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STRUCTURE OF THE PEPTIDE MOIETY OF VERTICILLIN

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The amino acid sequence of the peptide moiety of verticillin (I) — a phytotoxic metabolite of the causative agent of verticillium wilt of the cotton plant — has been determined. On the basis of the determination of the amino acid sequences of five short peptides isolated from the products of incomplete acid hydrolysis of oxidized desferriverticillin, the structure cyclo[L-glycyl-L-seryl-L-glycyl-(L-δ-N-hydroxyornithyl)$_3$] has been established for the peptide moiety of (I).

We have previously reported the presence in a culture liquid of the fungus *Vertillium dahliae* Kleb., the causative agent of wilt in the cotton plant, of phytotoxic metabolites causing the characteristic symptoms of the disease when they are introduced into the cotton plant [1, 2]. In the present paper we give the results of a study of the structure of the peptide moiety of verticillin (I) — one of these substances.

On the basis of information obtained previously concerning the elementary composition, qualitative reaction, physicochemical properties, and some chemical transformations, (I) has been assigned to the siderophores of the ferrichrome type — cyclic hexapeptides forming strong complexes with trivalent iron and containing in their molecule three residues of δ-N-acylated δ-N-hydroxyornithine and three residues of other small amino acids [3]. Analysis of the products of the complete acid hydrolysis of (I) and of desferriverticillin with 7 N hydrochloric acid and reductive hydrolysis with 50% hydriodic acid showed that the peptide moiety of (I) consists of serine, glycine, and δ-N-hydroxyornithine in a ratio of 1:2:3 [1]. Among the known siderophores, such an amino acid composition is possessed by ferricrocine [4]. However, the question of the sequence of amino acids in (I) and consequently its identity with the latter has remained unanswered.

To isolate (I) we used a method developed previously [5]. Desferriverticillin (II) was obtained by removing iron ions from (I) with the aid of 8-hydroxyquinoline [6]. To obtain peptide fragments we used incomplete acid hydrolysis, since the treatment of siderophores of the ferrichrome type with trypsin, chymotrypsin, or pepsin does not lead to the cleavage of this cyclopeptide [7]. The hydrolysis of (II) formed a complex mixture of ninhydrin-positive substances, probably because of the lability of the δ-N-hydroxyornithine. On the other hand, the known method for the catalytic conversion of the latter into ornithine [8] is inapplicable, since it is accompanied by the reduction of serine to glycine. In order to eliminate these difficulties, the oxidation of (II) was carried out with performic acid, leading to the quantitative conversion of the δ-N-hydroxyornithine into glutamic acid [6].

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When the products of incomplete acid hydrolysis of the oxidized (II) were separated by electrophoresis, eight ninhydrin-positive zones were obtained, and these were numbered in sequence in the direction from the cathode to the anode (P-1-P-8). The substances were eluted from the electrophoretograms and were purified by paper chromatography (PC). The homogeneity of the peptides was checked by determining the N-terminal amino acids and by the chromatography of their DNS derivatives. As a result, the eight fragments P-1-2, P-2-2, P-3-2, P-4-2, P-5-1, P-6-2, P-8-2, and P-8-3 (the second figure denotes the number of the zone on the chromatograms) were obtained, of which P-1-2, P-2-2, and P-6-2 were found from the results of amino acid analysis and the chromatographic mobilities of their DNS derivatives to be glycine, serine, and glutamic acid.

The N-terminal amino acids in the peptides obtained were determined by Gray's method [9], and the amino acid sequences by Edman degradation in the modification described by Vinogradova et al. [10]. The amino acid compositions, the N-terminal amino acids, and the structures of the peptide fragments of the oxidized desferriverticillin are as follows:

Peptide	Amino acid composition	Ratio of the amino acids	N-terminal amino acid	Structures
P-3-2	Ser, Gly	1:1	Gly	Ser-Gly
P-4-2	Glu, Gly, Ser	1:1:1	Ser	Glu-Gly-Ser
P-3-1	Gly, Glu	1:1	Glu	Gly-Glu
P-8-2	Glu	—	Glu	Glu-Glu-Glu
P-8-3	Glu	—	Glu	Glu-Glu
Oxidized (II).	Cer, Gly, Glu,	1:2:3	—	Gly-Ser-Gly | | Glu-Glu-Glu

The numbers of glutamic acid residues in peptide P-8-2 and P-8-3 were determined not only by Edman degradation but also in the following way. The products of incomplete hydrolysis of peptide P-8-2, after dansylation, were chromatographed on plates coated with silica gel using DNS-(glutamic acid) and the DMS derivatives of peptide P-8-3 as markers. Three spots were detected on the chromatograms, corresponding in their mobilities to the above-mentioned markers and to the DNS derivative of peptide P-8-2. A similar operation with peptide P-8-3 led to the appearance on the chromatograms of two spots corresponding to DNS-(glutamic acid) and the DNS derivative of peptide P-8-3.

On the basis of the results obtained it was established that the peptide moiety of verticillin has the structure of L-Gly-L-Ser-L-Gly-δ-N(OH)-Orn-L-δ-N(OH)-Orn-L-δ-N(OH)-Orn, i.e., a sequence of amino acids similar to that of ferricrocine [3].

EXPERIMENTAL

The following solvent systems were used for chromatography: 1) butan-1-ol—pyridine—acetic acid—water (15:10:3:12); 2) benzene—acetic acid (9:1); 3) formic acid—water (1.5:200); 4) chloroform—ethanol (98:2); 5) chloroform—ethanol—methanol (88.2:18:10); 6) methyl acetate—isopropanol—25% ammonia (9:4:6); 7) isobutanol—acetic acid—water (15:4:2); and 8) butan-1-ol—acetic acid—water (4:1:5).

Elimination of the Iron from Verticillin (I). A solution of 400 mg of (I) in 20 ml of water was mixed with 60 mg of 8-hydroxyquinoline in 10 ml of ethanol. The brown precipitate that had formed after the mixture had been allowed to stand in the cold for 1 h was separated off by centrifugation, and the supernatant was left in the refrigerator for two days. The resulting brown precipitate was separated off by centrifugation, and the supernatant was first extracted with chloroform (5 × 20 ml) to eliminate the excess of 8-hydroxyquinoline, and after this about 100 mg of wood charcoal was added to it, and this was then filtered off. The filtrate was lyophilized and the residue obtained was dissolved in 10 ml of glacial acetic acid. Traces of the brown precipitate were separated off by centrifugation. The solution was lyophilized, and the residue was dried over caustic soda in a desiccator in vacuum. This gave about 200 mg of desferriverticillin (II).

Oxidation of (II). The treatment of 200 mg of (II) with 20 ml of performic acid (16 ml of 80% formic acid was mixed with 4 ml of 25% hydrogen peroxide and the mixture was left for 0.5 h) was performed at −20°C for 24 h. Then the solution was diluted with water and was lyophilized.