

## QUANTITATIVE $^1\text{H}$ AND $^{13}\text{C}$ NMR SPECTROSCOPY OF LIGNINS ISOLATED FROM ASPEN WOOD BY VARIOUS METHODS

A. V. Rokhin,<sup>a</sup> L. V. Kanitskaya,<sup>b</sup> D. F. Kushnarev,<sup>a</sup>  
and G. A. Kalabin<sup>a</sup>

UDC 543.422.5:547.992.3

*A comparative analysis has been made of the chemical structures of aspen milled wood lignin, aspen dioxane lignin, and lignin from the explosive autohydrolysis of aspen wood.*

For the investigation of the transformations of lignin under various treatments, use is usually made of so-called mild-isolated lignins, which include organosoluble lignins: milled wood lignin (MWL) obtained by Björkman's method [1] and dioxane lignin (DL). It is considered that DL is little changed and resembles Björkman lignin to some degree [2].

In recent years a new process in delignification technology has been proposed — explosive autohydrolysis, in which intensive fragmentation of the polymeric structure of lignin takes place, leading to a comparatively mild and extremely complete elimination of lignin from wood raw material by washing with organic solvents or dilute solutions of alkali [3].

In the present paper we give results of a comparative investigation by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy of the chemical structures of lignins isolated from aspen wood by various methods: MWL obtained by Björkman's method, DL, and the lignin of an explosive autohydrolysate (LAH).

Procedures for the quantitative determination of functional groups and structure-forming fragments and bonds in lignin preparations with the aid of NMR have been described in detail in the literature [4-6]. However, for recording the  $^{13}\text{C}$  NMR spectra of lignin we have previously used only the IGD (Inverse Gated Decoupling) regime. In the present work we have used the CSE (Conventional Spin Echo) and GASPE (Gated Spin Echo) multipulse sequences, which enable quantitative subspectra of the primary and tertiary ( $\text{CH} + \text{CH}_3$ ) and the secondary and quaternary ( $\text{CH}_2 + \text{C}$ ) carbon atoms to be obtained without polarization transfer into account [7].

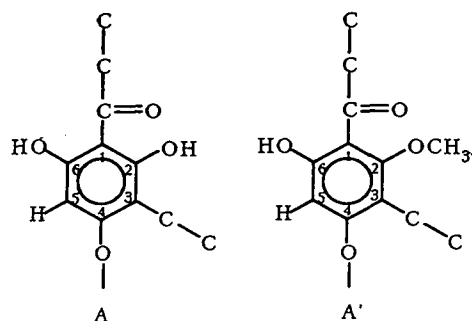
Table 1 gives the results of the quantitative analysis of the  $^1\text{H}$  NMR spectra of the lignins. The multipulse experiments permitted a more accurate determination of the numbers of carbon atoms in ketone and aldehyde groups, in olefinic fragments, in  $-\text{CHO}$  and  $-\text{CH}_2\text{O}$  groups, and in pinosresinol and phenylcoumaran structures (Table 2), and a refinement of the distribution of carbon atoms of aromatic rings in the MWL over the various types: carbon atoms bound to carbon atoms ( $\text{C}_{\text{ar}}-\text{C}$ ) and to hydrogen atoms ( $\text{CH}_{\text{ar}}$ ) (Table 3).

The number of  $\text{C}_{\text{ar}}-\text{C}$  bonds, which characterizes the degree of condensation of the lignin sample, was higher in the MWL than that determined from the synoptic  $^{13}\text{C}$  NMR spectrum [5]. The main contribution to the increase in this structural parameter was made by the signals of carbon atoms present in the 115-100 ppm region of the spectrum. We have reported the presence of such signals previously, but only in the lignins of herbaceous plants [8].

The signals of quaternary carbon atoms in aromatic rings in this region of the spectrum can be explained by the presence in the aspen lignin macromolecule of structures of the acronylin type [9] with  $\text{C}_{\text{ar}}-\text{C}$  bonds in the C-1 and C-3 positions and, simultaneously,  $\text{C}_{\text{ar}}-\text{OH}(\text{OR})$  bonds in the C-2, C-4, and C-6 positions of the aromatic ring (structure A). In this case, the chemical shifts (CSs) of C-1 and C-3 may have values of 99 and 104 ppm, respectively. If there is an  $\text{OCH}_3$  group in the C-2 position (structure A'), the CSs of the C-1 and C-3 atoms are 109.6 and 113.2 ppm, respectively, while in this case the signal of the  $\text{OCH}_3$  group itself is found in a weaker field — at 61-62 ppm [9].

---

a) Irkutsk State University. b) Irkutsk Institute of Organic Chemistry, Siberian Division of the Russian Academy of Sciences [IrIOKh SO RAN]. Translated from *Khimiya Prirodnikh Soedinenii*, No. 6, pp. 878-886, November-December, 1995. Original article submitted November 22, 1994.



In the subspectra of CH+CH<sub>3</sub> groups of aspen MWL there were similar signals, and their numbers, calculated to one aromatic ring (AR) are given in Table 2.

Signals of OCH<sub>3</sub> groups with CSs of 61-52 ppm have previously been observed repeatedly by many workers in the recording of the subspectra of lignins of various tree species by the DEPT (Distortionless Enhancement by Polarization Transfer) method [10]. However, no attention has been directed to their presence in the spectrum because of their low intensity. Nevertheless, resonance signals with a lower intensity for CH<sub>α</sub> of olefinic fragments in Ar-HC=CH-CHO structures have been reported [10].

To prove that the signals in the spectral range of 115-100 ppm for the aspen MWL relate to quaternary carbon atoms, and not to CH<sub>2</sub> groups of olefinic fragments, and those in the 61-62 ppm region to OCH<sub>3</sub> groups, we additionally used editing of the spectra by the DEPT procedure in which only carbon atoms bearing hydrogen are observed [10]. In the subspectrum of CH<sub>2</sub> groups we observed no resonance signals in the 115-100 ppm region, while in the subspectrum of CH<sub>3</sub> groups signals of OCH<sub>3</sub> groups were present in the 61-62 ppm region. From the number of carbon atoms having resonance signals in the 115-100 ppm region we determined the number of A + A' structures in the aspen MWL macromolecule referred to 100 ARs (see Table 3).

As can be seen from Table 2, the structure of aspen MWL contains a fairly large number of functional groups: C=O, C(O)H, and C(O)OR. Qualitative and quantitative analysis of the <sup>13</sup>C NMR spectrum of the MWL showed that the majority of α-C=O groups (ketonic and, possibly, ester groups) conjugated with an aromatic ring were present at S-rings. This was shown by the intense signals of carbon atoms at 106-105 ppm (C-2 and C-6 in S-rings) (Table 2) and the low intensity of the resonance signals in the spectral ranges of 123-124 and 113-112 ppm (C-6 and C-2 in G rings with α-C=O, respectively) [11].

Judging from the numbers of C-2 and C-6 atoms in S-fragments with α-C=O, in the MWL macromolecule there were not more than 14-15 of them per 100 ARs. If it is assumed that all the S-rings have C(O)OR groups but not C=O groups in the α-position, the remaining 57 C(O)OR groups are not conjugated with aromatic rings and may be present in either a β- or a γ-position relative to an aromatic ring. The presence of ester groups in lignins (particularly in the lignins of broad-leaved species) has been reported repeatedly [12, 13].

An indirect confirmation of the presence of a large number of ester groups in the lignins of broad-leaved tree species such as birch may be its mean structural formula calculated from the elementary composition and the results of functional analysis [14]: C<sub>9.34</sub>H<sub>7.04</sub>O<sub>2.07</sub>(OH<sub>phe</sub>)<sub>0.21</sub>(OH<sub>alk</sub>)<sub>1.04</sub>(OCO)<sub>0.33</sub>(OCH<sub>3</sub>)<sub>1.50</sub>.

In view of the fact that, according to Adler [15], the number of C<sub>ar</sub>-O-C bonds in birch lignin amounts to 0.74/PPU it can be calculated that 1.33 oxygen atoms per PPU still remain unidentified, while it has been reported [14] that there are no C(O)OH groups in birch MWL. Consequently, we are left to assume either that there is ~0.7 COOR to one structural unit or that there are 1.33 (!) C<sub>alk</sub>-O-C<sub>alk</sub> ether bonds per one structural unit of birch lignin.

On analyzing the <sup>13</sup>C NMR spectra of aspen MWL, attention is attracted by the large number of carbon atoms linked to oxygen atoms in side-chains (resonance signals in the 90-60 ppm region (Table 2). The total of -CHO and -CH<sub>2</sub>O groups in aspen MWL corresponds to ~3.9 carbon atoms per AR, which is close to figures that we have obtained previously [16], the difference between these two determinations not exceeding the experimental error. However, the distribution of carbon atoms over the -CHO and -CH<sub>2</sub>O groups differs considerably from other previous results [5] (Table 2). Thus there were only 1.8 CHO groups and 2.026 CH<sub>2</sub>O groups per aromatic ring in the aspen MWL macromolecule, while a fairly large proportion of these groups may have been present in CH<sub>2</sub>-O-CH<sub>2</sub> and C(O)O-CH<sub>2</sub> fragments of side-chains (<sup>13</sup>C CS 72-71 ppm). Thus, it may be assumed that some of the ester groups in the aspen MWL macromolecule link side-chains with one another.

TABLE 1. Distribution of Hydrogen Atoms over Functional Groups and Structural Fragments of the Lignins ( $f_x = I_x/I_{tot}$ ) from  $^1\text{H}$  NMR Spectra

Fragments	Lignins			Range of $^1\text{H}$ CSs ( $\delta$ , ppm) and assignments of the signals [4]
	MWL	DL	LAH	
$\text{H}_{\text{C}(\text{O})\text{OH}}$	0.0001	0.002	0.006	14.0–12.0 OH of carboxylic acids
$\text{H}_{\text{C}(\text{O})\text{H}}$	0.002	0.006	0.012	9.7–9.0 H in aldehydes*
$\text{H}_{\text{OH phe}}$	0.012	0.022	0.016	12.4–9.3 OH at C-4 G', H'
$\text{H}_{\text{OH phe}}$	0.003	0.014	0.026	9.3–8.4 OH at C-4 S', G <sup>†</sup>
$\text{H}_{\text{ar}}$	0.136	0.135	0.155	8.4–6.3 hydrogen atoms of aromatic rings
$\text{H}_{\text{O-alk}}$	0.716	0.590	0.338	6.3–2.9 CH, CHO, $\text{C}\bar{\text{H}}_2\text{O}$ , and $\text{CH}_3\text{O}$ in the $\alpha$ -, $\beta$ -, and $\gamma$ -positions to an aromatic ring, and carbohydrate hydrogen atoms
$\text{H}_{\alpha,\beta,\gamma}$	0.131	0.263	0.449	2.9–0.3 CH, $\text{CH}_2$ , and $\text{CH}_3$ in the $\alpha$ -, $\beta$ -, and $\gamma$ -positions to an aromatic ring

\*Determined from  $^1\text{H}$  NMR spectra recorded in chloroform-d solution.

<sup>†</sup>G' –hydrogen atoms of hydroxy groups at the C-4 carbon in structures with 5-5 bonds.

TABLE 2. Numbers of the Main Functional Groups and Fragments ( $n_x$ ) per AR in Aspen Lignins from  $^{13}\text{C}$  NMR Spectra

Fragments	Lignins			Range of $^{13}\text{C}$ CSs ( $\delta$ , ppm) and assignments of the signals [5, 6]
	WML	DL	LAH	
C=O	0.061	0.149	0.286	220–190 C=O in ketones
C(O)H	0.031	0.103	0.142	210–185 C=O in aldehydes
C(O)OH	0.034	0.074	0.155	185–164 C(O)O of carboxylic acids
C(O)O	0.685	0.260	0.244	185–164 C(O)O of esters
CH=CH	0.203	0.084	0.222	156–120 CH=CH Ar-CH=CH-C(O)H
$\text{C}_{\text{ar}}\text{-O}(1)$	0.173	0.241	0.092	164–156 C-4 H, H'
$\text{C}_{\text{ar}}\text{-O}(2)$	1.066	0.884	0.530	156–150 C-3/C-5 S, C-4 G, G'5-5
$\text{C}_{\text{ar}}\text{-O}(3)$	0.865	0.892	1.509	150–140 C-3/C-4 G, G'
$\text{C}_{\text{ar}}\text{-O}(4)$	0.580	0.620	0.560	134–138 C-4 S, S'
$\text{C}_{\text{ar}}\text{-C}(1)$	0.826	1.018	1.223	140–123 C-1 S, S', C-1 G, G'
$\text{C}_{\text{ar}}\text{-C}(2)$	0.217	0.179	0.182	123–119 C-1 H, H'
$\text{C}_{\text{ar}}\text{-C}(3)$	0.073	0.048	0.107	119–114 C-1/C-3 A
$\text{C}_{\text{ar}}\text{-C}(4)$	0.105	0.121	0.163	114–95 C-1/C-3 A'
$\text{CH}_{\text{ar}}(1)$	0.162	0.117	0.212	132–125 C-2/C-6 H, H'
$\text{CH}_{\text{ar}}(2)$	0.309	0.220	0.138	125–117 C-6 G, G'
$\text{CH}_{\text{ar}}(3)$	0.378	0.218	0.217	117–114 C-5 G, G', C-3/C-5 H, H'
$\text{CH}_{\text{ar}}(4)$	0.274	0.266	0.255	114–108 C-2 G, G'
$\text{CH}_{\text{ar}}(5)$	0.296	0.387	0.318	108–105 C-3/C-5 S, S' ( $\alpha\text{C}=\text{O}$ )
$\text{CH}_{\text{ar}}(6)$	0.673	0.653	0.446	105–102 C-3/C-5 S, S'
$\text{CH}_{\text{ar}}(7)$	–	0.073	0.048	102–95 C-5 C-5 A, A'
$\text{CHO}_{\text{alk}}$	1.869	0.998	0.644	90–64 $\text{C}_{\alpha,\beta}$ in ( $\beta\text{-O-4}$ )
$\text{CH}_2\text{O}_{\text{alk}}$	2.026	0.853	0.172	90–64 $\text{C}_{\gamma}$ in $\text{CH}_2\text{O-R}$
$\text{OCH}_3'$	0.100	0.165	0.215	64–58 C in Ar-OCH <sub>3</sub>
$\text{OCH}_3$	1.443	1.444	1.202	54–54 C in Ar-OCH <sub>3</sub>
$\text{CH}_{\beta-\beta}$	0.307	0.099	0.089	54–52 $\text{C}_{\beta}$ in ( $\beta\text{-}\beta$ )
$\text{CH}_{\beta-5}$	0.103	0.074	0.072	54–50 $\text{C}_{\beta}$ in ( $\alpha\text{-O-4}$ , $\beta\text{-5}$ )
$\text{C}_{\text{alk}}$	0.642	0.856	1.196	45–5 CH, $\text{CH}_2$ , and $\text{CH}_3$ aliphatic
$f_a$	0.461	0.538	0.570	164–102 $f_a = I_{\text{ar}}/I_{\text{tot}}$

The main difference of the  $^{13}\text{C}$  NMR spectra of the lignins of broad-leaved trees from those of the lignins of coniferous species consists in the presence of intense resonance signals in the spectral ranges of 160–150 and 107–102 ppm, which are characteristic, respectively for the C-3/C-5 and C-2/C-6 atoms of syringyl rings. And although the  $\text{C}_{\text{ar}}\text{-C}_{\text{ar}}$  signals fall into the 160–150 ppm range (C-3 in 5-5 bonds amounting to  $\sim 4$  per 100 rings), their number is not comparable with the number of S-structures, and they have therefore been ignored in the calculation of the number of S-fragments [5, 6]. The number of unesterified syringyl rings (S') was estimated by using the  $^1\text{H}$  NMR spectrum of aspen MWL recorded in HMPT- $\text{d}_{18}$  solution [4].

The numbers of bonds in the MWL characterizing the degree of condensation of the material ( $\text{C}_{\text{ar}}\text{-C}$ ) and the sum of the aryl-alkyl and aryl-aryl ether bonds ( $\text{C}_{\text{ar}}\text{-O-C}$ ) are given in Table 3.

TABLE 3. Numbers of Structural Fragments and Bonds ( $N_p$ ) in Aspen Lignins per 100 ARs

Types of fragments and bonds	Lignins			Relative error, %
	MWL	DL	LAH	
S+S' (S')	58(5)	62(18)	56(33)	10.3
G+G'	28	26	26	12.0
H+H'	5	4	4	13.0
A+A'	9	15	13	20.0
S( $\alpha$ C=O)	15	19	16	10.5
G( $\alpha$ C=O)	2	4	8	10.5
$\Sigma$ C <sub>ar</sub> -O	268	264	269	10.3
C <sub>ar</sub> -C	22	37	68	10.3
$\Sigma$ CH <sub>ar</sub>	210	193	163	10.3
C side-chains	532	269	202	10.3
C <sub>ar</sub> -OH	20	47	53	12.0
OCH <sub>3</sub>	144	144	120	9.5
OCH <sub>3</sub> '	10	17	22	10.3
C <sub>ar</sub> -O-C	94	56	74	14.0
C <sub>alk</sub>	64	86	120	9.5

TABLE 4. Amounts of the Main Functional Groups, wt. %

Functional groups	Lignins			Relative error, %
	MWL	DL	LAH	
C=O	0.6	1.7	3.5	6.9
C(O)H	0.3	1.3	1.8	6.9
C(O)OH	0.6	1.4	3.1	9.2
C(O)O-	11.1	4.9	4.7	9.6
OCH <sub>3</sub>	16.5	16.6	16.4	6.9
OCH <sub>3</sub> '	1.5	3.4	2.4	9.2
OH <sub>phe</sub>	1.6	3.4	4.0	9.2
OH <sub>al</sub>	10.3	5.8	2.6	20.0
OH <sub>carbohydrates</sub>	0.2	1.7	1.0	6.9
(CH+CH <sub>2</sub> +CH <sub>3</sub> ) of saturated carbohydrates	3.2	5.2	9.7	6.9
(COO+CH <sub>2</sub> O+CH <sub>3</sub> O) - nonlignin components	0.0	0.0	24.0	20.0

The number of C<sub>ar</sub>-C bonds in the aspen MWL was 22/100 ARs, which is close to the number of these bonds in beech lignin [17]. The number of C<sub>ar</sub>-O-C bonds in aspen MWL is somewhat greater than in birch and beech lignins [14, 17].

The aspen MWL also contained a small amount of unoxidized aromatic carbon atoms of side-chains — 64/100 ARs. They are found in all samples of lignins — from the woods of coniferous and broad-leaved species and from herbaceous plants [5, 6, 8]. Practically all lignin spectra have signals in the 39-14 ppm range. Is it possible to regard the unoxidized aliphatic fragments as "built into" the lignin macromolecule or must they be considered as difficulty separable fatty acid esters? May all COOR groups relate to difficulty separable fatty acid esters? Some may, but by no means all, since the number of COOR groups in the MWL amounted to 69/100 ARs (Table 2) and the sum of the C<sub>al</sub> atoms was only 64/100 ARs. Likewise, not all the COOR groups can bind lignin with carbohydrates, since in the MWL sample studied their amount was only about 1%.

The chemical structure of aspen DL differs appreciably from that of aspen MWL [5, 6]. The <sup>1</sup>H NMR spectrum of the aspen DL showed considerably more hydrogen atoms of phenolic OH groups and aldehyde groups and hydrogen atoms of saturated aliphatic chains than that of the MWL (Table 1). Quantitative analysis of the <sup>13</sup>C NMR spectra of the DL showed an increase in the number of C=O groups of ketones and aldehydes and a 2-fold fall in the number of COOR groups as compared with the MWL (Table 2). The degree of aromaticity of the DL was higher than that of the MWL because of the smaller proportion of side-chain carbon atoms (1.8-fold without taking into account the carbon atoms of unoxidized aliphatic chains and the carbon atoms of xylans). Consequently, in the isolation of DL from aspen wood the cleavage of ester bonds is possible, with the dissolution of fragments of lignin having side-chains shorter than those in the MWL (2.69 and 5.32 carbon atoms, respectively; Table 3). The increase in the number of phenolic OH groups (Table 3) shows a cleavage of C<sub>ar</sub>-O-C bonds. The number of the latter in the macromolecule of DL as compared with that of the MWL had decreased by 33/100 ARs. The degree of condensation of the aspen DL was almost twice that of the MWL, and there were 3 times fewer pinoresinol structures (Table 2). The numbers of S, G, H, and other fragments are given in Table 3.

It is possible that the differences in the structures of aspen DL and MWL noted above are a consequence not only of the more severe method of isolating the lignin but also of the fact that the yield of DL on the weight of the wood was substantially higher than the yield of MWL. Consequently, lignin separated from the wood most completely may also be the most representative in the structural respect. Thus, for example, it is well known that the Björkman lignin (MWL) of broad-leaved tree species has a lower S:G ratio than the whole lignin of the same wood [1, 2, 15]. We also observed this for the aspen MWL as compared with the aspen DL (Table 3). It is interesting to note that there were almost twice as many A + A' structures in the DL than in the MWL (Table 3): in the MWL the S:G:H:A ratio was 58:28:5:9, and the DL it was 62:26:4:8.

The main advantage of the high-temperature autohydrolysis of lignified plant raw material is the possibility of obtaining the main chemical components — cellulose, lignin, hemicelluloses — in relatively pure form. The yield of dioxane-extractable lignin after hydrolysis is always higher than on the isolation of dioxane lignin [3, 14]. It is known that on extraction with aqueous dioxane a definite part of the carbohydrate components is always extracted simultaneously and accompanies the lignin preparations. The same thing took place on the extraction of aspen wood that had been subjected to autohydrolysis. As found in [14], aspen LAH contains 5-7% of carbohydrates, while birch LAH, for example, contains 2.3% of carbohydrates. In addition, it has been established that a preparation of birch LAH contained a nonlignin component (~24%) "built into" the lignin macromolecule. The researchers concerned did not rule out the presence of methylated hemicelluloses, either [14].

Qualitative analysis of the  $^{13}\text{C}$  NMR spectra showed that the aspen LAH contained no appreciable amount whatever of carbohydrates. However, in the region of the  $^{13}\text{C}$  NMR spectrum from 75 to 65 ppm narrow intense resonance signals of  $\text{OCH}_2$  groups were observed in the C +  $\text{CH}_2$  subspectrum and narrow signals of  $\text{OCH}_3$  groups with CSs of 63-64 ppm in the CH +  $\text{CH}_3$  subspectra; narrow resonance signals with different intensities were also observed in the weak-field part of the spectrum (165-175 ppm) which may relate to C(O)OR and C(O)OH groups. An analysis of the literature [18] led us to the conclusion that such a "set" of resonance signals may belong to compounds of the type of  $\text{CH}_3-\text{O}-\text{CH}_2-\text{C}(\text{O})\text{OH}(\text{R})$ . The level of such nonlignin compounds in the LAH amounted to ~20 wt. % (Table 4).

Thus, as in the case of birch LAH [14], a high-molecular-mass preparation of aspen LAH contained a large amount of compounds of clearly nonlignin nature. We assume that such structures may be produced by the decomposition of carbohydrates.

The autohydrolysis of aspen wood is accompanied by the oxidation of the lignin side-chains to C=O and C(O)H groups. Their amount is greater than in aspen DL. G-Structures are oxidized predominately in the  $\alpha$ -position (Table 3). A large proportion of the C=O and C(O)O groups are present in the  $\beta$ - and  $\gamma$ -positions of the side-chain. The degree of condensation of the LAH was 3.2 times higher than that of the aspen MWL and 1.8 times higher than that of the aspen DL; i.e., in the LAH practically every aromatic ring had, in addition to a  $(\text{C}_{\text{ar}}-1)-\text{C}_{\alpha}'$  bond, one  $\text{C}_{\text{ar}}-\text{C}$  bond. The results of an NMR investigation of the degree of condensation of the LAH were indirectly confirmed by those of the alkaline nitrobenzene oxidation of aspen LAH: the yield of the main product — syringaldehyde — fell sharply in comparison with the MWL and amounted to 9.2 wt. %, as compared with 32.0 wt. %.

The number of  $\text{C}_{\text{ar}}-\text{O}-\text{C}$  bonds was comparable with their number in the DL macromolecule; consequently, in the high-temperature autohydrolysis process condensation ("cross-linking") reactions predominated over the cleavage of ether bonds. The greatest differences in the structures of aspen MWL, DL, and LAH were observed in the numbers of  $-\text{CHO}$  and  $-\text{CH}_2\text{O}$  groups in side-chains (Table 2). During the autohydrolysis of aspen wood, cleavage of the C-C bonds of the side-chains took place. As a result of this, in the LAH macromolecules there was an average of only 2 side-chain carbon atoms per aromatic ring. The amount of  $\text{OH}_{\text{al}}$  in the aspen LAH was 1.5 times less than in the DL, and 2.7 times less than in the MWL (Table 4). This agrees with the results for birch LAH [14].

The ratio of the main units in the LAH (S:G:H:A) 56:26:4:13 was close to their ratio in the aspen MWL. It must be mentioned that on the autohydrolysis of aspen wood the greatest changes were undergone by  $\text{C}_{\text{ar}}-\text{O}-\text{C}$  bonds at S-rings: more than half the S-rings were unesterified (Table 3). The degree of substitution of the S, G, and H rings after autohydrolysis had changed in comparison with the DL and MWL ( $\text{C}_{\text{ar}}-\text{C}$  bonds). In the S-rings, 39 C-2/C-6 atoms were substituted, while in the G- and H-rings a total of 55 were substituted. It must be borne in mind here that there were 20 fewer of these rings than of S-fragments. In the G- and H-rings the C-5 (G) and C-3/C-5 (H) positions were most highly substituted. In the G-rings the number of  $\alpha$ -C=O groups had become somewhat greater than in the aspen DL and MWL. Therefore the conclusion of Zakis et al. [14] that the S-PPUs underwent more profound changes than the guaiacyl units in autohydrolysis is not fully confirmed by our results.

Thus, the chemical structures of the macromolecules of aspen lignins isolated from the wood by various methods have been investigated by quantitative  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy. In addition to syringyl, guaiacyl, and *p*-hydroxyphenyl structures, structures of the acronylin type in amounts of 9-15 per 100 aromatic rings have been found in aspen lignin.

It has been established that a more severe method of isolating lignin from wood leads to an intensification of the oxidation of the side-chains to  $-\text{C}=\text{O}$ ,  $-\text{C}(\text{O})\text{H}$  and  $\text{C}(\text{O})\text{OH}$  groups, the cleavage of aryl-alkyl ether bonds with the formation of phenolic OH groups, and the cleavage of ester bonds, and also to condensation reactions of the polymeric molecule. In addition, cleavage of C-C bonds of side-chains takes place with the splitting out of aliphatic OH groups.

The chemical structure of dioxane lignin has changed so much that it cannot be considered as close in structure to aspen milled wood lignin. Only the lignin from explosive autohydrolysis is similar in the ratio of the S, G, and H fragments to the structure of the MWL.

## EXPERIMENTAL

Aspen MWL was obtained by Björkman's method [1], aspen DL by Pepper's method [2], and aspen LAH by the method given in [14]. The nitrobenzene oxidation of the MWL and the LAH was carried out by A. F. Gogotov of the Division of Wood Chemistry of IrIOKh SO RAN. The elementary compositions of the lignin preparations were, %: MWL: C 57.87; H 6.36; O 33.64; DL: C 59.55; H 5.91; O 34.54; LAH: C 62.25; H 6.11; O 31.64.

The spectra of the lignins were recorded on Bruker WP-200SY and Varian VXR NMR spectrometers. The conditions of recording the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra have been given in [4-6]. The relative error in the determination of the numbers of carbon atoms of various types from the  $^{13}\text{C}$  NMR spectrum (Table 2) ranged from 4.6 to 6.4%.

## REFERENCES

1. A. Björkman, *Ind. Eng. Chem.*, **49**, 1395 (1957).
2. D. Fengel and G. Verenger, *Wood (Chemistry, Ultrastructure, Reactions)*, Springer, New York (1984).
3. I. H. Lora and M. Wayman, *Can. J. Chem.*, **58**, No. 7, 669 (1980).
4. L. V. Kanitskaya, S. A. Medvedeva, S. Z. Ivanova, D. F. Kushnarev, B. Ri, V. A. Babkin, and G. A. Kalabin, *Khim. Drev.*, No. 6, 3 (1987).
5. A. V. Rokhin, L. V. Kanitskaya, A. N. Zakazov, A. F. Gogotov, D. F. Kushnarev, V. A. Babkin, and G. A. Kalabin, *Khim. Prir. Soedin.*, 277 (1993).
6. L. V. Kanitskaya, I. P. Deineko, D. F. Kushnarev, A. V. Klemper, and G. A. Kalabin, *Khim. Drev.*, No. 6, 17 (1989).
7. V. M. Polonov, G. A. Kalabin, D. F. Kushnarev, and V. P. Datsyshev, *Khim. Tverd. Topliva*, No. 4, 9 (1984).
8. A. V. Rokhin, L. B. Kanitskaya, D. F. Kushnarev, G. A. Kalabin, Kh. A. Abduazimov, L. S. Smirnova, and B. Kh. Pulatov, *Khim. Prir. Soedin.*, 798 (1994).
9. A. Patra and P. K. Mukhopadhyay, *J. Indian Chem. Soc.*, **40**, 265 (1983).
10. C. L. Chen and D. Robert, *Methods Enzymol.*, **161**, 137 (1988).
11. H. D. Ludemann and H. Nimz, *Makromol. Chem.*, **175**, 2393 (1974).
12. A. P. Lapan, V. A. Gordeeva, and A. V. Laptev, *Khim. Drev.*, No. 5, 96 (1975).
13. A. P. Lapan, V. B. Chekovskaya, M. V. Efimova, and V. A. Gordeeva, *Khim. Drev.*, No. 4, 60 (1978).
14. G. F. Zakis, P. P. Érin'sh, B. Ya. Neiberte, and S. V. Khokholko, *Khim. Drev.*, No. 2, 3 (1991).
15. E. Adler, *Wood Sci. Technol.*, **11**, 169 (1977).
16. L. V. Kanitskaya, I. D. Kalikhman, S. A. Medvedeva, I. A. Belousova, V. A. Babkin, and G. A. Kalabin, *Khim. Drev.*, No. 2, 3 (1991).
17. H. Nimz, *Angew. Chem.*, No. 9, 336 (1974).
18. H. O. Kalinowski, S. Berger, and S. Braun, *Carbon-13 Spectroscopy*, Wiley, New York (1988).