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The Effect of Alkali and Strong Acid on the Ultraviolet Absorption Spectrum of Lignin and Related Compounds[1]

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Ultraviolet absorption spectra of different lignin preparations have been determined in neutral, alkaline and 95% sulfuric acid solutions. The maxima of difference curves obtained by subtracting the spectrum of the neutral solution from those of the alkaline and strong acid solutions, respectively, are compared with the absorption bands in alkaline and strong acid solution of model compounds such as conidendrin, vanillin and veratraldehyde. It is shown that band displacements in these solutions, interpreted according to Doub and Vandenbelt, provide a method for separating the absorption of phenylpropane lignin units substituted with phenolic hydroxyl and α-carbonyl groups from that of the remaining lignin units. The contribution of these ionizable groups to the lignin spectrum in neutral solution is discussed.

The status of the interpretation of the ultraviolet absorption spectrum of lignin and related compounds has recently been discussed by Jones[2] and by Aulin-Erdtman,[3] who first reported the influence of the *p*H of the solvent on the ultraviolet absorption spectrum of several different lignin preparations. With model compounds containing one phenolic hydroxyl group either per one, or per two phenylpropane units, Aulin-Erdtman[3] has shown that the displacement toward longer wave lengths of the 280 mμ maximum in alkaline solution is roughly proportional to the phenolic hydroxyl content of these substances. Band displacements in alkaline solution of phenolic compounds, including hydroxyaldehydes and hydroxyketones, had been investigated previously by Morton and Stubbs[4] and by Lemon.[5] Spectrophotometric methods for the determination of vanillin, syringaldehyde and *p*-hydroxybenzaldehyde have been based upon this effect.[6,7] Recently, Doub and Vandenbelt[8] have demonstrated that ultraviolet absorption spectra of mono- and disubstituted benzene derivatives can be represented as a regular progression of bands resulting from the displacement of the absorption bands of benzene, and that these band displacements, including those due to ionization of the substituent groups, can be interpreted on the basis of the electronic interaction between substituent groups and benzene ring. Doub and Vandenbelt have applied this interpretation also to the band displacements in strong sulfuric acid solution,[8a] observed by Flexser, Hammett and Dingwall,[9] where ionization involves the addition of a proton to the substituent group.

The present investigation is concerned with the effects of alkali and of strong sulfuric acid on the ultraviolet absorption spectrum of lignin. These effects, interpreted on the basis of Doub and Vandenbelt's work, provide a method for separating the absorption due to phenylpropane units substituted with certain ionizable groups from that of the remaining lignin units, and thereby provide information regarding the structure of lignin which cannot be obtained from its rather unspecific spectrum in neutral solutions.

Results

It was found that the spectra of several lignin preparations in 95% sulfuric acid solution, as well as in alkaline solution, show band displacements toward longer wave lengths. Typical results obtained with western hemlock "native lignin" are shown in Fig. 1. Since these changes of the spectrum are almost completely reversible by neutralization, it may be assumed that the band displacements are due to ionization effects. If only a relatively small fraction of the total number of absorbing groups in the preparation is capable of ionization and, furthermore, if the absorption of the ionized groups is appreciably higher than that of the same groups in neutral solution, it should be possible to obtain an indication of the spectrum of the ionized groups by subtracting the absorption curve of the neutral solution from those of the alkaline and strong acid solutions, respectively. Difference curves for several lignin preparations (Figs. 2 and 3) obtained in this manner reveal three absorption bands, at approximately 250, 300 and 350 mμ. In order to determine the groups whose ionized forms might cause the appearance of these bands, the spectra of several simple model substances were determined under similar conditions. The absorption characteristics of these compounds are summarized in Table I.

According to Doub and Vandenbelt, the substituent groups of simple substituted benzene derivatives do not act as independent chromophores.[8a] These authors have shown convincingly[8b] that the absorption bands of such compounds are of benzenoid origin and are displaced by the effect of the substituent groups. The secondary, first primary and second primary bands[8] which result from the displacement of the absorption bands of benzene at approximately 250, 200 and 180 mμ, respectively, can be readily distinguished[8a] by their different intensity as well as by the different magnitude of the displacement caused by ionization of the substituent groups. For instance, the secondary band near 280 mμ of compounds such as eugenol and conidendrin with phenolic hydroxyl groups and no side-chain unsaturation conjugated with the benzene ring is shifted in alkaline solution to approximately 300 mμ with only a slight increase in inten-

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[1] Paper presented before the Division of Cellulose Chemistry at the 122nd meeting of the American Chemical Society, Atlantic City, N. J., 1952.

[2] E. J. Jones, Jr., *Tappi*, **32**, 311 (1949).

[3] G. Aulin-Erdtman, *ibid.*, **32**, 160 (1949).

[4] R. A. Morton and A. L. Stubbs, *J. Chem. Soc.*, 1347 (1940).

[5] H. W. Lemon, THIS JOURNAL, **69**, 2998 (1947).

[6] H. W. Lemon, *Anal. Chem.*, **19**, 846 (1947).

[7] J. E. Stone and M. J. Blundell, *ibid.*, **23**, 771 (1951).

[8] (a) L. Doub and J. M. Vandenbelt, THIS JOURNAL, **69**, 2714 (1947); (b) L. Doub and J. M. Vandenbelt, *ibid.*, **71**, 2414 (1949).

[9] L. A. Flexser, L. P. Hammett and A. Dingwall, *ibid.*, **57**, 2103 (1935).

sity. On the other hand, the first primary band near 280 mμ of compounds such as vanillin and acetovanillone in which the phenolic hydroxyl group is conjugated through the ring with an α-carbonyl group is shifted in alkaline solution to approximately 350 mμ with a more than twofold increase in intensity. The short wave length bands of both types of compounds are shifted in alkaline solution to a wave length near 245 mμ. When the spectrum of conidendrin in neutral solution is subtracted from that in alkaline solution, maxima at 298 and 250 mμ are obtained; by applying the same procedure to the spectra of vanillin, maxima at approximately 350 and 250 mμ are obtained.

From the results of the work of Doub and Vandenbelt it may be predicted that similar band displacements should result from the ionization in basic and in strong acid solution in the case of compounds in which an electron-releasing and an electron-attracting group, both capable of ionization, are opposed to each other in para position. In such compounds, ionization enhances the already existing tendency of the substituent groups for electron transfer to and from the ring and thus should result in a shift of the absorption to longer wave lengths.[8a] This prediction is confirmed in the case of *p*-hydroxybenzaldehyde. Ionization of this compound in basic solution, involving the loss of a proton from the phenolic hydroxyl group, and ionization in strong acid solution involving the addition of a proton to the carbonyl group, result in almost exactly the same displacement of the primary band from 283 mμ to 333 and 336 mμ, respectively. Similar band displacements in strong sulfuric acid solution are shown also by vanillin, acetovanillone and veratraldehyde (Table I). The last compound, lacking a free phenolic hydroxyl group, cannot ionize in alkaline solution; it shows the same spectrum in alkaline and in neutral solution. In strong sulfuric acid solution, on the other hand, the spectra of veratraldehyde and vanillin are almost identical. By neutralizing a strong sulfuric acid solution of veratraldehyde, and adding an excess of alkali, the unchanged spectrum, identical with that of the neutral solution, was again obtained showing that the compound had not been permanently altered in the strong acid.

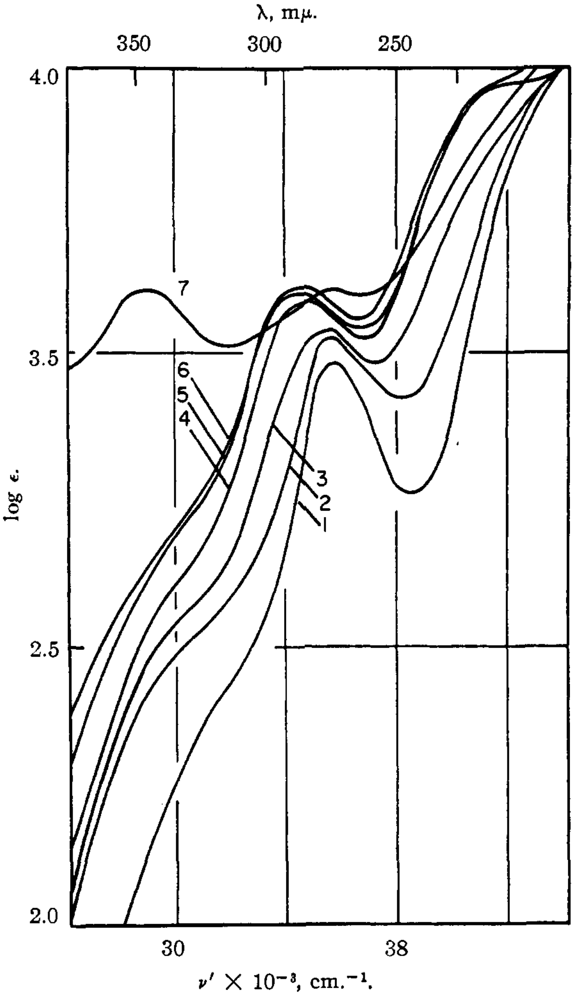

Fig. 1.—Ultraviolet absorption of western hemlock "native lignin" in (1) water, (2) *p*H 9.3, (3) *p*H 10.3, (4) 0.01 *N* NaOH, (5) 0.1 *N* NaOH, (6) *N* NaOH, (7) 95% H_2SO_4.

TABLE I

ULTRAVIOLET ABSORPTION CHARACTERISTICS OF LIGNIN MODEL COMPOUNDS

	Bands in neutral sol.[a]				Bands in alkaline sol.[b]				Bands in strong acid sol.[c]			
	Secondary		1st primary		Secondary		1st primary		Secondary		1st primary	
Compound	λmax[d]	ε × 10⁻³	λmax	ε × 10⁻³	λmax	ε × 10⁻³	λmax	ε × 10⁻³	λmax	ε × 10⁻³	λmax	ε × 10⁻³
Phenol	270[e]	1.50	210.5	6.20	287[e]	2.60	235	9.40	284	1.00	228	8.40
Guaiacol	274	2.15	214	5.80	290	3.30	239	7.90	279	2.55	240	7.90
Veratrole	272	2.30	222	7.00	...	...	...	...	280	3.60	246	10.2
Eugenol	279.5	3.80	224	11.2	295	4.30	245	8.90	...	...	...	...
Conidendrin[f]	284	3.37	~230[g]	6.80	298	4.50	245	8.30	286	2.20	230	7.8
									~300[g]	1.75	~250[g]	2.4
	1st primary		2nd primary		1st primary		2nd primary		1st primary		2nd primary	
p-Hydroxybenzaldehyde	283[h]	14.7	220	13.7	333	26.8	238	7.30	336	30.5	226	5.60
Vanillin	279	10.6	230	15.0	347	25.6	249	9.70	336	19.2	238	7.40
	310	9.20										
Veratraldehyde	278	11.1	230	15.6	...	...	...	...	341	20.2	239	9.20
	308	9.40										
Acetovanillone	276	10.3	228	16.0	345	21.5	246	9.20	331	16.3	240	8.90
	303	7.90										

[a] Solvent is water except where otherwise indicated. [b] Solvent is 0.1 *N* NaOH solution. [c] Solvent is 95% H_2SO_4. [d] Wave length given in mμ. [e] Doub and Vandenbelt (ref. 8a). [f] ε calculated per one-half mole; in neutral sol. solvent is 95% ethanol. [g] Point of inflection. [h] Solvent is 0.1 *N* HCl solution.

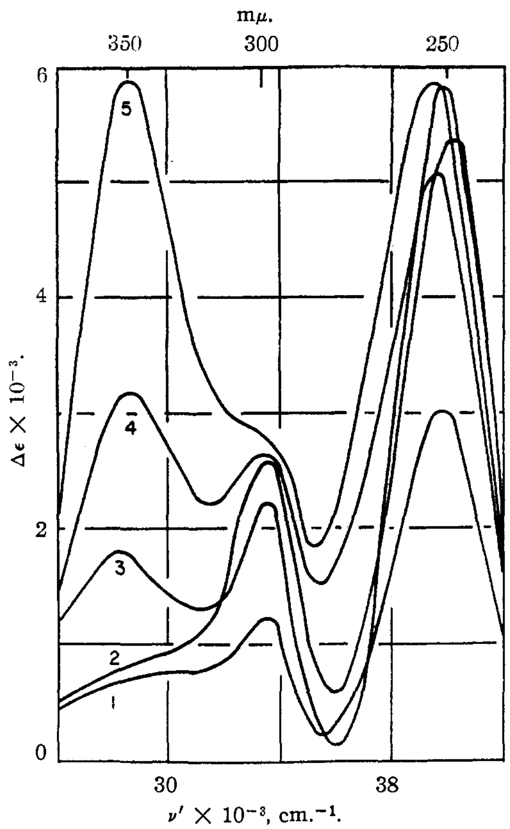

Fig. 2.—Curves obtained by subtracting the spectrum of the neutral solution from that of the alkaline solution of (1) lignin sulfonic acid (0.1 *N* NaOH), (2) western hemlock "native lignin" (0.1 *N* NaOH), (3) decayed western hemlock ethanol extract (0.1 *N* NaOH), (4) lignin sulfonic acid (refluxed 2 hr. in 5% NaOH), (5) lignin sulfonic acid (refluxed 8 hr. in 5% NaOH).

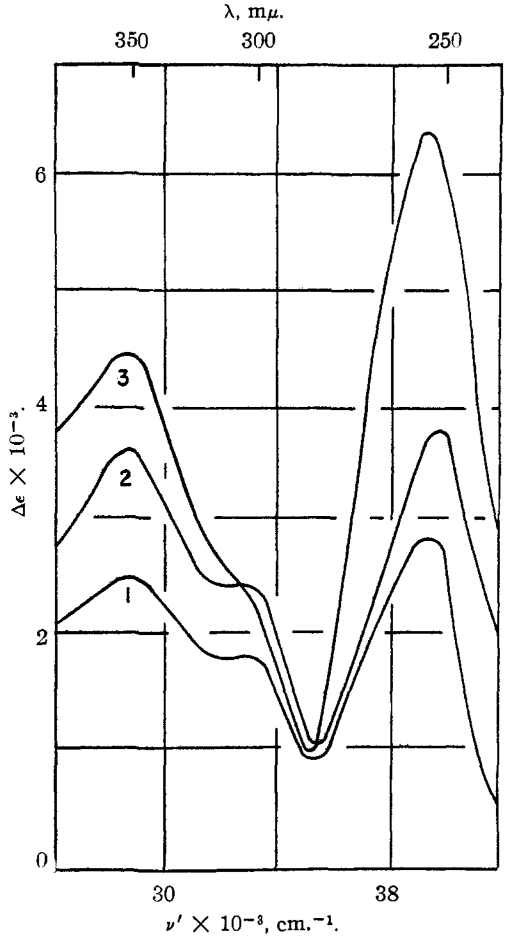

Fig. 3.—Curves obtained by subtracting the spectrum of the neutral solution from that of the 95% H_2SO_4 solution of (1) lignin sulfonic acid, (2) western hemlock "native lignin," (3) decayed western hemlock ethanol extract.

Ionization of conidendrin in 95% sulfuric acid solution results in partial displacement of the spectrum. By subtracting the spectrum of the neutral solution from that of the strong acid solution, maxima at approximately 300 and 250 mμ are obtained. Similar displacements are shown by the spectra of the strong acid solutions of phenol, guaiacol and veratrole (Table I). Under the influence of the strong acid, eugenol gave a strongly fluorescent solution with spectral characteristics similar to those of diisoeugenol.

Comparison of the three bands of the difference curves with the spectra of the model compounds leads to the following conclusions: (1) The band near 300 mμ, obtained from the alkaline solutions of the lignin preparations, is due to the ionization of a phenolic hydroxyl group. This conclusion is supported by the fact that a *pK* close to 10 was obtained by plotting the absorptivity maximum at 298 mμ of the difference curves from Fig. 1 against the *p*H of the solution. These values were $\Delta\epsilon \times 10^{-3}$ zero, 0.63, 1.35, 2.32, 2.64, 2.70, at *p*H values 6.3, 9.3, 10.3, 11.7, 12.7, 13.3, respectively. Due to a partial band displacement similar to that observed with conidendrin, the curves obtained from the strong acid solutions of the lignin preparations show a shoulder at this wave length. (2) The band near 350 mμ of the difference curves derived from the alkaline solutions appears to be due to the presence in these lignin preparations of phenolic hydroxyl groups conjugated through the ring with an α-carbonyl group. The increase in intensity of this band in the curves obtained from lignin sulfonic acid solutions treated under conditions where vanillin is actually formed[10] supports this conclusion (Fig. 2, curves 4 and 5). The maximum near 350 mμ in the difference curves derived from the strong acid solutions (Fig. 3) is due to the presence in these lignin preparations of carbonyl groups conjugated with etherified as well as free phenolic hydroxyl groups. (3) Ionization in both alkaline and strong acid solution of the groups discussed results in a shift of the short wave length band to the 230–250 mμ region of the spectrum. The composite effect of these short wave length bands of the different ionized structures gives rise to the 250 mμ band of the difference curves.

Discussion

The apparent shift toward longer wave lengths of the lignin maximum in alkaline solutions (Fig. 1) is seen to result from the increase in height with increasing *p*H of the solution of the phenolate ab-

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[10] G. H. Tomlinson and H. Hibbert, THIS JOURNAL, **58**, 348 (1936).

sorption at 298 mμ. The height of the 298 mμ band of the difference curves provides a sensitive measure of the phenolic hydroxyl content of different lignin preparations.[11] For instance, the presence of phenolic hydroxyl groups in the highly purified lignin sulfonic acid preparation is clearly revealed by the difference curve (Fig. 3, curve 1) although the spectrum of the alkaline solution itself did not show a perceptible shift of the maximum.

The height of the 350 mμ band of the difference curves from the alkaline and strong acid solutions provides a measure of α-carbonyl groups conjugated with free and etherified phenolic groups, respectively. As a result of the great intensity of the absorption of the ionized forms of the vanilloyl and veratroyl groupings, as little as one such grouping per 40–50 phenylpropane units should be detectable by this method. The lignin preparation from decayed western hemlock wood appears to contain an appreciably greater, the lignin sulfonic acid preparation a considerably smaller number of such carbonyl groups than western hemlock "native lignin."

Spectra of lignin preparations in alkaline and strong acid solutions provide not only considerably more information than the spectrum in neutral solution regarding the structure of some of the phenylpropane units; this information leads also to a new interpretation of the neutral spectrum. Since the data for the model compounds show that in neutral solution the first primary band of compounds with a conjugated carbonyl group is three to four times as high as the secondary band of compounds without that group, even a small number of lignin units with an α-carbonyl group will contribute appreciably to the 280 mμ maximum of the neutral lignin spectrum. This maximum is therefore most likely due to the composite effect of the first primary and the secondary absorption bands of such lignin units of different structure. Moreover, the first primary band of compounds with the vanilloyl or veratroyl structure has a second maximum in the 300–310 mμ region,[12] apparently due to the unsymmetrical substitution of the benzene ring with a methoxyl group in the meta position to the carbonyl group since it is not shown by compounds such as syringaldehyde.[5] This maximum may contribute to the higher absorption of the lignin preparations in this region as compared with model compounds such as conidendrin.

Experimental

Methods.—Spectra were obtained with a Model DU Beckman quartz spectrophotometer, matched 1-cm. cells being used. Molar absorptivities were calculated by dividing the absorbance readings by the molar concentration of the solutions. Concentrations of the lignin preparations were calculated on the basis of their methoxyl equivalent weight[13] (grams of total solids per 31.02 g. of methoxyl). Solutions were prepared by volumetric dilution (usually 1:50) of stock solutions of suitable concentration with distilled water, aqueous sodium hydroxide, and concentrated sulfuric acid. Stock solutions were made up either from solutions of known total solids content or from weighed quantities of the compounds. With substances not sufficiently soluble in water, dilutions were made from stock solutions in ethanol. In order to avoid temperature rises when making dilutions with concentrated sulfuric acid, both the aliquot of the stock solution and the acid were cooled with ice prior to and during the mixing operation. For all absorption measurements, solvent blanks were prepared in exactly the same manner as the solutions.

Materials.—The following lignin preparations were used in this investigation: A sample of western hemlock "native lignin,"[14] 14.8% methoxyl, obtained from Dr. F. E. Brauns, a sample of purified lignin sulfonic acid, 11.25% methoxyl, prepared by the Pulp Mills Research Group, University of Washington, by purification of sulfite waste liquor from a commercial pulping operation by exhaustive continuous dialysis[15]; and finally an ethanol extract of western hemlock wood naturally decayed by "brown rot." A finely ground sample of the decayed wood, 8.5% methoxyl, 46% soluble in 1% NaOH solution, was exhaustively extracted with ethanol at room temperature. Dilutions of this extract were used without further purification; the methoxyl content of the ethanol soluble fraction (8% of the decayed wood) was estimated to be 10.5%.

The model compounds were commercial preparations except for the samples of conidendrin and veratraldehyde which were prepared at this Laboratory by standard methods. Where further purification was necessary, samples were recrystallized or redistilled until melting point or ultraviolet absorption spectrum showed no further changes.

Neutralization of a Sulfuric Acid Solution of Veratraldehyde.—A solution of veratraldehyde (0.12 g./l.) in 95% H_2SO_4, prepared by diluting an ethanol solution of veratraldehyde with concentrated H_2SO_4, was added to a large excess of ice-cold water to which was added, while cooling, a sufficient amount of ice-cold dilute NaOH to make the final solution 0.1 N with respect to NaOH and 1.44×10^{-6} M with respect to veratraldehyde. The spectrum of this solution was identical with that of a distilled water solution of veratraldehyde.

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SHELTON, WASH.

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[11] After this paper was completed, phenolic hydroxyl group contents of several lignin preparations obtained by a similar method were reported by G. Aulin-Erdtman, *Svensk Papperstidn.*, **55**, 745 (1952).

[12] See Table I and also R. F. Patterson and H. Hibbert, THIS JOURNAL, **65**, 1862 (1943).

[13] G. Aulin-Erdtman, *Svensk Papperstidn.*, **47**, 91 (1944).

[14] F. E. Brauns, THIS JOURNAL, **61**, 2120 (1939).

[15] Q. P. Peniston and J. L. McCarthy, *ibid.*, **70**, 1324 (1948).