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AN INVESTIGATION OF THE LIGNINS OF HEALTHY AND WILT-AFFECTED COTTON PLANTS OF VARIETY TASHKENT-1 ACCORDING TO THE VEGETATION PERIODS

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UDC 547.458.84+549.927.2

Wilt — one of the most dangerous diseases of the cotton plant — causes great losses to cotton growers. In recent years, breeders have isolated new wilt-resistant varieties of the cotton plant Tashkent-1, -2, -3, and others [1], but with time they have also become subject to the action of wilt.

We have performed a comparative study of the dioxane lignins of healthy cotton plants and plants affected by the fungus *Verticillium dahliae* Kleb. of variety Tashkent-1 according to the vegetation period. The samples were collected on the territory of the F. Engels kolkhoz in the Srednechirchikskii region, Tashkent oblast. The characteristics of the samples — healthy stems of the early period (I), healthy and wilt-affected stems of the flowering period (II, III), and healthy and wilt-affected bolls and stems after the harvesting of the crop (IV-VII) — are given below (%):

Chemical component	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*
Ethanol–benzene extract	8,15	5,10	6,80	12,73	7,25	6,60	4,22
Aqueous extract	19,41	19,33	25,26	7,77	9,56	3,14	12,82
Komarov lignin	23,20	23,20	23,25	22,80	24,50	22,94	24,49
Cellulose	31,00	38,00	34,60	28,95	32,25	38,07	31,30
Ash substances	9,60	4,15	3,60	10,20	7,10	5,40	5,22

Thus, the amount of substances extractable by hot water from the welt-damaged samples of the cotton plant was greater than from healthy specimens, but the amount of substances extractable by ethanol–benzene (1:1) from the healthy specimens was greater. Apparently, under the action of the wilt fungi the cellulose and polysaccharides are broken down into lower-molecular-weight fractions and become water-soluble. This can explain the marked increase in water-extractive substances in wilt-affected specimens and the decrease in the amount of cellulose in them.

In wilt-damaged specimens of the cotton plant, the amount of Komarov lignin and ash substances increases. The increase in the amount of Komarov lignin can be explained, on the one hand, by the protective reaction of the plants to the fungus. On the other hand, investigations of recent years [2] have shown that the parisitic fungus is to be less sensitive to the toxic action of an excess of phenolic substances than the cotton plant: the phenolic substances cause necrotization and withering of the affected tissues of the host plant, which aggravates the growth and development of the fungus in the conducting vessels adjacent to these tissues.

The dioxane lignins of the cottonplant of variety Tashkent-1 (DLCT) from stems of the early period finely comminuted (0.25 mm) and pre-extracted with ethanol–benzene (1:1) and hot water (DLCT-I), from healthy and wilt-affected plants in the flowering period (DLCT-II and -III), from healthy and affected mature plants (DLCT-VI, and -VII), and from healthy and wilt-affected bolls (DLCT-IV and -V) were isolated by a modification of Pepper's method [3]

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 260-264, March-April, 1978. Original article submitted October 4, 1977.

0009-3130/78/1402-0217$07.50

under a current of nitrogen and were purified by reprecipitation in absolute ether [4]. All the lignins isolated formed dark blue amorphous powders readily soluble in the usual solvents for lignins. The elementary compositions, contents of functional groups, and yields of the lignins are given below (%):

Elements and Functional Groups	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*
Carbon	59,96	55,97	61,04	59,30	58,25	58,72	59,54
Hydrogen	5,44	5,24	5,63	5,30	5,21	5,12	5,63
Oxygen	34,60	35,29	33,33	34,65	36,54	36,16	34,82
Methoxy groups	8,51	11,96	13,36	13,27	12,64	12,60	15,54
Carbonyl groups	6,50	6,60	4,85	4,30	6,70	6,05	6,70
Total hydroxy groups	13,41	11,67	12,38	12,78	14,10	12,87	10,27
Amount of sugars	1,20	4,70	3,30	2,00	0,52	1,44	—
Yield on the plant	1,84	3,66	2,52	1,84	1,81	1,71	1,43
Yield on the Komarov lignin	7,93	15,77	10,83	8,07	7,38	6,97	5,30

There is information in the literature [5] that after the destruction of preparations of natural pine lignin and a lignin of ground pinewood by storage-rot fungi there was a higher content of methoxy groups. Thus, the results of analyses of DLCT-I to -VII show that the amount of methoxy groups is higher in the lignins isolated from the wilt-affected plants in the flowering period and from the mature stems after the harvesting of the crop and that *V. dahliae* may be provisionally assigned to the group of storage-rot fungi that act both on cellulose and on lignin.

The total amount of hydroxy groups in the stems in the flowering period and in the bolls was higher in the lignins from the plants affected by wilt, while for the mature stems the opposite pattern was observed.

The content of carbonyl groups in the lignins from the plants affected by wilt in the flowering period decreased in comparison with lignin isolated from a healthy plant, and at the end of vegetation the amount of these groups increased in the lignins isolated from the affected plants.

In all the lignins isolated from the affected plants, the amount of sugars was lower than in the lignins from the healthy plants. In the lignins from the stems of a more mature plant there were no carbohydrates bound to the lignin whatever, which can be explained, as indicated above, by the consumption of these hydrocarbons by the wilt fungus.

In all the specimens the yields of lignins were higher for the plants not affected by wilt.

On the basis of elementary and functional analysis, semiempirical formulas have been calculated for the phenylpropane structural units (PPSUs) of DLCT-I to -VII taking the sugar content into account:

DLCT-I, mol. wt. of one PPSU = 190.4

$$C_9H_{7,67}O_{2,01}(OCH_3)_{0,53}(OH_{al})_{0,3}(OH_{ph})_{0,66}(O_{CO})_{0,44}(OOH_{COOH})_{0,03} \times (O_{al-ar})_{0,34};$$

DLCT-II, mol. wt. of one PPSU = 193.8

$$C_9H_{6,24}O_{1,59}(OCH_3)_{0,78}(OH_{al})_{0,70}(OH_{ph})_{0,73}(O_{OC})_{0,28}(OOH_{COOH})_{0,08} \times (O_{al-ar})_{0,37};$$

DLCT-III, mol. wt. of one PPSU = 192.4

$$C_9H_{6,68}O_{1,28}(OCH_3)_{0,87}(OH_{al})_{0,77}(OH_{ph})_{0,60}(O_{CO})_{0,28}(OOH_{COOH})_{0,068} \times (O_{al-ar})_{0,40};$$

DLCT-IV, mol. wt. of one PPSU = 198.9

$$C_9H_{6,26}O_{1,16}(OCH_3)_{0,87}(OH_{al})_{0,82}(OH_{ph})_{0,66}(O_{CO})_{0,31}(OOH_{COOH})_{0,097} \times (O_{al-ar})_{0,35};$$

DLCT-V, mol. wt. of one PPSU = 202.5

$$C_9H_{6,18}O_{1,01}(OCH_3)_{0,83}(OH_{al})_{1,04}(OH_{ph})_{0,62}(O_{CO})_{0,48}(OOH_{COOH})_{0,12} \times (O_{al-ar})_{0,38};$$

DLCT-VI, mol. wt. of one PPSU=200.75

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

DLCT-VII, mol. wt. of one PPSU = 201.94

$$C_9H_{7,02}O_{1,01}(OCH_3)_{1,01}(OH_{al})_{0,73}(OH_{ph})_{0,49}(O_{CO})_{0,48}(OOH_{COOH})_{0,16} \times (O_{al-ar})_{0,51}.$$

It can be seen from the formulas that the number of methoxy groups per PPSU increased during the vegetation period from 0.53 to 1.01, while the number of OCH_3 groups in the lignins isolated from the wilt-affected plants was greater than in the lignins from the healthy plants, which, as mentioned above, is characteristic for lignins isolated from plants affected by storage-rot fungi [5].

The molecular-weight distribution (MWD) of DLCT-I to -VII was studied by gel chromatography on a column of Sephadex G-75. As the solvent and eluent we used DMSO. Eluograms of the gel chromatography of DLCT-I to -VII are given in Fig. 1. The molecular weights were calculated by using the coefficients found previously [6], and the number-average $\overline{M}_n$, weight-average $\overline{M}_w$, and molecular-weight-average $\overline{M}_z$ molecular weights were calculated by Chien's method [7]:

DLCT preparation	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_z$	$\overline{M}_n : \overline{M}_w : \overline{M}_z$
I	8820	18420	27670	1 : 2,08 : 3,14
II	6860	13190	17950	1 : 1,92 : 2,62
III	5520	9890	15100	1 : 1,80 : 2,74
IV	5870	10350	14900	1 : 1,76 : 2,54
V	5650	13120	19000	1 : 2,32 : 3,36
VI	3400	8000	12700	1 : 2,34 : 3,69
VII	3300	6150	13640	1 : 1,84 : 4,08

As can be seen from the figures given, the lignins isolated were polydisperse, the lignins from the wilt-affected specimens being of lower molecular weight than those from the healthy specimens. Apparently, the wilt fungi act destructively on lignin.

In the UV spectra of DLCT-I to -VII taken in aqueous dioxane and in methylcellosolve there are maxima at 280 nm and shoulders at 300-360 nm, which is characteristic for the dioxane lignins and USL ["ultrasonic lignin"] lignins of plants of the family Malvaceae [8-10].

The IR spectra of all the lignins of the healthy and wilt-affected plants have absorption bands at 3400 cm^{-1} corresponding to OH groups associated by hydrogen bonds; 2950 cm^{-1}, stretching vibrations of C–H bonds; 1725-1730 cm^{-1}, β-carbonyl groups; 1420, 1460, 2870,

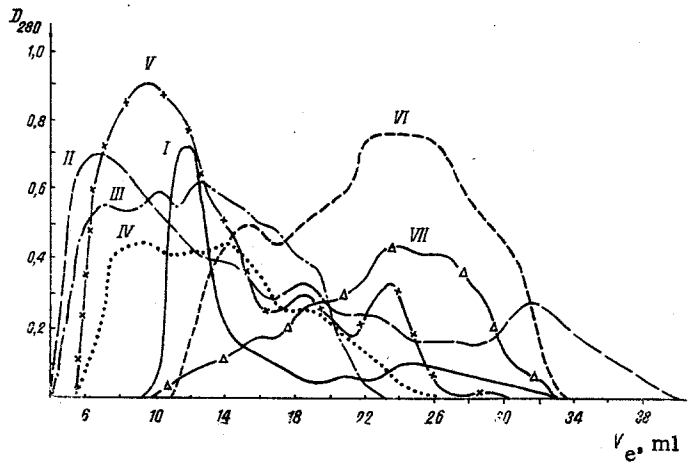

Fig. 1. Gel-chromatography curve: I) DLCT-I; II) DLCT-II; III) DLCT-III; IV) DLCT-IV; V) DLCT-V; VI) DLCT-VI; VII) DLCT-VII.

1335 cm^{-1}, C–H bonds in OCH_3 groups; 1600, 1520 cm^{-1}, skeletal vibrations of double bonds in aromatic rings; 1225-1320 cm^{-1}, phenolic OH groups; and 1125-1130 and 1040 cm^{-1}, various ether bonds.

There were no differences between the UV and IR spectra of the lignins isolated from the healthy and wilt-affected plants.

EXPERIMENTAL

The UV spectra were taken on a SF-16 spectrophotometer. The solvent for DLCT-I to -V was dioxane–water (9:1), and for DLCT-VI and -VII methylcellusolve–ethanol–water (2:1:1); λ_{max} 280 nm for all the lignins. DLCT-I, log ε 3.3990 (c $3.30 \cdot 10^{-4}$ M); DLCT-II, log ε 3.2994 (c $4.13 \cdot 10^{-4}$ M); DLCT-III, log ε 3.3922 (c $3.02 \cdot 10^{-4}$); DLCT-IV, log ε 3.3727 (c $3.01 \cdot 10^{-4}$); DLCT-V, log ε 3.1038 (c $2.96 \cdot 10^{-4}$); DLCT-VI, log ε 3.4788 (c $2.99 \cdot 10^{-4}$), DLCT-VII, log ε 3.4122 (c $3.13 \cdot 10^{-4}$).

The IR spectra were taken on a UR-10 instrument (tablets of KBr).

Gel chromatography was performed by a method described previously [11].

Isolation of Dioxane Lignin. The dioxane lignins were isolated by a modification of Pepper's method [3]. As compared with Pepper's procedure, we doubled the volume of solvent and performed extraction for 45 min. The yields of lignins were from 5 to 15% of the Komarov lignin (see above).

Methods of Determining Functional Groups. The methoxy, total hydroxy, phenolic hydroxy, and carbonyl groups were determined by methods given by Zakis et al. [12] and the sugar content by Bertrand's method [13].

SUMMARY

1. The dioxane lignins of healthy and wilt-affected cotton plants of variety Tashkent-1 have been isolated and have been studied comparatively in relation to the vegetation period. On the basis of elementary and functional analysis, semiempirical formulas of the dioxane lignins isolated (DLCT-I to -VII) have been determined and it has been established that the dioxane lignins isolated from the wilt-affected plants contain more OCH_3 groups per phenylpropane structural unit than the lignins of healthy plants.

2. The molecular-weight distributions of the dioxane lignins have been studied and it has been shown that all the dioxane lignins isolated are polydisperse, those of the affected plants to a greater degree.

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