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

Lignin. IV. Electrophoresis Study of Alkaline Cleavage Products from Lignin Sulfonates[1]

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An electrophoresis method has been used to study some products resulting from the reaction of lignin sulfonates with mercuric oxide and sodium hydroxide. Lignin sulfonates from hemlock, spruce and Douglas fir woods, as well as dialyzable and non-dialyzable fractions from mixed soft wood species were investigated. Several known cleavage products were characterized by mobility and ultraviolet absorption and the yields of these were estimated from the electrophoresis patterns. Several apparently new reaction products were also observed and the mobility and ultraviolet absorption spectra of these substances is reported.

Introduction

One approach toward elucidation of the structure of lignin is by study of the reaction and products of the alkaline cleavage of lignin. Many investigations of this type have been carried out[2] and of these the recent work of Pearl and Beyer[3,4] is of special interest because their cleavage of spruce calcium lignin sulfonate preparations with sodium hydroxide in the presence of mercuric oxide is reported to have brought about conversion of around 60%[3] of the weight of the initial lignin to ether-soluble products which consisted of such phenols as vanillin, vanillic acid, acetovanillone, guaiacol as well as other substances. Similar experiments except in the presence of nitrobenzene have been reported by Leopold.[5] Both investigators have illustrated the value of various chromatographic procedures for separating several substances from the complex mixture of reaction products obtained. In this investigation, alkaline cleavage in the presence of mercuric oxide has been further studied and use has been made of an electrophoresis procedure[6] to secure information relating to the nature and yield of the reaction products without isolating the individual products.

Experimental

Lignin Sulfonate Preparations.—Western hemlock (*Tsuga heterophylla*), Douglas fir (*Pseudotsuga taxifolia*), Sitka spruce (*Picea sitchensis*), Sugar maple (*Acer saccharum*) and Yellow birch (*Betula lutea*) woods were air-dried, made into sawdust, extracted in a Soxhlet apparatus with a 1:1 ethanol–benzene solution, washed with ethanol and then water, air-dried and then analyzed with the following results: 29.8, 30.4, 27.9, 18.7, 21.7% Klason lignin, and 4.56, 4.61, 4.58, 6.19 and 5.89% OCH_3, respectively. Delignification was conducted in a stainless steel autoclave with sulfurous acid–sodium bisulfite solutions usually containing 1.2% "combined SO_2" and 4.8% "free SO_2" (5.6% with hemlock), with a solution–wood ratio of ten, and with the temperature usually at 110° for one hour and then 130° for 11 hours. The cellulosic residues were filtered, and the filtrates were concentrated under a vacuum and then analyzed for reducing substances, total sulfur and methoxyl by methods described in a prior paper.[7] Diffusion coefficients for solutes absorbing ultraviolet radiation were estimated by a solution–gel method[8] and the results are given in Table I.

TABLE I

CHARACTERISTICS OF LIGNIN SULFONATE PREPARATIONS

Preparation	Reducing substances,[a] %		Total sulfur,[a] %		Methoxyl,[a] %		Diffusion coefficient,[b] mm.²/day	
	Orig.	Treat.[c]	Orig.	Treat.[c]	Orig.	Treat.[c]	Orig.	Treat.[c]
Hemlock	19.9	0.2	14.2	12.2	5.2	4.1	21	20
Douglas fir	24.6	.7	11.6	11.5	4.9	4.8	17	11
Spruce	22.0	.5	9.8	9.7	5.9	4.4	19	22
MD[d]	32.9	.5	5.7	7.2	5.4	6.1	24	24
MN[d]	2.8	..	5.2	..	11.5	..	7	..
Maple	23.9	.5	7.1	8.4	7.6	7.8	29	29
Birch	12.9	.7	6.2	7.7	6.8	7.8	27	29

[a] Reducing substances are calculated as glucose. Reducing substances, total sulfur and methoxyl are given as weight percentages of the total solids other than "free sodium hydroxide" which was determined by conductometric titration with hydrochloric acid. Sulfate calculated as sulfur was less than 0.2% of the total solids in all preparations. [b] Diffusion coefficients are given for the components in the preparations which absorb 2800 Å. ultraviolet radiation. [c] "Treated" indicates the preparation obtained after sorption on, and desorption from, an anion resin to remove sugars. [d] "MD" and "MN" signify mixed species dialyzable and non-dialyzable sulfonates, respectively.

The methoxyl recovered in these solutions amounted to 70, 66, 81 and 55% of that in the extracted hemlock, fir, spruce, maple and birch samples, respectively. Some experiments were also carried out using preparations made industrially from a mixture of hemlock and fir woods and then evaporated and dialyzed, as described in a prior paper,[9] to obtain sample MN, a non-dialyzable calcium lignin sulfonate fraction, and also sample MD, a dialyzable fraction comprising solutes which had diffused through the dialysis membrane (Table I).

To remove sugars, the solutions were permitted to flow through a Dowex 50-8X resin column to remove metal ion, and then into a Duolite A-2 anion-exchange resin column which had previously been washed with 1 *N* sulfuric acid, water, 1 *N* sodium hydroxide and then water until neutral. The outflow solutions and water washings were clear colorless liquids containing 70 to 80% of the input reducing substances. Adsorbed lignin sulfonates were displaced from the column by 1 *N* sodium hydroxide and analyzed (Table I). Methoxyl in the eluant was 73% of that added to the column for the fir, maple, birch and mixed species MD, and about 80% for the hemlock and 64% for the spruce. Nearly the same recoveries were obtained for substances absorbing ultraviolet light at 2800Å.

Alkaline Cleavage.—The reaction mixture usually consisted of sufficient lignin sulfonate solution to provide 2.80 g. of lignin (assuming 14.5 and 21.6% methoxyl for gymnosperm and angiosperm lignins, respectively), 16 g. of sodium hydroxide, the mercuric oxide precipitate obtained

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(1) Presented in part at Pacific Northwest Regional Meeting of the American Chemical Society, Corvallis, Oregon, June, 1952.

(2) F. E. Brauns, "The Chemistry of Lignin," Academic Press, Inc., New York, N. Y., 1952, pp. 546–560.

(3) I. A. Pearl, THIS JOURNAL, **71**, 2196 (1949).

(4) (a) I. A. Pearl and D. L. Beyer, *ibid.*, **76**, 2224 (1954); (b) **74**, 612 (1952); (c) *TAPPI*, **33**, 544 (1950); (d) **33**, 508 (1950).

(5) (a) B. Leopold, *Acta Chem. Scand.*, **6**, 44 (1952); (b) **6**, 49 (1952); (c) **6**, 55 (1952); (d) *Svensk. Kem. Tid.*, **64**, 1 (1952); (e) **64**, 18 (1952).

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

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

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

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

from 31.8 g. of mercuric acetate by the procedure of Lewis and Pearl,[10] and water to bring the final volume to 100 ml. In some experiments this mixture was vigorously refluxed in glass without mechanical stirring for several periods of time. Other experiments were conducted with mechanical stirring in a 500-ml. iron crucible fitted with a rubber stopper. Still other experiments were carried out in stainless steel bombs rotated end over end at about 25 r.p.m. in an oil-bath at 145°.

Reaction mixtures were cooled, centrifuged and the settled solids were washed twice with water. The clear solution and washings were combined, acidified with sulfur dioxide, and then exhaustively extracted with ether to yield an "acid fraction." The aqueous solution was acidified with sulfuric acid, sulfur dioxide was removed by vacuum aspiration at room temperature, and the solution was again extracted with ether to yield an "aldehyde fraction." The ether solutions were dried over anhydrous sodium sulfate and aliquots were used for determinations of the yield of ether-soluble substances and for examination by electrophoresis.

Electrophoresis.—Use was made of a previously described electrophoresis method[6] to estimate weight yields of identified reaction products (Table II and III) and also relative amounts obtained of products absorbing ultraviolet light (Table IV). An aliquot of the ether solution of a given acid or aldehyde fraction was added to a buffer solution (pH 7.3; 0.0264 M Na_2HPO_4 and 0.00575 M NaH_2PO_4) and the ether removed to provide an ether-soluble solid concentration of 80 to 400 mg./liter. The agar concentration in the Vycor electrophoresis tubes was usually 7 g./liter. From experiments with benzophenone, a neutral substance, the endosmotic mobility for the conditions used was found to be 0.23 cm.^{2}/volt hour. Using this value together with the experimental observations, the characteristic electrophoretic mobilities were computed for the various substances by dividing the net distance migrated by the time and the voltage gradient. For each substantially separated peak on the electrophoresis pattern, the ultraviolet absorption spectrum was also determined. To minimize the interference from the absorption of neighboring peaks, or from agar, a spectrum was determined by observing, at one position near the apex of the peak, the optical densities obtained for the wave lengths of interest when the spectrophotometer had been "balanced" at each of these respective wave lengths at another nearby position on the same peak. Similar experiments were carried out with several known pure substances and results are given in Table V.

TABLE II

YIELDS FROM CLEAVAGE OF NON-DIALYZABLE LIGNIN SULFONATES[a]

Time, hr.	Ether-sol. substances, %	Vanillin, %	Vanillic acid, %
6[b]	22	4.6	2.6
12	23	4.5	1.6
24	31	2.5	6.1
30	36	1.0	11.6
36	44	0.9	12.3
27	43	1.3	18.6
72	51	0	28.2
96	57	..	..

[a] Experiments were conducted with non-dialyzable mixed species lignin sulfonates, MN. Yields are given on a weight basis referred to unsulfonated lignin assumed to contain 14.5% methoxyl. [b] The first five experiments listed were conducted with the reaction mixture boiling under reflux but without mechanical stirring. In the last three experiments, the reaction mixture was strongly stirred mechanically.

TABLE III

YIELDS OF CLEAVAGE PRODUCTS FROM GYMNOSPERM LIGNIN SULFONATES[a]

Product	Pure species, %			Mixed species, %	
	Hemlock	Douglas fir	Spruce	Dial. = MD	Non-dial. = MN
Acid fraction					
Ether soluble	25.9	20.7	25.0	26.0	31.2
Vanillic acid	11.8	11.5	19.6	18.5	18.4
Vanillin	2.1	0.7	1.8	1.7	[b]
Acetovanillone	0.9	1.2	0.4	1.7	[b]
Aldehyde fraction					
Ether soluble	19.6	20.9	25.2	24.4	20.5
Vanillin	16.5	10.8	8.3	8.4	4.0
Vanillic acid	[b]	[b]	[b]	1.0	2.0
Total ether soluble	45.5	41.6	50.2	50.4	51.7
Total V + VA + AV[c]	31.3	24.2	30.1	31.3	24.4

[a] Yields are given on a weight basis referred to unsulfonated lignin assumed to contain 14.5% methoxyl. Reactions were conducted with mercuric oxide for 24 hours with vigorous mechanical stirring. [b] The reference product was either absent or the yield was too small to be reliably estimated. [c] "V + VA + AV" = sum of weight yields of vanillin, vanillic acid and acetovanillone.

TABLE IV

APPROXIMATE DISTRIBUTION OF ULTRAVIOLET ABSORPTION FOR CLEAVAGE PRODUCTS FROM GYMNOSPERM LIGNIN SULFONATES[a]

Product	Pure species, %			Mixed species, %	
	Hemlock	Douglas fir	Spruce	Dial. = MD	Non-dial. = MN
Acid fraction					
Vanillic acid	11.0	11.4	17.9	15.5	22.0
Vanillin	3.0	1.8	2.2	1.8	[b]
Acetovanillone	1.2	1.4	1.8	1.9	[b]
C	[b]	0.4	[b]	..	[b]
L	[b]	[b]	0.6	..	[b]
G	[b]	[b]	[b]	1.1	3.4
Aldehyde fraction					
Vanillin	19.5	12.9	9.7	13.6	5.8
5-Carboxyvanillin	3.0	2.1	3.4	[b]	1.8
Vanillic acid	[b]	[b]	[b]	1.2	1.5
A	2.1	[b]	1.4	1.3	0.9
B	5.0	4.9	3.0	[b]	3.5
F	[b]	[b]	[b]	[b]	0.5
H	[b]	[b]	[b]	4.1	[b]
I	1.0	[b]	1.6	[b]	[b]
R	[b]	2.6	[b]	[b]	[b]
S	[b]	1.6	[b]	[b]	[b]

[a] Distributions have been estimated by use of equation 3. [b] The reference product was either absent or the yield was too small to be reliably estimated.

When mobility and spectrum observation permitted identification of the compound giving rise to a peak in the electrophoresis pattern, and when the extinction coefficient of this compound was known for the conditions existing in the electrophoresis tube, the weight of the substance present was estimated by use of the Lambert–Beer law

$$(\text{O.D.}) = \log_{10}\left(\frac{I_0}{I}\right) = Elc = El\left(\frac{\mathrm{d}w}{S\,\mathrm{d}z}\right) \qquad (1)$$

where: O. D. = optical density; I_0 and I = intensity of incident and transmitted light beam; E = extinction coefficient, cm.^{2}/mg.; c = concentration, mg./cm.^{3}; and l = mean path length of light beam through the electrophoresis tube, cm. Now in dz cm. = differential length along the electrophoresis tube, there may be contained dw mg. = a certain weight of substance, at concentration = c = dw/S dz where S = cross-section area of solution contained in the electrophoresis tube. For the total length along the electrophoresis tube from the position z_1 cm., at which a peak appears, to the position, z_2 cm., at which the peak is no longer evident, the quantities E, l and S are nearly constant

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(10) H. F. Lewis and I. A. Pearl, U. S. Patent 2,433,227 (Dec. 23, 1947).

TABLE V

MOBILITY AND ULTRAVIOLET ABSORPTION OF CERTAIN PHENOLS AND UNKNOWN SUBSTANCES

Substance	Symbol	M.p., °C.	Mobility,[a] cm.^{2}/volt hr.	Ext. coef.,[b] cm.^{2}/mg.
Guaiacol	Gu	[c]	0.06	15.1
Acetovanillone	AV	115–116	.24	44.3
Unknown	R	.....	.46	..
Vanillin	V	80–81	.52	41.2
5-Acetylvanillin	5AV	120–121	.62	33.7
Dehydrodivanillin	DV	313–317	.65	81.5
Unknown	A	.....	.69	..
Unknown	S	.....	.71	..
Unknown	C	.....	.74	..
Ferulic acid	FA	168–170	.77	34.1
5-Acetylvanillic acid	5AVA	213–215	.77	10.1
Unknown	I	.....	.83	..
Vanillic acid	VA	207–208	.85	23.4
5-Carboxyvanillin	5CV	254–255	.93	46.1
Unknown	H	.....	.99	..
Unknown	B	.....	1.06	..
Unknown	L	.....	1.06	..
5-Formylvanillic acid	5FVA	232–233	1.20	40.9
Dehydrodivanillic acid	DVA	328–330	1.36	44.0
Unknown	F	.....	1.36	..
Unknown	G	.....	1.38	..
5-Carboxyvanillic acid	5CVA		1.51	8.51

[a] Mobilities recorded are in almost all cases the average of results of two or more experiments. Bath temperatures were usually 10 to 20° and mobilities have been corrected from the bath temperatures to 25° assuming a 2% increase in mobility for each 1° increase in temperature. Electrophoreses were conducted with *p*H 7.30 buffer solution using 0.7% agar concentration. [b] Extinction coefficients were taken at 2800 Å. in the phosphate buffer solution at *p*H 7.30. [c] Eastman Kodak Co. white label guaiacol.

so that from equation 1 the following integral expression can be written

$$w = \frac{S}{El}\int_{z_1}^{z_2} (\text{O.D.})\, dz \qquad (2)$$

The total substance weight, *w* mg. was obtained for the various experiments by graphical integration of equation 2 using the electrophoresis patterns consisting of a plot of optical density *versus* position in the tube as illustrated by Figs. 1 and 2. The value of "*l*" for each Vycor electrophoresis tube was obtained from separate optical density measurements on pure vanillin or vanillic acid under conditions such that "*w*" and "*E*" were already definitely known. From "*w*," and from the weight of material in the sample used for electrophoresis, and from aliquot and analytical relationships, estimates were made of the weight yields based on lignin of the substances identified in the electrophoresis patterns and results are given in Tables II and III.

For a peak observed in an electrophoresis pattern which could not be identified with a substance for which the extinction coefficient was known, the product $(wE)_x$ was calculated from the relation

$$(wE)_x = \frac{S}{l}\int_{z_1}^{z_2} (\text{O.D.})_x\, dz \qquad (3)$$

for the unknown substance, X. This was then compared with the sum of the (wE) products for all substances present in the electrophoresis tube which was calculated either as $\sum_1^n \frac{S}{l}\int (\text{O.D.})_i\, dz$, or from measurements of optical density and volume of the sample added to the electrophoresis tube. The result, as shown in Table IV, was an estimate of the proportion of the unknown substance present expressed as per cent. of the total ultraviolet absorption of the product substances in the electrophoresis tube, *i.e.*

$$\text{"Absorption \%" X} = \left[\frac{(wE)_x}{\sum_1^n (wE)_i}\right] 100 = \left[\frac{\int (\text{O.D.})_x\, dz}{\sum_1^n \int (\text{O.D.})_i\, dz}\right] 100 \qquad (4)$$

Ultraviolet spectra and extinction coefficients were determined with a Model DU Beckman spectrophotometer using aqueous solutions of *p*H 7.3 buffered with 0.02635 *M* Na_2HPO_4 and 0.00575 *M* NaH_2PO_4.

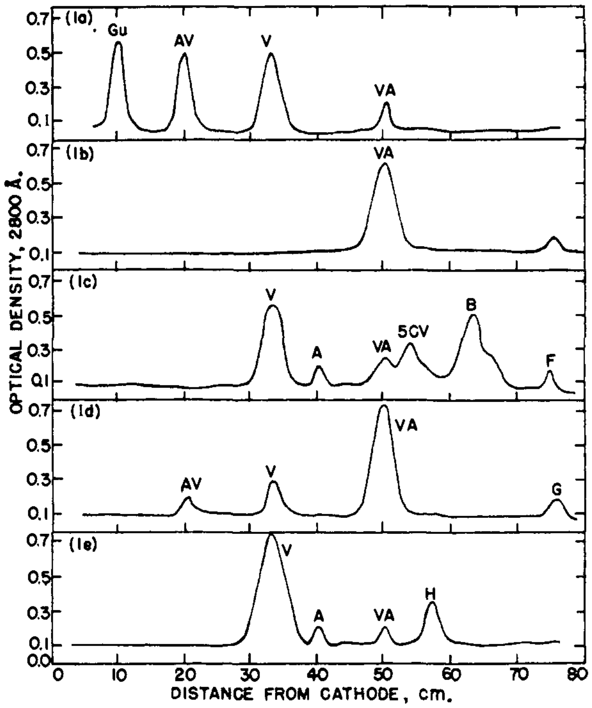

Fig. 1.—Electrophoresis patterns of cleavage products: (a) known mixture of pure phenols; (b) acid fraction and (c) aldehyde fraction of non-dialyzable lignin sulfonates, MN; (d) acid fraction and (e) aldehyde fraction of dialyzable lignin sulfonates, MD. Initial position of sample was at 30 cm. See Table V for significance of symbols.

Discussion

Several lignin sulfonate preparations were used in the experiments and some characteristics of these and other materials are shown in Table I. Sugars were removed by treating the solutions with an ion exchange resin which also irreversibly sorbed some 20 or 30% of the lignin sulfonates. However, diffusion coefficients for the lignins, except for Douglas fir, were about the same before and after the resin treatment indicating that the adsorbed lignins were not of particularly high or low molecular weight. The relatively low methoxyl and high sulfur content of the preparations indicates that other non-lignin material, probably carboxy and sulfonate sugar derivatives, remained present with the lignin sulfonates after the resin treatment. The higher methoxyl contents and

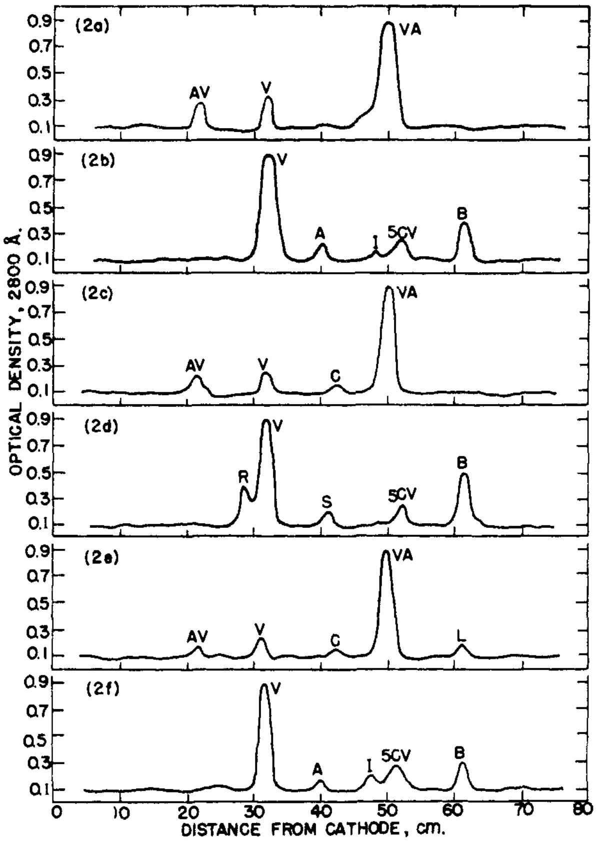

Fig. 2.—Electrophoresis patterns of cleavage products: (a) acid fraction and (b) aldehyde fraction of hemlock lignin sulfonates; (c) acid fraction and (d) aldehyde fraction of Douglas fir lignin sulfonates; (e) acid fraction and (f) aldehyde fraction of spruce lignin sulfonates. Initial position of sample was at 30 cm. See Table V for significance of symbols.

higher diffusion coefficients (or lower molecular weights) of the hardwood as compared with the softwood lignins can be noted in Table I.

Experiments were first carried out with a non-dialyzable lignin sulfonate fraction, MD, obtained from a mixture of hemlock and fir woods. An aqueous solution of this lignin was heated with sodium hydroxide and mercuric oxide for several periods of time. The yields of ether-soluble substances, obtained without stirring the reaction mixture, were measured and were found to increase with time to a maximum value of about 35% based upon the weight of the non-dialyzable or high molecular weight lignin sulfonates, or about 44% on the basis of the unsulfonated lignin assuming 14.5% methoxyl in this material (Table II). With vigorous mechanical stirring, the yield was more than 50% as previously found by Pearl.[3,4] As the time of reaction was increased, a larger proportion of the products appeared in the acid fraction.

The nature of the ether-soluble reaction products was studied by an electrophoresis method[6] and results are given in Tables III and IV, and in Figs. 1 and 2. To provide a basis for interpretation of these results, certain pure substances, some of which have been recognized as products obtained from this type of cleavage, were examined for mobility and ultraviolet absorption spectra and the results are shown in Table V and Fig. 3.

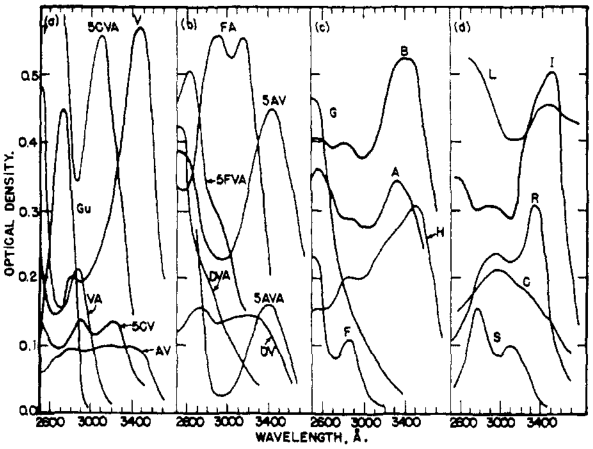

Fig. 3.—Ultraviolet absorption spectra of compounds observed by scanning electrophoresis tubes: (a) some phenols identified as cleavage products; (b) some pure phenols observed as standards; (c) and (d) unidentified substances observed as products of alkaline cleavage. Spectra were obtained for substances at 10 to 20 mg./l., concentration in *p*H 7.30 buffer agar gel. See Table V for significance of symbols.

In these experiments with pure phenols it was found that the mobility was easily reproducible, and both mobility and spectra were sufficiently unique to enable clear distinction of a given pure phenol to be made in all cases studied. The mobility of a phenol was often roughly predictable by assuming the property to be proportional to acid strength and inversely proportional to molecular size. From a knowledge of the extinction coefficient for a pure phenol in the buffered solution used for electrophoresis, the weight of the phenol present in the reaction product mixture was computed from measurements of the area under the appropriate electrophoresis peak. This procedure was found to be accurate within a few percentage when tested with measured quantities of guaiacol, acetovanillone, vanillin and vanillic acid which gave well separated peaks.

Typical electrophoresis patterns obtained from the non-dialyzable and dialyzable mixed species lignin sulfonate samples, MN and MD are shown in Fig. 1. In these patterns peaks are evident which have been definitely established, by mobility and ultraviolet absorption spectra measurements, to arise as a result of the presence of vanillin, vanillic acid, acetovanillone and 5-carboxyvanillin in the reaction product mixtures. In other patterns obtained after carrying out the electrophoresis for a shorter period of time, 5-carboxyvanillic acid has been identified. For the acid and aldehyde fractions of samples MN and MD, yields of vanillin, vanillic acid and acetovanillone were estimated from the electrophoresis data. The results in Table II show that the proportion of vanillic acid in the reaction product mixture increases and that of vanillin decreases as the time of reaction is increased.

Cleavage experiments were also carried out with lignin sulfonates prepared from Western hemlock, Douglas fir and Sitka spruce woods and the results are given in Fig. 2 and Table III along with other results from experiments with the mixed species dialyzable and non-dialyzable lignin sulfonates, MD and MN. The yields of the ether-soluble substances obtained from the various samples range from 40 to more than 50% and the total yields of vanillin, vanillic acid and acetovanillone amount to 25 to about 30%. These values are in the same range as those found by Pearl and Beyer,[3,4] and by Leopold,[5] who studied the nitrobenzene oxidation of lignin sulfonates. The total yields obtained from the hemlock, spruce and dialyzable mixed species lignin sulfonates are believed to be significantly higher than those obtained from the Douglas fir and the non-dialyzable lignin mixed species samples. This may arise because of the presence in these latter preparations of a higher proportion of linkages resistant to cleavage.

In the electrophoresis patterns there were evident several peaks which could not be identified with substances known to be lignin cleavage products and available to us. Each of these substances was designated by a letter, *e.g.*, A, B, F, etc., as indicated on Figs. 1 and 2, and was characterized in a preliminary manner by measurement of electrophoretic mobility (Table V) and by absorption spectrum (Fig. 3). Extinction coefficients of these compounds are unknown so that the proportions in which they were observed are represented in Table IV in units of percentage of the total ultraviolet absorption of the substances in the fraction examined by electrophoresis. Some of these substances may be those recently identified by Pearl and Beyer[3,4] or by Leopold,[5] and others may be new products of the alkaline cleavage of lignins. The substance B has recently been shown to be identical with vanilloylformic acid.[11]

In similar experiments with lignin sulfonates from maple (*Acer macrophyllum*), Ryan[12] with one of the authors has used the electrophoresis technique to establish the presence in the reaction product mixture of vanillin, vanillic acid, syringaldehyde and syringic acid. Several other substances were observed which were not identified, although evidence was obtained for the presence of acetovanillone and possibly syringoylformic acid.

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(11) D. W. Glennie, H. Techlenberg, E. Reaville and J. L. McCarthy, THIS JOURNAL, **77**, 2409 (1955).

(12) A. S. Ryan, M.S. Thesis, University of Washington, 1950.

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

Lignin. V. Vanilloylformic Acid from Alkaline Cleavage of Lignin Sulfonates[1,2]

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Alkaline cleavage of lignin sulfonates in the presence of cupric oxide yields a complex mixture of phenolic substances and partial separation of the components of this mixture has been achieved by means of anion-exchange resins. Some known cleavage products have been separated. In addition, a substance apparently heretofore unrecognized as a cleavage product has been isolated and shown to be vanilloylformic acid by comparison with authentic samples. Two methods for the synthesis of vanilloylformic acid have been developed to supplement those previously described in the literature.

Introduction

Previous studies[4-6] of the alkaline cleavage of lignin sulfonates in the presence of various oxidants have shown the reaction products to include low molecular weight phenols such as guaiacol, vanillin, acetovanillone, vanillic acid, 5-carboxyvanillin, 5-carboxyvanillic acid, 5-formylvanillin and 5-formylvanillic acid. These and other products of the reaction are obtained in complex mixtures and electrophoretic examinations of these mixtures have led to the detection in this Laboratory of several apparently new lignin cleavage products which have been characterized by electrophoretic mobility and ultraviolet absorption.[1] One of these has now been isolated in crystalline form and the object of the present paper is to describe the method used to accomplish its separation and to report evidence for its identity with vanilloylformic acid.

Discussion

Alkaline cleavage of the lignin sulfonates was carried out with a sodium hydroxide solution in the presence of cupric oxide under conditions similar to those employed by Pearl.[7] There resulted a dark colored solution which was filtered to remove brown solids, acidified, then extracted to obtain total ether-soluble products amounting to 22.7% of the lignin, assuming it to contain 14.5% methoxyl. The electrophoresis pattern[8] for these prod-

(1) For Paper IV of this series, see R. E. Davis, E. T. Reaville, Q. P. Peniston and Joseph L. McCarthy, THIS JOURNAL, **77**, 2405 (1955).

(2) Presented in part at Pacific Northwest Regional meetings of the American Chemical Society at Richland, Washington (1954) and Pullman, Washington (1953).

(3) Hooker Electrochemical Company Research Fellow.

(4) F. E. Brauns, "The Chemistry of Lignin," Academic Press Inc., New York, N. Y., 1952, pp. 546–562.

(5) I. A. Pearl, THIS JOURNAL, **72**, 2309 (1950).

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(7) I. A. Pearl and D. L. Beyer, *TAPPI*, **33**, 544 (1950).

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