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INFLUENCE OF INFECTION BY *Verticillium dahliae* ON THE CUTICULAR
LIPIDS OF THE LEAVES OF COTTON PLANTS OF VARIETY 175

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The composition of the cuticular lipids of young leaves of cotton plants of the wilt-resistant variety 175-F has been determined and it has been established that on artificial infection of the plant with cotton wilt (*Verticillium dahliae*) the component composition of these lipids did not change but the level of the 18:0, 18:1, and 18:2 acids in esters of fatty acids with alkanol, sterols, and triterpenols fell.

Continuing investigations of the metabolism of lipids in cotton plants infected with *Verticillium dahliae* Kleb., we have made an analysis of the cuticular lipids of young leaves of cotton plants of the wilt-resistant variety 175-F artificially infected with cotton wilt due to this organism.

For analysis we took healthy (I) and wilt-damaged (II) leaves of plants grown under artificial climate conditions. The yield of cuticular lipids (CLs) for samples (I) and (II) were 3.7 and 3.5 mg/g of dry substance (d.s.), respectively. The extracts of the CLs I and II were light brown and green, respectively. In the CLs of II we identified chlorophylls a and b and more polar chlorophyll-like compounds with the aid of TLC in system 1 by comparison with literature information [1], while these compounds were absent from the CLs of I. The presence of chlorophylls in the CLs of II was confirmed by absorption at $\lambda_{\text{max}}^{\text{acetone}}$ 662 nm in the UV spectrum of this extract and is apparently explained by a destruction of the integrity of the membranes as the result of the penetration of the infection into the photosynthetic tissues. To determine their composition, the CLs were separated by CC on silica gel followed by TLC in systems 2-5. The components were identified by the use of standards and from the results of mass-spectrometric analysis and of chemical transformations; the amount of each component was estimated gravimetrically (Table 1). The chlorophylls were eluted together with the residual lipids. As can be seen from Table 1, with respect both to the set of classes and to their quantitative amounts, the CLs of the two samples were almost identical.

According to mass-spectrometric results, the hydrocarbons of the CLs I and II consisted of a mixture of paraffinic and unsaturated homologues. In the case of the paraffins, the homologues from C_{27} to C_{33} (M^+ , m/z 380-464) were identified, the main ones being the C_{29} and C_{31} varieties. The unsaturated hydrocarbons had two, four, and five double bonds

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TABLE 1. Compositions of the Cuticular Lipids of Healthy (I) and Wilt-Affected (II) Leaves of a Cotton Plant of Variety 175-F

Lipid class	I	II
	mg/g d.s.	
Hydrocarbons	0,4	0,5
Esters of fatty acids with:		
fatty alcohols		
triterpenols	1,0	1,0
sterols	Tr.	Tr.
Triacyl glycerols	0,1	0,2
Free fatty acids	0,3	0,2
Fatty alcohols	0,9	0,6
Residual lipids	1,0	
Chlorophylls	—	1,0

TABLE 2. Fatty Acid Compositions of the Esters of Acids of Healthy (I) and Wilt-Affected (II) Leaves of A Cotton Plant of Variety 175-F

Acid	I	II	Acid	I	II
	weight, %			weight, %	
12:0	0,7	1,2	18:2	5,0	1,1
13:0	Tr.	Tr.	20:0	8,6	8,7
14:0	2,9	4,2	20:1	4,3	2,7
15:0	1,0	1,4	21:0	2,8	2,0
16:0	25,4	34,0	22:0	6,1	5,7
16:1	2,6	2,4	23:1	9,2	8,0
18:0	12,1	8,3	25:0	2,3	6,3
18:1	17,0	14,0			
Σ_{sat}				61,9	71,8
Σ_{unsat}				38,1	28,2

and consisted of the following components: for C_nH_{2n-2} — from $C_{17:2}$ to $C_{38:2}$ (M^+ m/z 236-530), the main ones being the C_{26} - C_{29} varieties, for C_nH_{2n-6} - $C_{26:4}$ (M^+ , m/z 358-540), the main ones being the C_{28} , C_{29} , and C_{30} varieties; and for C_nH_{2n-8} — from $C_{27:5}$ to $C_{39:5}$ (M^+ , m/z 370-358, the main varieties being C_{29} , C_{30} , and C_{31}).

The total amounts of esters of the two samples were subjected to severe alkaline hydrolysis, and the saponifiable and unsaponifiable fractions were investigated. The saponifiable fractions of the samples were analyzed by the GLC method in the form of methyl esters (Table 2). In the two samples the set of acids was the same and consisted of 15 components. However, the total amount of unsaturated acids in the damaged leaves was 10% lower than in the healthy leaves, mainly through a lowering of the level of the 18:2 and 18:1 acids. The amount of the 12:0, 16:0, and 25:0 acids in the damaged leaves was higher than in the healthy leaves, in contrast to the amount of the 18:0 acid which had likewise decreased.

In the unsaponifiable fraction of the esters of the CL acids of the two samples, fatty alcohols, triterpenols, and sterols were identified qualitatively.

According to mass-spectrometric results, the fatty acids of the esters of the CLs I and II consisted, just like the hydrocarbons, of a complex mixture of homologues of the paraffinic and olefinic series. In this mixture we identified nine primary alkanols from C_{24} to C_{32} ($(M-18)^+$, m/z 336-448) with a predominance of the C_{26} , C_{28} , and C_{30} compounds, and alkenols from $C_{17:1}$ to $C_{31:1}$ ($(M-18)^+$, m/z 236-432) with a predominance of the C_{17} , C_{18} , and C_{19} compounds).

The qualitative compositions of the free alcohols of the two samples were identical with those of the alcohols bound with the fatty acids.

The triterpene alcohols of the CLs I and II were represented by α - and β -amyrins, lup-eol, and cycloartenol. This identification was confirmed by mass spectroscopy [2].

Thus, the artificial infection of a cotton plant of the wilt-resistant variety 175-F by the pathogenic fungus Verticillium dahliae exerts no appreciable influence on the composition of the cuticular lipids of young leaves but affects the level of the 18:0, 18:1, and 18:2 acids bound with fatty and cyclic alcohols.

EXPERIMENTAL

UV spectra were taken on a Hitachi instrument in acetone, and mass spectra a MKh-1310 instrument with a SVP 5 system for the direct introduction of the sample, at an ionizing voltage of 70 V, a collector current of 80 μ A, a temperature of the ionization chamber of 150-170°C, and a temperature of the heating bulb of 130-140°C. GLC was carried out as described in [3].

The fatty acids were identified from their retention times relative to that of palmitic acid and from a graph of the dependence of the logarithm of the retention volumes on the number of carbon atoms of the methyl esters of fatty acids [6, pp. 287-293]. TLC was conducted on Silufol plates (Czechoslovakia) in the following solvent systems:

1) Hexane-acetone-benzene-isopronalol (69.5:25:4:1.5); 2) hexane; 3) hexane-diethyl ether (99:1); 4) hexane-diethyl ether (90:10); and 5) chloroform-benzene-diethyl ether (50:50:15).

For the artificial infection of the cotton plants we used the commonest biotypes of the fungus Verticillium dahliae A and B. The plants were grown in a hothouse in an artificial infection milieu to the phase of 3-4 true leaves and were then infected in root collars with a mixture of two biotypes at a concentration of conidial spores and endospores of $2.5 \cdot 10^4$ in 1 ml of inoculum. The infected plants were transferred to an artificial-climate chamber where a temperature-wilt milieu was created: illumination of 6-8000 lux, temperature of 24-26°C [4]. The first external symptoms of wilt in the affected plants of the experimental variants appeared in 6-8 days, and after 20-25 days the morbidity of the plants amounted to 80-90%. External symptoms of affection on the leaves were observed in the form of well-defined chlorotic spots and indications of wilting. The diseased plants were removed from the soil, and the chlorotic leaves were separated under laboratory conditions and the CLs were extracted from them immediately by brief treatment of the leaves with boiling chloroform [5]. The same treatment was carried out simultaneously with leaves from the healthy control plants.

Chlorophyll a (R_f 0.64 in system 1); UV spectrum, $\lambda_{\text{max}}^{\text{acetone}}$: 410, 504, 534, 610, 666 nm.

Chlorophyll b (R_f 0.55 in system 1); UV spectrum, $\lambda_{\text{max}}^{\text{acetone}}$: 410, 432, 520, 600, 654 nm.

On TLC in system 2, the hydrocarbons were separated into spots not revealed by iodine vapor (R_f 1.0) and spots revealed by this reagent (R_f 0.7-0.5). This fraction was isolated from the CLs by preparative TLC in system 3.

Severe saponification of the esters was carried out as described in [6, p. 817]. The fatty acids from the saponification products were methylated with diazomethane, and the methyl esters of the fatty acids were separated from the unsaponifiable products by preparative TLC in system 4.

On plates coated with silica gel impregnated with 20% of AgNO_3 , in system 5, the triterpene alcohols were separated into three spots, with R_f 0.65 (the main one), R_f 0.5 (traces), and R_f 0.3 (traces), corresponding in their mobilities to model samples of α - and β -amyriins, lupeol and cycloartenol.

Mass spectrum of the fatty alcohols, m/z : alkanols - $\text{C}_{24:0}$ - $\text{C}_{32:0}$: 336, 350, 364, 378, 392, 406, 420, 434, 448 ($M-18$)⁺; 308, 322, 336, 350, 364, 378, 392, 406, 402 ($M-46$)⁺; alkenols - $\text{C}_{17:1}$ - $\text{C}_{31:1}$: 236, 250, 264, 278, 292, 306, 320, 334, 348, 362, 376, 390, 404, 418, 432 ($M-18$)⁺; 208, 222, 236, 250, 264, 278, 292, 306, 320, 334, 348, 362, 376, 390, 404 ($M-46$)⁺ [7].

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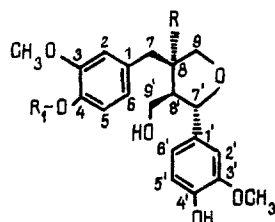
LIGNANS OF THE BARK OF *Syringa vulgaris*

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Two lignans have been isolated from the bark of *Syringa vulgaris* and identified: (+)-lariciresinol 4- β -D-glucopyranoside (I) and -olivil 4- β -D-glucopyranoside (II). This is the first time that glycoside (9) has been described.

We have previously reported the isolation from the bark of the common lilac (*Syringa vulgaris* L. family *Oleaceae*) of phenolic compounds [1] and of irodooids [2]. In a further study of the chemical composition of the bark of the common lilac, we have isolated two lignan glycosides (I and II). To establish the structure of the substances isolated we used UV, PMR, and mass spectroscopy and the results of chemical transformations, and also a direct comparison with authentic samples of the substances (compounds I and Ia).



- I. $R=H$; $R_1=\beta$ -D-Glc
- Ia. $R=R_1=H$
- II. $R=OH$; $R_1=\beta$ -D-Glc
- IIa. $R=OH$; $R_1=H$

Compounds (I) and (II) and also their aglycons (Ia) and (IIa), which were obtained on enzymatic analysis, formed a red coloration with diazotized sulfanilic acid (DSA), which is characteristic for phenolic substances. The fact that compounds (I) and (II) belonged to lignan monoglycosides was shown by their UV and PMR spectra and also by the mass spectra of the aglycons.

According to the PMR results, each of the two lignans contained two 1,2,4-substituted aromatic rings and two aromatic CH_3O groups. The acetylation of compounds (I) and (II) formed hexa- and heptaacetates, respectively, each of these derivatives having one aromatic acetoxy group (δ 2.30 in the PMR spectrum). It followed from the results of acetylation that the aglycon of (I) contained one free alcoholic OH group and that of (II) two such groups, one of them being of tertiary nature (singlet, δ 1.8, in the spectrum of the acetate). These facts permitted the assumption that compounds (I) and (II) were phenolic glycosides of lariciresinol and olivil. The 1H NMR spectrum of compound (I) was completely identical with that of (-)-lariciresinol 4- β -D-glucopyranoside, which we had isolated previously from

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