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QUANTITATIVE ^1H AND ^{13}C NMR SPECTROSCOPIES OF COTTONPLANT DIOXANE LIGNIN

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A comparative analysis has been made of the dioxane lignins of the stems, seedling shoots, and seed coats of the cotton plant. The ratio of p-hydroxyaromatic, guaiacyl, and syringyl units in the macromolecules of the lignins has been determined, and so have the amounts of functional groups and bonds. It has been established that the main difference between the dioxane lignins from seedling shoots and seed coats of the cotton plant, on the one hand, and the lignin from the woody stems, on the other hand is a lower content of syringyl and guaiacyl units in the former.

The dioxaane lignins from ripe stems (**1**), seedling shoots (**2**), and seed coats (**3**) of a cotton plant of variety 108F have previously been investigated by traditional chemical methods and also by IR and UV spectrocopies [1-3].

Lignins (**2**) and (**3**) have been studied to a very small extent and are interesting by virtue of the fact that the former is a lignin from the earliest stage of development of the cotton plant, while the formation of the latter takes place with no direct link to the cambial layer of the plant. It has been shown in [2, 3] that lignins (**2**) and (**3**) differ from the lignin from ripe stems of the cotton plant by a low content of methoxy groups and a high concentration of phenolic hydroxy groups.

The aim of our investigation was to determine the main differences in the structures of lignins (**1**)-(**3**) and to evaluate the amounts of the main functional groups and bonds and the ratio of *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units.

Figure 1 gives the ^1H NMR spectra of lignins (**1**)-(**3**) recorded in hexamethylphosphorotriamide-d_{18} (HMP — hexametapol) solution. The spectrum of lignin (**1**) shows two broadened resonance lines in the 10.7-9.3 and 9.3-8.5 intervals assigned to the resonance of the hydrogen atoms of the phenolic OH groups of H and G units and of S units, respectively [4]. The spectra of lignins (**2**) and (**3**) differ from that of (**1**) in the region of resonance of the hydrogen atoms of the OH groups of carboxylic acids (13.5-10.6 ppm) by the fact that the strongly broadened resonance line of the COOH group can be seen more clearly in them. Moreover, they show a very broad signal of phenolic OH groups. This indicates that in the structure of lignins (**2**) and (**3**) there are nonesterified aromatic fragments more diverse in structure than in (**1**). Table 1 shows the distribution of the hydrogen atoms over the functional groups and structural fragments of lignins (**1**)-(**3**).

The relative amounts of hydrogen atoms in aldehyde groups and aliphatic OH groups were estimated from a combination of the ^1H NMR spectra of the lignins recorded in HMP solution and with the addition to this solution of a small amount of trifluoroacetic acid (CF_3COOH), which, interacting with all the hydroxy groups, forms a common resonance signal of hydroxy groups in the 14-11 ppm interval and does not interfere with the estimation of the hydrogen atoms of COH groups [5, 6].

Analysis of the figures in Table 1 shows that, as compared with lignin (**1**), lignins (**2**) and (**3**) contained twice as many carboxy and phenolic OH groups, a somewhat smaller number of hydrogen atoms and aromatic rings and of CHO—, CH_2O—, and CH_3O— groups ($H_{O\text{-}alk}$) and larger numbers of hydrogen atoms in fragments not linked with oxygen atoms $H_{\alpha,\beta,\gamma}$ by factors of 6 and 2.5, respectively.

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TABLE 1. Distribution of the Hydrogen Atoms over the Functional Groups and Structural Fragments ($q_x = I_x/I_{tot}$)

Fragments	Lignins			Range of CSs, ppm	Assignment of the signals [4, 9]*
	(1)	(2)	(3)		
H_{CCOH}	0.006	0.008	0.003	10.5—9.0	H in Ar—CH=CH—C(O)H
H_{COOH}	0.004	0.007	0.006	13.0—10.5	OH in Ar—CH=CH—C(O)OH
$H_{OH\text{-}phen}$	0.014	0.036	0.020	12.4—9.3	OH at C-4 G'; H
$H_{OH\text{-}phen}$	0.013	0.025	0.022	9.3—8.4	OH at C-4 S'
H_{ar}	0.229	0.190	0.175	8.4—6.3	hydrogen atoms of aromatic rings
$H_{O\text{-}alk}$	0.642	0.394	0.636	6.3—2.8	CH, CHO, CH_2O, CH_3O in the α-, β-, and γ-positions
$H_{C_\alpha H_\beta \gamma}$	0.056	0.332	0.138	2.8—0.2	CH, CH_2, CH_3 in the α-, β-, and γ-positions
$H_{Ho\text{-}al}$	0.052	0.008	0.048		OH in alcohols

*S', G', H' — nonesterified syringyl, guaicyl, and *p*-hydroxyaromatic units.

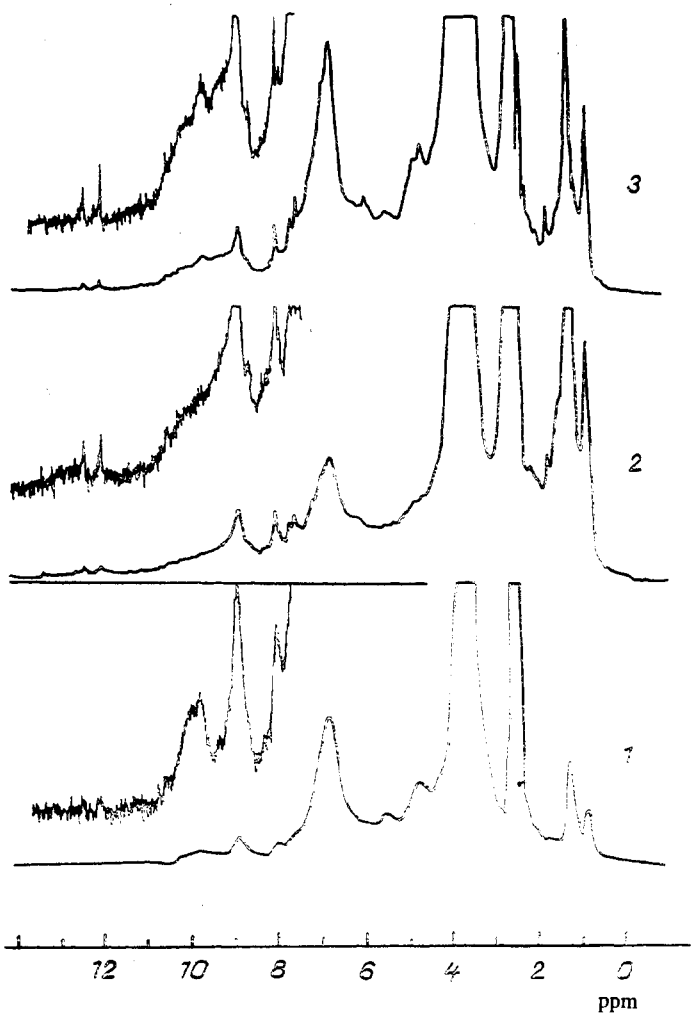


Fig. 1. ^1H NMR spectra of dioxane lignins (**1**)-(**3**) in hexamethylphosphorotriamide-d_{18} solution. Dioxane lignins (1) from the ripe stems of the cotton plant; (2) from seedling shoots of the cotton plant; 3) from seed coats of the cotton plant.

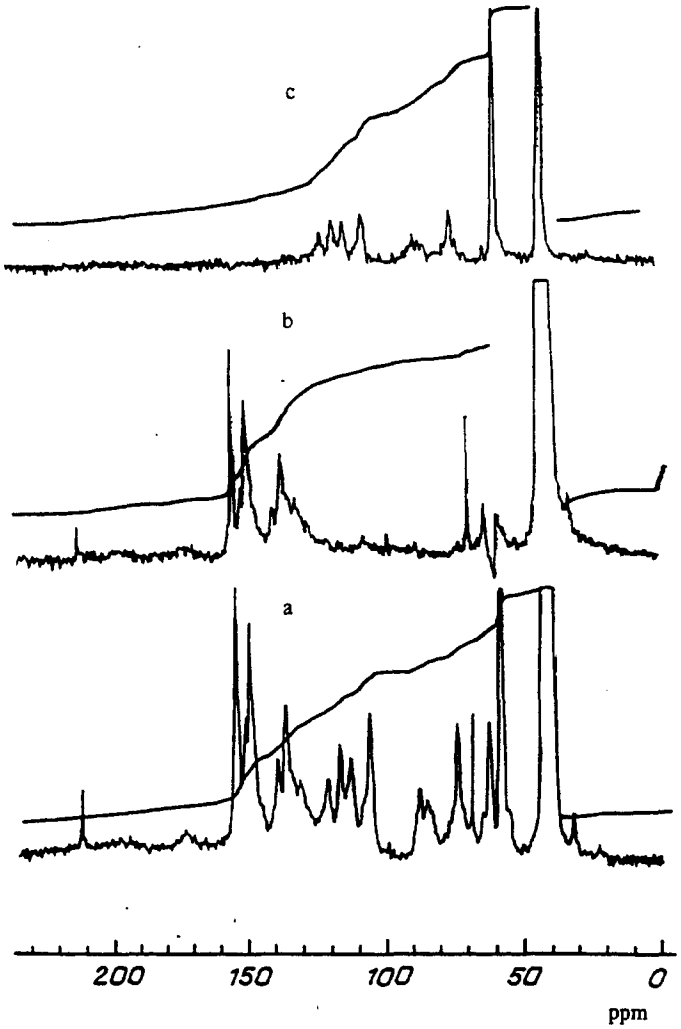


Fig. 2. ^{13}C NMR spectra of the dioxane lignin from ripe cottonplant stems (*a*), and the subspectra of secondary and quaternary (*b*) and primary and tertiary (*c*) carbon atoms in DMSO solution.

Thus, the qualitative and quantitative analysis of the ^{1}H NMR of the lignins under investigation permits a preliminary conclusion to be drawn: the lignin from the seedling shoots (**2**) is close in structure to the lignin from the seed coats of the cotton plant (**3**) and differs from the lignin from the ripe stems of the plant (**1**) by a higher degree of substitution of the aromatic rings with OH groups, a higher degree of oxidation of the side chains to —COH and —COOH groups, and a high content of structures uncharacteristic for lignin structures having unoxidized saturated side chains.

Figures 2-4 give the ^{13}C NMR spectra (*a*) and subspectra of the secondary and quaternary (*b*) and the primary and tertiary (*c*) carbon atoms of lignins (**1**)-(**3**) recorded in dimethyl sulfoxide-d_6 (DMSO) solution [7, 8].

On the basis of quantitative ^{13}C NMR spectra we calculated the relative numbers of carbon atoms in functional groups and fragments (P_x) and their number referred to one aromatic ring (n_x) from the formula

$$n_x = \frac{P_x \cdot 6}{f_a} = P_x \cdot k\,, \tag{1}$$

where $P_x = I_x/I_{tot}$ is the relative content of carbon atoms in the fragment of groups being determined; $f_a = I_{ar}/I_{tot}$ is the degree of aromaticity of the lignin; and the coefficient $k = 6/f_a$ [9].

The number of functional groups and fragments, f_a, and k are given in Table 2.

Let us dwell on an analysis of the ^{13}C NMR spectra of the lignin (**1**). The resonance signals in the spectral intervals of 154-150 ppm and 150-140 ppm indicate the presence of S- and G-units of the structure in the lignin [9, 12]. This is

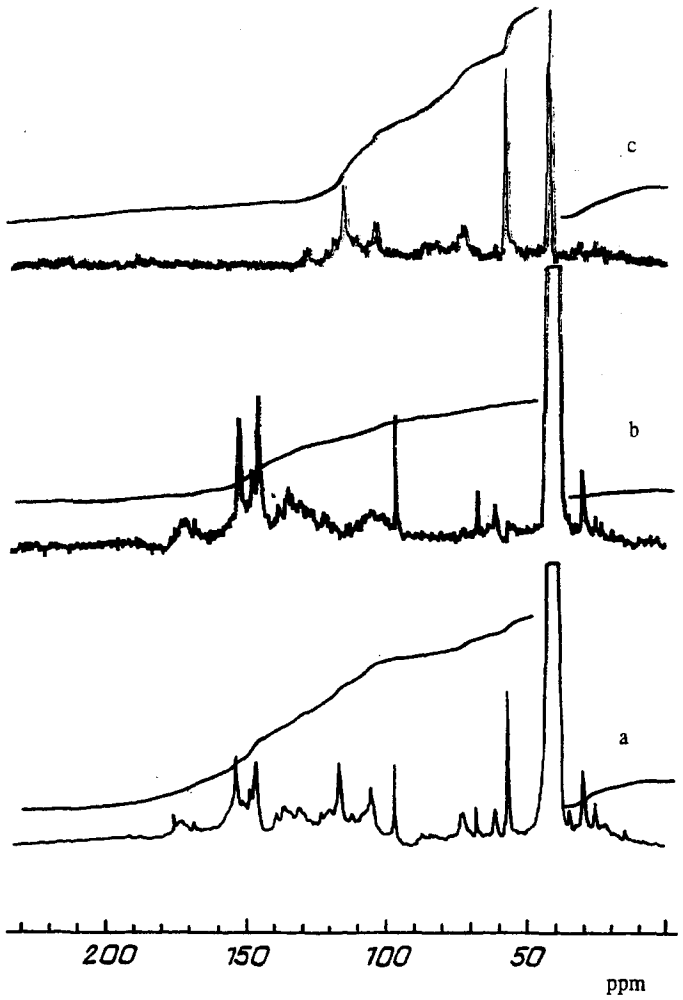
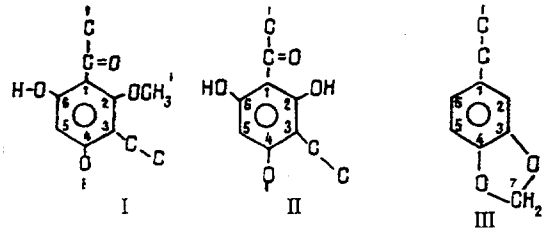


Fig. 3. ^{13}C NMR spectrum of the dioxane lignin from seedling shoots of the cotton plant (*a*) and the subspectra of the secondary and quaternary (*b*) and the primary and tertiary (*c*) carbon atoms in DMSO solution.

confirmed by the number of OCH_3 groups referred to one aromatic ring (Table 2). In the ^{13}C NMR subspectra (Figs. 2, 3, and 4, *a*, *b*, and *c*), the resonance signals of quaternary aromatic carbon atoms are observed in the range of CSs from 115 to 95 ppm and of OCH_3 groups in the range from 64 to 58 ppm, which are usually uncharacteristic for the spectra of lignins. On the basis of an analysis of the ^{13}C CSs of model compounds we came to the conclusion that these resonance signals may belong to the C-1/C-3 atoms of structures (I) and (II), to the OCH_3 group of structure (I) and to the C-7 atom of structure (III) [10]:



The use of the DEPT sequence convincingly showed the absence of structure (III) in the macromolecules of lignins (**1**)-(**3**) [11].

The known numbers of C-1/C-3 quaternary atoms in structures (I) and (II) and of atoms of OCH_3 groups enabled us to calculate the numbers of structures (I) and (II) associated with 100 aromatic rings (Table 3):

$$N_{C\text{-}1\,I,\,II}=[(n_{C\text{-}1,3\,I,\,II})/2 - (n_{OCH_3})]\cdot 100. \quad (2)$$

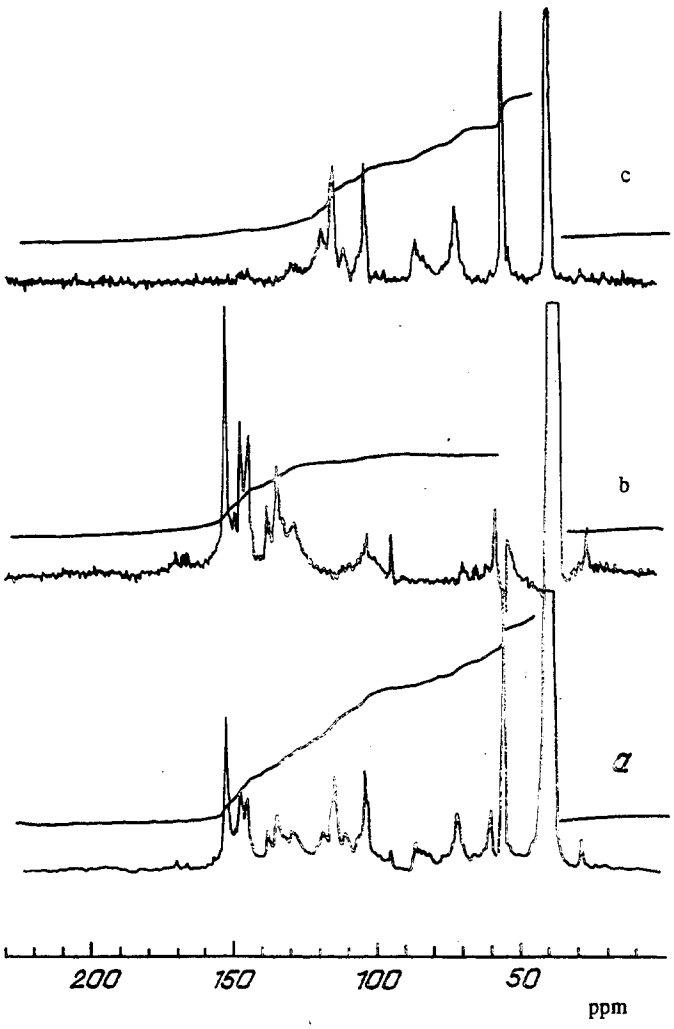
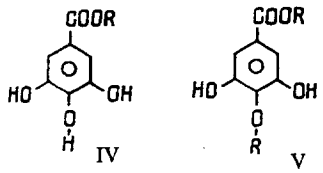


Fig. 4. ^{13}C NMR spectrum of the dioxane lignin from cottonplant seed coats (*a*) and the subspectra of the secondary and quaternary (*b*) and the primary and tertiary (*c*) carbon atoms in DMSO solution.

The greatest numbers of structures (I) and (II) were present in lignin (**2**), and a somewhat smaller amount of structures (I) in lignin (**3**), while in lignin (**1**) these structures were present in only small amounts (Table 3).

The intense resonance signals in the 146-145 ppm region in the ^{13}C ppm NMR spectra and the considerable number of phenolic OH groups indicated in the ^{1}H NMR spectra (Table 1) of lignins (**2**) and (**3**) showed that the macromolecules of these lignins contained structural units in which the C-3/C-4/C-5 positions are substituted by OR groups (R = H, Alk $\neq CH_3$) [10]:



The resonance signals of the hydrogen atoms of OH groups (at C-3/C-5) are masked by the signals of the OH groups of the S′ units, and the resonance signals of the OH groups at C-4 of structures (IV) and (V) are masked by the signals of the hydrogen atoms of the aromatic rings [13]. The number of carbon atoms bearing phenolic OH groups and also aldehydic and

TABLE 2. Numbers of the Main Functional Groups and Fragments (n_x) Referred to One Aromatic Ring in Cottonplant Lignins (1)—(3) from their ^{13}C NMR Spectra

Fragments	Lignins			Range of CSs, ppm	Assignment of the signals [9]
	(1)	(2)	(3)		
C=O	0.106	0.334	0.220	220—185	C=O in ketones
C=O-OH	0.082	0.117	0.035	210—185	C=O in Ar—CH=CH—C=O-OH
C=O-OH	0.052	0.101	0.078	185—164	C=O in carboxylic acids
C=O-O	0.365	0.774	0.425	185—164	C=O in esters
C_{ar}-O(1)	0.295	0.586	0.146	164—156	C-4 H, H', C-2,4,6,I,II
C_{ar}-O(2)	0.823	0.462	0.686	155—151	C-3,5 S
C_{ar}-O(3)	0.359	0.247	0.233	151—148	C-4 G
C_{ar}-O(4)	0.878	1.258	1.429	148—140	C-3, 4G, C-3G, C-3,5IV
C_{ar}-O(5)	0.234	0.136	0.207	138—134	C-4 S,S'
C_{ar}—C(1)	2.039	1.361	1.188	140—123	C-1 S,S', C-1 G,G'
C_{ar}—C(2)	–	0.258	0.178	123—118	C-1 H,H', C-1 IV, V
C_{ar}—C(3)	0.284	0.736	0.602	115—95	C-1,3 I,II
CH_{ar}(1)	0.029	0.158	0.125	135—125	C-2,6 H,H'
CH_{ar}(2)	0.240	0.285	0.318	125—117	C-6 G,G
CH_{ar}(3)	0.481	0.563	0.545	117—108	C-2,5 G,G', C-3,5 H,H'
CH_{ar}(4)	0.3–	0.234	0.342	108—102	C 2,6 S,S
CH=CH	–	–	0.090	156—140	CH=CH Ar—CH=CH—C=O-OH
CHO_{al}	1.318	0.760	0.625	90—64	$C_{\alpha,\beta}$ in β-O-4
CH_2O_{al}	1.082	0.830	1.091	76—59	C_γ in CH_2O—R
CH_3O	0.086	0.180	0.116	64—58	C in Ar—OCH_3 in I
CH_3O	1.305	0.614	0.873	58—54	C in Ar—OCH_3
$CH_{\beta-\beta}$	0.125	0.098	0.060	54—52	C_β in β—β
$CH_{\beta-5}$	0.038	0.064	0.035	54—50	C_β in β-O-4,β-5)
C_{al}	0.312	1.911	0.600	45—5	CH_2CH_3 in side-chains
ΣC_{al}	2.892	5.87	2.910	mean length of the side chain.	
f_a	0.560	0.522	0.592	164—102	$f_a = I_{ar}/I_{tot}$
k	10.710	11.500	10.130		$k = 6 \cdot f_a$

acidic carboxylic carbon atoms referred to one (n_x) aromatic ring can be calculated by using the relative numbers of hydrogen atoms (q_x) and the results of elementary analysis for [H] and [C] [9]:

$$n_x = \frac{q_x \cdot [H] \cdot 12 \cdot 6}{f_a \cdot [C]} = 72 \cdot \frac{q_x [H]}{f_a \cdot [C]}. \quad (3)$$

The numbers of H and H' units and of structures of types (IV) and (V) were calculated from the resonance signals in the 135-125 ppm region (C-2/C-5 of H and H' in the subspectra of tertiary carbon atoms) and 123-117 ppm region (C-1 of structures (IV) and (V) and of H and H' in the subspectra of quaternary aromatic carbon atoms) [9, 10]:

$$N_{IV,V} = [(n_{C\text{-}1}) - (n_{C\text{-}2/C\text{-}6\,H,H'})/2] \cdot 100. \quad (4)$$

TABLE 3. Numbers of Structural Fragments and Bonds (N_X) in the Cotton Plant Dioxane Lignins (1)—(3) Referred to 100 Aromatic Rings

Types of fragments and bonds	Lignins			Relative error, %
	(1)	(2)	(3)	
S+S'	45	23	34	10.3
G+G	41	15	19	9.5
H+H	1—2	8	6	6.7
Structure I	9	18	12	9.5
Structure II	6	19	19	9.5
Structures IV and V	–	17	11	9.5
Σ Cap—OH	37	89	68	9.0
Σ Cal—OH	142	73	89	9.1
Σ Cal—O—Cal	13	10	6	9.5
Σ Cal—O—Car	74	67	68	22.9
Σ Cal—O—C:al	4	17	18	40.3
$\Sigma\Sigma$ Car—O	259	269	270	9.5
$\Sigma\Sigma$ Car—C	232	207	197	9.5
$\Sigma\Sigma$ CH ar	109	124	133	6.7

From the numbers of structures (IV) and (V) it is possible to calculate the number of unesterified syringyl units (S):

$$S' = [(n_{C_{ar}-OHII}) - 2\cdot(n_{C\text{-}1\,IV,\,V})]\cdot 100. \quad (5)$$

Starting from the known number of OCH_3 groups and of syringyl units (C-3/C-5 of S — 155-150 ppm — and C-5 of S from the ^1H NMR spectra) we calculated the number of guaiacyl structures (G and G'):

$$G+G' = [(n_{OCH_3}) - 2\cdot(S+S')]\cdot 100. \quad (6)$$

The numbers of main structural units calculated from formulas (2)-(6) are given in Table 3. The ratio of S:G:H:I:II units in lignin (1) was as follows: 1:0.93:0.02:0.19:0.13. Lignins (2) and (3) also contained structures of types IV and V; the following ratio of units S:G:H:I:II:IV:V was obtained for them: 1:0.66:0.35:0.78:0.81:0.75, and 1:0.55:0.18:0.35:0.55:0.32, respectively.

Characteristic for lignins (1)-(3) was a very high degree of condensation ($n_{C_{ar}-C}$): to each aromatic ring in addition to C_1—C_α bonds there were 0.97-1.32 C_{ar}—C bonds, which corresponded to the degree of condensation of the lignin preparations (for comparison, the degree of condensation of ground sprucewood lignin is 0.39) [9]. The number of unsubstituted carbon atoms of aromatic rings ($n_{CH_{ar}}$) exceeded unity (Tables 2 and 3). The small amounts of S and G units in lignins (2) and (3), the considerable amounts of structures I and II, and also the high degree of condensation of the preparations indicated that the macromolecules of these lignins contained fragments of structures similar to condensed tannins (flavonoids) [15]. The numbers of aromatic hydroxy groups ($n_{OH_{al}}$) can be calculated from the difference between the numbers of oxygen atoms found in the elementary composition and the concentration of oxygen atoms in the lignin macromolecule determined directly by ^1H and ^{13}C NMR.

The numbers of aliphatic OH groups ($N_{OH_{al}}$) referred to 100 aromatic rings are given in Table 3. In lignins (2) and (3) $N_{OH_{al}}$ is little more than half as much as in lignin (1). This can be explained by a low content of CHO_{al} fragments in lignins (2) and (3) and CH_2O_{al} fragments in (2) corresponding to the α- and γ-positions of the side chains which are preferentially substituted by OH groups. In lignins (2) and (3) the α- and γ-positions are apparently oxidized to CO and COO groups (Table 2), which leads to a smaller number of aliphatic OH groups. The large number of COO groups and the low content of OH_{al}

TABLE 4. Amounts of the Main Functional Groups, % by Mass

Functional groups	Lignins			Relative error, %
	(1)	(2)	(3)	
COOH	1.1	1.2	0.5	6.9
C=O	1.4	4.1	3.1	6.9
COOOH	3.1	2.0	1.7	9.2
COOO–	7.5	14.9	9.5	9.6
OH_{phen}	3.4	5.8	5.3	9.2
OH_{al}	4.4	0.7	4.1	9.3
OCH_3	20.1	10.8	15.1	6.9

and also the high degree of condensation, particularly in lignin (2), suggests that the macromolecule of the lignin from seedling shoots contained units similar to hydrolyzable tannins [15]. However, the COO groups may not only bind the aromatic ring to a side chain, as in the hydrolyzable tannins, but also to two side-chains. There were considerably fewer such structures in lignins (1) and (3).

No little interest is also presented by a separate estimate of aryl—aryl and aryl—alkyl ether bonds in the lignin macromolecules. If it is assumed that the C_{al}—O—C_{al} bonds are represented mainly by resinol structures, knowing the number of C_{al}—OH bonds it is possible to calculate the number of C_{ar}—O—C bonds:

$$N_{C_{ar}-O-C_{al}}=\sum(N_{C_{al}-O})-(N_{C_{al}-OH})-(N_{C_{al}-O-C_{al}}). \quad (7)$$

Deducting from $\Sigma N_{C_{ar}-O}$ the number of methoxy (N_{OCH_3}) and phenol ($N_{C_{ar}NOH}$) groups, we obtain $N_{C_{ar}-O-C_{ar,al}}$:

$$N_{-O-C_{ar,al}}=\sum(N_{C_{ar}-O})-(N_{CH_3O})-(N_{C_{ar}-OH}). \quad (8)$$

In this way it is possible to calculate the number of C_{ar}—O—C_{ar} bonds, although with a considerable error (Table 3):

$$N_{C_{ar}-O-C_{ar}}=(N_{C_{ar}-O-C_{ar,al}})-(N_{C_{ar}-O-C_{al}}). \quad (9)$$

With the same numbers of C_{ar}—O—C_{al} and C_{al}—O—C_{al} bonds in lignins (1)—(3), lignins (2) and (3) differed by a higher number of C_{ar}—O—C_{ar} bonds [four times more than in (1) (Table 3)]. As we have already mentioned, lignin (2) was characterized by a considerable number of unoxidized saturated hydrocarbon side-chains ($\Sigma N_{C_{ar}} = 1.91$ per one aromatic ring). As a result of this, the mean structural formula (MSF) of a unit of lignin (2) differed widely from a PPU, unlike lignins (1) and (3):

(1) $C_{9.48}H_{11.89}O_{3.20}(OCH_3)_{1.39}$
(2) $C_{10.96}H_{13.05}O_{4.02}(OCH_3)_{0.79}$
(3) $C_{9.14}H_{10.59}O_{3.46}(OCH_3)_{0.99}$

Table 4 gives the concentrations of functional groups in lignins (1)-(3).

EXPERIMENTAL

The stems, seedling shoots, and seed coats of the cotton plant were ground, exhaustively extracted with alcohol—benzene (1:1 by volume), and washed with hot water. The preparation of the dioxane lignins and their elementary compositions have been given in [1-3].

^1H NMR spectra were recorded in the pulsed regime on a Bruker WR 200 SY spectrometer with a working frequency of 200.1 MHz. The width of the spectrum was 8 kHz, the relaxation delay 10 s and the pulse length 90°. The concentrations of lignins in solution amounted to 2-3%. Before the spectra of the samples were recorded we determined the intensity of the

residual signals of water in the solvent (HMP-d_{18}) [9]. To evaluate the amount of OH groups we used the addition of CF_3COOH to the solution under investigation [5, 6].

^{13}C NMR spectra were recorded on a Bruker WR200 SY NMR spectrometer with a working frequency of 50.3 MHz using noise decoupling from protons and a spectral width of 20 kHz after 6000-10,000 passages. The subspectra of the primary and tertiary and of the secondary and quaternary carbon atoms were obtained by the spin echo method with multiplet dephasing [modified GASPE (Gated Spin Echo), and CSE (Conventional Spin Echo)], and in all the experiments the noise decoupling was switched off during the relaxation delay, which amounted to 2.5 s [7, 8], the width of a pulse being 90°. The concentration of lignins in the DMSO solution was about 10%. Chromium tris-acetylacetonate in a concentration of 0.02 M was used as relaxant. ^{13}C NMR spectra were recorded by the DEPT (Distortionless Enhancement by Polarization Transfer) method on a Varian VXR 500S NMR spectrometer with a working frequency of 125.7 MHz and J = 140 Hz [11]. All the spectra were recorded at 25°C. The relative error of integration was 3-5%.

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