analyzer, Sweden). The results of the amino-acid analysis of the hydrolyzate were also used to calculate the amount of carbohydrates in the glycopeptide.

Electrophoresis in a thin layer of cellulose was used to separate the products obtained after two stages of the Edman splitting off of amino-acid residues in the glycopeptide T-1-1. Electrophoresis was performed on a 20 × 6 cm plate under the conditions described previously for peptide maps [2]; the spots were detected in UV light and with ninhydrin. Two fractions were found, and each was eluted with 10% acetic acid and, after evaporation in vacuum, hydrolyzed with 3 N HCl at 100°C for 3 h. The hydrolyzate was dried, kept in a vacuum desiccator over caustic soda, dissolved in water, and chromatographed on a plate with a thin layer of silica gel (6 × 6 cm) in the N-propanol–ethyl acetate–water (70 : 20 : 10 system), using orcinol to reveal the spots. The amino sugars in the hydrolyzate were revealed by the Elson–Morgan reaction.

SUMMARY

1. The 7S globulin of cotton seeds consists of eight polypeptide chains of two types differing by their contents of carbohydrates and of amide groups.

2. On the basis of the results of a study of the amino-acid sequences of the peptides obtained by cleaving the 7S globulin with trypsin and chymotrypsin, its complete primary structure has been put forward.

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METABOLITES OF THE PATHOGENIC FUNGUS *Verticillium dahliae*

VII. THE PHYTOTOXIC PIGMENT PKZh-1 FROM THE CULTURE LIQUID

L. N. Ten, O. S. Otroshchenko, N. N. Stepanichenko, and D. A. Inoyatova

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Many workers have reported the great importance of phytotoxins in plant diseases caused by microorganisms [1]. Among the phytotoxins are known substances of various chemical structures: peptides, glycosides, terpenoids, etc.

We have previously reported the presence in the culture liquid of the fungus *Verticillium dahliae* Kleb. of the Yangiyul' population of phytotoxic pigments – verticillins – belonging to the siderochrome group [2]. For their isolation we have developed a method using ion-exchange chromatography on CM-Sephadex C-25 and on DEAE-Sephadex A-25 [3].

We give here the results of a study of the phytotoxic pigment (PKZh-1) from the culture liquid of *V. dahliae*. It has been established that in a concentration of 100-150 μg/ml PKZh-1 causes the formation of chlorotic zones, necrotic spots, and the withering of the leaves of the cotton plant when the plants in the three-

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to five-leaf stage are immersed in an aqueous solution of the substance. Using isolated pea chloroplasts it has been shown that PKZh-1 inhibits cyclic phosphorylation, interfering with the transfer of electrons in the electron-transport chain [4]. It is possible that the cause of this is its interaction with the chloroplast membranes, as is indirectly confirmed by the increase in the permeability of artificial phospholipid membranes under the action of PKZh-1 on them [5]. The results obtained permit the conclusion that the compound isolated has an important role in wilt disease of the cotton plant.

The method of isolating the PKZh-1 has been described previously [5]. Its amount in the culture liquid is 1.2-1.3 mg/liter. To characterize the PKZh-1 and to check its homogeneity we used paper chromatography (PC) in various systems, gel chromatography on Sephadex G-25, and chromatoelectrophoresis.

In an acid hydrolyzate of PKZh-1 we found the following amino acids: aspartic acid, glutamic acid, threonine, serine, glycine, alanine, valine, leucine, isoleucine, cysteine, methionine, histidine, lysine, arginine, and tyrosine.

The results of a study of the hydrolysis products and spectral characteristics showed that PKZh-1 is a peptide.

From an acid hydrolyzate, by extraction with diethyl ether and purification by TLC we isolated a red compound (I) with mp 250°C (decomp.).

On the basis of its physicochemical properties (melting point, R_f values, and UV, IR, PMR, and mass spectra), (I) was identified as 3,6,8-trihydroxy-1,4-naphthaquinone, which was first isolated by Astill from Aspergillus citricus and called flaviolin [6]. Bell et al. isolated (I) in the free form from the mutant brm-1 in a study of the biosynthesis of melanins by the fungus V. dahliae [7].

Thus, in PKZh-1 we have found 3,6,8-trihydroxy-1,4-naphthaquinone which is probably responsible for the color of the PKZh-1 and is probably attached to a peptide chain.

EXPERIMENTAL

The spectra in the UV and visible regions were recorded on a Beckman model 25 UV spectrophotometer, the IR spectra on a UR-10 instrument (GDR), the PMR spectra on a Varian XL-100-15 multinuclear spectrometer, and the mass spectra on an MAT-311 mass spectrometer.

Physicochemical Properties. PKZh-1 consists of a dark red substance readily soluble in polar solvents and crystallizing from the solution in the form of extremely fine crystals. The crystalline structure was shown by electron diffraction and polarization microscopy.

Elementary composition (%): C—41.54, H—4.75, N—5.20, S—2.15, Fe—8.06.

The UV spectra of PKZh-1 (in ethanol) has absorption maxima at 205, 220, 270, and 320 nm (log ε 2.76, 2.73, 2.63, and 2.31, respectively). The position of the maximum in the visible region depends on the pH of the solution and changes from 420 nm in an acid medium (pH 2.0) to 490 nm (log ε 1.96) in a neutral medium (pH 7.0) and to 510 nm in an alkaline medium (pH 9.0), which is due to the presence of the naphthaquinone fragment in the PKZh-1.

The IR spectra has absorption bands at (cm^{-1}) 3400-3000 (broad), 1685 (w), 1640 (w), 1610, 1550, 1410, 1300, 1270, 1250, 1190, 1120, 1085, 1020, 890, 865, 810, 775.

The paper chromatography of PKZh-1 was performed on Filtrak FN No. 17 paper by the ascending method. The following R_f values were obtained in the given systems: distilled water, 0.80; acetone—water (80 : 20), 0.90; water-saturated butan-1-ol, 0.50; water-saturated ethyl acetate, 0.45; acetone—benzene—water (30 : 10 : 1), 0.60; butan-1-ol—hexane—acetone—water (20 : 9 : 10 : 10), 0.90; methanol—isoamyl acetate—water (25 : 14 : 1), 0.95; butan-1-ol—methanol—water (40 : 10 : 20), 0.90; petroleum ether, 0; benzene, 0.

Gel chromatography was performed on Sephadex G-25 (Pharmacia, Sweden). The PKZh-1 (20-25 mg) was deposited on a 1.0 × 50 cm column and was eluted with double-distilled water at the rate of 60 ml/h, 3-ml fractions being collected with the recording of their optical densities at 270 and 490 nm.

Chromatoelectrophoresis consisted of a combination of TLC on plates (20 × 20 cm) of Merck silica gel G (GFR) in the acetone—water—acetic acid—2N NH_4OH (15 : 5 : 1 : 2) system followed by electrophoresis in the perpendicular direction in the water—acetic acid—formic acid (135 : 3 : 1) system at a voltage of 700-800 V and a current strength of 10-12 A for 3-4 h. The PKZh-1 was detected on the plates in the form of a light yellow spot.

The acid hydrolysis of the PKZh-1 was performed in sealed tubes at 110°C for 24 h with 5.7 N hydrochloric acid that had been twice redistilled over $SnCl_2$. The amino-acid composition was determined on a Beckman model 119 analyzer.

Identification of 3,6,8-Trihydroxy-1,4-naphthaquinone (I). Substance (I) was extracted from an acid hydrolyzate of PKZh-1 with ether and was purified by TLC on cellulose in water-saturated n-butanol, R_f 0.90, which yielded it in the form of a red powder with mp 250°C (decomp), mol. wt. 206 (mass spectrometry); composition $C_{10}H_6O_5$.

Mass spectrum (temperature of the inlet system 120°C), m/e (%) 206 (M^+ 100), 178 (19), 150 (18), 137 (48), 136 (12), 109 (10), 108 (11), 69 (18).

UV spectrum, λ_{max} (in ethanol), nm; 215, 265, 310, 400, 450 (log ε 4.33, 4.18, 3.86, 3.38, respectively). The IR and NMR spectra were identical with those described by Bell et al. [7]. The PKZh-1 was tested for phytotoxicity on shoots of the cotton plant of variety 108-F in the three- to five-leaf stage by placing sections of the stem in a solution of predetermined concentration. Plants placed in mains water were used as controls.

SUMMARY

The phytotoxic pigment PKZh-1 that causes the characteristic symptoms of wilt in tests on cotton-plant shoots in a concentration of 100-150 μg/ml, has been isolated from the culture liquid of the fungus Verticillium dahliae Kleb.

On the basis of qualitative reaction, spectral characteristics, and a study of hydrolysis products, PKZh-1 has been assigned to the peptide group. A red substance identified as 3,6,8-trihydroxy-1,4-naphthaquinone has been isolated from an acid hydrolyzate of PKZh-1.

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