X-Pro-Y sections (where X and Y are any amino-acid residues). As an example we can give the fragments Ser^{159}-Pro^{160}-Cys^{161} in carboxypeptidase A [11], and Trp^{27}-Pro^{28}-Trp^{29} in α-chymotrypsin [12], assuming the B—R—R form, and also the Ile^{213}-Pro^{214}-Asp^{215} sections in carboxypeptidase A, realized in the form B—R—B.

SUMMARY

1. The optimum conformations of Ac-L-Ala-L-Pro-L-Ala-NHMe with eight possible systems of intramolecular hydrogen bonds have been calculated by the method of theoretical conformational analysis.

2. The structures found serve as canonical forms for the study of the conformational states of sections of peptide—protein systems with proline residues.

3. It has been shown that the interaction of adjacent residues in the structure of the compound mentioned play the dominating role.

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STRUCTURE OF GOSSYVERTIN — A NEW PHYTOALEXIN OF THE COTTON PLANT

A. K. Karimdzhanov, A. I. Ismailov, Z. S. Abdullaev, Sh. Yu. Islambekov, F. G. Kamaev, and A. S. Sadykov

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The phytoalexins, which arise in plant tissues damaged by phytopathogenic microorganisms, must be assigned to a special class of antibiotics [1]. Investigations of the chemical nature of the phytoalexins of the cotton plant infected by verticilliaceous wilt have only just begun, and there is fairly contradictory information in the literature. Thus, Bell [2, 3] regarded gossypol to be the main fungitoxic substance for the fungus. Zaki and Erwin [4] consider as phytoalexins of the cotton plant two compounds of phenolic nature which they isolated from an infected plant and which they called vergosin and hemigossypol. In investigations by Sadykov [5], it was shown that isohemigossypol — 8-formyl-1,2,7-trihydroxy-5-isopropyl-3-methylnaphthalene — isolated from plants infected with *Verticillium dahliae* Kleb. is also a phytoalexin of the cotton plant.

In addition to isohemigossypol, we have found in the stems of infected plants another series of compounds with the nature of phytoalexins, and in the present paper we give the results of a chemical study of one of these compounds.

Division of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR, Tashkent.
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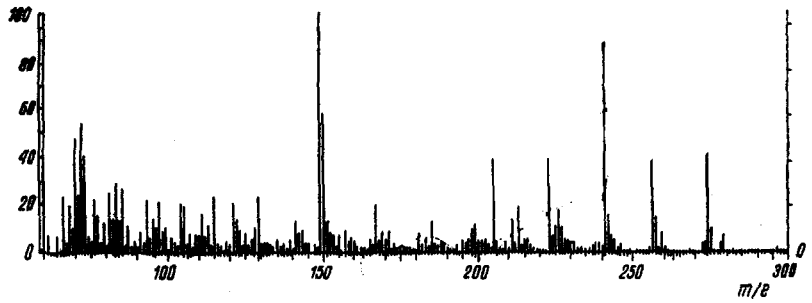


Fig. 1. Mass spectrum of gossyvertin.

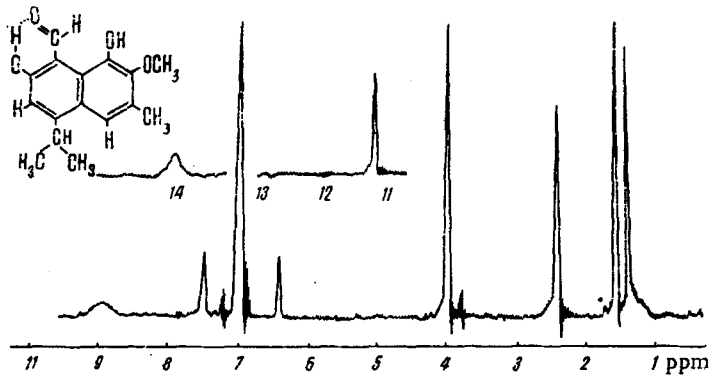


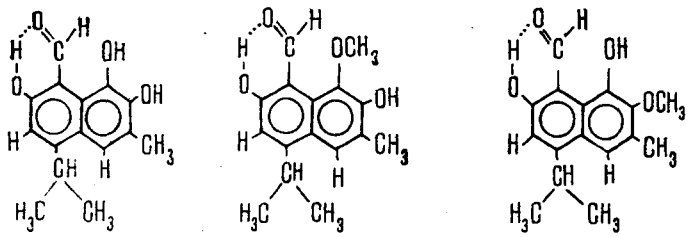
Fig. 2. PMR spectrum of gossyvertin.

From a chloroform extract of the stems of a wilt-infected cotton plant, after separation and chromatography, we succeeded in isolating a crystalline substance with mp 147-149°C. Its UV spectrum (ethanol) had absorption maxima at 223, 266, 350, and 385 nm.

Its mass spectrum (Fig. 1) has a strong peak of the molecular ion with m/e 274 (M^+) and a low-intensity peak with m/e 273 $(M-1)^+$, corresponding to the loss of a hydrogen atom. The fragmentation of the substance begins with the elimination of 15 mass units to form ions with m/e 259 ($M-CH_3$), 257 ($M-OH$), and 256 ($M-H_2O$), and an ion with m/e 213 ($M-CH_2O_2$). The formation of ions with m/e ($M-H_2O$) and ($M-OH$) and also the splitting off of 17 and 18 mass units from the ions with m/e 257 and 256 shows the presence of two hydroxy groups. The ions with m/e 231, 227, 214, 213, 199 and 185, 179, 167, and are formed by the elimination of CO, CHO, CHO_2, and CH_2O_2 groups from different fragments of the molecule. The loss of different groups of atoms in the fragmentation of a compound from the molecular ion and from various fragments of the molecule shows its existence in various tautomeric forms, which is characteristic for gossypol and isohemigossypol [5, 6].

The PMR spectrum of the substance in deuterochloroform is given in Fig. 2. The presence in the spectrum of the signals of the protons of an isopropyl group (1.43 ppm), a methyl group (2.35 ppm), and of an aromatic proton (7.45 ppm) and the proton of an aldehyde group (11.11 ppm), and also of two broad signals of hydroxy groups (14.2 and 9.0 ppm), shows that the substance isolated is an analog of gossypol [6, 8]. As compared with the latter, the PMR spectrum of the substance has the signal of an additional aromatic proton (6.66 ppm) and a methoxy group (3.90 ppm). This fact, and also the presence in the mass spectrum of the substance of an ion with m/e 274, shows that the compound isolated consists of one naphthalene nucleus with isopropyl, methyl, aldehyde, and two hydroxy groups. There is information in the literature on the isolation of a similar type of compound [4, 5] possessing fungicidal properties. In particular, we have reported the isolation of a new phytoalexin of cotton — isohemigossypol (I) — and the proof of its structure on the basis of experiments using the nuclear Overhauser effect (NOE) [5].

On the basis of the results of a comparison of the spectra of isohemigossypol and the compound under investigation (see Fig. 2), it may be concluded that the corresponding chemical shifts of the signals of the functional groups, and also of the two aromatic protons and of the proton of the aldehyde group participating in the formation of an intramolecular



I II III

hydrogen bond of the chelate type, have extremely similar values in these two compounds. Consequently, the newly isolated compound is a methoxy derivative of isohemigossypol, the methoxy group being either at C_1 (II) or at C_2 (III).

The main conclusions concerning the structure of the new substance are confirmed by the results of experiments with the NOE (Table 1).

In the case of structure (II), the proton of the aldehyde group is located close to the methoxy group and on irradiation of the latter a positive Overhauser effect should be observed. Since this is not the case, it may be concluded that the methoxy group is located at C_2, and the compound most probably has the structure (III).

Some increase in the intensity of the H_4 signal when the protons of the methoxy group are irradiated is apparently due to their meta position, and also to the fact that on irradiation of the methoxy group, because of the close values of the chemical shifts the methine proton of the isopropyl radical, which is sterically close to the H_4 proton, falls in the region of the action of the strong high-frequency field [9].

On the basis of UV and PMR spectroscopy and mass spectrometry, and also its physical properties, the most probable formula for the newly isolated substance is (III) — 8-formyl-1,7-dihydroxy-5-isopropyl-2-methoxy-3-methylnaphthalene. We propose to call the identified substance gossyvertin. Since gossyvertin is absent from healthy plants, it may be assigned to the class of phytoalexin-like compounds.

EXPERIMENTAL METHOD

For investigation we used diseased and healthy cotton plants of varieties having different resistances to verticilliaceous wilt (108-F, Tashkent-1, and Tashkent-3), which were gathered on the experimental plot of the Institute of Experimental Plant Biology of the Academy of Sciences of the Uzbek SSR in the mass flowering and fruit-bearing phases. A criterion in the selection of the diseased plants, apart from external symptoms on the leaves, was the browning of the xylem, and also the specific fluorescence of a longitudinal section in ultraviolet light.

For chromatography in columns we used type KSK silica gel and polyamide powder, and for thin-layer chromatography Silufol plates, with the following solvent systems: 1) benzene—methanol (95:5); 2) benzene—methanol (90:10); 3) benzene—methanol (85:15); 4) benzene—petroleum ether—methanol (50:40:5); 5) benzene—petroleum ether—methanol (95:95:5); 6) benzene—ethyl acetate (80:20); 7) benzene—ethyl acetate—acetic acid (75:24:1); 8) chloroform—methanol—benzene (95:2:3); 9) benzene—acetic acid—water—tridecane (32.5:7:0.3:15); 10) hexane—ethyl acetate—methanol (60:40:1).

TABLE 1

Group irradiated	Increase in the intensity of the following signals, %		
		H_4	H_6
	—	21	19
Ar-CH_3	—	27	—
O—CH_3	—	12	—

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

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

Isolation of Gossyvertin. The sum of the phytoalexin-like compounds of the cotton plant was obtained by the method developed previously [5]. From a concentrated chloroform extract of the stems of a cotton plant suffering from wilt, after treatment with petroleum ether, we obtained two fractions — soluble and insoluble in petroleum ether. On chromatography on Silufol plates in system 2, several spots giving a characteristic coloration with phloroglucinol were found. Of these, gossyvertin with R_f 0.44 was predominant.

For the isolation of gossyvertin, 15 g of the insoluble fraction was dissolved in chloroform, and the solution was mixed with polyamide powder. The resulting mixture, after evaporation of the solvent, was placed in a column (3.5 × 130 cm) $^3/_4$-filled with polyamide powder and was eluted successively with systems 4 and 5. Fractions of 30-40 ml were collected. When the column was washed with system 5, a series of fractions of eluate containing mainly gossyvertin were obtained. These fractions were combined, evaporated, and rechromatographed on a column of silica gel to free them completely from impurities. The column was eluted with system 6. This gave a number of fractions containing only gossyvertin. On thin-layer chromatography in solvent system 2, 5, 7, 8, 9, and 10, the substance gave a single spot in each case with R_f 0.44, 0.16, 0.67, 0.64, 0.18, and 0.82, respectively. After evaporation of the solvent and recrystallization from aqueous acetone, 50 mg of gossyvertin was obtained. It consisted of acicular crystals colored golden with a greenish tinge, mp 147-149°C (decomp.). Readily soluble in ethanol, acetone, and ethyl acetate, sparingly in benzene and petroleum ether, and insoluble in water. Gossyvertin reacts slowly with the phloroglucinol reagent, forming a bright crimson color, and in UV light it has a strong greenish-yellow fluorescence.

SUMMARY

A new phenolic compound — gossyvertin — has been isolated from the stems of a cotton plant infected with fungus *Verticillium dahliae* Kleb.; the structure 8-formyl-1,7-dihydroxy-5-isopropyl-2-methoxy-3-methylnaphthalene has been proposed for gossyvertin.

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