gel phase at various distances from the boundary.

Molecular weights were estimated from the diffusion coefficients measured for the lignin sulfonates by using a previously reported correlation[9] expressed as two empirical equations of the form

$$M = aD^{-b}$$

where the constants a and b have values of 5.4×10^{-14} and 8.1×10^{-7} and of 3.00 and 1.75, respectively, when the diffusion coefficient is above or below 18×10^{-7} cm.^{2}/sec., *i.e.*, when the molecular weight is below or above ten thousand.

Electrophoresis patterns were determined for the ultraviolet absorbing components present in the relatively low molecular weight fractions B-2, B-4, B-6 and B-8. The migration was conducted using a previously described procedure.[11] The solution was buffered with 0.12 *M* acetate buffer at about *p*H 4.6 and migration was carried out for 5 hours at 10–15° with 13.2 volts/cm. voltage gradient in a gel containing 0.7% purified agar with initial lignin sulfonate concentration at 250–350 mg./liter in the sample section. When obvious peaks were evident in the patterns obtained, electrophoretic mobilities have been calculated and corrected to 15° and are shown in Fig. 3.

Discussion

Samples A and B were fractionated by adding ethanol to precipitate the barium lignin sulfonates from aqueous solution onto the surface of cellulose or kieselguhr particles packed in a glass column, and then selectively dissolving the lignin sulfonate fractions by passing through the column aqueous solutions which were at first concentrated and later increasingly dilute with respect to ethanol (Tables I and II). The ultraviolet absorbance of each collected fraction was determined and the sum of these absorbances was found equal to that of the initial sample A, but not of the initial sample B of which about 10% was almost irreversibly sorbed on the kieselguhr column packing. Diffusion coefficients for the fractions were also determined and were used to estimate molecular weights of the components absorbing ultraviolet light. Similar examinations (Table III) were made of the sodium lignin sulfonate fractions obtained from samples C, D and E by a fractional reprecipitation method.

The cumulative percentages of ultraviolet absorbance have been plotted against the molecular weights indicated for the fractions, and lines have been drawn through the incremental results to obtain the integral molecular weight distribution curves shown in Fig. 1. The weight percentages of lignin sulfonates in the various molecular weight ranges are believed to be about the same as the indicated ultraviolet absorbance percentages because the absorptivity of lignin sulfonates with respect to ultraviolet light has been found in prior work[7,9] to be approximately constant over a wide range in molecular weights.

(10) P. K. Mulvaney, H. D. Agar, Q. P. Peniston and J. L. McCarthy, THIS JOURNAL, **78**, 1255 (1951).

(11) Q. P. Peniston, H. D. Agar and J. L. McCarthy, *Anal. Chem.*, **23**, 994 (1951).

TABLE II

SOLUTION FRACTIONATION OF BARIUM LIGNIN SULFONATE B

Fraction	Ethanol concn.[a]	Cum. u.v. collected,[b,c,d] %	D,[e] 10^7 cm.^{2}/sec.	Mol. wt.	Absorptivity ratios 2800 Å./min.	2800 Å./3100 Å
1	93	1.3	60	260	2.43	4.44
4	91	4.2	55	330	1.92	4.21
7	85	7.2	47	510	2.00	4.76
10	80	11.8	40	830	1.86	5.06
13	74	18.5	36	1,200	1.70	4.55
16	67	28.4	27	2,900	1.58	4.20
19	61	44.1	20	7,100	1.47	3.89
22	55	57.7	17	10,000	1.40	3.73
25	48	65.6	15	14,000	1.38	3.59
28	42	70.4	13	16,000	1.36	3.42
31	34	73.4	14	15,000	1.31	3.39
34	27	74.8	13	16,000	1.28	3.51
37	13	76.9	12	20,000	1.35	3.46
40	1	84.6	7.2	46,000	1.30	2.92
43	0	89.2	5.6	70,000	1.27	2.69

[a] The ethanol solution which had passed through the column was collected in increments each of about 750-ml. volume and the concentration of ethanol and the ultraviolet absorption in each was determined. Diffusion coefficients and absorptivity ratios were determined for every third fraction. [b] Cumulative ultraviolet is reported for 2800 Å. as percentage of the total original absorption of the sample. [c] The shift in the ultraviolet peak between *p*H 5 to 12 amounted to 125, 95, 85, 75, 45, 25, 15, 5 and 5 Å. for fractions 1, 4, 7, 10, 13, 16, 19, 22 and 25, respectively. [d] Cumulative percentages of reducing substances, based on the total originally present, amounted to 24, 48, 61, 70, 75, 78 and 81 for fractions 1, 2, 3, 4, 5, 7 and 9, respectively. [e] Diffusion coefficients were measured in 0.02 *M* NaCl at 25.2°.

molecular weight relationship which occurs with change in molecular weight.

The results indicate that the molecular weights of lignin sulfonates range from a few hundred to about one hundred thousand. The distributions of molecular weight were similar in samples A and B although the former was prepared under industrial conditions from mixed species of wood while the latter was obtained in the laboratory from an authentic sample of Western hemlock wood.

The distribution found for sample C, which was obtained by conducting the delignification in the laboratory for 6.5 hours at 135°, was also very similar to that observed for samples A and B, but the distributions found for D (135 hours, 90°) and E (20 hours, 135°) were somewhat different although the same procedure for fractionation was used for each of the three samples. In E, the acidic hydrolysis of the lignin polymer molecules[12] had apparently proceeded extensively because the proportion of the higher molecular weight lignins had substantially decreased and had given rise to an increased proportion of lignins in the other molecular weight ranges. However, for D, relatively high molecular weights were found over the whole range, perhaps because the acidic hydrolysis of the lignin polymer molecules had been minimized by conducting the delignification reaction at a low temperature.

Also shown in Fig. 1 are curves F and G which represent the results previously obtained in this Laboratory for two fractional precipitation separations of non-dialyzable lignin sulfonates. In both

TABLE III

REPRECIPITATION FRACTIONATION OF SODIUM LIGNIN SULFONATES C, D AND E

Fraction	Ethanol concn.,[a] vol. %	Sample C: Cum. u.v., %	Sample C: D,[b] 10^7 cm.^{2}/sec.	Sample C: Mol. wt.[c]	Sample D[a]: Cum. u.v., %	Sample D[a]: D,[b] 10^7 cm.^{2}/sec.	Sample D[a]: Mol. wt.[c]	Sample E: Cum. u.v., %	Sample E: D,[b] 10^7 cm.^{2}/sec.	Sample E: Mol. wt.[c]
1	90	10	50	440	7	34	1,400	11	48	490
2	85	24	35	1,200	16	28	2,500	23	33	1,500
3	80	30	32	1,700	25	23	4,600	32	26	3,200
4	75	46	23	4,500	37	17	10,000	94	11	22,000
5	70	70	15	13,000	74	12	19,000	99.0	9.5	28,000
6	65	93	11	23,000	78	11	21,000	99.6	8.8	32,000
7	60	100	6.3	58,000	98	6.3	55,000	99.9	7.6	41,000
8	55	..	..	....	100	4.7	96,000	100	5.6	70,000

[a] For sample D, the ethanol concentrations used were 90, 86, 82, 78, 74, 70, 65 and 60. [b] Diffusion coefficients reported here were measured in 0.02 *M* NaCl solution at 25.2°. [c] Ratios of absorptivities at 2800 to 2600 Å. for fractions 1–8 were found to be 1.90, 1.83, 1.72, 1.58, 1.53, 1.43, 1.23 and — for sample C; 2.14, 1.94, 1.79, 1.68, 1.54, 1.53, 1.46 and 1.36 for sample D; and 1.12, 1.11, 1.08, 0.99, 0.98, 0.88, 0.84 and 0.57 for sample E, respectively.

For samples A, C, D and E, average molecular weights calculated by weight averaging the molecular weights estimated for the individual fractions, *i.e.*, $\bar{M}_w = \Sigma w_i M_i$, were found to be 19,000, 13,500, 23,000 and 16,000. These may be compared with the values of 13,000, 10,000, 17,000 and 14,000, respectively, calculated by obtaining the D_A average for the fractions, *i.e.*, $D_A = [\Sigma w_i D_i^{-0.5}]^{-2}$, and then estimating a type of an average molecular weight by use of the equation given above in the Experimental part. Use of the weight average molecular weight is to be preferred because the average molecular weight obtained from use of the D_A average represents only in a complex way the influence of the change in the diffusion coefficient–

cases the proportion of lignin sulfonates which passed through the dialysis membrane has been taken into account so that the ordinate of the curve is the estimated cumulative weight percentage lignin based on the whole ultraviolet absorbing material brought from the wood into the aqueous solution. Curve F was obtained by carrying out the usual fractional precipitation procedure on barium lignin sulfonates using an ethanol–water system,[7] while curve G resulted from use of the improved "reprecipitation" procedure[9] also used for samples C, D and E. F and G differ somewhat from each other and from the other samples studied. To develop some understanding

(12) V. F. Felicetta and J. L. McCarthy, unpublished work.

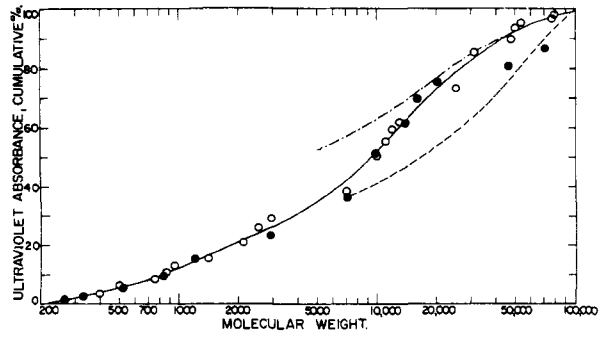

Fig. 1.—Integral molecular weight distribution curves for lignin sulfonate preparations: O, A fractions; ●, B fractions; - - -, sample F; and —·—, sample G.

of the reason for these differences, an investigation of the nature and kinetics of the reactions which control the distribution in molecular weights of lignins has been undertaken in this Laboratory.

For samples A and B, estimates were made of the proportion of copper-reducing substances calculated as glucose present in the fractions and these results are given in footnotes to Tables I and II. Most reducing material is separated with the fractions dissolved in solutions high in ethanol content and these reducing substances are known to be mostly sugars.[10,13] However in the fractions obtained later at lower concentrations of ethanol, there persists a small but appreciable reducing capacity which probably arises from the carbonyl groupings on the lignin sulfonates.

The ultraviolet absorption spectra of all lignin sulfonates in neutral aqueous solutions were generally similar and it was observed that the ratio of maximum absorptivity at about 2800 Å. to minimum absorptivity at about 2600 Å., or to the absorptivity at 3100 Å., changed in an ordered way with the molecular weights of the lignin sulfonates as shown in Fig. 2.

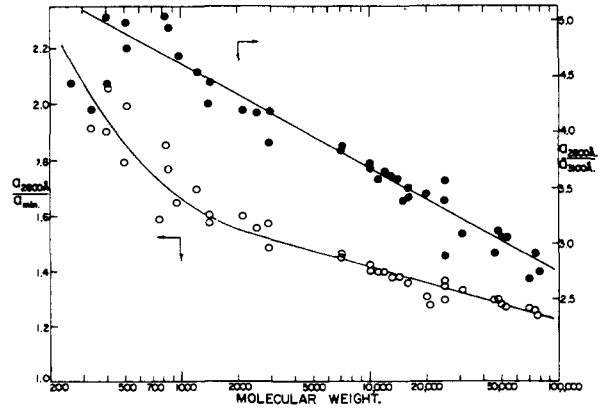

Fig. 2.—Relationship between absorptivity ratios and molecular weights for "A" and "B" fractions.

When the spectra of some of the A and B lignin sulfonates were determined in aqueous solution at *p*H 12 instead of *p*H 5 it was found that a bathochromic shift had occurred in the wave length of maximum absorption, as shown in the footnotes to Tables I and II. This type of shift was found by Lemon[14] to occur when various pure phenolic substances became ionized. Aulin-Erdtmann[15] found that similar shifts occurred with Braun's lignin, and with lignin sulfonates derived therefrom, but not with lignins representative of the total lignins in the wood. Recently Aulin-Erdtmann[15c] and Goldschmid[16] nearly simultaneously proposed that the absorption observed in neutral solution should be subtracted from that in alkaline solution to obtain a "difference spectrum," and they studied various known pure phenols and evaluated molar absorptivities for the difference spectra peaks presumably characteristic of the phenolate ion resonating systems. In view of these findings, we have calculated difference spectra by Goldschmid's method and used his "difference absorptivity" ($\Delta\epsilon$ = 4100 liters/mole cm.) to obtain estimates of the lignin sulfonate molecular weight equivalent to each non-conjugated phenolic hydroxyl group in several lignin sulfonates as follows; fractions A-4, -7, -11, -14, -17 and -21 yielded 420, 455, 740, 990, 1340, 1560 and fraction B-1, -4, -7, -10, -12, -15, -19, -22 and -25 yielded 360, 430, 430, 430, 430, 600, 830, 890 and 920. These data suggest that phenolic hydroxyls may be end groups on some lignin polymer branched chains.

Several fractions of the B lignin sulfonates in the lowest molecular weight range have been examined at *p*H 4.7 by an electrophoresis method[11] and the patterns obtained are shown in Fig. 3. For B-2 and B-4, four fairly narrow major peaks are evident which indicate the presence of four major

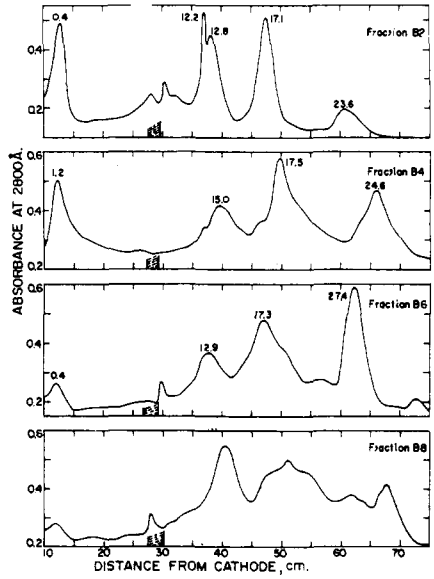

Fig. 3.—Electrophoresis patterns for low molecular weight lignin sulfonates. Cross hatched area denotes sample section. Numbers near peaks indicate experimental values for mobilities in cm.²/volt second at 15°.

(13) E. Hagglund, F. W. Klingstedt, T. Rosenquist and H. Urban, *Z. physiol. Chem.*, **177**, 248 (1928).

(14) H. W. Lemon, THIS JOURNAL, **69**, 2998 (1947).

(15) G. Aulin-Erdtmann, (a) *Tappi*, **32**, 160 (1949); (b) *Acta Chem. Scand.*, **4**, 1031 (1950); (c) *Svensk Papperstidn*, **55**, 745 (1952); (d) **56**, 287 (1953).

(16) O. Goldschmid, (a) THIS JOURNAL, **75**, 3780 (1953); (b) *Anal. Chem.*, **26**, 1421 (1954).

individual anionic substances, or groups of anionic substances, of characteristic electrophoretic mobilities. The peak to the left of the sample section arises as a result of the presence of neutral substances carried toward the cathode by endosmotic flow. In B-6 and B-8 the number of anionic components is found to increase as is indicated by the increasing number and decreasing sharpness of the peaks in the patterns compared with those observed for such pure substances as the products of condensation of formaldehyde with *p*-phenolsulfonate.[17] Since the average molecular weight of the components in these fractions is in the range expected for a mixture of monomeric, dimeric and trimeric lignin sulfonate molecules, efforts are now being made to isolate and identify some of these substances so that a better understanding will be at hand of the nature of the structural units and of the way they are combined in the lignin polymer.

(17) F. R. Stults, R. W. Moulton and J. L. McCarthy, *Chem. Eng. Prog. Symp. Ser.*, **48**, (4) 38 (1952).

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Lignin Structure. VIII.[1] Characterization of Ethanol Spruce Lignin Prepared by a New Method

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When spruce wood meal is treated with 0.2 *N* hydrogen chloride in a four to one mixture of chloroform and ethanol at 60°, 20% of the lignin is converted to ether-soluble oils and 60% to ether-insoluble powders. About 16% of the lignin remains in the wood. The lignin oils are somewhat low in methoxyl, in part because of carbohydrate impurities. The light tan powders, however, have a methoxyl to carbon ratio close to theoretical for a propylguaiacyl polymer and their average empirical formula can be calculated as $C_9H_{8.0}O_{2.6}(OC_2H_5)_{0.41}$. The average ethoxyl to phenol ratio is 1.16 or near the theoretical for simple alcoholysis of alkyl aryl ethers. If ethanol is subtracted from the empirical formula, the resulting formula is nearly identical with Erdtman's theoretical value for protolignin derived from data on lignin sulfonates.

Since lignin in wood, or the lignin remaining after wood polysaccharides have been dissolved, is insoluble in all solvents—presumably because of its network structure—it is important that some reaction used to obtain soluble products be thoroughly understood if the original structure is to be elucidated. After over a century of research on lignin, there is still no reaction suitable for its isolation from wood for which a balanced equation can be written with any assurance.[2] This article is a portion of a continuing study to determine whether or not lignin alcoholysis can be so described. It includes two independent preparations and characterizations of ethanol spruce lignin prepared under the relatively new conditions reported once previously.[3]

In essence, this preparation is a stepwise alcoholysis of Norway spruce wood meal in a good solvent system—anhydrous chloroform ethanol in a volume ratio of four to one—using 0.2 *N* hydrogen chloride as catalyst. The ethanol spruce lignins are isolated by filtration of the liquor, washing, concentration and precipitation into ether or preferably petroleum ether. Three or four fractions are obtained by successive treatments of the wood meal.

The lignin powders from a three or four step alcoholysis represent a minimum of 57% of the methoxyl of the original wood. The oils obtained from the precipitation liquors correspond to those from which Hibbert's school has previously obtained propylguaiacyl monomers.[4] These oils amount to about 25% of the original methoxyl. Less than 17% of the lignin (measured as methoxyl) remains in the residual wood meal. The ethanol lignin powders have been characterized by determination of methoxyl, ethoxyl and phenolic hydroxyl, by carbon and hydrogen analyses and by ultraviolet spectra.

An advantage of this preparation lies in the high yield of organic solvent-soluble lignin products corresponding to more than 80% delignification. The conditions of reaction—high dilution, low concentration, good solvent, absence of water and a stepwise isolation—have furthermore been chosen to lessen the probability of side reactions (Table I) and to simplify the interpretation of the reaction.

The ultraviolet absorption spectra of the three powders S 1, S 2 and S 3, obtained in one series of alcoholyses, are very similar and typical of ethanol spruce lignins.[5] They all exhibit a maximum at the 282 mμ region with nearly the same absorptivity (4.4–4.9 l. (mol. CH_3O)$^{-1}$ cm.$^{-1}$). From S 1 to S 3 the absorptivity gradually increases at the 250–270 and 290–320 mμ regions, rendering the 282 mμ maximum less sharp and possibly indicating increased structural complexity in the later fractions.

Similarly the analyses of the powders listed in Table II agree with each other within reasonable limits. The carbon and hydrogen values, while varying more than would be permissible for samples of a crystalline compound, are not greatly divergent considering that the products are amorphous mixtures of structurally related polymer fragments. The alkoxyl contents of the products

(1) Previous papers in this series include footnotes 3, 6, 7, reference 2 of Table I, THIS JOURNAL, **72**, 3838 (1950); *ibid.*, **75**, 707 (1953); "Chemistry in Canada," April, 1953, p. 35.

(2) The greatest headway on this problem seems to have been made on the technically important sulfonation of lignin, one excellent study of which has been reported by H. Erdtman, B. O. Lindgren and T. Petterson, *Acta Chem. Scand.*, **4**, 228 (1950). The extent of the concomitant hydrolysis, however, remains obscure.

(3) C. Schuerch, THIS JOURNAL, **74**, 5061 (1952).

(4) A. B. Cramer, M. J. Hunter and H. Hibbert, *ibid.*, **61**, 509 (1939).

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