THF with N^{α}-CTB-$N^{\omega,\omega}$-*bis*-CBO-arginine[3], via the mixed anhydride with trimethylacetic acid[3]. The resulting N^{α}-CTB-$N^{\omega,\omega}$-*bis*-CBO-arginyl-isoleucyl-tyrosine benzylester (IX) (85% yield; m.p. 164–166°; $[\alpha]_D^{20}$ −7°, c 0.6, DMF. *Anal.* Calcd. for $C_{49}H_{60}N_6O_{11} \cdot {}^1/_2H_2O$: C 64.1; H 6.7; N 9.2. Found C 64.3; H 6.8; N 8.8) was treated with HCl/AcOH 1.3*N* to give $N^{\omega,\omega}$-*bis*-CBO-arginyl-isoleucyl-tyrosine benzylester hydrochloride (X)[4] (yield 95%; $E_{1.9}$ = 0.66 Leu) which was condensed in DMF with CTB-phenylalanyl-O-acetyl-seryl-prolyl-phenylalanylazide, obtained by treatment at low temperature of the hydrazide VII with HCl/isoamyl nitrite[5]. The resulting N-CTB-phenylalanyl-O-acetyl-seryl-prolyl-phenylalanyl-$N^{\omega,\omega}$-*bis*-CBO-arginyl-isoleucyl-tyrosine benzylester (XI)[6] (40% yield; m.p. 160–163°; $[\alpha]_D^{20}$ −25°, c 1, DMF. *Anal.* Calcd. for $C_{77}H_{92}N_{10}O_{17}$: C 64.7; H 6.5; N 9.8. Found C 64.2; H 6.5; N 9.9) was treated with HCl/AcOH 1.3*N* to give the hydrochloride XII[4] (100% yield, $E_{1,9}$ = 0.60 Leu).

CBO-prolyl-prolyl-glycine (XIII)[7] (m.p. 103–108° ex AcOEt/pet. ether, $[\alpha]_D^{20}$ −80°, c 1, DMF. *Anal.* Calcd. for $C_{20}H_{25}N_3O_6 \cdot {}^1/_2H_2O$: C 58.2; H 6.4; N 10.2. Found C 58.2; H 6.4; N 10.1) was treated with HBr/AcOH to give the hydrobromide XIV which was condensed, without further purification, with *p*-nitrophenyl N^{α}-CBO-$N^{\omega,\omega}$-*bis*-CBO-argininate[8], in DMF with 2 equivalents of TEA, to give N^{α}-CBO-$N^{\omega,\omega}$-*bis*-CBO-arginyl-prolyl-prolyl-glycine[9] (XV) (60% yield; amorphous; $[\alpha]_D^{20}$ −50°, c 1, DMF. *Anal.* Calcd. for $C_{42}H_{49}N_7O_{11} \cdot {}^1/_2H_2O$: C 60.3; H 6.0; N 11.7; O 22.0. Found C 60.6; H 6.1; N 11.4; O 22.1) that was condensed in DMF, with DCCI, with phenylalanyl-O-acetyl-seryl-prolyl-phenylalanyl-$N^{\omega,\omega}$-*bis*-CBO-arginyl-isoleucyl-tyrosine benzylester hydrochloride (XII) to give N^{α}-CBO-$N^{\omega,\omega}$-*bis*-CBO-arginyl-prolyl-prolyl-glycyl-phenylalanyl-O-acetyl-seryl-prolyl-phenylalanyl-$N^{\omega,\omega}$-*bis*-CBO-arginyl-isoleucyl-tyrosine benzylester (XVI)[10] (41% yield; m.p. 125°; $[\alpha]_D^{20}$ −35°, c 1, DMF. *Anal.* Calcd. for $C_{114}H_{131}N_{17}O_{25}$: C 64.0; H 6.2; N 11.1. Found C 63.6; H 6.2; N 11.0).

The protected hendecapeptide XVI was treated overnight in pyridine with 10 equivalents of SO_3-pyridine complex; dilution with water and extraction with dichloromethane afforded the O-tyrosyl sulphate XVII (90% yield; m.p. 130–135°; $[\alpha]_D^{20}$ −18°, c 1, DMF. *Anal.* Calcd. for $C_{114}H_{131}N_{17}O_{28}S$: C 61.7; H 6.0; N 10.7; S 1.4. Found C 61.8; H 6.3; N 10.4; S 1.9) which was hydrogenated in MeOH 80% (Pd/C) at 5 atm pressure: the solution was next treated with NaOH *N*/10 until a permanent alkaline reaction was achieved. The solvent was evaporated and the residue purified by counter-current distribution in a BuOH/EtOH/AcOH/H_2O (5:1:1:8) system to give XVIII (50% yield; m.p. 240° dec.; $[\alpha]_D^{20}$ −63°, c 0.9, AcOH 95%; $E_{1.9}$ = 0.39 Arg; 0.82 Glu; $E^a_{1.9}$ = 0.58 Arg; 1.23 Glu. *Anal.* Calcd. for $C_{65}H_{93}N_{17}O_{17}S \cdot 2CH_3COOH$: C 53.9; H 6.6; N 15.5. Found C 53.8; H 6.5; N 15.6) which was found homogeneous and showed amino acid composition, behaviour towards tripsin and chimotripsin[11] and the same biological properties[12] as natural phyllokinin, thus confirming the formula deduced from degradative experiments[13].

Riassunto. Viene riportata la sintesi della arginil-prolil-prolil-glicil-fenilalanil-seril-prolil-fenilalanil-arginil-isoleucil-tirosina-O-solfato, un polipeptide identico per proprietà chimiche, fisiche e biologiche alla phyllochinina, analogo naturale della bradichinina.

L. Bernardi, G. Bosisio,
R. de Castiglione, and O. Goffredo

Istituto Ricerche Farmitalia, Milano (Italy), April 12, 1966.

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[10] The peptide was purified by chromatography on silica gel (eluent, the same as [6]).
[11] We are indebted to Dr. A. Anastasi for these assays.
[12] We are indebted to Prof. V. Erspamer for these assays.
[13] We wish to express our thanks to Dr. B. Camerino, Director of this Research Institute, for his sustained interest in this work.

Biochemical, Genetical Studies on Host-Parasite Relationship: Variation in Fusaric Acid Production with Different Carbon Sources[1]

Fusarium vasinfectum Atk., the causal agent of cotton wilt has been shown to utilize macromolecules as the sole carbon source with the help of adaptive hydrolytic enzymes, the production of which increases in the presence of the respective substrates or host tissue extracts[2]. Hence it was of interest to study how the various carbon sources affect the production of the wilt toxin fusaric acid by this pathogen; our results are presented in the present communication.

A pathogenic isolate of *Fusarium vasinfectum* Atk. was grown in Richards medium with various amendments as described elsewhere[2]. Krebs cycle intermediates were amended as follows: 0.30*M* citric acid, 0.42*M* succinic acid and 0.42*M* fumaric acid were dissolved in Richards medium without glucose, to supply 2% carbon equivalent as contained in 5% glucose present in Richards medium and adjusting the pH to 5.5 ± 0.2. 15-day-old culture filtrates were adjusted to pH 4.0, and extracted thrice with equal volumes of ethyl acetate[3]. The solvent was

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Carbon source (carbon content 2%, equivalent to 5% glucose in Richards medium)	Mycelial weight in mg	Total fusaric acid in mg	mg fusaric acid per mg dry mycelial weight[a]	Comparison of efficiency with the control (taken as 100%)
1. Glucose (Richards medium)	156.8	3.38	0.022	100
2. Starch	192.2	5.38	0.028	127
3. Cellulose	74.6	1.88	0.025	114
4. Casein	162.4	10.10	0.062	282
5. Egg albumin	172.0	11.22	0.065	295
6. Coconut oil	60.8	1.88	0.030	136
7. Olive oil	63.6	2.20	0.035	159
8. Yeast ribonucleic acid	13.0	0.32	0.024	109
9. Calf-thymus deoxyribonucleic acid	60.0	1.37	0.023	105
10. Citric acid	258.8	4.65	0.018	82
11. Succinic acid	374.6	4.95	0.013	59
12. Fumaric acid	366.2	4.95	0.014	64
13. Susceptible host extract	60.0	4.05	0.068	309
14. Resistant host extract	58.0	3.025	0.052	236

[a] Efficiency of fusaric acid production

evaporated using a current of air at room temperature and the residue dissolved in 10 ml of 80% ethanol. 0.2 ml of the samples were chromatographed for 18 h on Whatman No. 3 MM filter paper using the solvent system *n*-butanol, acetic acid and water (in the ratio 4:1:5) along with a set of standard solutions varying in concentration from 40–100 μg. The spots were marked under UV and eluted with 5 ml of 80% ethanol whose absorbancies were measured at 268 mμ, in a Beckman spectrophotometer (ZAHNER[4]).

It is interesting to note (Table) that the proteins support good growth and also fusaric acid production. The total fusaric acid production was maximum in their presence. This may be due to the fact that metabolic products of proteins available in high concentration probably promote the synthesis of fusaric acid, as it has been shown earlier that α- and β-alanines, glutamic acid, γ-amino butyric acid and serine stimulate fusaric acid production[5]. It may be that tryptophan contributes to the pyridine ring. No appreciable variation is observed in the efficiency of toxin production among the carbohydrates and nucleic acids. High growth in the presence of starch results in good yield of fusaric acid. Poorest toxin production is observed with the nucleic acids, probably due to poor growth. A slight increase in the efficiency of fusaric acid production observed in the presence of lipids may be due to the free availability of acetate, which was shown to be incorporated into fusaric acid by using labelled acetate[5]. Excellent growth in the presence of the Krebs cycle intermediates offers good yields of the toxin. However, the efficiency of production is found to be poor, thus indicating that these organic acids are preferentially utilized for growth purposes only.

The efficiency of fusaric acid production in the presence of autoclaved host extracts approaches that of the proteins. In spite of the poor growth, fusaric acid yield approaches that of the control, showing a high efficiency in toxin synthesis. Fusaric acid production in the presence of autoclaved extracts of both the resistant and susceptible hosts may be due to the denatured proteins that are available in these extracts. However, the difference observed may be due to the variation in their constitution which may have a relation to the resistance.

Zusammenfassung. Die Bildung von Fusarinsäure durch *Fusarium vasinfectum* Atk., bezogen auf das Trockengewicht, ist am grössten, wenn Proteine oder Auszüge aus Wirtspflanzen und am geringsten, wenn Säuren des Krebszyklus als Kohlenstoffquelle geboten werden.

A. SAMPATH NARAYANAN and
E. R. B. SHANMUGASUNDARAM

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Heterogeneity of Catalase in Blood of Heterozygous Cases of Acatalasia

Catalase can be isolated from small samples of hemolysate by gel-filtration on Sephadex[1] or by column chromatography on calcium phosphate-DEAE-cellulose complex gel[2]. Using the latter technique, 2 fractions can be obtained by extraction with 0.15 *M* sodium chloride solution (= fraction with index number 1) and 0.2 *M* secondary sodium phosphate solution pH 8.2 (= fraction with index number 2).

By applying this technique to samples of (a) normal human blood, (b) blood of a heterozygous, and (c) blood of a homozygous case of acatalasia, 6 different preparations of purified human red cell catalase are obtained. Analysis of their electrophoretic mobility on a mixture of

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