Lignin. XV. Preliminary Characterization of Several Low Molecular Weight Ligninsulfonate Mers[1]

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ABSTRACT: Gel chromatography fractionations of a gymnosperm ligninsulfonate sample in aqueous NaCl at 0.001 *M* and other molarities have separated about 14 substances or mixtures of substances amounting to about 17% of the weight of total ligninsulfonates. The uv spectra of most of these were about the same as that of the unfractionated sample. Sedimentation equilibrium molecular weights of the entities ranged from several hundred up to about two thousand. The entities appear to be the lower molecular weight members of the polymer series of ligninsulfonates.

To gain a better basis for understanding the structure of lignin,[3-5] more information is needed concerning the nature of the lower molecular weight members of the polymer series.

In this laboratory, special emphasis has been devoted to the sulfonate derivatives of lignins[6-8] because these can be obtained under quite mild conditions as water-soluble substances in almost quantitative yield relative to the lignin present in wood, and because increased understanding of the structure and properties of ligninsulfonates may encourage increased utilization of this material. The isolation and identification of two crystalline ligninsulfonates have been reported from this laboratory.[9-11] Parrish has reported isolation of three monomeric and possibly one dimeric ligninsulfonate from wattle wood.[12] Recently, some monomers of ligninsulfonates have been isolated and identified by Glennie and Mothershead.[13-15] Further fractionation studies using Sephadex gel chromatography have recently been conducted in this laboratory and reported.[16]

The present paper describes the partial isolation and preliminary characterization of certain low molecular weight ligninsulfonate mers or groups of mers.

Experimental Section

Sodium Ligninsulfonates (NaLS). The material investigated was obtained from gymnosperm wood sulfite spent liquor by selective precipitation with octadecyldimethylamine, followed by regeneration using sodium hydroxide. The preparation and analytical characterizations were carried out by Mr. Vincent F. Felicetta of the Georgia Pacific Co., Bellingham, Wash., and were described earlier.[16]

Dialyzate NaLS. NaLS (2 g) dissolved in 100 ml of distilled water was dialyzed against 400 ml of distilled water using a closed length of Visking cellophane tubing (length, 5 in.; i.d., 1.59 in.; wall thickness, 0.001 in.). Fractions of the dialyzate were collected after 23, 43, 43, and 296 hr, respectively; after each time period, the dialyzate was set aside and dialysis was continued with 400 ml of distilled water. The first three dialyzate fractions were mixed together and freeze-dried to yield 59% of the dialyzate NaLS. The fourth fraction and the undialysable material were freeze-dried separately and yielded 19 and 20% of NaLS, respectively. The dialysis was carried out at 26 ± 2°, the acidity of the first fraction was about pH 6.1.

Fractionations. Sephadex G-50 held in a glass column (i.d., 33.8 mm; gel height, 210 cm) was used for the fractionation according to procedures previously described.[16]

Characterizations. Molecular weights of ligninsulfonate fractions were estimated using a short-column sedimentation equilibrium procedure.[16,17]

Molecular weights of two ligninsulfonate fractions were also determined by using a mass spectrometer (MS-9, manufactured by Associated Electrical Industries). Five fractions of 10 ml each at peaks A and B were mixed together and freeze-dried. The mass spectra measurements on these fractions were taken at 200°, since at lower temperatures the sample did not volatilize. The volatilization at 200° probably was accompanied by substantial cracking of the molecules.

Ultraviolet absorption spectra were determined using Cary spectrophotometers (Models 11 and 14). The base line was adjusted for the range of 210–360 mμ with solvent in both compartments. Absorption spectra in neutral solutions were obtained on the eluted fractions just as they were obtained from the gel columns. Then the solutions were made alkaline to pH 12 with 1 *N* NaOH solution and the spectra of these solutions were determined. The peak heights

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(3) F. E. Brauns and D. A. Brauns, "The Chemistry of Lignin," Supplementary Volume, Academic Press, New York, N. Y., 1960.

(4) I. A. Pearl, "The Chemistry of Lignin," Marcel Dekker, Inc., New York, N. Y., 1967.

(5) J. Marton, Ed., "Advances in Chemistry Series," No. 59, American Chemical Society, Washington, D. C., 1966.

(6) J. Moacanin, V. F. Felicetta, W. Haller, and J. L. McCarthy, *J. Amer. Chem. Soc.*, **77**, 3470 (1955).

(7) E. Nokihara, M. J. Tuttle, V. F. Felicetta, and J. L. McCarthy, *ibid.*, **79**, 4495 (1957).

(8) C. H. Ludwig, B. J. Nist, and J. L. McCarthy, *ibid.*, **86**, 1196 (1964).

(9) V. F. Felicetta, D. Glennie, and J. L. McCarthy, *Tappi*, **50**, 170 (1967).

(10) S. W. Schubert, M. G. Andrus, C. Ludwig, D. Glennie, and J. L. McCarthy, *ibid.*, **50**, 186 (1967).

(11) S. W. Schubert and J. L. McCarthy, *ibid.*, **50**, 202 (1967).

(12) J. R. Parrish, *Tetrahadron Lett.*, **11**, 555 (1964).

(13) D. W. Glennie and J. S. Mothershead, *Tappi*, **47**, 356 (1964).

(14) J. S. Mothershead and D. W. Glennie, *ibid.*, **47**, 519 (1964).

(15) D. W. Glennie, *ibid.*, **49**, 237 (1966).

(16) P. R. Gupta and J. L. McCarthy, *Macromolecules*, **1**, 236 (1968).

(17) K. E. Van Holde and R. L. Baldwin, *J. Phys. Chem.*, **62**, 734 (1958).

TABLE I
ELUTION AND MOLECULAR WEIGHT CHARACTERISTICS FOR LS PEAK MATERIALS

Peak	Expt[a]	K_d[b]	M_w[d]	SD, %
A	1[c]	1.30	339; 429; 413; 360; 350	0.6; 1.9; 2.4; 0.8; 2.6
	2	1.22	538; 714; 722; 832	1.7; 0.6; 2.5; 2.4
B	1[c]	1.15	385; 403; 429; 391; 372	1.0; 0.7; 2.8; 1.0; 0.5
	2	1.08	513; 691; 874	
C	1[c]	1.04	437; 443; 515; 519; 473; 598	4.7; 0.7; 1.4; 1.3; 0.6; 1.5
	2	0.97	805; 730	4.3; 1.6
D	1[c]	1.01	787; 812; 823; 858; 829; 779; 854; 913; 875	1.1; 0.7; 2.6; 0.7; 1.6; 4.7; 2.3; 0.5; 1.5
	2	0.93	701; 815	2.1; 1.6
E	1[c]	0.77	720; 722; 847; 786; 775; 744; 793; 793	0.8; 0.7; 2.8; 1.8; 0.5; 1.1; 0.9; 1.4
	2	0.70	856; 844	5.1; 1.2
F	1	0.74		
	2	0.67	709; 696	1.0; 1.6
G	1	0.67		
	2	0.60	953; 1025	2.0; 1.6
H	1	0.64	907; 934; 1046; 978; 964	0.7; 0.4; 2.7; 1.3; 0.8
	2	0.58	1126; 1127	3.3; 0.9
I	1[c]	0.53	1064; 1298; 1335; 1323; 1272; 1165	1.0; 1.2; 2.4; 0.5; 1.3; 0.9
	2	0.46	1130; 1247	1.3; 1.5
J	1[c]	0.42	1428; 1536; 1624; 1647; 1675	1.1; 1.8; 2.7; 1.0; 1.6
	2	0.38	1293; 1483	1.9; 2.2
K	1[c]	0.34	1687; 1876; 1919; 1963; 1889	0.7; 0.6; 1.7; 0.8; 0.8
	2	0.30	1845; 1796	2.9; 2.6
L	1	0.28		
	2	0.25	1982	0.7
M	1	0.24		
	2	0.21	2143; 2383	1.5; 1.3
N	1	0.20		
	2	0.18		

[a] For expt 1 and 2, V_0 = 520 and 490 ml, respectively. [b] Mean values of K_d for LS peak materials are shown in Table III. [c] For the expt 1 runs designated, the first five values of M_w given were obtained at successively increasing centrifugal fields of approximately 65,000, 95,000, 140,000, 165,000, and 195,000 times gravity. [d] The molecular weights are presented as printed out by IBM digital computer. The first two figures are significant.

above the base lines were used to evaluate the absorption ratios at the peaks.

Definitions and Symbols. These are the same as given in our prior paper.[16]

Results and Discussion

In our recently reported studies[16] of the Sephadex gel fractionation of a certain purified sodium ligninsulfonate sample, a number of "peaks" were evident in the concentration of NaLS in the eluates. These peaks, however, appeared at somewhat different elution volumes, V_e, and were different in relative magnitude, depending on the molarity of sodium chloride solution used as the eluent.

This effect appears clearly in Figure 1, which shows segments of the elution curves obtained in the fractionation experiments which were conducted with eluents consisting of aqueous sodium chloride solutions at the indicated molarities. It was established experimentally that these elution curves were generally reproducible.

Based on the evidence given in our prior paper,[16] we have attributed the observed differences in elution behavior primarily to the changes which are presumed to occur in the effective radii of ligninsulfonate molecules as a result of changes in eluent electrolyte concentration.

In the present investigation it was desired to fractionate NaLS under conditions which would yield mers, or groups of mers, which were separated as nearly completely as possible. Thus further fractionation experiments were carried out with Sephadex G-50 using a dialyzate NaLS which was the lower molecular weight portion of the previously studied NaLS sample and comprised about 59% of the weight of the initial material. The elutions were conducted with 0.001 M NaCl, since in prior experiments the peaks seemed best defined and separated when this electrolyte concentration was used.

Figure 2 gives an example of the resulting elution curve which shows a striking array of peaks in NaLS concentration as judged by absorption of ultraviolet radiation at 280 mμ. To facilitate discussion, these peaks have been arbitrarily assigned letters, with progress through the alphabet in inverse order of their elution from the gel column and, presumably, in direct order of increase in molecular weight. The value of $K_d = (V_e - V_0)/V_i$ associated with each peak was calculated from knowledge of the elution volume for the peak, V_e, the void volume, V_0, and the imbibed liquid, V_i = 1250 ml. The results for two separate experiments are given in Table I. Since moderately good agreement is usually found in corresponding cases, average values of K_d characteristic of each peak substance are reported.

Estimates of the molecular weights of the NaLS in the fraction at the peaks in concentration were made using an ultracentrifuge sedimentation equilibrium technique, and these results are also given in Table I. In certain cases determinations were made at several

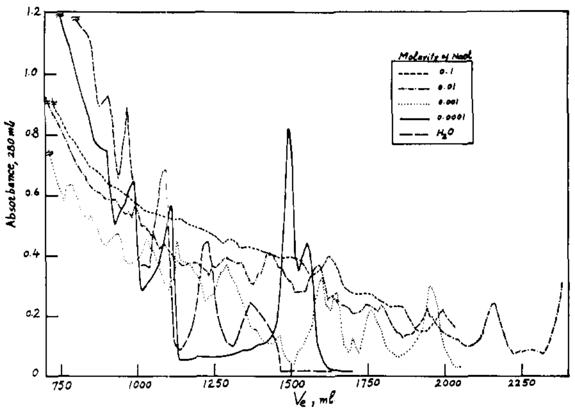

Figure 1. Segments of elution curves for unfractionated NaLS at several electrolyte concentrations showing the difference in resolution of components.

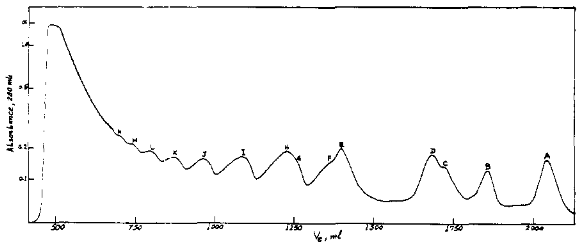

Figure 2. Absorbance of dialyzate NaLS *vs.* volume of eluent (0.001 *M* NaCl) from Sephadex G-50 gel column (experiment 2, V_0 = 490 ml).

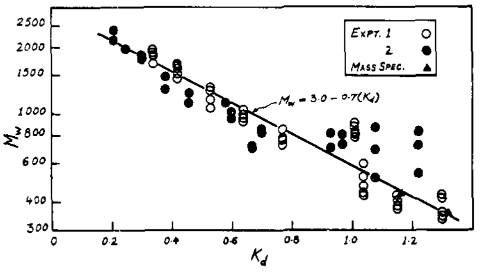

Figure 3. Experimental molecular weights *vs.* K_d for NaLS peak substances.

levels of centrifugal field strength, but no significant influence was ascertained. In general, the reproducibility in the molecular weight measurements was judged to be moderately good except for the NaLS samples with apparent molecular weights below about 600–800, as is shown in Figure 3 where all individual results are plotted together. On this graph, two molecular weight measurements are also included which were obtained by use of a mass spectrometer technique. To secure "smoothed" values of the molecular weight for the peak NaLS samples, a straight line was drawn relative to the points shown in Figure 3. The equation for this line is $M_w = 10^{3.0-0.7(K_d)}$. The fit is less than good where $K_d > 0.9$ and adsorption is suggested.

For each ultracentrifuge molecular weight measurement, the percentage standard deviation in molecular weight, %SD, was determined by the procedure given in our prior paper.[16] As shown in Table I, these values ranged from about 0.5 to 3.0 and suggest that only a small degree of polydispersity exists in the NaLS fractions separated as peak substances.

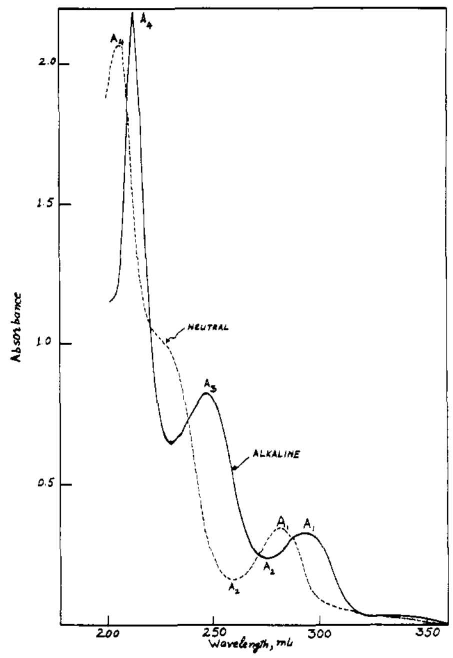

Figure 4. Peak substance C ultraviolet absorption spectra showing absorbance values used for ratios of Table II and Figure 5.

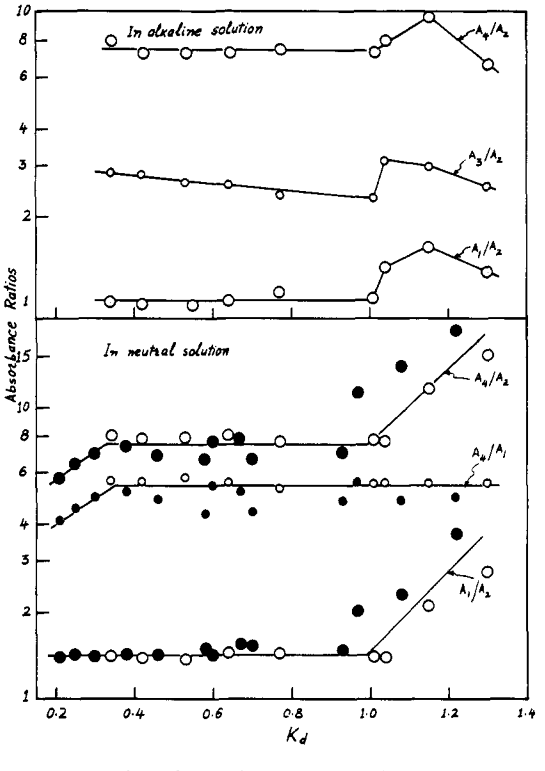

Figure 5. Ratios of absorbances for lignin peak substances in neutral and alkaline solutions: ○, expt 1; ●, expt 2.

To provide further preliminary characterization of the NaLS peak substances, ultraviolet absorption spectra were determined in neutral and alkaline media. The results are shown in Table II in terms of the absorbance ratios defined in Figure 4. These ratios are plotted *vs.* K_d in Figure 5. In general, the spectral patterns and

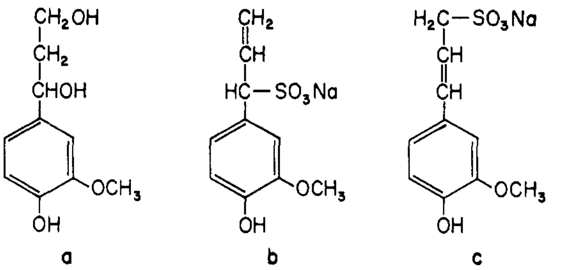

Figure 6. *Structures of lignin-type monomer and monomer sulfonates.*

Table II

Ratios of Ultraviolet Radiation Absorbance[a] at Certain Wavelengths for LS Peak Materials

	Neutral solution			Alkaline solution		
Peak	A_1/A_2	A_4/A_2	A_4/A_1	A_1/A_2	A_4/A_2	A_3/A_2
A	3.2	19.0	5.6	1.3	7.0	2.6
B	2.3	12.0	5.6	1.6	10.0	3.0
C	1.6	8.0	5.6	1.4	8.0	3.1
D	1.5	7.6	5.6	1.1	7.6	2.3
E	1.5	7.6	5.6	1.1	7.6	2.5
F	1.5	7.6	5.6			
G	1.5	7.6	5.6			
H	1.5	7.6	5.6	1.1	7.6	2.6
I	1.5	7.6	5.6	1.1	7.6	2.7
J	1.5	7.6	5.6	1.1	7.6	2.8
K	1.5	7.5	5.6	1.1	7.6	2.9
L	1.5	6.6	4.6			
M	1.5	5.6	4.3			
N						

[a] Absorbances were taken at the wavelength of the maximum or minimum values shown for each particular peak material, as is indicated approximately in Figure 4. These are the smoothed values read from Figure 5.

the intensity ratio parameters are approximately constant except in the range of $K_d > 0.9$, where unexplained departures from molecular weight correlations were also observed.

As a beginning toward rationalization of the present results, we suggest that the peaks reflect the presence of NaLS mers (or groups of mers) constructed of two types of structural units. Such units might arise, for example, from the two kinds of monomers[9-11]—one sulfonated and the other unsulfonated—as shown in Figure 6. The molecular weights of A and B or C are 198 and 266, respectively. Perhaps the sulfonated and unsulfonated structural units usually alternate, and the polymer molecular weight may be estimated by simple summing of the weights of the presumed monomers. If the mers do not alternate, then additional combinations may be predicted.

On this basis, and considering only alternation of mers, the system set forth in Table III has been developed, and "calculated" molecular weights are shown for the speculative structures up to the 10-mer.

Smoothed values of the molecular weights found for the LS peak substances are also shown in Table III in association with the respective mers with similar calculated molecular weights. Each presumed mer up to the 10-mer is represented by one or more LS peak substances, except the monomers which may have existed but have not been recovered in the amine precipitation step used to prepare the purified NaLS used in this investigation. That two or three LS peak substances are found in the molecular weight range calculated for a particular mer seems to reinforce the expectation arising from previous work that several types of monomers and dimers and analogous structural units may exist. The mean K_d values shown in Table III for the LS peak substances reflect the effective size of the respective components as derived from their behavior in the gel column.

Table III

Calculated *vs.* Experimental Molecular Weights and Other Characteristics of Ligninsulfonates

Calculated			Experimental			
Mer	Structure	Mol wt[a]	LS peak	Mol wt[b]	K_d[c]	Wt %
1	a	198				
	b, c	266				(0.0)[d]
2	a_1b_1	464	A	340	1.27	1.7
			B	450	1.12	1.1
			C	540	1.01	1.1 (3.9)
3	a_2b_1	662	D	570	0.97	1.5
	a_1b_2	730				(1.5)
4	a_2b_2	928	E	860	0.74	1.1
			F	900	0.71	1.0
			G	1020	0.64	1.0 (3.1)
5	a_3b_2	1126	H	1080	0.61	1.2
	a_2b_3	1194				(1.2)
6	a_3b_3	1392	I	1310	0.50	1.8 (1.8)
7	a_4b_3	1590	J	1530	0.40	1.4
	a_3b_4	1658				(1.4)
8	a_4b_4	1856	K	1760	0.32	1.1
			L	1950	0.27	0.9 (2.0)
9	a_5b_4	2054	M	2100	0.23	1.0
	a_4b_5	2122				(1.0)
10	a_5b_5	2320	N	2250	0.19	1.1 (1.1)

[a] Molecular weights of polymers resulting from additive combinations of presumed monomers a and b or c to yield structures indicated. [b] These values of molecular weight are "smoothed" values taken from the correlating line shown in Figure 3. [c] These values of K_d are averages of the two experimental values given in Table I. [d] Within the parentheses are given the estimated weight percentages of the LS mers.

The approximate percentage of the initial NaLS sample which corresponds to each LS peak substance and to each presumed mer is also shown in Table III. A total of about 17% of the beginning material is estimated to be present as peak substances. The data suggest that the even-numbered mers are present in a larger proportion than the odd-numbered mers.

Research is continuing toward more extensive separation and characterization of these lower molecular weight ligninsulfonates.

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