A STUDY OF THE LIGNIN OF THE COTTON PLANT OF THE SUBSPECIES *mexicanium*

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p-Hydroxybenzoic, vanillic, ferulic, and syringic acids, and also p-hydroxybenzaldehyde and vanillin, have been found in an ethanol–benzene extract of the stems of cotton plants of the subspecies *mexicanium*. The dioxane lignin (DLA) has been isolated by Pepper's method, the semiempirical formula of its phenylpropane structural unit and its molecular-mass distribution have been established, and its UV, IR, and PMR spectra have been recorded. The DLA has a comparatively low molecular mass and a lower degree of polydispersity than the dioxane lignin of the cotton plant of variety Tashkent-1, while the amounts of functional groups in them coincide, with the exception of a lower amount of carboxy groups in the DLA.

The wild variety of the cotton plant ssp. *mexicanium* is not subject to attack by wilt and is the ancestor of widely cultivated "Tashkent" wilt-resistant varieties of cotton, and therefore the study of its lignin and the comparison of the results obtained with the properties of the lignin of healthy and wilt-deseased cotton plants of the variety Tashkent-1 (DLCT-VI) [1] is of particular interest.

We have investigated ripe stems of the cotton plant ssp. *mexicanium* gathered on the experimental plot of the Zaitsev Institute of Breeding and Seed Production of the Academy of Sciences of the Uzbek SSR in November, 1979. As a preliminary treatment, the raw material (ground to dimensions of 0.25 mm) was subjected to extraction with ethanol–benzene (1:2) for 48 h and then with hot water. The yield of substances extracted by the ethanol–benzene, calculated on the absolutely dry raw material was 4.08%. From the ethanol–benzene extract, a 5% solution of sodium bicarbonate extracted acids with a yield of 1.05% on the total, and 6% sodium bisulfite solution extracted aldehydes with a yield of 5.8%. The composition of the total aldehyde and acid material was studied by the GLC method. The following acids were identified:

Substance	% in the mixture	% on the total extractive substances
p-Hydroxybenzoic acid	24.7	0.26
Vanillic acid	9.3	0.10
Syringic acid	1.7	0.02
Ferulic acid	17.4	0.18

Among the total aldehydes, a large amount of p-hydroxybenzaldehyde and traces of vanillin were found.

Thus, monomeric structural compounds of lignin may be present in the plant tissue in the free state.

The amount of Komarov lignin in the cotton plant of the ssp. *mexicanium* taking the moisture content into account was 28.2%, and the amount of cellulose 27.9%. As we can see, the amounts of lignin and cellulose in this case were approximately equal, while in cultivated varieties of the cotton plant the proportion of cellulose is considerably higher [1, 2].

We studied the dioxane lignin, which is close to the natural lignin, isolated from

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powdered samples of the cotton plant ssp. *mexicanium* by a modification of Pepper's method [3] (DLA). The yield of DLA amounted to 12.5% of the Komarov lignin. The preparation was purified by two reprecipitations from aqueous dioxane (1:9) solution in absolute ether. The DLA consisted of an amorphous cherry-red powder readily soluble in the usual solvents for lignins. It had the following elementary and functional compositions (%): C 59.81%; H 6.21%; O 33.98; OCH_3 12.82; CO 5.9; OH_{tot} 13.18; OH_{phe} 5.74. Its carbohydrate content was less than 0.5% and was not taken into account in the calculation of the semiempirical formula of the phenylpropane structural unit. The DLA had the following semiempirical formula (the formula of the dioxane lignin of ripe stems of cotton plants of the Tashkent-1 variety is given for comparison):

DLA, molecular mass of 1 phenylpropane structural unit (PPSU) 197.08

$$C_9H_{8,13}O_{1,15}(OCH_3)_{0,81}(OH_{ph})_{0,66}(OH_{al})_{0,87}(O_{CO})_{0,41}(OOH_{COOH})_{0,04}(O_{al\text{-}ar})_{0,34}$$

DLCT-VI, molecular mass of 1 PPSU 200.75

$$C_9H_{6,22}O_{1,21}(OCH_3)_{0,82}(OH_{ph})_{0,63}(OH_{al})_{0,88}(O_{CO})_{0,46}(OOH_{COOH})_{0,17}(O_{al\text{-}ar})_{0,37}$$

A comparison of the semiempirical formulas of the elementary units of the dioxane lignins isolated from the cotton plant of ssp. *mexicanium* and the stems of a late period of vegetation of the Tashkent-1 variety of the cotton plant shows that they differ by their hydrogen contents, while the numbers of functional groups per 1 PPSU in them basically agree, but there are fewer acidic groups in the DLA.

To investigate its molecular mass distribution, the DLA was subjected to gel chromatography on Sephadex G-75 using dimethyl sulfoxide as eluent and solvent. The eluograms and the integral and differential molecular-mass distribution curves are shown in Figs. 1 and 2.

The number-average ($\overline{M}_n$), mass-average ($\overline{M}_w$), and mean ($\overline{M}_z$) molecular weights were calculated by using the coefficients found previously [7]: $\overline{M}_n$ = 2,900; $\overline{M}_w$ = 5,700; $\overline{M}_z$ = 11,700; $\overline{M}_n:\overline{M}_w:\overline{M}_z$ = 1:1.91:3.92. The DLA is monomodal and has a lower molecular mass and a smaller degree of polydispersity than the DLCT-VI.

The UV spectrum taken in aqueous dioxane had a maximum at λ 280 nm and a shoulder at 300-360 nm.

The values of the relative optical densities of the absorption bands in the IR spectrum of the DLA relative to the aromatic band (1520 cm^{-1}) calculated by the method of Karklin' and Erin'sh [4] are given below:

	Frequency cm^{-1}	Relative optical density
OH groups and associated hydrogen bonds	3420	1,1105
Stretching vibrations of C–H bonds	2945	0,5155
C–H bonds in OCH_3 groups	2860	0,2636
	1470	1,0609
	1430	0,7763
	1330	0,9870
β-Carbonyl groups	1725	0,3344
Skeletal vibrations of double bonds in aromatic rings	1615	1,1371
	1520	1,0000
Phenolic OH groups	1230	1,1560
Ester/ether bonds	1130	1,6161
	1040	0,9045

In the IR spectrum of the DLA there are all the main absorption bands characteristic for lignins, but with different values of the relative optical densities.

A quantitative interpretation of the PMR spectrum of the acetylated DLA was made as described in a previous paper [5]. The value of one proton was calculated on the basis of the percentage of methoxyls (zone IV^a) and their number in the semiempirical formula of the phenylpropane structural unit. The results of the calculation are given below:

Zone of chemical shifts	Type of protons	ppm	No. of protons per C_9
I	Aromatic protons	2.0–3.7	2.00
II	β-Vinyl and biphenyl ether protons of the side chain	3.7–4.3	0.43
III	Protons of coumarane structures	4.3–4.8	0.31
IV		4.8–7.5	7.25
	IV[a] methoxyl protons	6.3–7.0	2.43
	Remaining α-, β-, and γ-protons of the side chain		4.82
V	Protons of aromatic acetoxy groups	7.5–7.9	2.43
VI	Protons of aliphatic acetoxy groups	7.9–8.5	3.18
VII	Highly screened protons of CH_3 and CH_2 groups	8.5–9.5	0.63
	Total		16.23

The total number of protons not in oxygen-containing groups calculated from the NMR spectrum (8.19) corresponds to the amount of such hydrogen in the semiempirical formula (8.13). The number of aromatic and aliphatic OH groups according to the spectral results (0.81 and 1.06) are somewhat higher than those given in the formula (0.66 and 0.87), but in both cases the numbers of aliphatic groups are greater.

EXPERIMENTAL

The dioxane lignin was isolated by a modification of Pepper's method [3]. The yield amounted to 12.5% of the Komarov lignin.

The functional groups were determined and the semiempirical formula was calculated by handbook methods [9].

The GLC analysis of the combined aldehydes was performed on a Chrom-4 chromatograph under the following conditions: stainless steel column 1.2 m long filled with 4% of PEGA on Chromaton N-AW-DMCS (0.250–0.315 mm), temperature 175°C, carrier gas He, 35 ml/min. The combined acids were analyzed in the form of the methyl esters as described by Dalimova et al. [6].

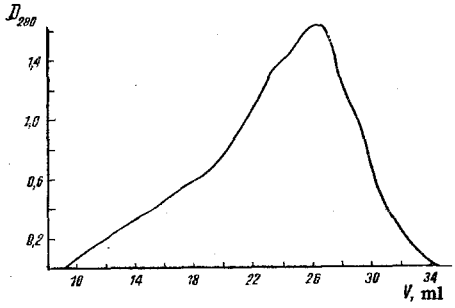

Fig. 1

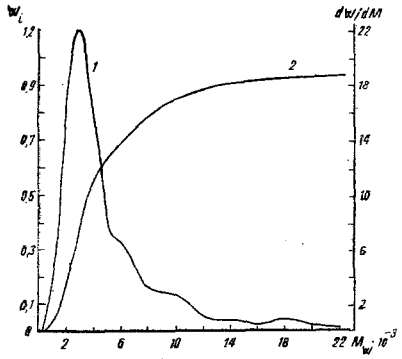

Fig. 2

Fig. 1. Eluogram of the molecular-mass distribution of the DLA.

Fig. 2. Differential (1) and integral (2) molecular-mass distribution curves of the DLA.

Gel chromatography was performed on Sephadex G-75 using dimethyl sulfoxide as eluent and solvent. The number-average, mass-average, and mean molecular weights were calculated as described in the handbook [8] using the values of the coefficients given by Alekseev et al. [7].

UV spectra were taken in aqueous dioxane (1:9) on a SF-26 spectrophotometer. λ_{max} 280 nm, lg ε 3.3952 (c $2.21 \cdot 10^{-4}$); IR spectra were taken on a UR-20 instrument (in tablets with KBr); and the PMR spectrum of the acetylated DLA on a JNM-4h-100/100 MHz spectrometer at room temperature with c 10–12% by mass; 10 — HMDS; solvent $CDCl_3$; calculation as described by Veksler et al. [5].

SUMMARY

1. The composition of the acids and aldehydes of an ethanol–benzene extract of the stems of the cotton plant of spp. *mexicanium* has been studied and it has been shown that they contain p-hydroxybenzoic, vanillic, ferulic, and syringic acids, and also p-hydroxybenzaldehyde and vanillin.

2. The dioxane lignin of the cotton plant of ssp. *mexicanium* has been isolated and its semiempirical formula has been established. It has been found that the amounts of the main functional groups in the lignin isolated and in the dioxane lignin of the cotton plant of the Tashkent-1 variety coincide, with the exception of a smaller number of carboxy groups in the *mexicanium* DLA.

3. On the basis of the molecular mass distribution established it has been shown that the dioxane lignin of the cotton plant ssp. *mexicanium* has a comparatively low molecular mass (2900) and a low degree of polydispersity (0.91).

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