p-*Hydroxybenzoate Groups in the Lignin of Aspen* (Populus tremula).

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The lignin of aspen wood contains *p*-hydroxybenzoate groups. They occur singly, involving aliphatic hydroxyl groups of the "native lignin," and account for about 10% of its weight.

"Native lignins," the soluble analogues of lignins which are the aromatic substances of high molecular weight associated with woodiness in plants (Percival, *Ann. Reports*, 1942, **39**, 142; Brauns, "The Chemistry of Lignin," Academic Press Inc., New York, 1952), have an ultraviolet absorption spectrum in neutral solvents (Brauns, *J. Org. Chem.*, 1945, **10**, 211; Kudzin and Nord, *J. Amer. Chem. Soc.*, 1951, **73**, 690; Enslin, *J. Sci. Food Agric.*, 1952, **4**, 318; Farmer, *Research*, 1953, **6**, 47) with a characteristic band at 275—285 mμ indicative of an *O*-substituted benzene ring (Jones, *Techn. Assoc. Papers, TAPPI*, 1949, **32**, 311). This band shifts to longer wavelengths and greater intensity in alkaline media, owing to ionisation of phenolic groups (Aulin-Erdtman, *Svensk Papperstidning*, 1952, **44**, 745; Goldschmid, *J. Amer. Chem. Soc.*, 1953, **75**, 3780).

The absorption spectrum of native lignin from aspen differs from those described above by having no defined maximum in neutral solvents (Brauns, Buchanan, and Leaf, *ibid.*, 1949, **71**, 1297). In the present work this lignin was found to show an abnormally large band at 295 mμ in alkaline solution. In 0·01N-sodium hydroxide this remained unchanged, but in N-sodium hydroxide it was slowly replaced by a broader band at 280 mμ. After treatment with N-alkali the lignin was separated into three fractions: A, insoluble in water at pH 4; B, soluble in water at pH 4 but not extracted by ether; and C, both soluble in water at pH 4 and extracted by ether. In alkali, fraction C had a broad absorption band at 280 mμ, thus resembling *p*-hydroxybenzoic acid, whereas fractions A and B showed absorption spectra of the type hitherto considered typical of lignins (Jones, *loc. cit.*). Spectra of the fractions are given in Fig. 1.

Examination of fraction C on paper chromatograms, using as solvents benzene–acetic acid–water and butanol–water (buffered paper), revealed that it was a complex mixture. *p*-Hydroxybenzoic acid, vanillic acid, syringic acid, and ferulic acid (4-hydroxy-3-methoxycinnamic acid) were identified on the chromatograms by comparison with known specimens. The related acids, *p*-coumaric acid and sinapic acid (4-hydroxy-3 : 5-dimethoxycinnamic acid), were not detected. Recrystallisation of fraction C yielded *p*-hydroxybenzoic acid (6·9% yield from the native lignin; the extinction of fraction C at 280 mμ corresponds to 12·2% of *p*-hydroxybenzoic acid, but other acids detected also absorb at this wavelength).

The absorption spectrum and the absence of material soluble in ether both indicate that free *p*-hydroxybenzoic acid is not present in native lignin, and the ease with which *p*-hydroxybenzoic acid is liberated suggests that it is present in an ester linkage. This could be formed either as lignin-O·CO·C_6H_4·OH (I), or as lignin-CO·O·C_6H_4·CO_2H (II), or as a depside-like polymer, produced by self-esterification of *p*-hydroxybenzoic acid, combined or physically associated with the lignin. To test these possibilities, the absorption spectra and rates of alkaline hydrolysis of three esters of *p*-hydroxybenzoic acid which simulate these types of linkage were compared with that of native lignin, as shown in the Table.

	Absorption max. in aq. NaOH (mμ)	Half-time of hydrolysis at 18° (min.)	(concn. of NaOH)
p-Acetoxybenzoic acid	228 •	1·2	0·01N
4-*p*-Hydroxybenzoyloxybenzoic acid	304	4·5	N
Methyl *p*-hydroxybenzoate	295	17	N
Aspen native lignin	295	39	N

• Measured in 1% sodium hydrogen carbonate.

The figures for *p*-acetoxybenzoic acid clearly rule out consideration of a linkage of type II, whereas the absorption maxima of native lignin and of methyl *p*-hydroxybenzoate are

at identical wavelengths. The absorption of the terminal phenolic nucleus of 4-*p*-hydroxybenzoyloxybenzoic acid shows a bathochromic shift of 9 mμ due to the adjacent aromatic

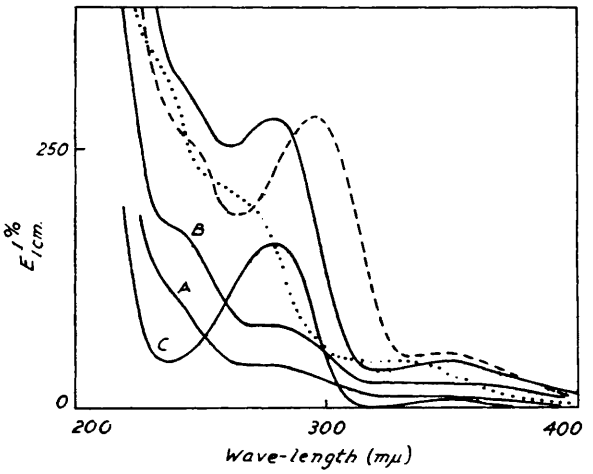

FIG. 1. *Absorption spectra of aspen native lignin.*

. . . . In MeOH.
– – – – In aqueous 0·01N-sodium hydroxide before hydrolysis.
——— In aqueous N-sodium hydroxide after hydrolysis.
A, B, and C are the fractions described in the text.

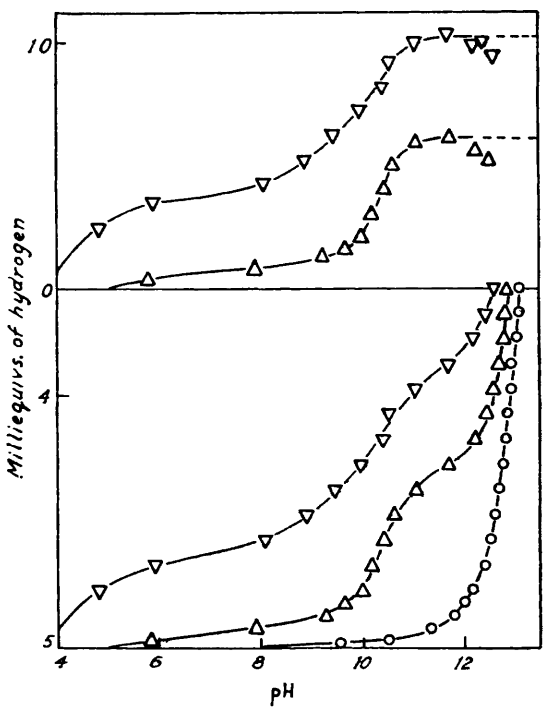

FIG. 2. *Hydrogen-binding curves (upper curves), and titration curves (lower curves), of aspen native lignin* (300 *mg.*).

Upper curves : △ Lignin before hydrolysis; ▽ lignin after hydrolysis.
Lower curves : ○ Sodium hydroxide (5 milliequivs.); △ with lignin before hydrolysis; ▽ with lignin after hydrolysis.

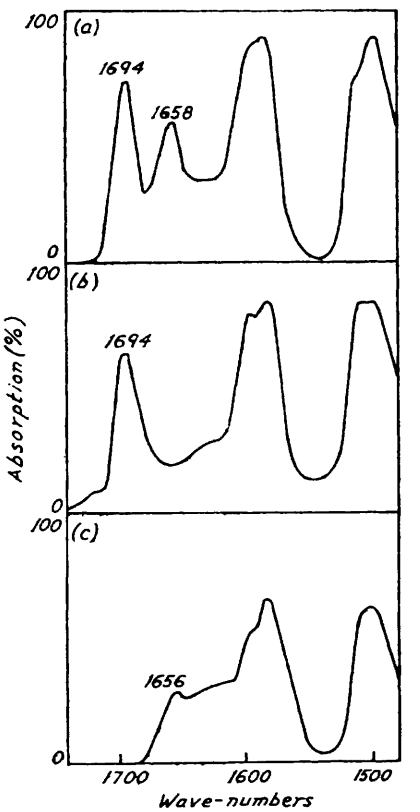

FIG. 3. *Infrared spectra of aspen native lignin in dioxan* (*c*, 10·0).

(*a*) Aspen native lignin.
(*b*) Aspen native lignin reduced by sodium borohydride.
(*c*) Aspen native lignin hydrolysed in aqueous sodium hydroxide.

nucleus, and *p*-hydroxybenzoate esters of phenolic groups of lignin would presumably show a similar shift. Since native lignin does not exhibit this shift, it is probable that the *p*-hydroxybenzoic acid molecules are linked individually by ester linkages of type I to

aliphatic hydroxyl groups of the lignin molecule. The rate of hydrolysis of the various compounds tends to confirm this suggestion. Hydrolysis of aspen native lignin in N-sodium hydroxide, measured spectrophotometrically, consists of a rapid stage (half-reaction time, 39 min.) ending at 69% hydrolysis, and a slow stage resulting in total hydrolysis in about 24 hours. The Table shows that this rate of hydrolysis is approached most closely by methyl *p*-hydroxybenzoate. The slower hydrolysis of lignin may be a consequence of its high molecular weight and the inaccessibility of some of its ester linkages.

The type of linkage postulated received confirmation from the titration curves of aspen native lignin, in the early and in the final stages of alkaline hydrolysis (Fig. 2). The inflexion at pH 11·5 denotes commencement of the process, phenoxide + H^+ ⟶ phenol; and the "hydrogen-binding curves" at this point (Parke and Davis, *Analyt. Chem.*, 1954, 26, 642) measure the total acidic groups (phenolic and carboxylic) present. As lignin separated at about pH 10·4, the dissociation constants of the phenolic groups cannot be derived from the titration curves. Since precipitation of lignin is completed by pH 7, and since the phenolic acids hydrolysed from the lignin are still in solution, the hydrogen-binding curves in this region measure the free carboxylic groups present. The free *p*-hydroxybenzoic acid was also determined spectrophotometrically. The resulting analysis of hydrogen-binding groups is shown below in milliequivalents per g. of lignin :

	Phenolic groups	Carboxylic groups	Free *p*-hydroxybenzoic acid
After 15 min. in N-NaOH	1·8	0·2	0·1
After 24 hr. in N-NaOH	2·1	1·2	0·9
Increase on hydrolysis	0·3	1·0	0·8

Thus there is initially one equivalent of phenolic hydroxyl per 550 g. of lignin, and half of this is in *p*-hydroxybenzoate groups. The increase in free phenolic groups on hydrolysis is small, confirming that the hydroxyl groups involved in esterification are mostly aliphatic. The increase in carboxyl groups on hydrolysis exceeds the *p*-hydroxybenzoic acid released; this may be due to the presence of acetate, derived from acetaldehyde split off the lignin (unpublished work).

The infrared absorption spectrum of aspen lignin in the 5·5—6·5-μ region (Fig. 3) confirms the presence of *p*-hydroxybenzoate groups. There are two bands due to carbonyl-stretching at 1694 and 1658 $cm.^{-1}$, and also two doublets in the regions of 1600 and 1500 $cm.^{-1}$ which are characteristic of phenyl nuclei. The 1694- and 1658-$cm.^{-1}$ bands were each selectively eliminated, the former by alkaline hydrolysis in N-sodium hydroxide, and the latter by reduction with sodium borohydride in 0·01N-sodium hydroxide. This suggests that the 1694-$cm.^{-1}$ band is due to an ester-carbonyl, and the 1658-$cm.^{-1}$ band to an aldehyde- or ketone-carbonyl group. A study of the spectra of analogous simple substances confirms these assignments. The carbonyl-stretching bands of methyl *p*-hydroxybenzoate, acetovanillone, 4-hydroxy-3-methoxycinnamaldehyde, and 3 : 4-dimethoxycinnamaldehyde were at 1694, 1657, 1660, and 1661 $cm.^{-1}$, respectively.

Native lignin is only one of several fractions in the cold-alcoholic extract of wood. The ether-soluble fraction, rejected during isolation of native lignin, was found to resemble native lignin closely in its ultraviolet absorption in 0·01N-sodium hydroxide, suggesting that it also contains *p*-hydroxybenzoate groups. In addition, the residual wood, after exhaustive extraction with alcohol, gave 1·4% of *p*-hydroxybenzoic acid when boiled with N-sodium hydroxide. In this respect, as in others (Brauns, *J. Amer. Chem. Soc.*, 1939, 61, 2120), the ether-soluble, the alcohol-soluble, and the insoluble fraction of lignin resemble one another so much as to suggest a common origin in the plant. The identification of *p*-hydroxybenzoate groups represents the first demonstration of a methoxyl-free aromatic nucleus in aspen lignin.

Experimental

Infrared spectra were measured on a single-beam spectrometer in dioxan solutions (10% for lignin, 7% for model substances) of 0·01-mm. thickness between sodium chloride plates.

Native Lignin.—Newly felled aspen branches (6" diam.) were stripped of bark, phloem, and

pith, immediately reduced to sawdust, and stored at −25° until used. Sawdust (1 kg.) was continuously extracted with cold methanol, and the extract distilled under reduced pressure, leaving an aqueous suspension of toffee-like solid. The solid was washed, dried *in vacuo*, and extracted with methanol (dried; 75 c.c.), leaving a residue (discarded). The resulting solution was run slowly into ether (dried, peroxide-free; 2·5 l.) with vigorous stirring. Lignin, precipitated as a pale cream-coloured powder, was centrifuged, washed with ether, and stored in a current of dry air until free from solvent (yield, 5·5 g.). The product, readily soluble in methanol and aqueous alkali, became partly insoluble in methanol after prolonged drying *in vacuo* (Found, in dried material: OMe, 21·7%).

Hydrolysis of Lignin.—Lignin (100 mg.) in 2·3N-methanolic potassium hydroxide (5 c.c.) was kept for 24 hr., then diluted with methanol (5 c.c.) and poured into water (80 c.c.) containing acetic acid (3 c.c.). The precipitate was centrifuged (fraction A), and the supernatant liquid was extracted with ether (giving fractions B and C). The ultraviolet absorption of each fraction was measured in N-sodium hydroxide.

In an experiment with lignin (300 mg.), fraction C, after evaporation and recrystallisation from hot water (1·5 c.c.) yielded *p*-hydroxybenzoic acid (20·6 mg.), m. p. 208—210°. Vacuum-sublimation raised the m. p. to 215—216° (authentic m. p. 216—217°; mixed m. p. 215—217°) (Found: C, 61·1; H, 4·4. Calc. for $C_7H_6O_3$: C, 60·9; H, 4·4%).

Paper Chromatograms.—The descending method on Whatman No. 1 paper was used. For detection of spots, chromatograms were sprayed with diazotised *p*-nitroaniline in sodium acetate (Swain, *Biochem. J.*, 1953, **53**, 200).

Hydrolysis Rates.—These were measured spectrophotometrically by the increase in extinction at 280 mμ (*p*-acetoxybenzoic acid), or by the decrease in extinction at 304 mμ [methyl *p*-hydroxybenzoate, 4-(*p*-hydroxybenzoyloxy)benzoic acid, lignin].

Hydrolysis of p-*acetoxybenzoic acid*, m. p. 187—190°; $c = 5 \times 10^{-4}$ g./100 c.c. in 0·01N-sodium hydroxide.

Time (sec.)	50	100	150	200	350
Hydrolysis (%)	34·5	64·5	85·6	95·5	100

Hydrolysis of 4-(p-*hydroxybenzoyloxy*)*benzoic acid* (Fischer and Freudenburg, *Annalen*, 1910, **372**, 47), m. p. 270°; $c = 7 \times 10^{-4}$ g./100 c.c. in N-sodium hydroxide.

Time (min.)	4	10	18	35	95
Hydrolysis (%)	41·2	77·4	86·3	94·4	100

Hydrolysis of methyl p-*hydroxybenzoate*, m. p. 124—128°; $c = 7 \times 10^{-4}$ g./100 c.c. in N-sodium hydroxide.

Time (min.)	5	10	18	35	95
Hydrolysis (%)	19·3	35·3	54·6	33·0	100

Hydrolysis of aspen native lignin, $c = 2{\cdot}3 \times 10^{-3}$ g./100 c.c. in N-sodium hydroxide.

Time (min.)	5	12	25	49	95	153	180	1200
Hydrolysis (%)	6·8	14·4	27·3	41·7	58·5	66·8	68·2	100

Titration Curves.—These were obtained using a "Doran alkacid" sealed glass electrode, a calomel microelectrode with wick-type liquid junction, and a "Cambridge portable" pH-meter. Lignin (300 mg.), N-sodium hydroxide (5 c.c.), and water (2 c.c.) were titrated with N-hydrochloric acid, admitted through an immersed capillary into a cell with a magnetic stirrer. After each addition of acid, precipitated lignin was allowed to redissolve before a reading was taken. At pH 10·4, lignin failed to redissolve.

Hydrolysis of Wood.—Aspen wood, after exhaustive extraction with cold methanol, was reduced in a ball-mill to pass a 200-mesh sieve. Powdered wood (200 mg.) in N-sodium hydroxide (200 c.c.) was shaken at room temperature or boiled under reflux. Samples were withdrawn at stated times and centrifuged, and the supernatant liquids (15 c.c. each) were shaken with ether (100 c.c.) containing acetic acid (2 c.c.). The ether extracts were washed once with water (100 c.c.) and extracted with N-sodium hydroxide (10 c.c.). The *p*-hydroxybenzoic acid contents were measured by the extinction at 280 mμ. The standard recovery was determined on pure *p*-hydroxybenzoic acid. Yields at room temperature were 0·38 and 0·72% after 4 and 24 hr. respectively.

Extraction of wood at 100°.

Time (min.)	15	30	60	120
Yield of *p*-hydroxybenzoic acid (%)	1·23	1·32	1·39	1·44

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