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DESTRUCTION OF ASPEN WOOD BY THE FUNGUS *Phanerochaete sanguinea* QUANTITATIVE ^1H AND ^{13}C NMR SPECTROSCOPIES OF BIOLOGICALLY DEGRADED LIGNIN

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The lignin of mechanically ground aspen wood and lignins isolated from aspen wood attacked by the fungus Phanerochaete sanguinea *have been investigated by quantitative ^1H and ^{13}C NMR spectroscopies. It has been shown that the biodestruction of the lignin takes place through the cleavage of alkyl–aryl and aryl–aryl bonds and is accompanied by demethylation (demethoxylation) reactions, and the oxidation of C_α and C_γ atoms. In addition to reactions in which the C—C bonds are cleaved, the formation of ether bonds has been observed. An interconnection has been shown between the variations in the amount of functional groups, fragments, and the bonds in biolignins and the loss in mass of the wood. A method is proposed for evaluating the carbohydrate content in lignin preparations using the NMR method.*

The microbiological destruction of wood is a biological process widely distributed in nature which is performed by natural associations of microorganisms. The destruction of lignin takes place predominately under the action of basidial fungi causing storage rot. Attempts to use the phenomenon of delignification for practical purposes has been stimulated by the study of the chemical reactions accompanying the biodestruction of lignin.

Several approaches are used in the investigation of the processes involved in the biodestruction of lignin: the study of the composition and structure of the low-molecular-mass products formed as the result of biodestruction of lignin; the study of the reactions involved in the biochemical transformation of compounds modeling monomeric and dimeric fragments of lignin; and the study of the structure of the residual biolignin. The first two approaches have been fairly well developed and are widely used [1-3]. The last, which enables information to be obtained directly about the biolignin is being actively developed at the present time [4-12].

It has been widely established that the biodestruction of lignin is an oxidative process. In biooxidation, for example, by the fungus *Phanerochaete chrysosporium* the content of carboxy and carbonyl groups rises and that of methoxy and aliphatic hydroxy groups falls [4-7].

The use of a complex of chemical, chromatographic and spectroscopic (IR, UV, NMR) methods of analysis and radioisotopic labels has deepened our knowledge about the reactions accompanying the biodestruction of lignin. Using synthetic lignin from the dehydrocondensation of ethanol and polyguaiacol labeled with ^{14}C as an example, it has been shown that the fungi of storage rot are capable not only of demethoxylating but also of destroying rings, bringing about the breakage of $C_\alpha-C_\beta$ bonds, and oxidizing terminal hydroxy groups [8-11]. With the aid of ^{13}C NMR spectroscopy it has been possible to reveal the dominating role of the oxidative cleavage of $C_\alpha-C_\beta$ bonds over processes involving the destruction of aromatic rings [12].

The present paper continues a series of investigations in the field of the development of a technology for the biodelignification of cellulosic materials using the fungus *Phanerochaete sanguinea* [13-15]. The aim of the work consisted of an

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TABLE 1. Characteristics of Lignin Preparations (1) and (2)-(9) Isolated from Healthy and Biologically Degraded Wood (mass %)

Lignin	Wood mass losses	Elemental composition				OCH_3	Carbohydrates.
		C	H	O	ashes		
1	—	57.87	6.36	33.61	2.15	16.88	0.7
2	0	55.50	6.42	35.31	2.77	14.63	0.7
3	1.5	52.40	5.93	38.37	3.30	11.94	10.4
4	2.8	51.10	6.20	40.60	2.10	11.08	17.5
5	6.4	48.90	5.70	43.20	1.20	8.56	22.0
6	9.6	49.70	6.04	41.36	2.90	9.39	19.1
7	13.0	51.98	5.76	42.26	0.00	—	17.2
8	17.2	50.90	6.10	42.99	0.01	9.09	19.0
9	23.0	48.39	6.49	45.12	0.00	8.85	25.0

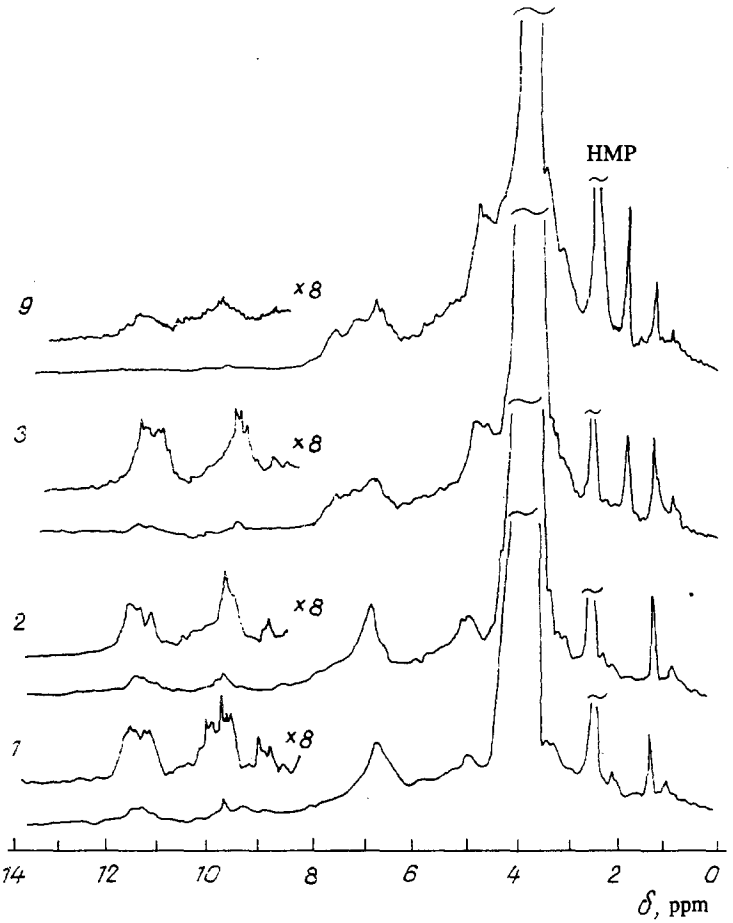

Fig. 1. ^1H NMR spectra of lignin (1) and biolignins (2), (3), and (9), recorded in HMP-d_{18}.

investigation by the method of quantitative ^1H and ^{13}C NMR spectroscopies of the process of degradation of the lignin of the aspen (*Populus tremula*) under the action of the fungus *Ph. sanguinea*. Aspen wood previously extracted with ethyl alcohol was subjected to the action of the fungus *Ph. sanguinea* for different times of incubation. The samples of the biolignin preparations (2)-(9) were isolated from biologically destroyed wood with different mass losses (WMLs). As follows from the analytical results given in Table 1, with an increase in the WML in biolignins (2)-(9) the proportion of oxygen rose and the methoxy group content fell. Similar results have been observed for biolignins obtained after the action of other basidiomycetes such as *Phanerochaeate chrysosporium* [4]. Biolignins (2)-(9), unlike (1), isolated from healthy wood, contained considerable amounts of carbohydrates (Table 1). However, no free carbohydrates were found in solution of the biolignins, and only after acid hydrolysis were xylose, glucose, and trace amounts of glucuronic acid identified. It is obvious that the carbohydrates were chemically bound to the lignin, since attempts to free the lignin from the carbohydrate fraction [16] proved unsuccessful and were accompanied by large losses of the initial biolignin.

TABLE 2. Distribution of the Hydrogen Atoms Over the Structural Fragments of the Lignin Preparations (1)-(9) (in fractions) (^{1}H NMR)

Structural fragment	Lignin									Range of CSs, δ, ppm from TMS, assignment
	1	2	3	4	5	6	7	8	9	
$\Sigma H_{OH,\ phen.}$	0.017	0.021	0.022	0.023	0.024	0.018	0.013	0.012	0.009	12.5—8.7; hydrogen atoms of phenolic OH groups
H_{OH}	0.003	0.003	0.002	0.001	0.003	0.003	0.000	0.000	0.000	9.3—8.7; hydrogen atoms of phenolic OH groups of S′ fragments and G′-fragments with β-5 and 5—5 bonds
H_{ar}	0.136	0.156	0.126	0.106	0.100	0.194	0.102	0.173	0.111	8.7—6.3; hydrogen atoms of aromatic rings
$H_{o\text{-}alk}$	0.718	0.717	0.751	0.775	0.773	0.619	0.720	0.695	0.741	6.3—2.8; hydrogen atoms of CH, CH_2, and CH_3 groups bound with an oxygen atom and olefinic fragments
H_{alk}	0.131	0.105	0.101	0.095	0.099	0.155	0.165	0.118	0.138	2.8—0.5; hydrogen atoms of CH, CH_2, and CH_3 not bound with an oxygen atom

G′, S′ — guaiacyl and syringyl fragments not substituted in the OH_{phen} groups.

TABLE 3. Numbers of Carbon Atoms in Structural Fragments of Lignins (1-9) per Aromatic Ring

Structural fragment	Lignin									Range of the spectrum (^{13}C NMR) ppm from TMS, assignment [22—28]
	1	2	3	4	5	6	7	8	9	
C=O	0.130	0.211	0.400	0.750	0.542	0.422	0.883	1.242	0.756	210—190; C=O, CHO
COO	0.403	0.621	0.903	1.469	0.987	1.145	1.721	1.945	1.315	185—163; acid and ester groups
C_{ar}—O (1)	0.117	0.105	0.133	0.137	0.123	0.120	0.139	0.130	0.108	162; C4 H,H'
(2)	0.052	0.152	0.311	0.306	0.139	0.196	0.465	0.605	0.036	160—154; C4 H; C3, 4 G, G' with α-CO; CHα in HC=CHCHO
(3)	1.066	0.984	1.081	0.857	0.956	1.039	1.085	0.948	0.926	154—151; C3, 5 S
(4)	0.572	0.820	0.562	0.704	0.618	0.603	0.620	0.392	0.900	151—140; C3, 4 G,G'; C3, 5 S'
(5)	0.539	0.418	0.548	0.444	0.478	0.512	0.543	0.474	0.400	135—134; C4 S,S'
ΣC_{ar}—C	1.184	1.254	0.903	0.811	1.133	0.844	1.069	0.782	1.262	140—125; C1 G,G'; C1 SS' 119; C1 H,H'
CH_{ar}	0.572	0.539	0.592	0.735	0.478	0.753	0.496	0.752	0.396	131; C2, 6 H,H' 125—117; C6 G,G'
CH_{ar} (1)	0.805	0.761	0.800	0.720	0.770	0.781	0.714	0.818	0.756	117—110; C3, 5 H,H'; C2, 5 G,G'
ortho (2)	1.050	0.972	1.154	0.887	0.925	0.843	0.902	0.982	1.152	108—103; C2, 6 S,S'
CHO	2.751	1.946	2.621	2.188	2.436	2.487	2.216	2.583	2.569	90—65; $C_\alpha C_\beta$ in α-O-4, β-O-4; Cγ в β-β; CH_α, $CH_{2\alpha}$ in—COOCα—
CH_2O	1.379	0.867	1.126	0.704	1.141	1.025	0.915	0.948	1.008	65—60; C_γ in β-O-4, β-1, β-5
CH_3O	1.444	1.300	1.318	1.041	0.987	1.341	1.271	1.128	1.387	60—65; OCH_3
CHβ	0.650	0.128	0.311	0.490	0.586	0.331	0.279	0.327	0.216	54—52; Cβ in β-1, β-β
C_{alk}	0.325	0.671	0.474	0.275	0.092	0.060	0.108	0.180	0.432	35—10; CH, CH_2, and CH_3 groups not bound with an oxygen atom
C1	0.000	0.000	0.163	0.413	0.432	0.301	0.372	0.327	0.630	103—91; C'1 of xylans
C2,3,4	0.000	0.000	0.489	1.240	1.295	0.903	1.116	0.981	1.890	78—70; C'2, C'3 and C'4 of xylans
C5	0.000	0.000	0.163	0.413	0.432	0.301	0.372	0.327	0.630	63—62; C'5 of xylans
CH_3	0.000	0.000	0.148	0.145	0.215	0.241	0.155	0.180	0.360	21.1; CH_3 of an acetyl group
f_a of the preparation	0.461	0.512	0.405	0.392	0.389	0.389	0.387	0.367	0.333	162—103; proportion of carbon atoms of aromatic rings of the preparation
f_a of the lignin	0.461	0.512	0.438	0.462	0.467	0.457	0.440	0.417	0.424	proportion of carbon atoms of the aromatic rings of the lignins (without the carbon atoms of the xylans and acetyl groups)

H, G, S — p-hydroxyphenyl, guaiacyl, and syringyl fragments substituted in the OH_{phen} group; H', G', S' — the corresponding fragments not substistuted in the OH_{phen} group.

TABLE 4. Amounts of Carbohydrates and Acetyl Groups in the Lignin Preparations (1-9) (mass-%) (^{13}C NMR)

Carbohydrate, acetyl group	Lignin								
	1	2	3	4	5	6	7	8	9
Xylans (Xy)	0.0	0.0	7.2±0.3	17.0±0.7	17.0±0.7	12.3±0.5	15.6±0.7	12.6±0.5	21.0±0.9
Acetyl groups (Ac)	0.0	0.0	2.6±0.1	2.3±0.1	3.4±0.1	3.9±0.1	1.9±0.1	2.7±0.1	5.2±0.2
ΣXy,Ac	0.0	0.0	9.7	19.4	20.4	16.2	17.5	15.4	26.2
(Xy:Ac).	—	—	(1:0.9)	(1:0.4)	(1:0.5)	(1:0.8)	(1:0.4)	(1:0.6)	(1:0.6)

TABLE 5. Number (N) of Main Structure-Forming Bonds and Fragments in Lignins (1-9)per 100 Aromatic rings (^{13}C NMR)

Bond, fragment	Lignin									Remarks
	1	2	3	4	5	6	7	8	9	
OH_{phen}	29±2.9	37±3.7	40±4.0	51±5.1	50±5.0	39±3.9	27±2.7	28±2.8	25±2.5	Phenolic OH groups
$\Sigma C_{ar}—O—C$	60±13	81±18	98±21	117±25	82±18	73±16	131±28	130±28	76±17	Sum of all the aryl-alkyl and aryl-aryl ether bonds
$C_{ar}—C$	18±2	25±2	0	0	13±1	0	7±0.6	0	26±2	Sum of all the $C_{ar}—C$ bonds determining the degree of condensation of lignin
$C_1—C_\alpha$	100	100	90±8	81±7	100	84±7	100	78±7	100	Bond of the C_1 atom of an aromatic ring with a propane chain
ΣS,S'	58±6	53±5	54±5	43±4	48±5	52±5	54±5	47±5	49±5	Syringyl units
ΣG,G'	28±3	24±3	24±3	18±2	3±0.3	32±4	19±2	35±4	39±5	Guaiacyl units
ΣH,H'	14±0.6	10±0.4	13±0.6	14±0.6	12±0.5	12±0.5	14±0.6	13±0.6	11±0.6	*para*-Hydroxyphenyl units
G*	0	13±2	9±1.3	25±4	37±5	6±0.9	13±2	5±0.8	0	3,4-Dihydroxyphenyl units.

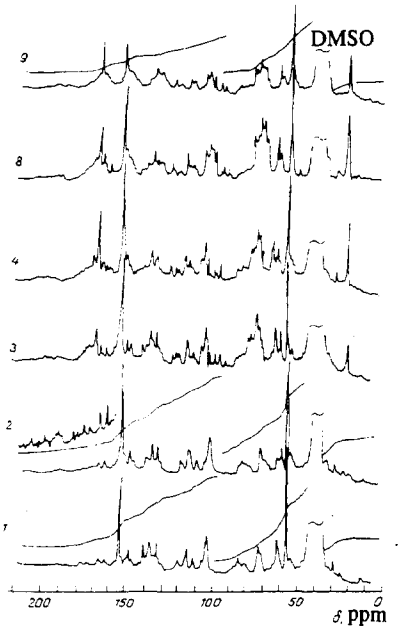

Fig. 2. ^{13}C NMR spectra of lignin (1) and of biolignins (2), (3), (4), (8), and (9) recorded in (dimethyl sulfoxide)-d_6 solution.

A distinguishing feature of the ^1H NMR spectra (Fig. 1) of all the biolignins was a resonance signal with a chemical shift (CS) of 1.93 ppm. This signal may be assigned to the resonance of hydrogen atoms of the methyl group in an acetyl fragment [17] while its intensity was the greater the higher the carbohydrate content of the biolignin preparations (Table 1). Consequently, it may be assumed that the carbohydrates of the biolignins were acetylated. In the region of the resonance of the hydrogen atoms of phenolic OH groups (12.6-8.5 ppm) [18] of biolignins (2)-(9), the resonance signals of the OH groups of syringyl fragments had undergone the greatest changes (9.3-8.5 ppm). In the spectra of biolignins with WMLs of 13-23% their intensities had fallen to zero (Table 1 and Fig. 1). The reason for this phenomenon may be reactions taking place at the OH groups of syringyl rings.

It must be mentioned that the spectra of the biolignins, like the spectrum of lignin (1), lacked resonance signals of hydrogen atoms of carboxy groups (14-11 ppm) [18].

The distribution of the hydrogen atoms over the structural fragments of lignin preparations (1)-(9) is given in Table 2. However, these facts do not enable us to judge changes in the structure of the lignins themselves, since the amounts of carbohydrates in the preparations were dissimilar (Table 1), and an analytical calculation of the distribution of the hydrogen atoms over the structural fragments of the lignins with allowance for the carbohydrate content is required. The use of the results of a chemical determination of the amount of carbohydrates for this purpose would apparently not be quite correct, since it is impossible to take into account the degree of acetylation of the carbohydrates. Therefore, in future we shall use the results of quantitative ^1H NMR only as auxiliary information for calculating amounts of individual structural fragments of the lignins in combination with the results of ^{13}C NMR spectroscopy and elemental composition.

The ^{13}C NMR spectra (Fig. 2) of biolignins (3-9) differed from the spectra of the lignin of healthy wood and of biolignin (2) by the presence of intense signals in the ranges of 102-91, 77-69, and 64-62 ppm belonging to the carbon atoms of a carbohydrate, especially xylose (C′-1; C′-2, C′-3, C′-4; and C′-5, respectively [9]), and also the presence in the strong-field region of narrow resonance signal of a methyl group with a CS of 21.1 ppm, close in intensity to a signal with a CS of 169.7 ppm belonging to the carbon atom of the C=O group of an acetyl fragment [20] (Table 3). This confirmed our assumption of the presence of an acetylated carbohydrate component, represented by a xylan, in biolignins (3-9).

The resonance signals of the anomeric carbon atoms of the xylan (C′-1) (102-91 ppm) were characteristic and did not overlap with the resonance signals of other carbon atoms of the xylan and the carbon atoms of the lignins. This enabled us to take intensities as a basis for the interpretation in the calculation of the amounts of carbohydrates in the lignin preparation (3-

9). The content of acetyl fragments was determined from the integral intensities of the CH_3 groups with a CS of 21.1 ppm. The amount of carbohydrates, calculated from the ^{13}C NMR spectra and elemental composition, agreed well with the results of chemical analysis (Tables 1 and 4). This indicates the possibility of using the ^{13}C NMR method for evaluating the carbohydrate component in lignin preparations.

Knowing the amounts of carbohydrates and acetyl groups, it was possible to determine the degree of acetylation of the carbohydrate moieties of the biolignins (Table 4). Calculation showed that a lignin–carbohydrate complex with a high degree of acetylation of the carbohydrate moiety of the preparation (Cbh:Ac = 1.09) had been isolated from the wood with a low WML (1.5%). In preparations (4), (5), and (7-9), this ratio amounted to 1:(0.4-0.6), which is characteristic for the xylans of the wood of deciduous species [21].

The assignment of the resonance signals in the ^{13}C NMR spectra of the lignins was made in the light of literature information [19, 22-25]. As the basis of quantitative calculations from the ^{13}C NMR spectra we took the proportion of carbon atoms of aromatic rings (f_a) (Table 3) [26-28] equivalent to the six carbon atoms of the aromatic ring. The number of functional groups and fragments associated with one aromatic ring was calculated from the formula

$$n_i = q_i * 6/f_a,$$

where q_i is the proportion of carbon atoms of the i-th functional group of fragments.

The form representing the quantitative information on the structure of lignins as calculated "per one aromatic ring" and not "per one phenylpropane unit" is more correct, since during the biodegradation of wood the structure of the lignin undergoes such profound changes that the mechanical transfer of classical ideas on the elementary structural phenylpropane unit of lignin to biolignin appears to us to be a matter of doubt.

Analysis of the results obtained (Tables 3 and 5) showed that with a rise in the value of WML the content of functional groups in biolignin varied within wide limits. No direct relationship was observed between the changes in the quantitative characteristics of the biolignins and the increase in WML. Thus, for example, the total number of CO and COO groups rose in the interval of WMLs from 0 to 2.8%, fell in the interval of WMLs from 6.4 to 9.6%, and rose again in the WML interval from 13.0 to 17.2% (Table 3). The number of phenolic OH groups in the biolignins rose in the WML interval from 0 to 6.4% and fell at WML > 9.6%. The number of methoxy groups fell in the WML interval from 0 to 6.4% and rose at WML > 9.6%.

Similar features of the change in the amounts of elementary units of the biolignins were characteristic not only for functional groups but also for other parameters describing the structure of lignin, especially for the $C_{ar}-O-C$ and $C_{ar}-C$ bonds (Table 5). There was also a change in the ratio of the main structural units of the biolignins – syringyl (S) and guaiacyl (G): at WML > 9.6% the proportion of G units became 7-10% higher, and the proportion of S-units lower, than in the lignin from healthy wood (Table 5). It may be assumed that at WML > 9.6% it was predominantly guaiacyl-containing lignin that was isolated from the wood.

Thus, on the basis of what has been said above the biodegradation of aspen wood by the fungus with *Phanerochaete sanguinea* may be arbitrarily separated into two stages: at WMLs from 0 to 9.6% we observe a change directly in the structure of the lignin (1) under the action of the fungus, while at WML > 9.6% the changes bear a summary nature connected both with the biodegradation of (1) and with the appearance of a qualitatively different lignin liberated from the deeper layers of the cell wall and the middle lamellas. Consequently, the variation in the amount of structural elements of the lignins under investigation must be considered by dividing the biolignins into two groups (2-5) and (6-9).

In the ^{13}C NMR spectra of biolignins (2-9), the intensity of the resonance signals of the carbon atom of the CO groups of ketones and aldehydes (190-210 ppm) and of ester groups (163-185 ppm) increased in comparison with the spectrum of lignin (1) (Fig. 2). With an increase in the resonance signals of CO and COO groups, there was a rise in the intensities of the signals with CSs of 149, 123, 113-112, and 108-107 cm, assigned, respectively, to the C-3, C-2, and C-6 signals of the G-fragments and the C-2-C-6 atoms of the S-fragments with an α-CO group [23-25]. This permits us to assert the occurrence of oxidative processes in the biodegradation of lignin by the fungus *Ph. sanguinea*, and this directly in the α-position of the propane chain.

The quantitative analysis of the ^{13}C NMR spectra (Table 3) of the biolignins showed that with an increase in the content of CO and COO groups the number of C_γ atoms fell. Consequently, it may be assumed that the decrease in the content of CH_2O groups is connected with oxidative processes which affect not only the C_α but also the C_γ atoms. In view of

the fact that in the ^1H NMR spectra of the biolignins there were no signals of the hydrogen atoms of carboxy groups, it may be assumed that oxidation was accompanied either by a decarboxylation reaction or by an esterification reaction.

The decrease in the content of OCH_3 groups showed the occurrence of demethylation (demethoxylation) reactions in biolignins (2-5) (Table 3). As a result, a decrease in the amount of S- and G-units in these lignins and a simultaneous rise in 3,4-dihydroxy-substituted fragments was observed (Table 5). It is possible that the demethylation reactions form one of the reasons for the increase in the number of phenolic OH groups (Table 5).

The numbers of carbon atoms of aromatic rings linked with a carbon atom at the side-chain or with a neighboring aromatic ring – $\Sigma SC_{ar}-C$ bonds (Table 3), calculated from the intensities of the signals present in the 125-140 ppm region, was lower in biolignins (3-8) than the initial lignin (1) and, in the majority of cases amounted to less than 1 per aromatic ring (Table 3). Consequently, one of the possible pathways of the biodegradation of lignin is realized through the cleavage of alkyl-aryl and aryl–aryl bonds, including C_1—C_α bonds, while for the majority of basidial fungi the cleavage of the C_α—C_γ bond of the propane chain is the most characteristic [4, 5].

In parallel with the cleavage of C_{ar}—C bonds a reaction takes place that leads to the formation of ether bonds: the small content of C_{ar}—O—C bonds in the biolignins had risen 1.3- to 2-fold in comparison with the lignin (1) (Table 5). It is not excluded that the formation of ether bonds leads to an increase in the molecular mass of the biolignins: the change in the molecular mass of the biolignins [15] correlates with the changes in the numbers of C_{ar}—C—O bonds (Table 5).

The proportion of carbon atoms of aromatic rings (the degree of aromaticity of the lignin) in biolignins (2-9), calculated with allowance for the acetylated xylan present, scarcely changed with a rise in the WML (Table 3). On the one hand, this may indicate the absence of oxidative cleavage of the aromatic rings. On the other hand, the degradation of an aromatic ring may take place simultaneously with the cleavage of alkyl–aryl bonds [29]. Then, not only the cleaved aromatic ring but also the side chain would have passed into solution. At the same time, f_a of the high-molecular-mass fraction of the lignin did not change. It is possible that an answer to the question of tbe presence or absence of oxidative cleavage of the aromatic rings under the action of the fungus *Ph. sanguinea* on aspen wood can be given only by additional investigations.

Particular interest is presented by the presence of a large amount of ester bonds in the biolignins. The assumption that the ester bonds are lignocarbohydrate bonds was not confirmed by quantitative calculations: if, from the total number of carbon atoms having resonance in the 185-163 ppm region, we deduct the number of carbohydrate acetyl groups, then to one carbohydrate ring in the biolignin there will be 5-6 ester groups (Table 3). The appearance of ester bonds in the biolignins could be explained by the degradation of aromatic rings, leading to the formation of oxalates and cyclic carbonates (29]. However, this is also inadmissible, since f_a in the biolignin had fallen by only a few percentage points, while the amount of ester groups had increased by a factor of 1.5-4.8 (Table 3). If we take into account the facts that the number of $CH-O_{\alpha,\beta}$ and H_2C-O groups in the biolignins had fallen by a factor of 1.2-2 and the number of C_β atoms in $\beta-\beta$, $\beta-1$, and $\beta-5$ bonds had fallen by a factor of 1.1-5.0, while the relative proportion of carbon atoms of aromatic rings in the biolignins had scarcely changed, it is possible once again to come to the conclusion that one of the pathways for the formation of ester bonds may be the oxidation of the carbon atoms of the side chain to COOH groups, followed by an esterification reaction.

It is also impossible to deny the possibility that the presence of ester groups in the biolignins is connected with the liberation, as the result of the action of the fungus *Ph. sanguinea*, of lignin blocks containing ester bonds, the presence of which in aspen lignin has been reported frequently [30, 31]. In addition to this, the point of view exists that the ester bonds appear after biotreatment as the result of the interaction of the COOH groups of lignin with lipid structures of the fungus [4].

Thus, lignins isolated from healthy aspen wood and aspen wood infected by the fungus *Ph. sanguinea* have been investigated by quantitative ^1H and ^{13}C NMR spectroscopies. The numbers of functional groups, fragments, and bonds in the lignins associated with one aromatic ring have been determined. It has been established that biolignins differ from healthy wood by a higher content of carbonyl and ester groups. It has been shown that the biodegradation of lignin is accompanied by demethylation (demethoxylation) reactions, C_α and C_γ oxidation, and the cleavage of alkyl–aryl and aryl–aryl bonds, including C_1—C_α bonds. The action of the fungus is accompanied not only by degradation reactions but also by reactions leading to the formation of ether and, possibly, ester, bonds. The hypothesis has been expressed that the change in the structure of lignin at high mass losses (more than 10%) is connected both with the biodegradation of the lignin and with the liberation of a quantitatively new lignin from the deeper layers of the cell wall and the middle lamellas. A method of calculating the amount of carbohydrates in lignin preparations based on quantitative ^{13}C NMR spectra and elemental compositions has been proposed.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded on a Bruker WP-200 SY spectrometer with working frequencies of 200.1 MHz (^1H) and 50.13 MHz (^{13}C). The widths of the spectra were 7000 Hz (^1H) and 20,000 Hz (^{13}C). ^{13}C spectra with noise decoupling from protons was recorded after 1000 passages for 20-30% solutions of the lignins in (methyl sulfoxide)-d_6. Noise decoupling was switched off during the relaxation delay, which amounted to 2.5 sec. Chromium acetylacetonate in a concentration of 0.02 M was used as relaxant. ^1H NMR spectra were recorded for 2-5% solutions of the lignins of hexamethylphosphorotriamide-d_{18} (HMP) with a preliminary evaluation of the number of hydrogen atoms of water solvated by the solvent. The delay between pulses was 4 sec. The relative error of integration was 3%.

In the assignment of the signals in the ^1H and ^{13}C spectra we used information given in the literature [26-28]. The quantitative analysis of the spectra was performed by using an approach proposed in [28]. The results of quantitive spectroscopy were treated statistically. The numbers of functional groups, fragments, and bonds in the lignins obtained by calculation are given with an indication of the relative errors of their determination.

Isolation of the Lignins. Aspen wood in the form of 3 × 2 × 0.5 cm blocks was extracted with ethyl alcohol and was then dried in the air and incubated with the fungus *Ph. sanguinea* under the conditions given in [32]* for a week (lignin 2, WML 0.0%) and for six months. In the final samples, blocks with close WMLs were combined 1.5 ± 0.5% (lignin) 3; 2.8 ± 1% (4); 6.4 ± 1% (5); 9.6 ± 1.5% (6); 13.0 ± 1.5% (7); 17.2 ± 2% (8), and 23.0 ± 3.5% (9).

The lignins of the healthy (1) and biologically degraded (2-9) woods were isolated by the procedure of [33]. A partially evaporated aqueous dioxane solution of the lignin was precipitated in a five-fold volume of diethyl ether–ethyl alcohol (10:1, v/v). The lignin precipitate was filtered off and reprecipitated under the same conditions and was then dried in vacuum without heating.

The amounts of methoxy groups in the lignins were determined by the method of [34], and the carbohydrate content on a FÉK-56 photoelectrocolorimeter from the absorption at λ490 nm after the reaction with phenol and sulfuric acid. The qualitative compositions of the carbohydrates were determined by paper chromatography after the acid hydrolysis of the biolignins [33].

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