THE BOND OF LIGNIN WITH WOOD POLYSACCHARIDES. I

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At present, most chemists do not deny the existence of a covalent bond between the lignin and a carbohydrate fraction of wood, namely the hemicelluloses [1–3]. A bond between the lignin and the cellulose is discussed in a small number of papers and is regarded as unlikely [4]. Considerable differences arise in the elucidation of the types of bonds between the components of wood [4]. The complex of lignin with xylan has been studied in more detail, but no unambiguous conclusions concerning the type of bond were made [5].

This paper gives the results of the isolation and preliminary investigation of a lignin-hemicellulose complex (LHC).

When absolutely dry wood of the Scotch pine Pinus sylvestris (chips with dimensions of 1–2 mm freed from resin with ethanol–benzene) were treated with a solution of dry hydrogen chloride in absolute acetic acid at −10 to 10° C, about 30% of the wood passed into solution. Preliminary experiments established the following facts: in the first place, at a concentration of hydrogen chloride below 7% not all the hemicelluloses were able to pass into solution; in the second place, an increase in the concentration of hydrogen chloride above 8% did not increase the yield of LHC; and in the third place model test on xylan showed that at a concentration of hydrogen chloride of 7–8% there was no cleavage of the glycosidic bonds or humification of the polysaccharide (chromatography, UV spectroscopy).

The lignin-hemicellulose complex was isolated from the acetic acid solution by precipitation with water and subsequent extraction with chloroform and precipitation with hexane. The LHC formed a white amorphous substance soluble in acetone, dioxane, and dimethylformamide but insoluble in alcohols and carbon tetrachloride; it softened on heating (at about 100° C). Chromatography on Whatman MM paper in the butanol–acetic acid–water (4 : 1 : 5) system gave two spots with R_f 0 and 0.82. Thin-layer chromatography in the same system also gave two spots, with R_f 0 and 0.62. The eluates of all the spots absorbed only at 280 mμ (SF-4A, dioxane), which is characteristic for lignin-containing compounds.

Table 1 gives the component compositions of the initial wood, the residue after the extraction of the LHC, and the LHC. The lignin was determined by the sulfuric acid method in Komarov's modification [6], and the carbohydrates in the hydrolysates by paper chromatography (Tables 1–4).

It can be seen from the tables that about one third of the lignin passes into solution and so do all the hemicellulose, regardless of the method of isolation. The passage of the LHC into the solution is probably explained by the fact that under the conditions of treatment acetylation of the lignin and hemicelluloses takes place (with HCl as catalyst), which makes the LHC soluble in acetic acid.

To obtain preliminary information on the structure and composition of the LHC, hydrolysis was carried out at pH 2.5 and the substance was also treated with 0.5 N NaOH and with a 1 N solution of sodium methoxide in methanol by Zemplen's method [7].

Table 2 gives the yields and some characteristics of the residues after the treatments mentioned. The solubility of the LHC in organic solvents is completely lost.

The content and some characteristics of the lignins and carbohydrate components of the initial wood, the LHC, the residue after the extraction of the LHC, and the LHC treated under various conditions are given in Table 3, and the compositions of the mother solutions after the treatment of the LHC at pH 2.5 and with 0.5 N NaOH and 1 N CH_3OH are given in Table 4. After the alkaline treatment the polysaccharides were precipitated with ethanol and hydrolyzed.

On acid hydrolysis, about 4% of the hemicellulose passed into solution; the remainder stayed in the lignin (see Tables 2–4). Since hemicelluloses are soluble in water, their precipitation (especially that of the partially hydrolyzed

Table 1

Component	Initial Wood	Residue	LHC
	% on the weight of the absolutely dry wood		
Lignin	28.86	25.89 18.85	28.63* 10.01**
Carbohydrates****			
Glucose	50.45	54.61 48.12	5.59 1.96
Galactose	1.78	—	5.16 1.80
Mannose	9.89	3.62 3.14	19.42 7.05
Arabinose	0.76	—	2.13 0.75
Xylose	3.96	2.66 2.30	4.72 1.90
Uronic acids***	+	+	+
Acetyl groups	3.61	12.42	32.18
Methoxyl groups	4.67	4.59 3.28	6.22 1.74

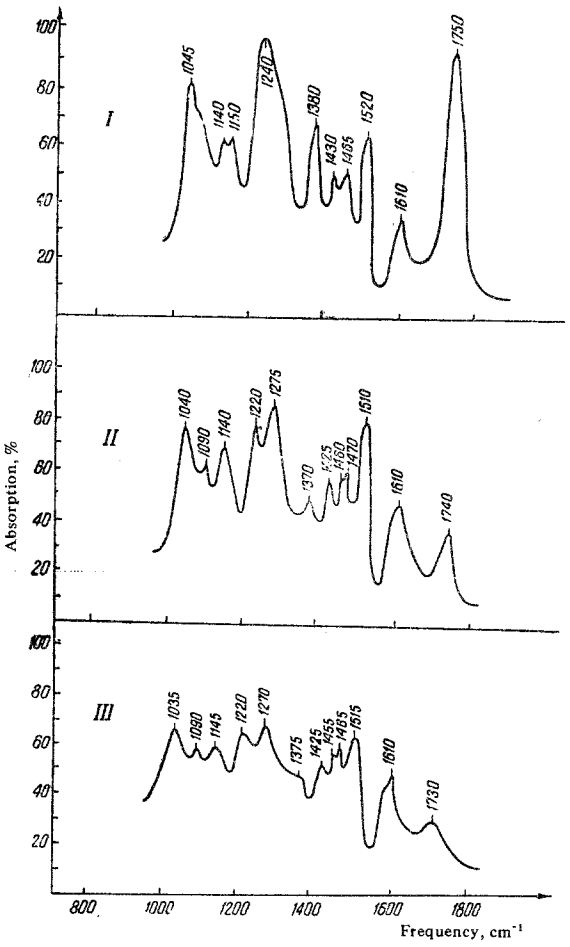
*On the residue and the LHC; **on the initial wood;
***here and in the subsequent Tables the carbohydrates are calculated to polysaccharides; ****not identified.

Table 2

Treatment	Residue	C	H	OCH_3	CH_3CO
		%			
Initial LHC	—	54.11	5.64	6.22	32.18
Hydrolysis, pH 2.5	56.00	57.60	6.19	12.15	—
NaOH, 0.5 N	28.66	65.30	6.09	15.31	—
CH_3ONa, 1 N	40.00	58.50	6.14	11.01	—

Table 3

Sample	In % on the initial absolutely dry wood									
	lignin	content in the lignin			hydrolysate					
		C	H	OCH_3	glucose	galactose	mannose	arabinose	xylose	uronic acids
Initial wood	28.86	64.29	6.48	15.32	50.45	1.78	9.89	0.76	3.96	+
LHC	10.01	65.41	6.48	15.53	.96	1.80	7.05	0.75	1.90	+
Residue	18.85	65.20	5.98	15.10	48.12	—	3.14	—	2.30	+
Residue of the LHC after hydrolysis, pH 2.5	9.97	65.31	6.40	15.30	1.53	1.60	5.07	0.27	1.39	+
NaOH, 0.5 N	9.83	65.30	6.09	15.31	—	—	—	—	—	—
CH_3ONa, 1 N	10.08	64.94	6.43	15.66	1.04	—	1.24	—	0.08	—


I
1045
1140
1150
1240
1380
1430
1495
1520
1610
1730
II
1040
1090
1140
1220
1275
1370
1425
1460
1470
1510
1610
1740
III
1035
1080
1145
1220
1270
1375
1425
1455
1465
1515
1610
1730
Absorption, %
100
80
60
40
20
800
1000
1200
1400
1600
1800
Frequency, cm⁻¹

material) is unlikely. Consequently, it may be concluded that the bond of the lignin with the hemicelluloses is stronger under conditions of acid hydrolysis than the glycosidic bond between the carbohydrate residues.

Table 4

Mother solution	Glucose	Galactose	Mannose	Arabinose	Xylose	Uronic acids
	% on the weight of the absolutely dry wood					
After hydrolysis, pH 2.5	0.27	0.55	2.02	0.40	0.47	—
NaOH, 0.5 N*	1.80	1.85	7.00	0.60	1.77	+
Chromatography of the mother solutions without hydrolysis	—	—	—	0.14	0.07	—
After hydrolysis with CH_3ONa, 1 N*	0.77	1.64	5.01	0.69	1.68	+
Chromatography of the mother solutions without hydrolysis	—	—	—	0.10	0.05	—

*Polysaccharides precipitated with ethanol and hydrolyzed.

When the LHC was treated with 0.5 alkali, all the polysaccharides passed into solution while under the action of sodium methoxide part of the glucose, mannose, and xylose remained bound to the lignin (see Table 3). This fact permits the assumption of two possible types of bond between the hemicelluloses and the lignin. In addition, when the mother liquors from the alkaline treatment (0.5 N NaOH and 1 NCH_3ONa) were chromatographed, monosaccharides, xylose and arabinose, were found (see Table 4). Thin-layer chromatography showed no acetyl derivatives of these carbohydrates. Their origin is still not clear, but they are probably bound to the lignin.

The figure gives the IR spectra (UR-10 instrument, tablets with KBr) of the LHC (I), the LHC treated with sodium methoxide (II), and the sulfuric acid lignin of the initial wood (III).

The absence of absorption bands at 1750 cm^{-1} and 1240 cm^{-1} [8, 9] for the deacetylated LHC (II) shows the complete removal of the acetyl groups. We ascribed the appearance of a band at 1740 cm^{-1} [9, 10] to the cleavage of the ester bond between the carboxyl groups of the lignin and the hydroxyl groups of the hemicelluloses on treatment with sodium methoxide. In the spectrum of sulfuric-acid lignin (III) this band (1730 cm^{-1}) [9, 10] is less intense, which is obviously due to partial decarboxylation during isolation. The band at 1275 cm^{-1} [8, 11], which is characteristic for aliphatic-aromatic ethers, is found in sample II with the absorption of methoxyl groups and the ether bond formed through the phenolic hydroxyl of the lignin and the glycosidic hydroxyls of the hemicelluloses. The greater intensity of absorption for sample II as compared with III with a lower content of methoxyl groups (see Tables 2 and 3) we ascribed to the second type of bond.

Thus, the results of the preliminary investigations (see Tables 2–4 and the figure) permit the assumption of two types of bond between lignin and hemicelluloses: an aromatic ether (phenyl-glycosidic bond) and an aromatic ester bond formed by the carboxyl groups of the lignin and the hydroxyls of the hemicelluloses.

EXPERIMENTAL

Chromatography was carried out on Whatman MM paper, "M" paper of the Volodarskii Leningrad mill, and an alumina (activity grade II). The following systems of solvents were used: 1) butan-1-ol–water–acetic acid (4 : 1 : 5) and 2) ethyl acetate–pyridine–water (4 : 1 : 5).

The carbohydrates were revealed with aniline phthalate and the aromatic compounds with diazotized sulfanilic acid [12]. In the case of thin-layer chromatography, the zones were revealed by spraying with conc H_2SO_4.

Isolation of the LHC. To 1 g of absolutely dry (vacuum, P_2O_5, 72 hr) deresined wood cooled to −5° C was added 20 ml of a solution of dry hydrogen chloride in absolute acetic acid and 0.3 ml of acetic anhydride, cooled to −5° C. The concentration of the hydrogen chloride was 7.9 wt. %. The mixture was kept at −10 to 12° C for 48 hr. After thawing, the residue was filtered off and carefully washed with acetic acid on the filter. The filtrate was poured into 500 ml of ice water containing NaOH in the amount necessary to neutralize the hydrogen chloride. The precipitate that deposited was extracted with chloroform, and the chloroform solution was dried over Na_2SO_4, evaporated in vacuum to a volume of 10–15 ml, and poured into 100 ml of hexane. The precipitate was filtered off, washed with hexane, and dried in vacuum over P_2O_5 and paraffin wax. This gave 0.35 g (35%) of LHC; without acetyl groups 28%. Found, %:

C 54.11; H 5.64; OCH_3 6.22; CH_3CO 32.18.

Thin-layer chromatography on alumina in system 1 gave two spots, with R_f 0 and 0.62, and on paper in system 1 again two spots, with R_f 0 and 0.85. Treatment with aniline phthalate did not reveal any reducing sugars. The UV spectra of the eluates of the spots showed absorption maxima in the 280 mμ region (dioxane).

The carbohydrate composition of the LHC was determined by hydrolysis [6]. The lignin was separated, the mother solution was desalted [13], and the carbohydrates were separated in system 1 (see Tables 1 and 2).

The residue on the filter after the separation of the LHC was carefully washed with ethanol and water and was dried at 105° C. Yield 0.87 g (87%); without acetyl groups, 72%. Found, %: OCH_3 4.59; CH_3CO 12.42.

The lignin and the carbohydrate composition were determined as for the LHC. The carbohydrates were separated in system 2 (see Tables 1 and 3).

Acid hydrolysis of the LHC. A 1-g sample of the LHC was heated in a sealed tube with 25 ml of citrate buffer, pH 2.5, at 100° C for 15 h. The precipitate was filtered off, washed with water, and dried in vacuum over P_2O_5. The yield was 0.56 g (56%). Found, %: C 57.60; H 6.19; OCH_3 12.15.

The lignin and the carbohydrate composition were determined as for LHC.

The carbohydrates were separated in systems 1 and 2 (see Table 3).

The mother solution was neutralized in ammonia to pH ~7, and the carbohydrates were separated in system 1 (see Table 4).

Treatment of the LHC with 1 N sodium methoxide. A 1-g sample of the LHC was dissolved in 2.5 ml of absolute chloroform. To the solution cooled to −2 to 3° C was added 2.5 ml of a cold 1 N solution of sodium methoxide in methanol with vigorous stirring. After 15–20 min, 2.5 ml of ice water and then 0.2 ml of glacial acetic acid were added to the reaction mixture. The precipitate that deposited was separated off and was washed with chloroform and water. Yield 0.40 g (40%). Found, %: C 58.50; H 6.14; OCH_3 11.01. The lignin and carbohydrate composition were determined as described above. The carbohydrates were separated in system 1 (see Table 3).

In the mother solution the chloroformic and aqueous layers were separated. The chloroformic layer gave no residue. The aqueous layer was chromatographed in system 1 (see Table 4). The polysaccharides from the aqueous layer were precipitated with a fivefold amount of ethanol. The precipitate was centrifuged and hydrolyzed as described by Komarov [6]. The carbohydrates were separated in system 1 (see Table 4).

Alkaline treatment of the LHC. A 1-g sample of the LHC was treated with 0.5 N NaOH [14]. After neutralization to pH ~7, the precipitate was filtered off, washed with water, and dried over P_2O_5. Yield 0.2866 g (28.66%). Found, %: C 65.30; H 6.09; OCH_3 15.31.

The polysaccharides from the mother solution were precipitated with ethanol, hydrolyzed by Komarov's method [6], and chromatographed in system 2 (see Table 4).

CONCLUSIONS

1. A method has been proposed for the isolation of a lignin-hemicellulose complex, and its composition has been established.

2. The hypothesis has been put forward of the possibility of two types of bonds between the lignin and the hemicelluloses: an aliphatic-aromatic ether (phenyl glycoside) bond and an aliphatic-aromatic ester bond.

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