INVESTIGATION OF THE DIHYDROXYLIGNIN OF *Althaea rhyticarpa*

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Althaea and the cotton plant belong to the family Malvaceae, but, in contrast to the cotton plant, which is an annual and cultivated plant, *Althaea* is a perennial and wild plant. In view of this, their comparative study is a matter of interest.

Althaea grows widely in the territory of central Asia and, in particular, in Uzbekistan it is represented by eight species. The species that we investigated, *Althaea rhyticarpa* [1], belongs to the most widespread species. It was collected in the territory of the Bostandyk region of Tashkent oblast in September (after the stem had lignified).

The dioxane lignin (DLA) from a finely ground (0.25 mm) powder of the *Althaea* roots that had previously been extracted with ethanol—benzene (1:1) was isolated by Pepper's method [2]. The yield was about 5% on the weight of the initial plant or 16.4% on the Komarov lignin. The DLA formed a light brown amorphous powder readily soluble in aqueous solutions of alkalis, moist dioxane, dimethyl sulfoxide (DMSO), and 90% acetic acid, and less readily soluble in n-butanol and water.

The lignin purified by Björkman's method [3] contained 2.4% of carbohydrates [4]. The elementary composition of the DLA and the amounts of the main functional groups in it were calculated with allowance for the presence of carbohydrates in percentages and atomic units (AU) per phenylpropane unit (PPU): C 58.64; H 6.48; O 34.88. The amounts of functional groups in the *Althaea* DLA are given below:

Functional groups	Amount, %	AU/PPU
Methoxy	16.15	1.07
Total hydroxy	11.24	1.36
Aliphatic hydroxy	7.70	0.93
Phenolic hydroxy	3.54	0.43
Carbonyl	6.22	0.52

Semiempirical formulas of the phenylpropane structural unit were calculated (mol. wt. 206.1):

$$C_9H_{10{,}03}O_{3{,}42}(OCH_3)_{1.07}$$

$$C_9H_{8.67}O_{1.94}(OCH_3)_{1.07}(OH_{phen})_{0.43}(OH_{alip})_{0{,}93}(O_{CO})_{0{,}52}.$$

The composition of the *Althaea* DLA differed from that of the DLA from cotton-plant stems:

$$C_9H_{8{,}26}O_{3{,}28}(OCH_3)_{1{,}002}$$

$$C_9H_{6.86}O_{1.62}(OCH_3)_{1.002}(OH_{phen})_{0.40}(OH_{alip})_{0.97}(O_{CO})_{0.21}(COOH)_{0.045}$$

by its larger amounts of hydrogen and carbonyl groups.

The UV spectrum of the *Althaea* DLA taken in ethanol gave a band characteristic for an aromatic ring, λ 280 nm.

The IR spectrum (tablets with KBr), which is very close to the spectrum of the DLA from cotton-plant stems, showed the bands characteristic of a substituted benzene ring (1615, 1520,

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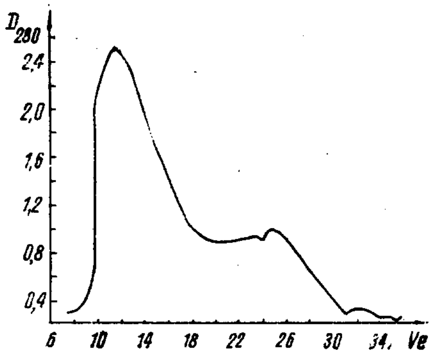

Fig. 1

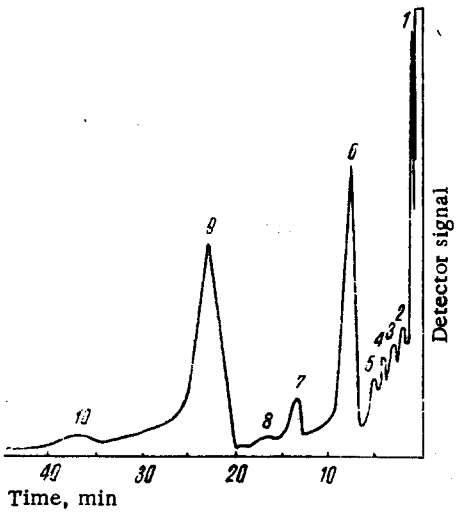

Fig. 2

Fig. 1. Curve of the gel chromatography of *Althaea* DLA.

Fig. 2. Chromatogram of the products of the nitrobenzene oxidation of *Althaea* DLA: 1) p-hydroxybenoic acid; 2) ferulic acid; 6) vanillin; 8) p-hydroxybenzaldehyde; 9) syringaldehyde.

1450 cm^{-1}), and of hydroxy (3400 cm^{-1}), carbonyl (1710 cm^{-1}), and ether (1280, 1230, 1040 cm^{-1}) groups.

The polydispersity of the *Althaea* DLA was studied by gel filtration on Sephadex G-75. DMSO was used as the eluent. The gel chromatograms of this lignin (Fig. 1) showed that, like the DLA from cotton-plant stems [5], it is bimodal, the high-molecular-weight fraction (mol. wt. 17,000-20,000) predominating at 66%, and the low-molecular weight fraction (mol. wt. 2000-4000) amounting to 34%. The molecular weights of these fractions were calculated by using the factors found previously [6].

To study the structure of the *Althaea* DLA we used alkaline nitrobenzene oxidation [7]. The products of nitrobenzene oxidation investigated by the GLC method [8] are given below:

Product	*Althaea* (plant)*	*Althaea* DLA	Cotton-plant stem DLA
Vanillin	2.36	12.85	18.00
Acetovanillone	0.93	-	0.73
Ferulic acid	0.89	0.79	0.31
Vanillic acid	-	1.01	-
p-Hydroxybenzaldehyde	0.50	0.72	0.58
p-Hydroxybenzoic acid	0.35	1.17	0.34
Syringaldehyde	3.08	32.13	10.30
Syringic acid	-	1.67	-

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*The yields of aldehydes are given as calculated on the initial weight of the plant and that of the lignin, respectively.

On the basis of the results given, it may be concluded that the *Althaea* DLA (Fig. 2) and the cotton-plant stem DLA contain all three types of structural units of lignin, namely: guaiacyl, from which vanillin, vanillic and ferulic acids, and acetovanillone are formed; p-coumaryl, from which p-hydroxybenzaldehyde and p-hydroxybenzoic acid are formed; and syringyl, from which syringaldehyde and syringic acid are formed.

On the basis of the results of the nitrobenzene oxidation, it was established that in the *Althaea* DLA the terminal structural units are distributed in the following way: p-coumaryl 3.76%, guaiacyl 29.11%, and syringyl 67.14%. Thus, guaiacyl units predominate in the cotton plant DLA and syringyl units in the *Althaea* DLA. Consequently, the *Althaea* DLA is less condensed than the cotton-plant lignin, which is also confirmed by the lower molecular weight of the *Althaea* DLA than of the cotton-plant lignin [5].

EXPERIMENTAL METHOD

The chemical composition of the lignified stems of the *Althaea rhyticarpa* was as follows:

Component	Percentage on the absolutely dry weight
Komarov lignin	30.66
Pentosans	27.35
Methoxy groups	3.60
Substances extractable by ethanol–benzene (1:1)	10.55
extractable by ether	3.12
soluble in 1% NaOH	48.17
soluble in hot water	14.67
Cellulose	27.50

Isolation of the Dioxane Lignin. The system for the isolation of the DLA consisted of two units connected in series: extraction, neutralization, and concentration. All the operations were performed in a current of nitrogen. The extraction flask was charged with 15 g of finely ground *Althaea* stems which had previously been extracted with a mixture of ethanol and benzene (1:1), and this was extracted with 730 ml of dioxane–water (9:1) containing 13.5 ml of concentrated hydrochloric acid at 90°C for 30 min. Then in the second unit the dioxane solution was neutralized with sodium bicarbonate and was concentrated under vacuum at 40°C. The residue was dissolved in aqueous dioxane (9:1) and precipitated in absolute ether. The yield was 5% on the weight of the initial plant.

Procedures for Determining Functional Groups. The methoxy groups were determined by the method of Vieböck and Schwappach [9], and the total hydroxy groups by the method of Verley and Bolsing [10]. The phenolic hydroxy groups were found by comparing the amounts of methoxy groups in the lignin and in the lignin methylated with diazomethane. The aliphatic hydroxy groups were calculated by the difference between the total amount of hydroxy groups and the phenolic group. The carbonyl groups were determined by the method of Gierer and Soderberg [11].

Gel Chromatography. A 0.5% solution of the DLA in DMSO (0.3-0.5 ml) was charged into a column (1.2 × 50 cm) of Sephadex G-75 equilibrated with DMSO. The rate of elution was 6-8 ml/h. Fractions with a volume of 1 ml were collected, and the concentration of lignin in them was determined on an SF-4 spectrophotometer at 280 nm. To determine the "free" volume (the volume of solvent between the granules of gel), a solution of dextran blue with a molecular weight of 2,000,000 in DMSO was passed through the column. The volume of DMSO that eluted the dextran blue was taken as the "free" volume. The calculation made use of the basic formula of chromatography using the factors found previously [6].

Nitrobenzene Oxidation of the Lignin. A mixture of 1 g of lignin, 1 ml of nitrobenzene, and 10 ml of 8% caustic soda solution was heated in an autoclave at 180°C for 2 h. After the end of the reaction, the solution was separated from the precipitate, and the latter was washed with a small amount of water. The reaction mixture together with the wash waters was extracted with ether to eliminate the products of the reduction of the nitrobenzene. The aqueous layer was acidified to pH 2.5 with concentrated hydrochloric acid and was again extracted with ether. The dried ethereal solution was evaporated, and the dry residue was dissolved in ethanol and investigated by GLC.

The nitrobenzene oxidation of the plant was performed as described by Lepold [7].

Conditions of GLC. To determine the aldehydes we used a "Tsvet-4" chromatograph with a flame-ionization detector and a meter stainless-steel column with an internal diameter of 4 mm filled with 4% of poly(ethylene adipate) (PEGA) on Chromaton NAW (60-80 mesh). The temperature of the column was 206°C and that of the evaporator 250°C. The vanillic and syringic acids were determined on a "Khrom-4" instrument with a flame-ionization detector using a 250 × 3 cm column containing 4% of PEGA + 1% of H_3PO_4 on Chromaton coated with acid (0.100-0.125 mm). The temperature of the column was 205°C and the rate of flow of the carrier gas 40 ml/min. The volume of the sample was 2 μl.

On the chromatograms, the products of nitrobenzene oxidation were identified by their retention times and by the addition of markers. Quantitative evaluation was performed by the area-normalization method.

SUMMARY

1. The developed semiempirical formula for the dioxane lignin from the stems of *Althaea rhyticarpa* has been found from the results of elementary and functional analyses.
2. It has been shown that the lignin consists of high- and low-molecular-weight fractions.
3. It has been shown by the nitrobenzene oxidation method that p-coumaryl, guaiacyl, and syringyl structural units are present in the lignin.

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STUDY OF THE LOW-MOLECULAR-WEIGHT ULTRASONIC LIGNIN FROM *Pinus sylvestris*

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An ultrasonic lignin (USL) has previously been obtained from *Pinus sylvestris* (Scotch pine) with the aid of ultrasound; its properties are very close to those of the Björkman lignin but differ from it by its low molecular weight [1]. We have isolated the USL from the plant mentioned by the method described [2]. The preparation obtained, after purification according to Björkman [3], proved to be identical with that characterized previously [1]. The results of an investigation of its molecular-weight distribution (MWD) on a column of Sephadex G-75 showed that the lignin is bimodal. The high-molecular-weight fraction (weight-average molecular weight 10,000) makes up 27% and the main, low-molecular-weight fraction 65% (weight-average molecular weight 1600). To fractionate the USL we used dialysis through a semipermeable membrane (Cellophane) against distilled water. After dialysis, the MWD of the lignin had changed insignificantly.

It may be considered that the USL that we had isolated consisted mainly of low-molecular-weight molecules.

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