ISOLATION OF HEMIGOSSYPOL QUINONE FROM A COTTON PLANT INFECTED WITH WILT

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One of the mechanisms of the immunity of plants to phytopathogenic microorganisms is the capacity of the host plant for forming, in response to infection, antibiotic substances that are practically absent from the intact tissues – phytoalexins [1]. In the stems of the cotton plant affected by verticillosis a number of gossypol-like compounds phytotoxic to the conidia of the causative fungus of the wilt have been found which have been assigned to the phytoalexins [2, 3].

Bell et al., have shown the structure of three phytoalexins of the cotton plant – hemigossypol, 6-methoxy-hemigossypol, and 6-detroyhemigossypol [4]. We have previously reported on the isolation of two phytoalexins from the xylem tissue of a cotton plant infected with the fungus Verticillium dahliae Kleb. and have demonstrated their structure – isohemigossypol and gossyvertin [5, 6]. In addition to these compounds, several other polyphenols absent from healthy plants have been found in the stems of a diseased plant. In this paper we give the results of the isolation of one of the gossypol-like substances of the cotton plant that have been mentioned above and of a study of its structure.

From a chloroform extract of the stems of a cotton plant affected with wilt after separation and chromatography we succeeded in isolating a crystalline substance with mp 160-162°C, λ_{max} (chloroform) 242, 270, 313, and 408 nm. Its mass spectrum contains the intense peak of the molecular ion with m/e 274 (M^+). The PMR spectrum of the substance (in $CDCl_3$) (Fig. 1) shows the following signals: Intense doublet at 1.36 ppm, which corresponds to the six protons belonging to the methyl groups of an isopropyl radical (J = 7 Hz); a three-proton doublet at 2.07 ppm (J = 2 Hz), belonging to the protons of a methyl group; a signal consisting of seven lines with their center at 4.1 ppm, ascribed to the methine proton of the isopropyl radical; two broad signals at 6.46 and 12.9 ppm which disappear on exchange with deuterium, showing that they belong to OH groups; and a signal at 10.66 ppm, assigned to the proton of an aldehyde group. Also characteristic for this compound is the presence of a one-proton quartet at 6.78 ppm with a coupling constant of 2 Hz. Double-resonance experiments showed that the presence of the quartet is due to spin–spin coupling of this proton with the protons of the methyl group resonating at 2.07 ppm. This fact shows that this proton and the methyl group are in the ortho position with respect to one another.

In view of the molecular weight (274), the presence of a chelate intramolecular hydrogen bond, and the change in the chemical shift of the aldehyde proton comparison with its position in the spectra of gossypol and other derivatives studied previously, and also on the basis of biogenetic considerations, literature information, and the UV, PMR, and mass spectra, and the results of a study of some physicochemical properties, the most probable structure for the substance isolated, which we have called hemigossypolone, is 8-formyl-6,7-dihydroxy-5-isopropyl-3-methyl-1,4-naphthoquinone.

Information exists showing the higher fungitoxicity of oxidized polyphenols than of their reduced forms [1, 7-10]. This leads to the assumption that in the attack of plants polyphenols are oxidized by oxidizing enzymes, especially polyphenol oxidase, with the formation of highly toxic substances more actively suppressing the development of the pathogen and of enzymes inhibiting it. Consequently, some phenols may act on the parasite through the quinone forms.

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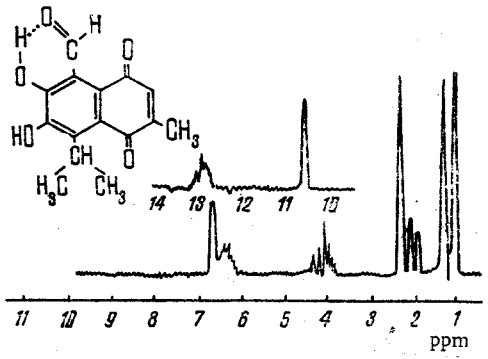

Fig. 1. PMR spectrum of hemigossypolone.

Since hemigossypolone is a quinone, it possesses a high fungitoxicity. According to the results of a determination of its fungitoxicity with respect to the fungus V. dahliae at a concentration of 6 μg/ml, hemigossypolone is capable of completely suppressing the growth of the fungal spores. ED_{50} for hemigossypolone is 1.5 μg/ml.

A comparative study of the fungitoxicity of gossypol and other gossypol-like compounds isolated from the stems of the cotton plant infected with wilt – isohemigossypol and gossyvertin – has shown that hemigossypolone has the highest fungitoxicity for the causative agent of verticilliaceous wilt of the cotton plant. The strong inhibiting action of the quinone once again confirms the protective role of the polyphenol – polyphenol oxidase system.

EXPERIMENTAL

The stems, freed from bark, of diseased and healthy cotton plants of variety 108-F gathered in the experimental plot of the Institute of Experimental Plant Biology in the phase of mass flowering and fruit bearing were investigated.

For column chromatography we used silica gel of types KSK and Silpearl, and for thin-layer chromatography Silufol plates, and the following solvent systems: 1) petroleum ether–diethyl ether (95 : 5); 2) petroleum ether–diethyl ether (90 : 10); 3) benzene–methanol (95 : 5); 4) benzene–methanol (90 : 10), 5) petroleum ether–diethyl ether–concentrated HCl (70 : 30 : 0.3); 6) benzene–petroleum ether–ethyl acetate (40 : 40 : 20).

The substances on the chromatogram were revealed with a 1% ethanolic solution of phloroglucinol in 2 N hydrochloric acid, with 1% ferric chloride solution, and with concentrated sulfuric acid.

The UV spectra were taken on a Beckman model 25 spectrometer, the PMR spectra on a Varian XL-100 spectrometer, and the mass spectra on a Varian MAT-3011 instrument.

Isolation of Hemigossypolone. The sum of the gossypol-like compounds was obtained by the method used previously [5, 6]. An ethereal solution of this material was treated several times with 2% borax solution. The resulting aqueous solution was acidified with HCl to pH 3 and was exhaustively extracted with ether. The purified total material after evaporation of the solvent was transferred to a column (3.5 × 130 cm) filled with type KSK silica gel and was eluted successively with systems 1 and 2. Fractions of 100-120 ml were collected. A number of fractions containing mainly hemigossypolone was obtained, and these were combined, evaporated, and chromatographed on a column of Silpearl silica gel in order to eliminate impurities completely. The column was eluted first with pure petroleum ether and then with system 1. This gave a series of fractions containing only hemigossypolone. On thin-layer chromatography in solvent systems 3, 4, 5, and 6 the substance gave a single spot in each case with R_f 0.68, 0.69, 0.56, and 0.70, respectively.

After evaporation of the solvent and recrystallization from ether, 120 mg of hemigossypolone was obtained in the form of dark yellow acicular crystals with mp 160-162°C (decomp.). It dissolves readily in acetone and diethyl ether, sparingly in benzene and petroleum ether, and does not dissolve in water.

Determination of the Fungitoxicity of Hemigossypolone. To determine its fungitoxicity, hemigossypolone was dissolved in ethanol, and then a series of dilutions with definite concentrations was prepared using a 1% solution of glucose in 0.04 M K phosphate buffer, pH 6.2. Each dilution was mixed with the same volume of a growth medium containing 0.04 M K phosphate buffer (pH 6.2), 1% of glucose, and 3% of agar at 50°C and the mixture was poured into Petri dishes. An aqueous suspension of spores of the fungus V. dahliae obtained after its growth on a potato medium for 10-14 days was deposited by means of a pipet on the surface of the agar medium. The spores were incubated at 26°C for 18 h. Spores having a germ tube longer than the diam-

eter were regarded as having germinated. The experiments were performed in duplicate, and 200 spores were recorded in each Petri dish.

SUMMARY

From the stems of the cotton plant infected with the fungus Verticillium dahliae Kleb. has been isolated a quinone derivative of hemigossypol – hemigossypolone – for which the structure 8-formyl-6,7-dihydroxy-5-isopropyl-3-methyl-1,4-naphthoquinone is proposed, and its fungitoxicity has been determined.

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MASS-SPECTROMETRIC STUDY OF THE SESQUITERPENE LACTONE GROSSMISIN AND ITS DERIVATIVES

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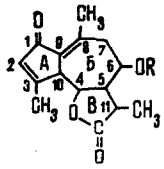
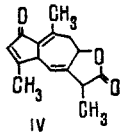
The structure of grossmisin isolated from the epigeal part of Artemisia caucasica Willd. (A. grossheimii Krasch. ex Poljak) (Caucasian wormwood) has been established previously [1]. We have studied the fragmentation under electron impact of grossmisin (I), its deuterium analog (II), and its derivatives acetylgrosssmisin (III) and anhydrogrossmisin (IV) in order to establish the main laws of the dissociative ionization of these compounds due to the presence of the structural elements characteristic for them.



I. R=–H
II. R=–D
III. R=–COCH$_3$



IV

The mass spectra of compounds (I-IV) areshown in Fig. 1. The appearance of ions in the mass spectrum of grossmisin (I) in the region of high masses and of the main ions in the region of moderate masses is shown in the scheme, and their accurate masses and elementary compositions are given in Table 1. The main direction of fragmentation of (I) is the elimination by the ion M^+ of an H_2O molecule (m/e 244) followed by the ejection of a CO group (m/e 216) or a CH_3 radical (m/e 229). A side direction of the decomposition of (I) is associated with the loss by the molecular ion of (I) of CH_3 and OH radicals (m/e 247 and 245, respectively).

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