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COTTON PLANT LIGNINS

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This paper generalizes the results of studies on lignins isolated from the stems, bolls, and seed hulls of cotton plants of a number of varieties as functions of the vegetation periods of the plants. The UV, IR and ^1H and ^{13}C NMR spectra of the lignins have been studied. The dioxane lignin (DLA) and the natural lignins have been subjected to nitrobenzene oxidation and to cleavage with sodium in liquid nitrogen. A structure of the lignin macromolecules consisting of 18 phenylpropane structural units has been done up. Information is given on the use of lignins in agriculture, the food industry, and the paint and varnish industry. The anticorrosion and sorption properties of hydrolysis and modified hydrolysis lignins have been studied.

The lignins of the cotton plant have been little studied. This relates above all to little-changed lignins close to the natural lignins. As is known, these include Björkman, Brauns, and Pepper lignins [1-3].

N. N. Shorygina and K. R. Niyazov isolated Björkman lignins (MWL) from ripe cotton plant stems and cotton bolls [4]. On the basis of elemental and functional-group analyses they calculated the following empirical formula for them:

$$\text{Stem MWL}: C_9H_{7.56}C_{1.51}(OCH_3)_{1.17}(OH_{tot})_{1.36}$$
$$\text{Boll MWL}: C_9H_{7.58}O_{1.52}(OCH_3)_{1.19}(OH_{tot})_{1.28}$$

It was impossible to characterize the MWL from cotton seed hulls because of its low yield.

The molecular masses of the lignins were determined, and they were subjected to nitrobenzene oxidation and to cleavage with sodium in liquid ammonia [4, 5]. The oxidation and cleavage products were found to include substances with guaiacyl and syringyl structures. The process of isolating MWLs is lengthy and laborious, and the yields of lignins are low. In view of this, Pepper's method and one involving ultrasound have been used for the isolation of lignins [6].

However, the results of a study of the lignin obtained with the aid of ultrasound led to the conclusion that during isolation under the action of ultrasound the syringyl structures of the lignin are demethoxylated and the C3 side-chains are destroyed [7, 8].

Isolation of Dioxane Lignins from the Cotton Plant

As already mentioned, one of the methods of isolating lignin from plants in a little-changed form and with good yield is Pepper's method [3]. It is based on the fact that lignin is extracted by aqueous dioxane (1:9) in the presence of catalytic amounts of hydrochloric acid with heating under a current of nitrogen. It has been confirmed with various plants that the lignin obtained by this method is very similar to the Björkman lignin [9-15].

In order to obtain lignin from ripe cotton plant stems in unchanged form we have used a method perfected by V. M. Reznikov the essence of which is that all the operations, beginning with extraction and ending with precipitation of the lignin in water, are carried out under a current of nitrogen. To study the dynamics of the process, we performed the stepwise extraction of lignin for one, two, and three hours [16], so that the lignin being isolated did not undergo changes due to its

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TABLE 1. Yields and Characteristics of the Fractions

Fraction	Yield, % on the Komarov lignin	Carbohydrates, %	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_z$	$\overline{M}_n/\overline{M}_w$	E_{280}
F-I	21.7	3.05	8800	12800	18880	2.28	2900
F-II	13.0	1.0	8370	16730	21380	2.0	2970
F-III	7.4	–	13900	20000	23560	1.44	3020

TABLE 2. Yields and Molecular Masses of the DLA from Stems of a Tashkent-1 Cotton Plant

Vegetation period	Lignin, % of Komarov lignin	DLA, % of the plant	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_z$	$\overline{M}_n/\overline{M}_w$
Early	23.2	1.84	8800	18400	27780	2.08
Flowering	23.2	3.66	6800	13200	17950	1.92
Late	22.94	1.71	3400	8000	12700	2.34

lability during extraction. The solvent was changed every hour. In this way, three fractions of lignin were obtained: F-I (extraction for 1 h), F-II (2 h), and F-III (3 h). The fractions obtained were purified by reprecipitation in water from dioxane solutions.

On the basis of elemental and functional-group analyses, the empirical formulas for 1 PPSU were calculated for all the fractions:

F-I

$$C_9H_{8.02}O_{0.58}(OCH_3)_{1.23}(OH_{ph})_{0.44}(OH_{al})_{0.96}(O_{co})_{0.22}(OOH_{COOH})_{0.085}(O_{ar\text{-}al})_{0.56}$$

F-II

$$C_9H_{7.96}O_{0.49}(OCH_3)_{1.23}(OH_{ph})_{0.43}(OH_{al})_{1.0}(O_{co})_{0.23}(OOH_{COOH})_{0.064}(O_{ar\text{-}al})_{0.57}$$

F-III

$$C_9H_{7.78}O_{0.52}(OCH_3)_{1.14}(OH_{ph})_{0.51}(OH_{al})_{0.99}(O_{co})_{0.23}(OOH_{COOH})_{0.097}(O_{ar\text{-}al})_{0.49}$$

It can be seen from the formulas that with an increase in the time of acidolysis the content of methoxy groups in the DLA decreased and that of phenolic hydroxy, carboxy, and carbonyl groups increased. It is obvious that prolonged hydrolysis causes demethoxylation in DLA. The increase in the amount of carboxy groups is probably connected with the saponification of ester groups of the lignin during the process of acidolysis.

As can be seen from Table 1, during extraction the molecular masses of the DLA roughly doubled, and in the third fraction there were no carbohydrates bound to the lignin. This means that the low-molecular-mass fractions of the lignin, as the most soluble, are extracted first. The high-molecular-mass fractions become soluble in the later stages of isolation. It is not excluded that the increase in molecular mass may be a consequence of polymerization of the lignin being isolated under the more prolonged action of the temperature and the acid under the acidolysis conditions. After acidolysis for three hours, all the bonds of the lignin with carbohydrates had been hydrolyzed and, as can be seen from Table 1, the total yield of all the DLA fractions amounted to 42.1% of the Komarov lignin.

It is known [17] that, in the isolation of lignin from plants, small molecules from small pores of the plants are first liberated and the larger ones pass into solution only when the pores have considerably increased in size. Consequently, the lignins from each of the three fractions were isolated from different parts of the cell walls.

Change in Cotton Plant Lignin over the Vegetation Periods

The lignin in cell walls arises as a result of biosynthesis, and its production concludes the process of formation of the cell wall [18]. The complexly branched lignin molecule in the cell wall is produced in an already formed swollen carbohydrate matrix in the form of a rigid three-dimensional network [19]. There are reports in the literature that the content and chemical composition of the lignin change as the plant grows. This has been shown for the case of many plants belonging to different families: potato seedlings (fam. Solanaceae), rye stems and reeds (fam. Gramineae), and wood [20-22]. At the same time, as the plant continues to grow the lignin already formed scarcely changes, but different lignins are synthesized at different periods

TABLE 3. Amounts and Molecular Masses of the DLAs from Several Cotton Plant Varieties

Variety	Lignin content	Yield of DLA	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_z$	$\overline{M}_w/\overline{M}_n$
	% of the Komarov lignin					
Mexican	28.2	12.5	2900	5700	11700	1.91
Tashkent-1	22.94	7.4	3400	8000	12700	2.34
Tashkent-6	25.6	10.76	4400	9700	17800	2.2
AN Bayaut-2	23.01	6.1	3400	7000	12900	2.06
S-4880	–	16.9	2800	7400	15000	2.64
108-F	26.6	8.2	6760	15190	20280	2.64
S-6030	21.2	9.5	5350	10100	–	1.89

TABLE 4. Yields and Characteristics of the Lignins from Cotton Bolls and Seed Hulls

Material	Yield of lignin	Yield of DLA	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_z$	$\overline{M}_w/\overline{M}_n$
	% of the Komarov lignin					
Bolls of the variety Tashkent-1	22.8	8.07	5890	10350	14900	1.76
Bolls of the variety AN Bayaut-2	27.5	5.6	3000	7000	12800	2.33
Bolls of the variety 108-F	25.2	10.0*	3600–3200			
Seed hulls of variety 108-F	23.8	17.0	26000–27000			

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*Figures for MWL.

of development of the plant. In the early stages of vegetation the lignins have a low degree of methoxylation. As the plant grows, more highly methoxylated lignins are synthesized. Experiments with radioactive CO_2 have shown that methoxylation of the lignin molecule is not a stepwise process [23].

We have made an investigation of the change in lignin according to the vegetation period for two medium-fiber varieties of the cotton plant — 108-F and Tashkent-1 [24, 25]. This work was performed more completely with the Tashkent-1 variety. We investigated the stems of a cotton plant in an early vegetation period (2-4 true leaves) and in the flowering period, and stems gathered after the harvesting of the raw cotton. DLA was obtained from all the samples by Pepper's method.

The high content of Komarov lignin in the stems of the cotton plant in the early vegetation period is apparently explained by their pronounced contamination with humification products [26, 27].

It can be seen from Table 2 that the MM of the DLA from ripe stems was lower than that of lignin from the early vegetation period.

For a more detailed study, we isolated DLA with the low molecular mass M_w = 4900 and a degree of polydispersity of 1.53 from two-week shoots of a 108-F cotton plant with a yield of 0.3% [28].

Empirical formulas were derived from elemental and functional-group analyses:

DLA from the stems of a Tashkent-1 cotton plant in the early vegetation period:

$$C_9H_{7.67}O_{2.01}(OCH_3)_{0.53}(OH_{ph})_{0.66}(OH_{al})_{0.3}(O_{co})_{0.44}(OOH_{COOH})_{0.03}\ (O_{ar\text{-}al})_{0.34}$$

DLA from two-week shoots of a 108-F cotton plant:

$$C_9H_{9.24}O_{1.51}(OCH_3)_{0.53}(OH_{ph})_{0.78}(OH_{al})_{0.48}(O_{co})_{0.36}(O_{ar\text{-}al})_{0.22}$$

DLA from the stems of a Tashkent-1 cotton plant in the flowering period:

$$C_9H_{6.24}O_{1.59}(OCH_3)_{0.78}(OH_{ph})_{0.73}(OH_{al})_{0.7}(O_{co})_{0.28}(OOH_{COOH})_{0.08}\ (O_{ar\text{-}al})_{0.27}$$

DLA from ripe stems of a Tashkent-1 cotton plant:

$$C_9H_{6.22}O_{1.21}(OCH_3)_{0.82}(OH_{ph})_{0.63}(OH_{al})_{0.88}(O_{co})_{0.46}(OOH_{COOH})_{0.17}\ (O_{ar\text{-}al})_{0.37}$$

It can be seen from the formulas given that as the plants grew the lignin became more highly methoxylated. In the lignin from a plant in the late vegetation period the C_3 side-chain was more highly hydroxylated. That is to say, in different stages lignins are synthesized that differ from one another both by the substitution in the aromatic nuclei and by the structure of the C_3 side-chain.

Study of the Lignins from Different Varieties of Cotton Plants

In investigations to find new thin-fiber and medium-fiber varieties of the cotton plant, emphasis is placed on increasing crop yield and improving the quality of the fiber and on early ripening properties and resistance to disease.

Lignin plays a large role in the life of plants, especially annuals. It fulfills a multiplicity of functions, the main ones being mechanical — imparting rigidity to the lignified plant cortex; chemical — imparting to the tissues resistance to the action of oxidants, radiation, etc.; and biochemical — protection of the plant from the action of pathogenic microorganisms (bacteria, fungi) [29].

A change in the plant during selection should probably be reflected in the structure of the lignin. We have therefore studied the lignins of ripe cotton plant stems of several varieties: the primordial wild form of the Mexican cotton plant *Gossypium hirsutum* L. ssp. *mexicanum*, var. *nervosum* (Mauer), the medium-fiber varieties 108-F, Tashkent-1, Tashkent-6, S-4880, and AN Bayaut-2, and the thin-fiber variety S-6030.

DLAs were obtained from all the plant samples and were subjected to comparative study [30-33]. As can be seen from Table 3, the highest amount of lignin was present in the stems of the Mexican cotton plant, and the lowest amount in the thin-fiber variety S-6030. The yields of DLA from the stems of the different varieties ranged from 6.1 to 16.9% (of the Komarov lignin). This means that it is possible to isolate only an inconsiderable part of the lignin from cotton plant stems by mild acid hydrolysis.

The DLAs isolated differed sharply in molecular mass and degree of polydispersity. The DLA with the lowest molecular mass was that from the Mexican cotton plant stems.

Empirical formulas were calculated on the basis of elemental and functional-group analyses:

Mexican cotton plant DLA

$$C_9H_{8.13}O_{1.15}(OCH_3)_{0.81}(OH_{ph})_{0.66}(OH_{al})_{0.87}(O_{co})_{0.41}(OOH_{COOH})_{0.04}(O_{ar\text{-}al})_{0.34}$$

Variety Tashkent-1 DLA (see above)

Variety Tashkent-6 DLA

$$C_9H_{7.96}O_{0.62}(OCH_3)_{0.99}(OH_{ph})_{0.28}(OH_{al})_{1.33}(O_{co})_{0.31}(OOH_{COOH})_{0.06}(O_{ar\text{-}al})_{0.42}$$

Variety AN Bayaut-2 DLA

$$C_9H_{8.76}O_{2.31}(OCH_3)_{0.81}(OH_{ph})_{0.49}(OH_{al})_{0.55}(O_{co})_{0.39}(OOH_{COOH})_{0.03}(O_{ar\text{-}al})_{0.51}$$

Variety S-4880 DLA

$$C_9H_{7.81}O_{0.58}(OCH_3)_{1.31}(OH_{ph})_{0.24}(OH_{al})_{0.92}(O_{co})_{0.56}(OOH_{COOH})_{0.03}(O_{ar\text{-}al})_{0.76}$$

Variety 108-F DLA (see above)

Variety S-6030 DLA

$$C_9H_{8.7}O_{0.59}(OCH_3)_{1.18}(OH_{ph})_{0.29}(OH_{al})_{1.19}(O_{co})_{0.32}(O_{ar\text{-}al})_{0.71}$$

As can be seen, all the DLAs differed from one another by the numbers of functional groups both in the aromatic nuclei (OCH_3, OH_{ph}) and in the CH_3 side-chain (OH_{al}, CO, COOH). Consequently, the lignins of different cotton plant varieties differ structurally.

TABLE 5. Yields and Molecular Masses of the Fractions of Cotton Plant Stem DLA [37]

Fraction	Yield, %	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_w/\overline{M}_n$
Initial DLA	100	4200	12000	2.9
I	19.8	15000	21800	1.45
II	5.80	11200	19000	1.70
III	22.5	7500	12000	1.60
IV	12.8	3700	5700	1.50
V	5.70	3200	4100	1.30
VI	12.6	2200	3000	1.4

TABLE 6. Komarov Lignin Content, % of the Plant

Plant organ and vegetation period	Plants	
	healthy	affected by wilt
Stems, flowering	23.20	23.25
Stems, late period	22.94	25.12
Bolls, late period	22.80	24.50
Seed hulls	26.80	28.70

TABLE 7. Molecular Masses and Degrees of Polydispersity of the DLAs

Source of isolation of the DLA	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_z$	$\overline{M}_w/\overline{M}_n$
Healthy stems, flowering, Tashkent-1	6800	13200	17950	1.92
Diseased stems, flowering, Tashkent-1	5500	9900	15100	1.80
Healthy bolls, Tashkent-1	5900	10350	14900	1.76
Diseased bolls, Tashkent-1	5650	13100	19000	2.32
Ripe healthy stems, Tashkent-1	3400	8000	12700	2.34
Ripe diseased stems, Tashkent-1	3300	5150	13640	1.94
Ripe healthy stems, S-6030	5350	10100	–	1.89
Ripe diseased stems, S-6030	3800	8200	16400	2.12

The lignin of the wilt-resistant Mexican cotton plant proved to be soluble in water, less condensed, and of lower molecular mass. As in the case of polyphenols, these properties are probably responsible for the protective functions of cotton plants of this species. This is the reason why the wild Mexican cotton plant is widely used for breeding wilt-resistant varieties.

Lignins of Cotton Bolls and Seed Hulls

The bolls of the cotton plant pass through a shorter (by several months) cycle of development than the stem. The hulls of cotton seeds pass through an even shorter cycle. And the lignin in these objects is synthesized in a still shorter time. The roll of the cotton seed hull is very important for preserving the viability of the embryo and its growth. The lignin of cotton seed hulls must be distinguished structurally from the lignin of other parts of the cotton plant since its formation has no direct connection with the cambial layer of the plant.

The DLAs of ripe bolls of cotton plants of the varieties Tashkent-1 and AN Bayaut-2, the MWLs of ripe bolls of the variety 108-F [4], and the DLAs of the seed hulls of a 108-F cotton plant have been studied (Table 4). The most complex material proved to be the cotton seed hulls — in an attempt to isolate MWL from it by Björkman's method we came up against difficulties in its purification. When an acetic acid solution of the lignin was poured into water, it did not precipitate like all other lignins but remained in a colloidally dispersed state [4].

Empirical formulas were calculated on the basis of elemental and functional-group analyses:

DLA of cotton bolls of the variety Tashkent-1 [34]

$$C_9H_{6.26}(OCH_3)_{0.87}(OH_{ph})_{0.66}(OH_{al})_{0.82}(O_{co})_{0.31}(OOH_{COOH})_{0.1}(O_{ar\text{-}al})_{0.34}$$

DLA of cotton bolls of the variety AN Bayaut-2 [35]

$$C_9H_{8.34}O_{2.06}(OCH_3)_{0.76}(OH_{ph})_{0.56}(OH_{al})_{0.72}(O_{co})_{0.49}(O_{ar\text{-}al})_{0.44}$$

DLA from cotton seed hulls of the variety 108-F [34]

$$C_9H_{8.08}O_{0.53}(OCH_3)_{0.84}(OH_{ph})_{0.69}(OH_{al})_{1.31}(O_{co})_{0.4}(O_{ar\text{-}al})_{0.31}$$

In a comparison of the results obtained with the corresponding figures for the DLAs of the stems of the same varieties it can be seen that the DLA of the Tashkent-1 bolls differed little from the DLA of stems of the same variety, while the DLA of the AN Bayaut-2 bolls was more hydroxylated than the DLA of the stems of the same variety.

The DLA of the cotton seed hulls was the most highly hydroxylated of all the lignins studied, 1.31 OH groups being found in the side-chain. The higher degree of hydroxylation is probably responsible for the capacity of the lignin of the cotton seed coat for swelling during the growth of the seed. Of all the lignins studied, the DLA of the cotton seed hulls had the highest molecular mass.

Fractionation of Lignin by Molecular Mass

It is known that DLAs are polydisperse. Study has shown that lignins differing both in molecular mass and in the structure of the PPSUs are synthesized in different periods of growth of the plant. For a deep study of polydispersity, the DLA from ripe cotton plant stems was fractionated according to molecular mass by sequential precipitation from dioxane solutions with ether. This is the so-called triangular method [36], by which a small number of fractions of different molecular masses are obtained. The separation yielded six fractions with successively decreasing molecular masses, since, with an increase in the volume proportion of precipitant, lignin fractions with ever smaller molecular weights are deposited (Table 5).

The polydispersities of the fractions ranged from 1.4 to 1.7. The low-molecular-mass fractions V and VI were the more homogeneous. A comparison of the $\bar{M}_w$ and $\bar{M}_n$ values shows that the chosen method of fractionation is fairly effective for DLA, since the molecular masses of the fractions differ appreciably.

Empirical formulas for the fractions were drawn up on the basis of elemental and functional-group analyses:

I. $C_9H_{7.76}O_{0.88}(OCH_3)_{1.38}(OH_{ph})_{0.37}(OH_{al})_{0.98}(O_{co})_{0.2}(OOH_{COOH})_{0.059}(O_{ar\text{-}al})_{0.63}$

II. $C_9H_{8.9}O_{1.05}(OCH_3)_{1.2}(OH_{ph})_{0.44}(OH_{al})_{0.92}(O_{co})_{0.2}(OOH_{COOH})_{0.063}(O_{ar\text{-}al})_{0.56}$

III. $C_9H_{8.11}O_{0.98}(OCH_3)_{1.36}(OH_{ph})_{0.44}(OH_{al})_{0.92}(O_{co})_{0.23}(OOH_{COOH})_{0.061}(O_{ar\text{-}al})_{0.56}$

IV. $C_9H_{7.44}O_{0.66}(OCH_3)_{1.34}(OH_{ph})_{0.43}(OH_{al})_{0.93}(O_{co})_{0.22}(OOH_{COOH})_{0.067}(O_{ar\text{-}al})_{0.57}$

V. $C_9H_{7.38}O_{0.82}(OCH_3)_{1.36}(OH_{ph})_{0.44}(OH_{al})_{0.91}(O_{co})_{0.22}(OOH_{COOH})_{0.071}(O_{ar\text{-}al})_{0.56}$

VI $C_9H_{7.56}O_{0.91}(OCH_3)_{1.38}(OH_{ph})_{0.47}(OH_{al})_{0.81}(O_{co})_{0.32}(OOH_{COOH})_{0.093}(O_{ar\text{-}al})_{0.53}$

It can be seen that the fractions differed with respect to the number of functional groups in a PPSU, these being present both in the aromatic nuclei (OCH_3, OH_{ph}) and in the side-chain (OH_{al}, CO, COOH). The low-molecular-mass fractions were more highly methoxylated than the high-molecular-mass ones but had fewer aliphatic hydroxyls.

Lignins from the Stem of a Cotton Plant Affected by Wilt

Verticillium wilt is the most widespread disease of the cotton plant, causing great losses to the cotton-growing industry. Medium-fiber varieties of the cotton plant are attacked by the fungus *Verticillium dahliae*, and thin-fiber varieties by *Fusarium oxysporum*.

It is known from the literature [38-40] that in a fungal attack it is not individual enzymes that exert their action but a whole system of enzymes destroying both the carbohydrate and the lignin parts of wood. Under the action of the fungus, a change in the functional groups of the lignin takes place — oxidation of terminal alcohol and aldehyde groups to carboxy and carbonyl groups and also demethylation and demethoxylation of the lignin. The fungal hyphae break down the syringyl structural units of the lignin faster than the guaiacyl units. Under the action of white rot and brown rot fungi, the liberation of CO_2 takes place most readily from methoxy groups, and then from the side-chain, and with the greatest difficulty from the

TABLE 8. Spectral Characteristics of DLAs from Various Sources [45]

Source of the lignin	λ_{max}	ε_{280}	OCH_3/C_9
Pine wood	277–282	3880	–
Ripe stems of a 108-F cotton plant	277	3100	1.01
Early vegetation-period stems of a 108-F cotton plant	275–280	3100	0.47
Flowering-period stems of a 108-F cotton plant	280	2700	0.73
Ripe bolls of a 108-F cotton plant	280	2800	0.74
Seed hulls of a 108-F cotton plant	280–282	4700	0.84
Ripe stems of a Tashkent-1 cotton plant	277	3100	0.82
Early-period stems of a Tashkent-1 cotton plant	280	2500	0.53
Flowering-period stems of a Tashkent-1 cotton plant	280	2000	0.78
Ripe bolls of a Tashkent-1 cotton plant	280	2350	0.87
Ripe stems of an AN Bayaut-2 cotton plant	280	2900	0.81
Ripe bolls of an AN Bayaut-2 cotton plant	280	3000	0.76

TABLE 9. Numbers of Protons in the PPSUs of the Dioxane Lignins of Cotton Plants of Different Varieties

Zone	Variety of cotton plant					
	108-F	Tashkent-1	AN Bayaut-2	S-4880	S-6030	Mexican
I	2.34	1.66	2.92	1.83	2.80	2.70
II	0.58	0.40	0.44	0.36	0.46	0.43
III	0.32	0.20	0.71	0.27	0.33	0.31
IV	6.12	5.74	6.23	6.93	5.60	7.25
IVa	3.69	2.46	2.43	3.93	3.54	2.43
	2.43	3.28	3.80	3.03	2.06	4.82
V	0.95	1.01	1.46	1.13	1.18	2.43
VI	2.90	2.62	1.59	2.56	3.57	3.18
VII	0.36	1.21	0.22	1.10	1.54	0.63
Total	13.58	12.87	13.57	14.18	14.51	16.23

TABLE 10. Products of the Alkaline Nitrobenzene Oxidation of Cotton Plant Stem DLAs (% on the DLAs)

Variety	Numbers of the substances identified*										
	1	2	3	4	5	6	7	8	9	10	11
Tashkent-1	–	1.2	–	–	11.4	–	0.2	0.1	5.7	–	0.1
Mexican	0.2	0.3	–	0.7	1.0	4.4	0.3	–	0.4	4.3	–
Tashkent-6	0.2	0.5	–	1.7	1.9	5.8	0.4	–	0.4	5.6	–
S-488	–	0.4	0.2	0.7	15.2	5.1	–	–	10.4	0.7	–
AN Bayaut-2	–	0.4	–	–	18.7	3.9	–	0.9	11.9	–	2.4
S-6030	0.3	0.1	1.3	0.1	3.2	13.9	–	–	1.0	7.6	–

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*1) *p*-Hydroxybenzaldehyde; 2) *p*-hydroxybenzoic acid; 3) *p*-coumaric acid; 4) guaiacol; 5) vanillin; 6) vanillic acid; 7) acetoguaiacol; 8) ferulic acid; 9) syringaldehyde; 10) syringic acid; 11) sinapic acid.

aromatic ring. The biological degradation of lignin under the action of a fungus depends on the molecular mass of the lignin: the lower this is, the faster does decomposition proceed.

The yield of products of the alkaline nitrobenzene oxidation of wood that has been subjected to the action of a fungus falls, which shows the occurrence of condensation processes. The rate of degradation of lignin depends on the type of fungus, the temperature, the pH of the medium, and the sources of nitrogen and vitamins added to the nutrient medium, and also on the oxygen content.

However, there is very little information in the literature on the specific action of wilt. It is known that a *Fusarium* fungus is capable of breaking down individual lignin fragments subjected to its action more rapidly than *Verticillium* [41].

The action of wilt on the stems of cotton plants of the varieties Tashkent-1 (medium-fiber) and S-6030 (fine-fiber) has been studied. In the early vegetation period, damage to the cotton plant by wilt was insignificant. The disease developed intensively during the budding and flowering period and continued until the end of vegetation, damaging both the stems and the bolls.

TABLE 11. Products of the Cleavage of the DLA from Cotton Plant Stems by Sodium in Liquid Ammonia (% on the DLA)

Variety and vegetation period	Numbers of the products identified*														
	12	13	14	15	4	5	16	17	18	19	20	21	22	23	24
108-F, early period	0.20	–	–	–	0.05	0.25	0.04	0.03	–	1.69	0.21	–	1.16	0.12	–
108-F, flowering period	–	0.02	–	–	0.02	1.38	0.44	–	0.21	3.58	0.33	–	3.95	0.07	–
108-F ripe stems	0.01	0.02	–	–	0.04	2.35	0.21	–	0.53	6.45	0.81	0.57	1.87	0.37	–
Tashkent-1, early period	0.15	–	–	1.12	2.10	4.47	1.20	–	–	7.28	0.80	–	7.92	–	–
Tashkent-1, flowering period	–	–	0.4	1.0	3.84	9.96	0.28	–	–	7.08	3.10	1.0	9.92	–	0.88
Tashkent-1, ripe stems	6.66	–	0.22	0.22	–	–	0.18	0.62	–	7.16	1.02	–	8.54	–	–
Mexican, ripe stems	0.02	–	0.05	0.03	0.01	0.66	–	0.08	0.02	1.66	0.6	0.2	3.51	–	–
Tashkent-6, ripe stems	–	–	0.14	–	0.02	0.68	–	0.1	0.01	1.65	0.94	0.1	1.56	–	–
S-4880, ripe stems	–	0.28	0.26	–	–	2.30	–	–	0.16	3.95	0.09	0.16	5.89	–	–
AN Bayaut-2, ripe stems	–	–	–	0.98	0.31	–	–	0.39	–	4.43	0.56	–	3.61	–	–
S-6030, ripe stems	–	–	–	0.65	0.26	–	–	0.38	–	6.08	0.9	–	2.8	–	–

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*12) phenol; 13) *p*-hydroxyphenylethane; 14) *p*-hydroxyphenylpropane; 15) 3-(*p*-hydroxyphenyl)propan-1-ol; 4) guaiacol; 5) vanillin; 16) vanillyl alcohol; 17) guaiacylethane; 18) 1-guaiacylethanol; 19) guaiacylpropane; 20) 1-guaiacylpropan-1-ol; 21) 3-guaiacylpropan-1-ol; 22) syringylpropane; 23) 1-syringylpropan-1-ol; 24) 3-syringylpropan-1-ol.

TABLE 12. Numbers of Fragments and Bonds in 100 Aromatic Nuclei of DLA

Types of fragments and bonds	Dioxane lignins		
	1	2	3
S, total	45	23	34
G, total	41	15	19
H, total	1–2	8	6
Structure 1	9	18	12
Structure 2	6	19	9
Structures 3 and 4	–	17	11
N C_{ar}-OH	37	89	68
N C_{al}-OH	141	73	69
N C_{al}-O-C_{al}	13	10	6
N C_{ar}-O-C_{al}	74	67	68
N C_{ar}-O-C_{ar}	4	17	18
N C_{ar}-O	259	269	270
N C_{ar}-C	232	207	197
N CH_{ar}	109	124	133

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*S) Syringyl; G) guaiacyl; H) *p*-coumaryl structures.

TABLE 13. Numbers of Fragments and Groups in Lignin Formulas

Fragments and groups	Lignin			
	reed [80]	spruce [81]	beech [75]	cotton plant
Total PPSUs	14	18	15	18
p-Coumaryl	2	2	–	0.35
Guaiacyl	4	16	7	7.3
Syringyl	8	–	8	7.9
Structure 1	–	–	–	1.5
Structure 2	–	–	–	1.0
Pinoresinol	3	1	1	1
Furocoumarin	1	1	1	–
OH_{phen}	6	6	5	6.6
OH_{alip}	10	18	13	25
CO_{ketone}	2	3	3	1.9
$CO_{aldehyde}$	1	1	–	1.4
TotalCH_{arom}	31	46	30	19.5
$C_{al}-O-C_{al}$	7	3	4	2.2
$C_{ar}-O-C_{ar}$	–	–	–	0.72
$C_{ar}-O-C_{al}$	7	10	9	13.4
COOH	–	–	–	0.93
COOR	–	–	–	6.6
$CH_{\beta-\beta}$	6	4	4	2.25

Healthy and wilt-affected samples of Tashkent-1 cotton plants were gathered during the flowering period and after harvesting, and corresponding samples from the thin-fiber variety S-6030 after harvesting. In the case of the Tashkent-1 variety it was shown that in the wilt-affected cotton plant the content of lignin (Komarov lignin) had increased (Table 6).

The same phenomenon was traced in the case of the thin-fiber cotton plant. The content of Komarov lignin in the healthy stems was 24.17%, and in the wilt-affected stems 25.16%.

We obtained DLAs from all the cotton plant samples, and their yields were always lower in the infected plants. It is obvious that wilt damage to a cotton plant changes the ratio of the components and decreases the amount of that part of the lignin that can be extracted by aqueous dioxane.

A study of the molecular-mass distribution (MMD) of the DLAs from healthy and wilt-affected plants showed that all the DLAs were polydisperse, those from the wilt-affected plants being more highly polydisperse than those from healthy plants (Table 7). A comparison showed that under the action of wilt the molecular masses of the DLA decreased at first (flowering period), but it increased in the fully fungus-damaged stems. It is likely that wilt acts on lignin first in a destructive and then in a polymerizing fashion.

Empirical formulas of all the DLAs were calculated on the basis of elemental and functional-group analyses. Analysis showed that in the case of the Tashkent-1 variety the DLAs from the wilt-affected plants contained a larger number of OCH_3 groups than the DLAs from the healthy plants. In the DLAs of wilt-affected stems of S-6030 cotton plants the OCH_3 content was lower than in the DLAs from healthy stems:

DLA of wilt-affected stems of a Tashkent-1 plant (flowering)

$$C_9H_{6.68}O_{1.28}(OCH_3)_{0.87}(OH_{ph})_{0.6}(OH_{al})_{0.77}(O_{co})_{0.28}(OOH_{COOH})_{0.07}(O_{ar\text{-}al})_{0.4}$$

DLA of wilt-affected bolls of a Tashkent-1 plant

$$C_9H_{6.18}O_{1.01}(OCH_3)_{0.83}(OH_{ph})_{0.62}(OH_{al})_{1.04}(O_{co})_{0.4}(OOH_{COOH})_{0.12}(O_{ar\text{-}al})_{0.38}$$

DLA of ripe wilt-affected stems of a Tashkent-1 plant

$$C_9H_{7.22}O_{1.14}(OCH_3)_{1.12}(OH_{ph})_{0.42}(OH_{al})_{0.82}(O_{co})_{0.45}(OOH_{COOH})_{0.24}(O_{ar\text{-}al})_{0.58}$$

DLA of ripe wilt-affected stems of a S-6030 plant

$$C_9H_{8.13}O_{1.77}(OCH_3)_{0.98}(OH_{ph})_{0.29}(OH_{al})_{1.65}(O_{co})_{0.4}(O_{ar\text{-}al})_{0.71}$$

Developed empirical formulas of the DLAs from healthy samples of the corresponding plants are given above.

In all the DLAs from the wilt-affected plants the total oxygen content was always higher than in the DLAs of the corresponding healthy samples, which shows the oxidative nature of the action of wilt on lignin. In the case of fusarium wilt, a fall in the amount of OCH_3 groups probably takes place through a demethoxylation of the syringyl components, as can be seen from the products of alkaline nitrobenzene oxidation and of cleavage by metallic sodium in liquid ammonia. In the case of verticillium wilt, a rise in the amount of OCH_3 groups is possible in three cases: if phenolic OH groups are methylated, if the content of syringyl structures increases, and if aliphatic OH groups in the side-chain are methylated.

If phenolic OH groups were methylated, then the products of nitrobenzene oxidation should include methyl ethers of aromatic aldehydes, ketones, acids, phenols, and phenolic alcohols — products of oxidative and reductive cleavage. But thin-layer chromatography with "marker" ethers of the above-mentioned substances did not show their presence. In the products of the oxidative and reductive cleavage of the DLAs, the content of substances with syringyl structures had not increased. To confirm the possibility of the methylation of the lignin side-chain under the action of wilt, we studied the action of fungi on coniferin. As a result, it was found that no methylation of coniferin took place, and the coniferin underwent oxidative cleavage. Consequently, the increase in the amount of methoxyls does not take place through methylation of the side-chains of the DLA. Thus, the increase in the amount of methoxy groups in the DLA of wilt-affected DLA is probably connected with a rise in the amount of guaiacyl structures.

UV and IR Spectra of Lignins

In the UV spectra of all the lignins that we have studied there was an aromatic maximum at 275-285 nm, and in some samples there was a second maximum, less strong and sometimes in the form of a shoulder, at 310-330 nm. The position of the first maximum depends on the degree of substitution of the benzene ring. Syringyl components have a maximum at 271-275 nm, *p*-coumaryl components at 275-277 nm, and guaiacyl components at 280-286 nm. The intensity of this maximum also depends on the nature of the aromatic components concerned [42]. If the intensity is expressed as the extinction calculated to the molecular mass of a PPSU, the aromatic components of lignin form the series guaiacyl > *p*-coumaryl > syringyl. If there is aryl–aryl linkage, the position of the maximum shifts into the long-wave region and its intensity increases sharply. If, however, the aromatic components are bound by aryl–O–aryl bonds, the position and intensity of the maximum change insignificantly [43, 44].

The appearance of a second maximum in the far UV region of the spectrum most frequently indicates the presence of C=O group conjugated with aromatic rings in the lignin molecule. A comparative study has been made of the UV spectra of DLAs. Cotton plant lignins are constructed of structural units of three types: guaiacyl, syringyl, and *p*-coumaryl. In all their spectra, a maximum at 275-280 nm and a shoulder at 320-340 nm are observed. For our preparations we determined the coefficient of molar absorption at 280 nm calculated to the molecular mass of a PPSU (E_{280}).

As can be seen from Table 8, the extinctions in all the DLAs studied, except for that from cotton seed coats, was considerably lower than for the pinewood DLA because of the presence of syringyl and *p*-coumaryl structures in them. The chromophoric groups in the lignins of the different varieties of cotton plants differed considerably, and this was reflected in the changed molar extinctions in their UV spectra.

A very high extinction was observed in the UV spectrum of the DLA from the cotton seed coats which was probably due, in considerable degree, to structures with linked aromatic nuclei.

The IR spectra of all the lignins studied were similar and consisted of the contours of a group of overlapping absorption bands. There were all the bands characteristic for natural lignins, but their intensities were different for each preparation. The interpretation of the absorption bands in the IR spectra of the lignins was made in accordance with the literature [46, 47].

It was impossible to judge the intensities of the bands visually. For a comparative study, therefore, we used the method involving the base line of an internal standard and the calculation of the relative optical densities of the absorption bands (RODs) by the method of [48]. As the internal standard we used a band at 1515 cm^{-1} corresponding to the vibrations of the benzene rings in lignin, and the base lines were drawn through the absorption minima at 1800-700 and 3800-2750 cm^{-1}. From the RODs of the bands in the IR spectra of lignins it is possible to estimate the number of corresponding functional groups in DLAs.

PMR Spectra of DLAs

The PMR method began to be used for the characterization of lignin preparations in the 1960s [49]. It has proved to be extremely informative and is widely used for the study of lignin. Spectra were taken in a JNM-4H-100/100 MHz spectrometer in deuterochloroform. The DLA was acetylated beforehand in order to improve solubility and to obtain information on phenolic and aliphatic hydroxyls.

The interpretation of the chemical shifts of the protons in the PMR spectra of lignins was made in accordance with the literature [50-52]. For this, the lignin spectra were divided into seven regions corresponding to definite types of protons. There were no signals below 2 ppm in the PMR spectra of the lignins studied. The boundaries of the first zone were therefore selected in the interval of 2.0-3.7 ppm. According to the literature, natural lignins contain insignificant amounts of α-vinyl groups (about 0.06 per C_9) [51]. It may therefore be considered that it is mainly aromatic protons that appear in the first zone.

β-Vinyl and benzyl ether protons in the C_3 side-chain appear in the second zone, at 3.7-4.2 ppm. The third zone, at 4.2-4.8 ppm, contains the signals of protons present in coumarane structures. In the fourth zone, at 4.8-7.5 ppm, are shown protons of methoxy groups and the α-, β-, and γ-protons of the C_3 side-chain. Methoxy protons appear clearly in all the spectra, and it is easy to distinguish the boundaries of their zone, in the 6.0-6.8 ppm region. The protons of phenolic acetoxy groups appear in the fifth zone, at 7.5-7.9 ppm, and the protons of aliphatic acetoxy groups in the sixth zone, at 7.9-8.4 ppm. In the seventh zone, at 8.4-9.5 ppm, are located the signals of highly screened protons possibly belonging to aliphatic methyl and methylene groups.

For the quantitative evaluation of PMR spectra it is customary to calculate the number of protons in an elementary C_9 unit of the lignin from analytical results [50] or to use the internal-standard method [53]. We made a quantitative calculation of the spectra starting from the numbers of methoxy groups in the lignins, referred to C_9.

The total integral intensity of the signals of all the protons was taken as 100%, and we then determined the percentage of the intensity due to the methoxy protons. The percentages of the intensity corresponding to each interval of the integral curve and corresponding to a definite type of protons were determined similarly. Using empirical formulas, from the numbers of methoxy groups per C_9 unit we calculated the area of the signal of the OCH_3 groups as percentages per proton. Starting from these results, we determined the numbers of protons in each region. And although the PMR spectra of the lignins differed little in general shape, from the numbers of protons in the various regions it was possible to draw conclusions concerning differences in the structures of the lignins. For illustration we give the distribution of protons in the PPSUs of the DLAs for different varieties of cotton plant (Table 9).

It can be seen from Table 9 that the DLAs from the different varieties differed by the numbers of protons of definite types. It is possible to judge the degree of substitution of the aromatic ring in a lignin PPSU from the number of free aromatic protons (zone 1) and the number of OCH_3 groups. The lowest degree of substitution was found in the aromatic nuclei of the DLA from the AN Bayaut-2 variety of cotton plant. At the same degree of methoxylation of the DLAs from the AN Bayaut-2 and Mexican cotton plants (0.81 OCH_3) the aromatic nuclei of the DLA from the Mexican plant were more highly substituted.

The smaller the degree of substitution, the greater the number of free hydrogens and the higher the reactivity of the DLA in substitution reactions at the aromatic ring.

We calculated the numbers of α- and γ-hydroxy groups from the characteristics of the PMR spectra. All the acetyl fragments substituting aliphatic OH groups appeared in zone VI. The differentiation of α- and γ-hydroxy groups was made from the results of a comparison of their number with the number of protons in zone II. From the numbers of protons in zone III it was possible to draw the conclusion that the proportion of coumarane structures was small in all the DLAs studied.

The use of the PMR method for the study of the lignin of a 108-F cotton plant fractionated according to molecular mass enabled us to obtain valuable information on condensed aromatic nuclei [54] and to show that their amount decreased in the low-molecular-mass fractions [55].

The identification and calculation of aliphatic acetyl and benzylacetyl protons showed that in all the fractions there was a hydroxy group in the α-position to the aromatic ring in every second PPSU. These hydroxyls were not detected by other methods. The higher degree of condensation of the high-molecular-mass fractions was also shown by the presence of protons belonging to phenylcoumarane structures. Their amount was higher in the high-molecular-mass fractions. Thus, the use of the PMR method for studying lignin has given additional information on the structures of the DLAs studied.

Alkaline Nitrobenzene Oxidation of Lignin

One of the most effective chemical methods for the chemical study of lignin is alkaline nitrobenzene oxidation. From the products of nitrobenzene oxidation we can judge the presence of lignin in plants, the types of lignin, and its quantitative level.

Oxidation conditions have been developed by several authors, and the best were adopted: temperature 160-180°C, 4% aqueous alkali, time 3 h. The process consists of two stages: hydrolysis of the ether bonds of the lignin and subsequent oxidation of the liberated fragments of the lignin molecule to aldehydes and acids [57].

The products of the oxidation of the lignins of coniferous wood always contain phenolic aldehydes and phenolic acids with aromatic nuclei of the guaiacol type. Among the oxidation products of the wood of broad-leaved trees are substances with guaiacyl and syringyl nuclei. The composition of the products of the oxidation of bushy and herbaceous plants has proved to be complex: in addition to those mentioned above, *p*-coumaryl components have also been detected.

Alkaline nitrobenzene oxidation has been widely used in the study of native and isolated cotton plant lignins.

As has been stated above, no substances of *p*-coumaryl nature have been detected in the monomeric products of the decomposition of MWL from the stems, bolls, and seed hulls of the cotton plant [4]. This situation does not agree with the fact that the cotton plant is a representative of the bushy dicotyledonous plants for which the presence of *p*-coumaryl structures is characteristic.

In view of this, we have made a detailed study of the total products of the alkaline nitrobenzene oxidation of the native lignin of a cotton plant gathered in the early vegetation period (2 cotyledons) [58]. In addition to vanillin and syringaldehyde, a third aldehyde was isolated by two-dimensional preparative chromatography on gypsum–silica gel (1:13) plates in the methanol–benzene (1:9) system. It was identified by UV and mass spectra as *p*-hydroxybenzaldehyde. The 2,4-dinitrophenylhydrazone obtained from the third aldehyde was identical with the 2,4-dinitrophenylhydrazone of an authentic specimen of *p*-hydroxybenzaldehyde [59]. Thus, the presence in cotton plant lignin of a third type of structural units — *p*-coumaryl — has been shown experimentally for the first time. Subsequently, compounds with a *p*-coumaryl structure were found by the GLC method in all the products of the oxidation and cleavage of cotton plant lignins.

The alkaline nitrobenzene oxidation of all the substances was carried out under identical standard conditions. The monomeric oxidation products were analyzed by GLC [60] (Table 10).

It can be seen from Table 10 that substances with *p*-coumaryl, guaiacyl, and syringyl structures were detected in the lignins of all the plants. From the results of a study of the changes taking place in the lignin with the growth of the cotton plant and from the products of nitrobenzene oxidation, it can be seen that in all the lignins the main structures were the guaiacyl type, followed by syringyl and *p*-coumaryl [60a].

The total amount of oxidation products increases with the growth of the plant. This can be seen clearly for the native lignins and for the DLAs. It follows from this that less condensed lignin is synthesized in the plants as growth proceeds than in the early period. A considerable increase in the total amount of substances with the syringyl structure also shows a lower degree of condensation of the lignin in the late vegetation period.

TABLE 14. Amino Acid Composition of the Adsorbed Protein in the Premix Carrier (after hydrolysis)

Amino acid*	Content, %
1. Aspartic	10.0
2. Threonine	4.4
3. Serine	5.0
4. Glutamic	13.0
5. Proline	11.0
6. Alanine	6.0
7. Glycine	3.9
8. Valine	6.0
9. Methionine	1,0
10. Isoleucine	1,7
11. Leucine	12.0
12. Tyrosine	3.7
13. Phenylalanine	4.6
14. Histidine	2.8
15. Lysine	3.8
16. Arginine	6.0
17. Cysteine	1.0

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*The essential acids are underlined.

A comparison of the oxidation products for different vegetation periods shows that the amount of syringyl components was always higher in the DLA than in the natural lignins. It is obvious that, on mild acid hydrolysis, weakly condensed lignin enriched with syringyl structures is extracted from the plant first.

A comparison of the products of nitrobenzene oxidation obtained from the lignin of different varieties of cotton plant show that, in all cases, 20-30% — i.e., a considerable proportion — of these total products were identified. It can be seen from the ratio of the three types of structures in the nitrobenzene oxidation products that in all cases the guaiacyl derivatives were the main ones and the amount of the syringyl derivatives was always less (0.56-0.66 in relation to the guaiacyl derivatives), and that of the *p*-coumaryl derivative always the least. From the total yields of oxidation products and the amounts of structures of different types in them, the most highly condensed and most enriched with *p*-coumaryl units was the DLA from the stems of the Mexican cotton plant. The total yield of oxidation products was lowest for the DLA of the Mexican cotton plant and highest for the DLA from the variety AN Bayaut-2, which shows different degrees of condensation of the DLA.

Cleavage of the Lignins with Metallic Sodium in Liquid Ammonia

In the study of lignins, decomposition with metallic sodium in liquid ammonia has been recognized as an effective method for the determination of aromatic fragments and the structure of the C_3 side-chain. This method was developed by N. N. Shorygina and her colleagues [61]. It was shown that reduction with metallic sodium in liquid ammonia leads to the cleavage of ether bonds. As also in nitrobenzene oxidation, we have to deal with monomers obtained in the cleavage of the uncondensed parts of the lignin macromolecule. In addition to information on the structure of the aromatic rings, in this case it is possible to find out the positions of hydroxyls in the C_3 side-chain. It is impossible to deduce the numbers of primary and secondary OH groups in the lignin molecule since, in the first place, the reaction always takes place in a heterogeneous system and nonquantitatively, and, in the second place, a secondary reaction leading to arylpropanes always occurs.

It has been shown on models [62, 63] that the OH groups of the phenolic alcohols in the products are not of secondary origin but were present in the same positions in the lignin molecule. No substances with a β-OH group in the phenylpropane side-chain have been found among the cleavage products. In the cotton plant lignins the majority of structural units are probably linked by β-aryl-alkyl bonds.

It must be mentioned that among the cleavage products have been found substances with one and two carbon atoms in the side-chain: vanillin, vanillyl alcohol, guaiacylethane, and 1-guaiacylethanol. So far it has not been definitely established whether these substances are formed by the cleavage of C—C bonds during decomposition or are lignin structures [64]. It has been shown that vanillyl alcohol is produced in good yield from a number of models in such reactions [63]. According to our hypothesis, vanillin is split off from structural fragments of the lignin molecule to which it is linked by an ether bond. It has been established [66] that vanillin is not completely reduced even on prolonged contact with sodium in liquid ammonia.

TABLE 15. Maximum Levels of Adsorption by HLs and Modified HLs

Preparation	Maximum adsorption, mg/g
Cotton seed hull HL	115.3
Wood HL	65.0
H_2O_2-oxidized HL	58.4
Willstätter lignin from pine wood	53.5
Rice husk HL	51.2
Piperidinomethylated HL	47.5
Chlorinated HL	45.1
Demethylated HL	40.0
Phosphorylated and piperidinomethylated HL	39.5
Nitrolignin	34.5
Sulfolignin	25.0
Nitrosulfolignin	8.5

Table 11 shows the products of the cleavage of lignins by metallic sodium in liquid ammonia. Just like the products of nitrobenzene oxidation, the cleavage products contained substances with three types of structures: *p*-coumaryl, guaiacyl, and syringyl.

On studying the changes in the lignin during the growth of the plant it can be seen that the quantitative ratios of the types change, a tendency being observed to an increase in the amount of syringyl and a decrease in the amount of *p*-coumaryl structures relative to guaiacyl structures. The total yield of monomeric phenols increases as the plant grows. This obviously shows an increase in the amount of aryl-alkyl ether bonds and a decrease in the degree of condensation of the lignins in the late vegetation period. It is known that the capacity of the structural units for condensation decreases in the series: *p*-coumaryl, guaiacyl, syringyl. It is therefore obvious that the lignins of ripe stems contain a larger amount of the more methoxylated units.

Analysis of the products of the cleavage of the DLAs of different varieties of cotton plant has shown the lowest yields of monomeric phenols in the case of the Tashkent-6 and Mexican varieties, the yields for the other varieties being 2-2.5 times higher. This fact correlates well with the oxidation products. The DLAs for the Tashkent-6 and Mexican varieties proved to be the most highly condensed according to the oxidation products, as well.

In a study of the products of cleavage of the cotton plant lignins by metallic sodium in liquid ammonia, we isolated fractions of dimers on a column of Sephadex LH-20 using ethanol–water (9:1) as eluent [66]. Dehydroisoeugenol and 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propan-1-ol were identified in them by GLC [67]. The detection of the latter (a dimeric alcohol with a C–O–C bond) confirmed the hypothesis that on cleavage with sodium in liquid ammonia the C–O–C bonds in lignin are not cleaved quantitatively. The detection of dehydroisoeugenol indicates the presence of such a fragment in the lignin macromolecule.

Structures of this type are readily synthesized in an acid medium from α-phenolic alcohols [68, 69] — cleavage products of lignin — during their isolation from the reaction mixture. In a study of the condensation of such phenolic alcohols we obtained dimers consisting of two guaiacyl units (dehydroisoeugenol), two syringyl units, and syringyl and guaiacyl units [70]. These dimers had a single type of structure (oxindane) and differed from one another by the numbers of methoxy groups in the aromatic nuclei. The condensation of 1-(*p*-hydroxyphenyl)propan-1-ol gave a dimer not of the oxindane series, 1,3-di(*p*-hydroxyphenyl)-2-methylpent-1-ene. Here condensation of the phenolic alcohol formed took place only through the phenylpropane side-chain without affecting a proton from the aromatic nucleus [71]. Thus, the probability of the formation of such dimeric fragments during the synthesis of lignin in plants cannot be excluded.

^{13}C NMR Spectroscopy and the Scheme of Construction of a Fragment of the Cotton Plant Lignin Macromolecule

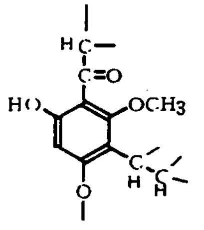
A large amount of information on the structure of lignin is given by ^{13}C NMR spectroscopy. Material on the recording, interpretation, and calculation of the ^{13}C NMR spectra of lignins has accumulated in the literature [72-76].

We have taken, interpreted, and calculated the ^{13}C NMR spectra of DLAs from a cotton plant of the 108-F variety: 1) from ripe stems; 2) from shoots; 3) from seed hulls. For each DLA we recorded the spectrum in DMSO-d_6 and two subspectra: of primary and tertiary, and of secondary and quaternary, carbon atoms. Interpretation was carried out in accordance with [77]. On the basis of quantitative ^{13}C NMR spectra we calculated the relative numbers of carbon atoms in functional groups and fragments and the number of them associated with one aromatic ring [78].

TABLE 16. Protective Effects of Lignin Preparations

Preparation	Concentration of additive, wt. %	Rate of corrosion, g-m	Protective effect
Control	–	468.8	–
Nitrochlorolignin	1.0	74.0	84.5
Nitrolignin	0.8	135.5	71.6
Chlorolignin	1.0	215.6	54.9
LUP	10	230.9	51.5
H_2O_2-oxidized chlorolignin	1.0	61.4	87.6
DMFA-soluble part of LUP	1.0	220.9	53.4
H_2O_2-oxidized nitrosulfolignin	1.0	309.3	35.2

In the subspectra of all three lignins we observed resonance signals from quaternary aromatic carbon atoms in the 115-95 ppm interval and of OCH_3 groups at 64-58 ppm, which are not characteristic for the spectra of lignins. Analysis of the chemical shifts (CSs) of model compounds led to the conclusion that these resonance signals can be ascribed to the C-1 and C-3 atoms of structures (**1**) and (**2**) and to the OCH_3 group of structure (**1**).



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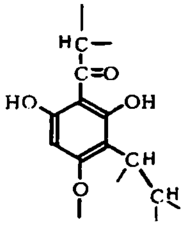

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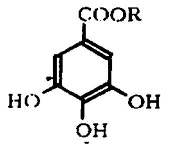
Such structures had not previously been detected in lignins. Their numbers were small in the DLAs from ripe stems but were fairly considerable in the DLAs of shoots and seed hulls. In the DLA from shoots, the length of the side-chain was not C_3 (as is usual for lignins) but far greater (about 6).

In the lignins of the shoots and seed hulls, intense resonance signals in the 146-145 region of the ^{13}C NMR spectra showed the presence of structures in which the C-3,4,5 positions were substituted by OR groups (R = H, CH_3) [79]. These are structures (**3**) and (**4**).



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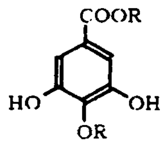


4

The greatest number of such structures was found in the shoot lignin. Thus, the lignins of shoots and seed hulls of the cotton plant are characterized by the presence of structures that are not typical for the lignin from ripe stems.

Using the results of elemental analysis (percentages of C and H) and methods proposed in the literature [73], we have calculated the numbers of fragments and bonds in the dioxane lignins (**1-3**) per 100 aromatic rings. From this it is possible to calculate the ratios of the main structural units in the DLAs:

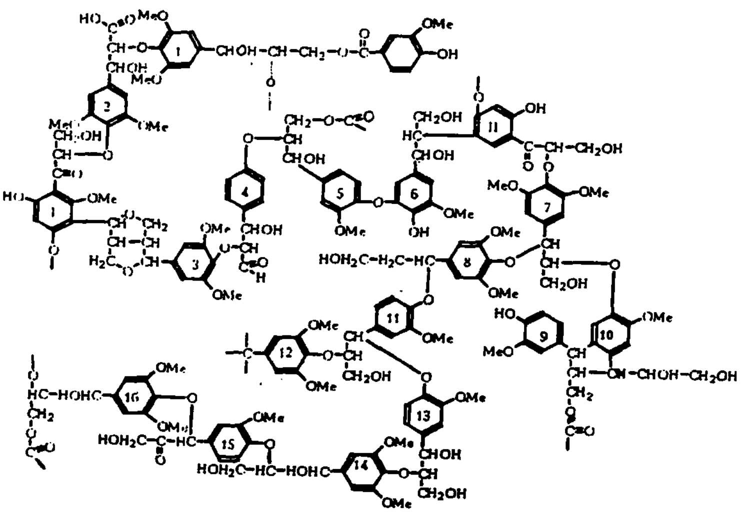
DLA of ripe stems: S:G:H:I:II = 0.93:0.02:0.19:0.13

DLA of shoots: S:G:H:I:II:III:IV = 1:0.66:0.34:0.78:0.81:0.74

DLA of hulls: S:G:H:I:II:III:IV = 1:0.55:0.18:0.34:0.54:0.32

A high degree of condensation ($C_{ar}-C$) was characteristic for all the cotton plant lignins. To each aromatic ring, in addition to the C_1-C bond there are 0.97-1.32 $C_{ar}-C$ bonds. For comparison, in spruce MWL the degree of condensation is only 0.39 [74].

On the basis of the ^{13}C NMR results and a chemical study of the DLA of ripe stems of a 108-F cotton plant, the following scheme of the structure of an average fragment has been drawn up:



Schematic structure of a fragment of the cotton plant lignin macromolecule.

It follows from the developed empirical formula of DLA given above that the molecular mass of one PPSU is 205 c.u. It has been shown by quantitative ^{13}C and ^{1}H NMR that the average formula of a DLA unit is very close to the composition of a PPSU $C_9H_{11.89}O_{3.2}(OCH_3)_{1.39}$ [79] and its molecular mass is also close to that of a PPSU calculated from the results of chemical methods of analysis and amounting to 220 c.u. Consequently, it will be justified to use the results of calculations from NMR spectra of the numbers of structural parameters of the cotton plant relating to 1 (or 100) aromatic rings in the DLA macromolecule to draw up an average scheme of the construction of the lignin macromolecule from PPSUs. Starting from the value of $\overline{M}_w$ it is possible to calculate the number of PPSUs in the DLA macromolecule. It will consist of 58 PPSUs. To draw up a formula, we selected 18 PPSUs, making up 1/3 of the DLA macromolecule. The numbers of structural fragments and bonds for the 18 PPSUs were calculated from the results of NMR spectroscopy and are given in Table 13. For comparison, this table also gives the numbers of fragments and bonds in the average structural formulas of spruce, reed, and beech lignins.

It was established from the results of alkaline nitrobenzene oxidation and ^{13}C NMR spectra that the main PPSUs of DLA are guaiacyl and syringyl structures [80]. There are very few *p*-coumaryl structures in it.

The main types of bonds in the DLA, as in all lignins, are $C_{ar}-O-C_{al}$ and $C_{ar}-C$.

A distinguishing feature of cotton plant DLA is the presence of structures **1** and **2** and of a large number of ester bonds and carboxy groups. The possibility of the substitution of aromatic rings, as in structures **1** and **2**, follows from the qualitative and quantitative analysis of ^{13}C NMR spectra [78]. The pinoresinol ring is included in the formula also on the basis of NMR results. The presence of carboxy groups was conformed by two independent methods: chemical and ^{13}C and ^{1}H NMR. The presence of these groups in kenaf DLA has been shown by alkaline hydrolysis [82]. The diisoeugenol structure has been included in the formula on the basis of its detection in the products of the cleavage of the cotton plant with metallic sodium in liquid ammonia [68].

USE OF LIGNIN

Three biochemical and hydrolysis factories are operating in Uzbekistan, and in them a large amount of lignin accumulates each day [83]. Part of it is used for the preparation of activated carbon, and the remainder is used as fuel or discarded in dumps [84, 85].

D. I. Mendeleev wrote: "In chemistry there are no wastes but there is unused raw material." Finding ways of using lignin, the products of its cleavage, and modified products will create the possibility of working by a waste-free technology for the chemical factories and will consequently solve a serious ecological problem.

Nitrogen-Containing Lignin Derivatives as Plant-Growth Stimulators

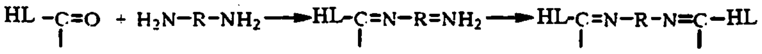
As a product of organic origin, lignin improves the physicochemical properties and the water-air regime of the soil [86] and it stimulates the vital activity of soil microorganisms and thereby creates conditions for the better growth and development of plants [87, 88]. Lignin is one of the sources of formation of humic substances in the soil [89]. The use of lignin in saline soils promotes the better washing out of poisonous water-soluble salts from the fields and protects the soil from water and wind erosion [91-93].

The study of the action of some lignin derivatives (ammonium salts of lignin polycarboxylic acids (APCs), quinone nitropolycarboxylic acids (QNPAs) and lignin stimulating fertilizers (LSFs)) on cotton and other agricultural crops has shown that they lead to an increase in the yield of raw cotton by 300-600 kg/ha [94, 95]. As is known, some lignin cleavage products and precursors (vanillin, pyrocatechol, vanillic acid, ferulic acid, and others) exert an influence on the growth and development of agricultural crops and trees [90].

In order to obtain cheaper and more active products, we have prepared a number of known and new derivatives of hydrolysis lignin (HL) and of chlorolignin (CL) by their interaction with amines: ammonia, urea, thiourea, and *o*-, *m*-, and *p*-phenylenediamines [96, 97].

The reaction of HL with amines takes place mainly through the β-carbonyl groups of the lignin. This has been confirmed by the reactions of urea and of *o*-, *m*-, and *p*-phenylenediamines with model compounds having α- and β- carbonyl groups [96].

The formation of a dimer is possible:



$$\mathrm{HL{-}C{=}O + H_2N{-}R{-}NH_2 \longrightarrow HL{-}C{=}N{-}R{=}NH_2 \longrightarrow HL{-}C{=}N{-}R{-}N{=}C{-}HL}$$

In the interaction of HL with ammonia, a reaction also takes place with the carboxy groups of the lignin to form ammonium salts. M. I. Chudakov and his colleagues consider the interaction of HL with ammonia through other lignin functional groups, as well, to be possible [16].

The reaction of chlorolignin with amines takes place in two directions — through the interaction of the amino groups with the carbonyl groups of the chlorolignin, and then through the replacement of chlorine by amino groups:

$$\mathrm{CL{-}Cl + H_2N{-}R{-}NH_2 \rightarrow CL{-}NH{-}R{-}NH_2{\cdot}HCl}$$
$$\mathrm{2\,CL{-}Cl + H_2N{-}R{-}NH_2 \rightarrow CL{-}NH{-}R{-}NH{-}CL}$$

The products of the reaction of CL with amines contain more nitrogen than the products obtained from HL [14].

All the compounds obtained have been tested as growth stimulators for cotton and other agricultural crops [96].

Lignin is not toxic for the human and animal organism [99, 100] but is biologically active. Under the action of lignin, microbiological processes in the soil are intensified: the number of bacteria increases 2.2-fold and that of actinomycetes 1.5- to 1.6-fold. At the same time, the number of pathogenic bacteria falls by 20% [101]. Lignin is therefore recommended as an organomineral fertilizer for raising the productivity of the soil in the growing of cotton, rice, potatoes, and other agricultural crops.

Factory hydrolysis lignin contains sulfuric acid residues. When the lignin is treated with ammonia, the sulfuric acid residues are first neutralized with the formation of ammonium sulfate, and then the ammonia enters into reaction with the functional groups of the lignin.

Two methods of obtaining ammoniated HL by treating HL with a dilute solution of ammonia have been developed in IKhRV AN RUz [Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan] [102, 103]. Both methods have been adopted by the Yangiyul' and Chimkent hydrolysis factories.

Ammoniated lignin from cotton seed hulls has the following elemental and functional compositions (%): C, 52.55; H, 6.2; O, 37.75; N, 2.05; OH, 5.11; OCH_3, 3.15; CO, 5.15. Part (0.5%) of the total nitrogen in the ammoniated lignin is present in the form of ammonium sulfate, which is readily digested by plants, and the remainder (1.5%) is chemically bound with the lignin. As the ammoniated lignin decomposes in the soil, the nitrogen chemically bound with it is gradually assimilated by plants. In addition, the ash of ammoniated lignin has been found to contain more than 25 macro and trace elements (%): Ca, 0.05, S, 0.25, Mg, 0.02, Al, 0.01, Fe, 0.02, Mn, 0.0006, Ni, 0.00122, Co, 0.0003, Ti, 0.002, V, 0.0003, Cr, 0.0015, Mo, 0.00037, Na, K, 0.05, Ba, 0.0045, Pb, 0.0003, Zn, 0.0002, and others that are also necessary for the growth and development of plants [104].

It has been established that the addition to soil of 10 tonnes/ha of ammoniated lignin in 2-3 days accelerates the maturation of the cotton plant. A smaller loss of fruit elements and an increase of up to 400 kg/ha in the yield of raw cotton on a typical sierozem and up to 600 kg/ha on virgin soils has been reported [105].

The use of a mixture of the antiwilt preparations Olgin or Uzgen and ammoniated lignin (100 kg/ha and 10 t/ha, respectively) reduces damage due to cotton wilt by 30-40% and decreases the amount of infection in the soil [106]. The combined use of Trikhodermin (wilt antagonist) with ammoniated lignin (120 kg/ha and 10 t/ha), respectively, leads to a decrease in the attack of the cotton plant by wilt and to an increase in the yield of raw cotton to 400-600 kg/ha [107]. The use of ammoniated lignin gives an increase in the yields of tomatoes and cabbages by 4800 and 5900 kg/ha and of potatoes by 2300 kg/ha, and at the same time the amounts of starch, sugar, and vitamin C in the products rise [107]. The use of ammoniated lignin in rice-growing gave a yield increase of 2700-5200 kg/ha [108].

The yield of kenaf bast increased by 300 kg/ha and that of the fiber in the bast by 9-12% [109].

Preparation "Roslin"

The new preparation Roslin, which possesses growth-stimulating and fungicidal activity, has been created from acrylates and modified hydrolysis lignin [110]. It has a low toxicity (LD 8400 mg/kg) and is not harmful to plants [111].

Roslin has the following elemental composition: C, 48.99; H, 5.05; N, 8.34; O, 36.16; Na, 1.5%. Its molecular mass is 50,000 to 60,000. It is supplied in the form of a dark brown odorless 10% aqueous solution with Ph 7-8.

At the present time, Roslin has been approved for wide use in agriculture by the Uzbekistan State Chemical Commission.

When cotton seeds, especially bare seeds, were treated with Roslin (100-600 g/ha, depending on the method of treating the seeds), the standard density of sowing the seeds, especially bare seeds, fell to 30% and the consumption of seed dressing to 50%.

The use of Roslin promotes intensive growth of the radicles of cotton plant seedlings, which favors the simultaneous appearance of uniform shoots not only under favorable but also under extreme conditions.

Analysis of the results obtained over a number of years in field conditions has shown that Roslin is a plant-growth regulator with a binary type of action. Thus, in low doses (100 g/ha) it stimulates the growth and development of the cotton plant and increases its resistance to unfavorable environmental conditions. In comparatively high doses (1200 g/ha) it suppresses the causative agents of root rot and wilt. The preparation prevents the shedding of fruit organs and thereby increases the yield of raw cotton to 3800 kg/ha and the oil content of the seeds to 1% [112].

Preparation Vanillin Azine

A number of preparations based on lignin cleavage products have been obtained in Ikhrv AN Ruz and sent for biological trials [113]. One of them is vanillin azine. In low doses, vanillin itself promotes the growth and development of plants, while in high doses it is an inhibitor [115].

Vanillin azine is obtained by condensing vanillin with hydrazine sulfate [113]. The potassium salt of vanillin azine is a yellow, odorless, nontoxic powder with LD_{50} 2500 mg/kg. It is readily soluble in water, alcohol, chloroform, and aqueous solutions of alkalies. Its composition is $C_{16}H_{14}N_2O_4K_2$ and its molecular mass is 376.

The results of trials have shown that the resowing treatment of cotton seeds in a solution of a vanillin azine salt at a concentration of 0.001% improves the germination of the seeds. A 0.01% solution of the preparation is used for coating seeds [116]. Under the influence of the preparation, the field germination rate of the seeds is 13.7% higher than in a control. The use of the preparation accelerates the budding phase and the ripening of the cotton bolls. The total yield of raw cotton increases by 2300 kg/ha [116].

Protein Premix for Poultry Farming

A deficiency of protein and amino acids in the poultry ration is one of the main reasons for a fall in the productivity of the birds. The development of the combined fodder industry depends on the production of premixes — mixtures of a group of biologically active substances (vitamins, antibiotics, free amino acids, proteins, carbohydrates, trace elements) with some carrier or other [118, 119]. As the carrier of the premix we have proposed to use purified and activated hydrolysis lignins from cotton seed hulls and rice husks, since they are good adsorbents and contain large amounts of trace elements. According to the literature, a fodder supplement for laying hens with trace elements improves the egg production of the hens, hatchability, and the live weight of day-old chicks [119]. The second starting material for obtaining a premix consisted of sedimented brewer's yeast. No less than 5-7 tonnes of this waste, containing 350-450 kg of protein substances, is produced every day in the breweries of Tashkent.

To improve the absorption properties of the hydrolysis lignins, they were activated with sodium hydroxide solution. Lignin washed with water to neutrality, dried, and ground was used to obtain the premix carrier. Brewer's yeast unsuitable for brewing was added to the lignin flour obtained, and the mixture was stirred mechanically for an hour. Then the lignin-yeast concentrate was separated on a filter, dried in the air, and ground in a disk mill. Not only was the hydrolysis lignin a sorbent for the protein substances from the yeast suspension but it also promoted the better separation of the solid part of the premix. The protein content of the liquid yeast suspension ranged between 40 and 65%. It must be mentioned that the value of the protein is determined by its content of essential amino acids [120, 121].

The replacement of the usual combined fodder by the protein premix in the raising of chicks exerted a positive influence on the main indices of their productivity. The average daily increase in weight of the chicks amounted to 13.79-17.93%, and the number reaching an age of 140 days exceeded the control by 2.22-3.4%.

Nutritional Vanillin

Vanillin is widely used in the food and perfumery industries as a flavoring and aromatizing agent, and also for the manufacture of drugs [122].

In view of the capacity of the redox catalyst anthraquinone for intensifying cleavage between the structural units of lignin [123], we have used it in the oxidation of cotton plant stems and seed hulls and also hydrolysis lignin [124]. In the presence of anthraquinone, the yield of oxidation products increased but a complex mixture of products related to the *p*-coumaryl, guaiacyl, and syringyl structural units of cotton plant lignin was formed. Moreover, under the action of anthraquinone a demethylation of the syringyl structural units of the lignin took place with the formation of *p*-coumaryl structural units. In view of the unsuitability of using cotton plant lignin for obtaining vanillin, further work was carried out with spent wash concentrates from the wood of coniferous trees, which consists of guaiacyl structural units and is a raw material for industrial processing [125].

The action of anthraquinone and its derivatives on the wood mass is based on the conversion of the lignin into low-molecular-mass phenolic compounds. In the initial stage of digestion, anthraquinone oxidizes the terminal groups of the cellulose and hemicellulose chains to stable aldonic acid groups and is itself reduced to anthrahydroquinone. Subsequently, it acts as a reducing agent and accelerates the delignification process by intensifying the cleavage of alkyl–aryl bonds between the structural units of the lignins. The low-molecular-mass fragments of lignosulfonates that are produced are a source of formation of vanillin in the process of oxidizing a spent wash concentrate with atmospheric oxygen in an alkaline medium in the presence of anthraquinone.

Laboratory work on the production of vanillin used 0.5-1% of anthraquinone on the weight of the dry matter in the spent wash concentrate. The oxidizing agent was atmospheric oxygen. The highest yield was achieved on the addition of 0.1% of catalyst on the weight of the spent wash concentrate. The yield of vanillin rose by 48% as compared with the control. Under industrial conditions, the maximum yield of vanillin increased by 30.55% in comparison with experiments in the absence of anthraquinone. At the same time, the quality and color of the vanillin improved considerably [126].

The use of anthraquinone under industrial conditions eliminates the formation of emulsions at the stage of extracting the vanillin.

The method of obtaining vanillin from lignosulfonates using anthraquinone as catalyst was introduced into the Says' Pulp and Paper Combine, Leningrad oblast, in 1983.

Sorbents

One of the promising directions for the use of hydrolysis lignins is the preparation of cheap active sorbents from them. The capacity of hydrolysis lignins for sorbing many classes of compounds is widely known [127]. Aqueous solutions are always used in these processes. In aqueous solutions and some polar solvents, HL swells strongly. Solvent molecules penetrate into the lignin molecule and change its sorption properties. In nonpolar solvents, lignin does not swell and its properties do not change appreciably.

The sorption properties of HL in relation to the highly polar components of crude cotton seed oil have been studied with the use of hexane as solvent. It is known that the pigment composition of crude cotton seed oil includes tocopherols, components of gossypol and flavonoid natures, and products of their transformations arising in the processing of the cotton seeds.

The sorption properties of the HLs from cotton seed hulls, sawdust, and rice husks and of modified cotton seed hull Hls have been studied. The adsorption process was investigated under static conditions at room temperature.

The kinetics of the sorption process were studied for unmodified Hls from cotton seed hulls, wood, and rice husks. It was found that in the initial stage the magnitudes of the adsorption of all three Hls differed insignificantly. After isotherms of the adsorption of polar components on the Hls had been plotted, the maximum levels of adsorption were calculated. In terms of the maximum levels of adsorption, the unmodified Hls can be arranged in the series: cotton seed hull HL > wood HL > rice husk HL.

The above-mentioned lignins were obtained by one and the same method, but the maximum adsorption levels of the resulting preparations differed greatly. The main role here is probably played by the structure of the cell walls of the initial plant material and its internal surface, which is liberated on the hydrolytic destruction of the carbohydrates, and, consequently, the hydrolysis lignins obtained had considerable differences in the structures of their active centers. When the cotton seed hull HL was subjected to any modification the maximum adsorption of the resulting preparations decreased sharply (2- to 10-fold) [128].

After their adsorption properties had been examined, the Hls were used for purifying technical plant oils. All the Hls were first washed with water and treated with organic solvents, since, when technical Hls are washed with water, residues of sulfuric acid, reducing substances, and phenols are eluted [129], while organic solvents eliminate resinous substances [130].

A solution of the oil in a nonpolar organic solvent was brought into contact with the Hls and modified Hls (MHLs) at various ratios of the weights of the lignin and the oil until a clear solution of the latter had been obtained, which was separated from the lignin by filtration through a paper filter. The lignin was washed several times with the same solvent, the completeness of the desorption of the lipid components being monitored by TLC.

Our results show that the clarification of apricot-kernel, peach-kernel, and grape seed oils by all the lignins investigated except the nitrosulfolignin took place at the same ratio of lignin to oil — 2:1 (w/w). The clarification of these oils on nitrosulfolignin required a lignin:oil ratio of 3:1.

For pomegranate seed and cotton seed oils this magnitude was between 3:1 and 6:1, the sorption properties of the MHLs being expressed somewhat better in the clarification of cotton seed oil, for which a MHL:oil ratio of 4:1 was sufficient, as compared with one of 5.6:1 for the unmodified Hls. This positive effect did not depend on the method of modifying the Hls.

Unlike the stone-fruit oils, the pomegranate seed and cotton seed oils were clarified by oxidized HL, in spite of the fact that the maximum adsorption of this HL was the highest among the MHLs. All the oils except the cotton seed oil were better clarified by such MHLs as the demethylated and phosphorylated piperidinomethylated lignins, which had small, but close, values of their maximum adsorption [131]. The adsorption properties of the other MHLs in relation to the components of the plant oils investigated were comparable with one another.

Thus, the sorption of the pigmentary components of crude plant oils by lignin is largely determined by the nature of the lipophilic pigments. It is known that crude cotton seed oil contains lipophilic pigments of gossypol nature [132]. The color of grape seed oil is due to the presence of native and modified forms of chlorophyllous and anthocyan pigments [133]. There is no information in the literature on the pigment composition of crude apricot-kernel, peach-kernel, and pomegranate seed oils. The polar lipids of plant oil are phospho- and glycolipids.

All the lignins investigated were capable of retaining triacylglycerols having polyenic fatty acids with nonconjugated double bonds feebly, free fatty acids more strongly and polar lipids and lipophilic compounds, including pigmentary substances, still more strongly [134]. The refining of technical oils on one and the same lignin can be carried out repeatedly (up to 50 times) without activation of the lignin [133].

New Anticorrosion Paint and Varnish Materials Based on Lignin

Every year, because of corrosion, tens of millions of tonnes of metal structures are melted down prematurely [135]. At the present time, the provision of the national economy of the CIS with means of protection from corrosion amounts to 40%.

Several derivatives of cotton seed hull Hls have been obtained and tested as corrosion inhibitors: nitrolignin, chlorolignin, a lignourea preparation, nitrosufolignin, a product of the oxidation lignin with hydrogen peroxide, etc. [136]. Empirical formulas have been drawn up for all the preparations on the basis of elemental and function-group analyses:

Cotton seed hull HL

$$C_9H_{11.48}O_{3.88}(OCH_3)_{0.37}(OH_{\mathrm{tot}})_{0.59}(O_{\mathrm{co}})_{0.55}(OOH_{\mathrm{COOH}})_{0.12}$$

Nitrochlorolignin

$$C_9H_{10}O_{22.35}(OCH_3)_{0.1}(OH_{\mathrm{tot}})_{0.24}(O_{\mathrm{co}})_{0.84}\,N_{0.24}\,Cl_{1.4}$$

Chlorolignin

$$C_9H_{8.52}\,O_{2.47}(OCH_3)_{0.3}(OH_{\mathrm{tot}})_{0.67}(O_{\mathrm{co}})_{0.57}\,Cl_{0.57}$$

Lignourea preparation (LUP)

$$C_9H_{12}O_{3.94}(OCH_3)_{0.18}(OH_{\mathrm{tot}})_{0.64}(O_{\mathrm{co}})_{0.09}\,N_{0.45}$$

Nitrolignin

$$C_9H_{9.81}\,O_{2.91}(OCH_3)_{0.2}(OH_{\mathrm{tot}})_{0.31}(O_{\mathrm{co}})_{0.72}\,N_{0.26}$$

DMFA-soluble part of LUP

$$C_9H_{11.78}O_{1.57}(OCH_3)_{0.19}(OH_{\mathrm{tot}})_{0.65}(O_{\mathrm{co}})_{0.19}\,N_{0.78}$$

Product of the interaction of chlorolignin with urea

$$C_9H_{10.07}O_{3.09}(OCH_3)_{0.31}(OH_{\mathrm{tot}})_{0.37}(O_{\mathrm{co}})_{0.32}\,N_{0.8}\,Cl_{0.031}$$

The influence of the lignin derivatives on the corrosion of steel was determined by the generally adopted gravimetric method [137]. The investigations showed that the greatest protective effect was possessed by nitrolignin, nitrochlorolignin, chlorolignin and hydrogen-peroxide-oxidized lignin.

The presence in HL and its modified derivatives of a considerable amount of phenolic hydroxyls and carboxy, carbonyl, nitro, chloro, and amino groups is responsible for their ability to form complexes with oxides and hydroxides of iron [138].

On the basis of nitrolignin, we, together with the Pigment NPO [Scientific Production Combine] (St. Petersburg), have created in the form of an aqueous dispersion the anticorrosion primer LÉPVA-0011 for metal structures and means of transport [139].

The primer LÉPVA-0011 is recommended for use as a protective coating for articles of wood, concrete, and metal used in a moist atmosphere, oils, or gasoline. It may also be used as a primer in combination with amide, vinyl chloride, and other paint and varnish materials. The use of the undercoat-primer LÉPVA-0011 improves the working conditions of those applying it, permits the labor involved to be reduced (application of two coats instead of three or four), and extends the duration of the protection of metal structures 1.5- to 2-fold.

Trials of the primer LÉPVA-0011 on buildings and in mechanical engineering, shipbuilding and ship repair enterprises have given positive results. In 1988 the Kharkov paint and varnish factory mastered the technology of producing the primer LÉPVA-0011 and delivered an experimental-industrial batch of it.

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