STUDIES IN THE AMINO ACID COMPOSITION OF *FUSARIUM* MYCELIUM

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INTRODUCTION

With the advent of the toxin theory of wilting in tomato caused by *Fusarium lycopersici* (Gäumann, 1951), much attention has been focussed on the production of these toxins both *in vitro* and *in vivo* (Gäumann *et al.*, 1952, 1953; Fluck and Richle, 1955; Sanwal, 1955; Lakshminarayanan and Subramanian, 1955; Kalyanasundaram and Venkata Ram, 1956). Recently, Fluck and Richle (1955) from chromatographic studies of amino acid metabolism of *F. lycopersici* concluded that certain amino acids (alanine and citrullin) might be the precursors of the phytotoxin fusaric acid produced by the pathogen. These observations have been further strengthened by the results obtained by Sanwal (1955).

Application of the paper partition chromatographic technique for the detection of amino acids, sugars, vitamins, etc., is being made widely in biological sciences and has been recently utilized in taxonomical studies (Bidwell *et al.*, 1952; Buzzati-Traverso and Rechnitzer, 1953; Mansford and Raper, 1956). In the present investigation the free and bound amino acids of *Fusarium* mycelium of twenty-two species were studied to determine whether any association existed between the formation of these acids and the taxonomic position of these species. This work was also carried out to study the amino acids synthesized by Fusaria as a possible guide to their ability to produce toxins from these amino acid precursors.

MATERIALS AND METHODS

Biological material.—Fusaria were obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland, and from these monoconidial cultures were made and maintained on potato-dextrose agar for at least ten cultural generations prior to being employed for investigation. The species selected for investigation represented nine different taxonomic groups (Wollenweber and Reinking, 1935): *F. buharicum*, *F. culmorum* and *F. sambucinum*, section Discolor; *F. caucasicum*, *F. javanicum* and *F. solani* (Martiella); *F. lateritium* (Lateritium); *F. bulbigenum* var. *lycopersici*, *F. conglutinans*, *F. lini*, *F. orthoceras*, *F. oxysporum*, *F. udum* and *F. vasinfectum* (section Elegans); *F. chlamydosporum*, *F. poae* and *F. sporotrichioides* (Sporotrichiella); *F. equiseti* and *F. scirpi* (Gibbosum); *F. dimerum* (Eupionnotes); *F. semitectum* (Arthrosporiella); *F. moniliforme* (Liseola).

Culture methods.—Mycelium inoculum was obtained from cultures grown on Richard's medium for two weeks at 25–29°C. Further incubation resulted in onset of autolysis in many species accompanied by decrease of free amino acids present.

Culture extraction.—The fungal mycelium was extracted with 70 per cent ethyl alcohol at room temperature (25–29°C.) for 24 hours, centrifuged and the clear supernatant layers were then concentrated *in vacuo* to dryness. Chromatographic separation of the amino acids in the extract made with 70 per cent ethanol was found to be very unsatisfactory due to interference by other salts and therefore desalting of the extract was found necessary. This was achieved with good results

by extracting the amino acids in 5 ml. of a mixture containing 6 parts of *n*-butanol and 4 parts of phenol as advocated by Verghese (1956).

Determinations of the bound amino acids were made on the cellular material which was previously extracted with 70 per cent ethanol. The material was dried in desiccator *in vacuo*, ground to a fine powder and 500 mg. of the cellular material of each of the species were hydrolyzed by boiling for 24 hours in 35 ml. of 6 *N* HCl under a reflux condenser. The hydrolyzate was then concentrated to 2-3 ml. volume under reduced pressure at 70°C. and kept in a desiccator over KOH *in vacuo* to remove the last traces of HCl. The amino acids in the hydrolyzate were extracted with 5 ml. of the *n*-butanol phenol mixture for chromatography.

Culture analysis.—The extracts containing the free and bound amino acids were analyzed by paper partition chromatography (Consden, Gordon and Martin, 1944), employing essentially the uni- and 2-dimensional chromatographic methods (Block *et al.*, 1952). Chromatograms were run on Whatman No. 1 filter paper employing *n*-butanol : acetic acid : water (4 : 1 : 5) and phenol : water (3 : 1) as solvents for the first and the second dimensions, respectively. After development, chromatograms were first air dried, sprayed with 0·2 per cent ninhydrin in acetone and heated at 65°C. for 20–30 minutes to increase the intensity of the colour spots. The identity of the spots was established by comparing the R_f values of the amino acids in a known mixture with those in the extract, by the characteristic colour of the spots and by superimposing known amino acids over spots of the experimental material prior to irrigating the chromatograms.

Visual comparison of the chromatograms was made based on the fact that the size and colour intensity of the spots is largely a function of the concentrations of the compounds (Dent, 1948), and the relative amounts of the amino acids present are given on the basis of such comparisons.

Results

The free amino acids detected in the mycelium of the *Fusarium* species are shown in Table I. The following free amino acids were found in the mycelial extract of all the species: aspartic acid, glutamic acid, threonine, alanine, glutamine, lysine, arginine, valine, phenylalanine and tyrosine. In addition, the culture extracts of all the species, excepting *F. equiseti*, *F. scirpi* and *F. dimerum*, contained serine and glycine, whilst proline was detected in the mycelium of *F. javanicum*, *F. conglutinans*, *F. orthoceras*, *F. lini*, *F. lycopersici* and *F. udum*. With the exception of *F. chlamydosporum*, *F. sporotrichioides*, *F. equiseti*, *F. scirpi*, *F. dimerum* and *F. semitectum*, all the other extracts contained cystine.

Gamma-amino butyric acid was detected in the extract of *F. moniliforme* (Fig. 1), *F. vasinfectum*, *F. chlamydosporum* and *F. sporotrichioides*. An unknown substance giving a yellow colour after ninhydrin spray, spot 16 (Fig. I), was detected in the extracts of *F. moniliforme*, *F. sambucinum*, *F. lateritium*, *F. vasinfectum*, *F. sporotrichioides*, *F. equiseti* and *F. scirpi*. Another unknown, spot 17 (Fig. 1), was present in the chromatograms of *F. moniliforme*, *F. vasinfectum* and *F. sporotrichioides*.

Quantitative differences in the amino acid content in the free form in the mycelium were apparent even within species belonging to the same taxonomic group viz., in *F. javanicum* and *F. solani* (section Gibbosum) (Table I.), although the overall amino acid composition was similar. Marked quantitative as well as qualitative dissimilarities in the amino acids present in the mycelium of Fusaria belonging to different taxonomic groups were observed (Table I).

The bound amino acids found in the culture of the *Fusarium* species (Table II) were: aspartic acid, glutamic acid, serine, glycine, threonine, alanine, lysine, arginine, proline, valine, leucine and/or isoleucine (with the exception of *F. oxysporum*), phenylalanine and tyrosine (excepting *F. sambucinum* and *F. chlamydosporum*).

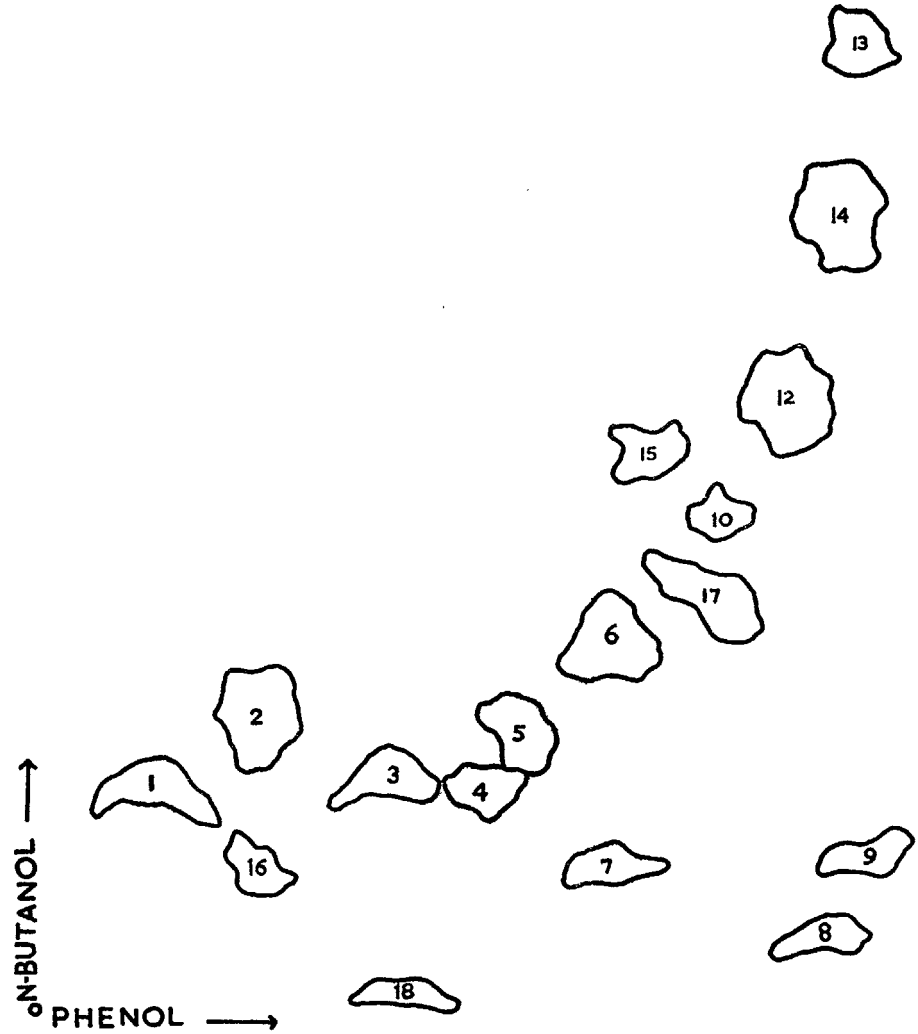

FIG. 1. Free amino acids occurring in the mycelium of *Fusarium moniliforme*. [Identity of spots in Figs. 1 & 2: (1) Aspartic acid, (2) Glutamic acid, (3) Serine, (4) Glycine, (5) Threonine, (6) Alanine, (7) Glutamine, (8) Lysine, (9) Arginine, (10) Gamma-amino butyric acid, (11) Proline, (12) Valine, (13) Leucine and/or isoleucine, (14) Phenylalanine, (15) Tyrosine, (16) Unknown, (17) Unknown, (18) Cystine].

Spot 10 in the chromatogram of *F. sambucinum*, *F. culmorum* (Fig. 2) and *F. buharicum* corresponded in position to gamma-amino butyric acid, while another unknown, spot 16, which gave a yellow colour with ninhydrin, was present in the extracts of *F. culmorum* (Fig. 2), *F. solani*, *F. javanicum*, *F. lateritium*, *F. equiseti* and *F. scirpi*. Differences in the chromatographic pattern of bound amino acids in mycelium of Fusaria belonging to different as well as the same taxonomic group seemed to be more quantitative than qualitative (Fig. 3, Table II).

Simonart and Chow (1954) stated that the amino acid composition of the mycelium of *Aspergillus oryzae* was considerably influenced by the carbon source in the substrate. To test this in Fusaria, mycelium of *F. moniliforme* harvested

TABLE I

Showing the free amino acids present in the mycelium of Fusaria

Fusarium spp.	Aspartic acid	Glutamic acid	Serine	Glycine	Threonine	Alanine	Glutamine	Lysine	Arginine	Gamma-amino butyric acid	Proline	Valine	Leucine and/or isoleucine	Phenylalanine	Tyrosine	Cystine	Unknown†	Unknown‡
F. sambucinum	*x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	
F. culmorum	x	x	x	x	x	xx	x	x	x			x	x	x	x	x		
F. buharicum	x	x	x	x	x	xx	x	x	x			x	x	x	x	x		
F. solani	x	x	x	x	x	xx	x	x	x			x		x	x	x		
F. caucasicum	x	x	x	x	x	xx	x	x	x			x		x	x	x		
F. javanicum	xx	xx	x	x	x	x	x	x	x		x	x		x	x	x		
F. lateritium	x	xx	x	x	x	x	x	x	x			x		x	x	x	x	
F. orthoceras	x	x	x	x	x	xx	x	x	x		x	x	x	x	x	x		
F. oxysporum	x	x	x	x	x	xx	x	x	x			x	x	x	x	x		
F. conglutinans	x	x	x	x	x	xx	x	x	x		x	xx	x	x	x	x		
F. lini	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		
F. bulbigenum var. *lycopersici*	x	x	x	x	x	xx	x	x	x		x	x	x	x	x	x		
F. udum	x	x	x	xx	xx	xx	x	x	x		x	x	x	x	x	x		
F. vasinfectum	x	x	x	x	x	xx	x	x	x	x		x	x	x	x	x	x	x
F. sporotrichioides	x	x	x	x	x	xx	x	x	x	x		x	x	x	x		x	x
F. chlamydosporum	x	x	x	x	xx	xx	x	x	x	x		x	x	x	x			
F. poae	x	x	x	x	x	x	x	x	x			x	x	x	x			
F. equiseti	x	x			x	xx	x	x	x			x		x	x		x	
F. scirpi	x	x			x	xx	x	x	x			x		x	x		x	
F. dimerum	x	x			x	xx	x	x	x			x		x	x			
F. semitectum	xx	xx	x	x	x	xx	x	x	x			x		xxx	x			
F. moniliforme	x	x	x	x	x	xx	x	x	x	x		x	x	xx	x	x	x	x

* The number of x signs indicate the relative amounts of each amino acid.
† Spot 16 in Fig. 1 which gave a yellow colour with ninhydrin.
‡ Spot 17 in Fig. 1 which gave a purple colour with ninhydrin.

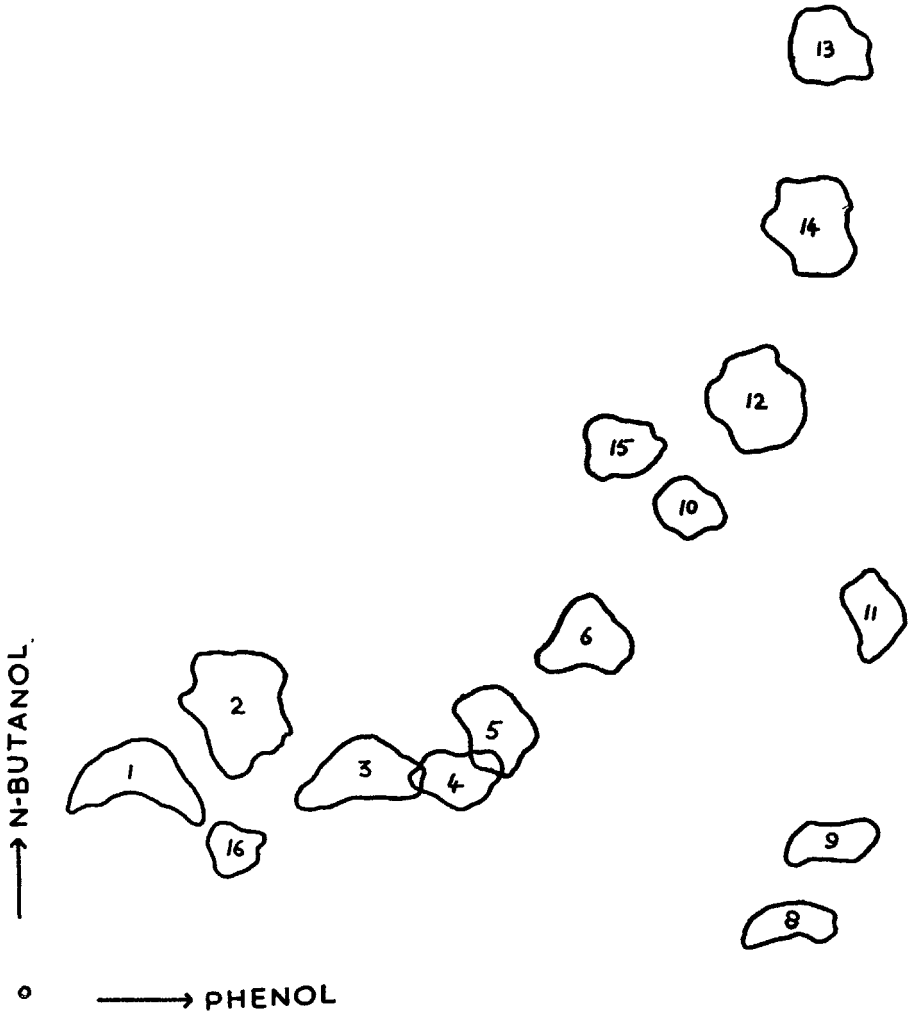

FIG. 2. Bound amino acids occurring in the mycelium of *F. culmorum*.

from a two week-old culture grown on Richard's medium containing 50 g./l. arabinose, glucose, fructose, starch and sucrose was examined for free amino acids present (Table III). Gamma-amino butyric acid was detected in extracts of mycelium harvested from sucrose and fructose cultures only, while threonine and tyrosine were found in extracts of glucose, fructose and sucrose cultures only. Two unknown ninhydrin positive substances were detected in extracts of glucose and sucrose and not in the other cultures.

DISCUSSION

Earlier work of Fluck and Richle (1955) and Sanwal (1955) indicated the possibility of the production of the phytotoxin fusaric acid from certain amino acids synthesized by *F. lycopersici*, particularly alanine and citrullin. In the present investigation on the amino acids synthesized by twenty-two species of Fusaria, alanine was found to occur in free and bound forms in all the species. Of these

TABLE II

Showing the bound amino acids present in the mycelium of Fusaria

Fusarium spp.	Aspartic acid	Glutamic acid	Serine	Glycine	Threonine	Alanine	Lysine	Arginine	Gamma-amino butyric acid	Proline	Valine	Leucine and/or isoleucine	Phenylalanine	Tyrosine	Unknown*
F. sambucinum	†xx	xx	x	x	xx	xx	x	x	x	x	x	x	xx†	x	x
F. culmorum	xx	xx	x	x	xx	xx	x	x	x	x	xx	xx	xxx	x	
F. buharicum	xx	xx	x	x	xx	xx	x	x	x	x	xx	xx	xxx	x	
F. solani	xx	xx	x	x	xx	xx	x	x		x	x	x	xx	x	x
F. caucasicum	xx	xx	xx	xx	xx	xxx	xx	xx		x	xx	xxx	xxx	x	
F. javanicum	xx	xx	x	xx	xx	xx	x	xx		x	xx	xxx	xxx	x	x
F. lateritium	xx	xx	x	xx	xx	xxx	x	xx		x	xx	xx	xxx	x	x
F. orthoceras	xx	xx	x	xx	xx	xx	x	x		x	xx	x	xxx	x	x
F. oxysporum	x	xx	x	x	xx	xx	x	x		x	x		x	x	
F. conglutinans	xx	xx	x	x	xx	xx	x	x		x	x	x	xx	x	
F. lini	xx	xx	x	x	xx	xxx	x	x		x	xx	x	xxx	x	
F. bulbigenum var. *lycopersici*	xx	xx	x	x	xx	xxx	x	x		x	xx	x	xxx	x	
F. udum	xx	xx	x	x	xx	xxx	x	x		x	x	x	xxx	x	
F. vasinfectum	xx	xx	xx	xx	xx	xxx	xx	xx		x	x	x	xxx	x	
F. sporotrichioides	xx	xx	xx	xx	xx	xxx	x	x		x	xx	xx	xxx	x	
F. chlamydosporum	xx	x	x	x	x	x	x	x		x	xx	x	xx		
F. poae	xx	xx	xx	xx	xx	xxx	x	x		x	xx	xx	xxx	x	
F. equiseti	xx	xx	x	x	x	xx	x	x		x	xx	x	xxx	x	x
F. scirpi	xx	xx	x	x	x	xx	x	x		x	xx	x	xxx	x	x
F. dimerum	xx	xx	x	x	xx	xx	x	x		x	xx	x	xxx	x	
F. semitectum	xx	xx	x	x	xx	xx	x	x		x	xx	x	xxx	x	
F. moniliforme	xx	xx	x	x	x	x	x	x		x	x	x	xxx	x	

* Spot 16 in Fig. 4 which gave a yellow colour with ninhydrin.
† The number of x signs indicate the relative amounts of each amino acid.

studied, the ability to synthesize fusaric acid *in vitro* has been reported only in *F. vasinfectum, Gibberella fujikuroi* (*F. moniliforme*), *F. lycopersici* and *F. orthoceras* (Venkata Ram, 1956). Plausibly, the other species investigated are unable to convert the alanine and other amino acid precursors into fusaric acid through the absence of specific enzyme systems concerned in the process. The production of

TABLE III

Showing the amino acids (free) detected in the mycelium of F. moniliforme *grown on various carbon sources*

Carbon sources	Aspartic acid	Glutamic acid	Serine	Glycine	Threonine	Alanine	Lysine	Arginine	Gamma-amino butyric acid	Glutamine	Valine	Leucine and/or isoleucine	Phenylalanine	Tyrosine	Cystine	Unknown*	Unknown†
Arabinose	xx	xx	x	x	x	xx	x	x		x	x	x	xx	x	x	x	x
Glucose	xx	xx	x	x	x	x	x	x		x	x	x	xx	x	x	x	
Fructose	xx	xx	x	x		xxx	x	x	x	x	x	x	xx		x		
Starch	xx	xx	x	x		xx	x	x		x	x	x			x		
Sucrose	xx	xx	x	x	x	xx	x	x	x	x	x	x	xx	x	x	x	x

* Gave a yellow colour with ninhydrin.
† Gave a purple colour with ninhydrin.

fusaric acid, therefore, seems to bear little relationship with the amino acid synthesis in Fusaria.

Chromatographic studies of amino acids and other constituents of plant and animal species undertaken from a taxonomic point of view have yielded largely negative results. However, Work and Dewey (1953) examining the chromatograms of hydrolyzates of 118 micro-organisms found that the presence of α, ϵ-diaminopimelic acid was specific to certain taxonomic groups of bacteria and algae only. Similarly, Buzzati-Traverso and Rechnitzer (1953) were able to obtain a positive correlation between amino acid content and the taxonomic position of certain fishes.

DeVay (1954) working with *Ustilago zeae* failed to obtain any correlation between amino acids synthesized in culture and their sex and pathogenicity, whilst Murray and Zscheile (1956) showed that amino acid synthesis is not a limiting factor in chlamydospore production in *Tilletia caries*. Similarly, it was observed here that no relationship existed between amino acid content and the taxonomic position of a particular *Fusarium* species. Indeed, variations both quantitative as well as qualitative with free and bound amino acid pattern of the fungal mycelium were as much between species of different taxonomic groups as in species within the same group. These results are in line with the very recent findings of Mansford and Raper (1956) who demonstrated that no correlation existed between the taxonomic position and the amino acid content of many of the higher and lower plant forms studied by them.

As shown in Table III, there is evidence to indicate that the ability to synthesize amino acids in culture is considerably influenced by the carbon source present in the substrate, thus confirming the observations previously reported in *Aspergillus oryzae* (Simonart and Chow, 1954).

Summary

Quantitative and qualitative differences were found in the free amino acid content of cultures of twenty-two species of *Fusarium* but were not correlated with the taxonomic position of the species. Only slight quantitative differences were found in the bound amino acid content of the different species tested. Fifteen known amino acids and one unknown ninhydrin positive substance were identified as occurring both in the bound and free forms in the *Fusarium* mycelium; in addition, another unknown substance was detected in the free form. Qualitative changes in the carbon sources present in the substrate considerably influenced the free amino acid composition of the mycelium of *F. moniliforme*. *Fusarium* mycelium harvested from a two week-old culture in Richard's medium contained aspartic acid, glutamic acid, serine, glycine, threonine, alanine, glutamine, lysine, arginine, valine, leucine and/or isoleucine, phenylalanine, tyrosine in the free form in the majority of the species, whilst in addition to these and with the exception of glutamine, proline was always detected in the bound form. Gamma-amino butyric acid was found to occur in the free and bound form in certain species.

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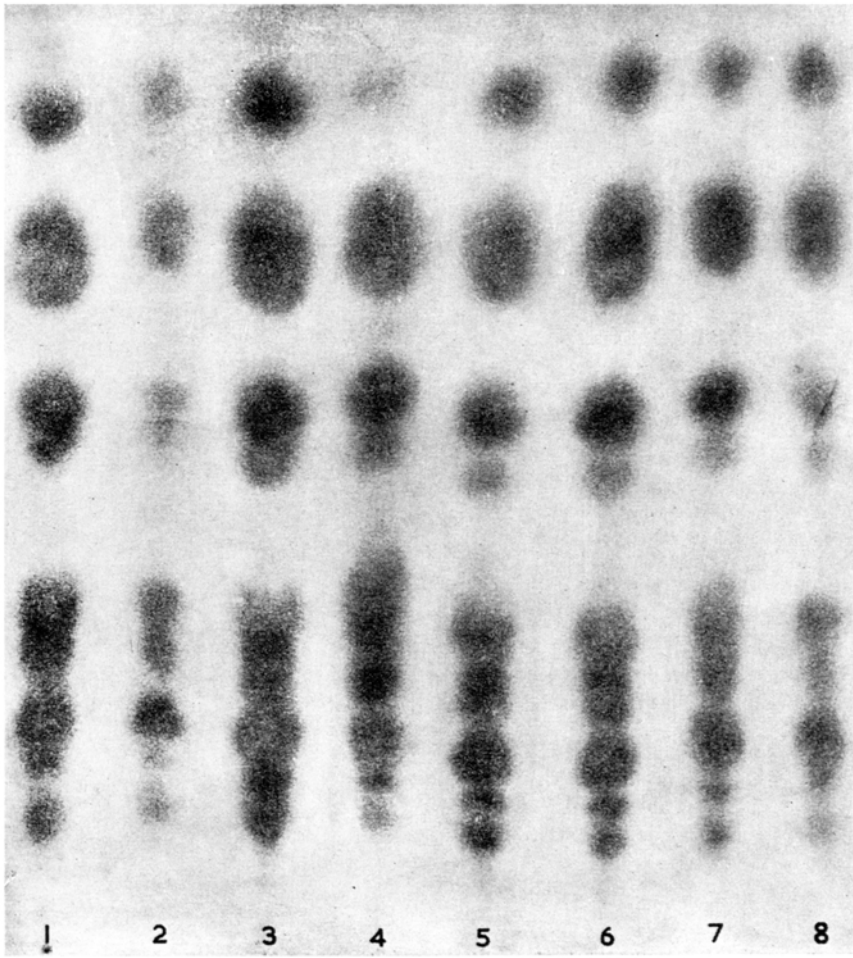

FIG. 3. Unidimensional chromatogram of bound amino acids detected in the mycelium of (1) *F. sporotrichioides*, (2) *F. chlamydosporum*, (3) *F. poae*, (4) *F. equiseti*, (5) *F. scirpi*, (6) *F. semitectum*, (7) *F. dimerum*, (8) *F. moniliforme*.

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