

following N-terminal amino acids appeared: aspartic acid, glycine, alanin, lysine and methionine. The glutamyl and tyrosyl residues did not show any significant change during the digestion time used. As the value of tyrosine was practically constant during the fibrinogenolysis, the released amounts of the N-terminal amino acids were expressed in relation to tyrosine (see Fig. 1). When the digestion of fibrinogen had proceeded so far that only traces could be coagulated with trombin, approximately one mole lysyl, two moles each of glycylyl, aspartyl and methionyl, and three moles alanyl residues were formed from one mole of fibrinogen.

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An Antibiotic Related to the Streptothricin Group

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From an unidentified actinomycete No. 4279 isolated from a soil sample collected in North Zealand, Denmark, in 1953 we have succeeded in isolating a crude substance with antibiotic activity against a wide range of bacteria and fungi. This substance has properties very similar to those described by Schaffner, Rangaswami and Waksman^{1,2} as regards the mycothricin complex though it differs from the latter in certain chemical respects. Optimum yield was obtained after 36 h at

25–28° in a medium containing the extractive material from 40 g of soy-meal per l in an aerated and stirred fermentator.

A crude preparation of the active substance was obtained from the broth by adsorption on activated carbon at pH 7–8 and elution with 80 % ethanol at pH 2.7. Purification was carried out (a) by treating a 20 % aqueous solution of the substance at pH 2.7 with activated carbon and subsequent precipitation by means of 10 volumes of acetone or ethanol and (b) by adsorption on Amberlite IRC-50 at pH 8 and elution with dilute hydrochloric acid; the active fractions were then neutralized with Amberlite IR-4B and evaporated *in vacuo* or, in some cases, lyophilized.

The substance is readily soluble in water and slightly soluble in ethanol. It is insoluble in chloroform, ether, benzene, toluene and *n*-butanol. Paper chromatograms developed with dilute ethanol at pH 3.0 showed the following R_F values: 0.38 (ethanol:water, 80:20), 0.70 (ethanol:water, 70:30). The total activity of the substance can be precipitated by ammonium reineckate, methyl orange, and picric acid, whereas only a fraction of the total activity can be precipitated by flavianic acid (pH between 5.0 and 5.5).

The following tests were negative: Ninhydrin, Pauly, Sakaguchi, Hopkins-Cole, Fehling, Tollens, Molisch, 9-hydrazinoacridin for hydrophilic aldehydes³. The biuret reaction was dubious. With sodium periodate the substance gave formaldehyde.

On hydrolysis in 2 N hydrochloric acid an amino acid and a sugar were formed.

Minimum inhibitory concentration

Test organism	$\mu\text{g/ml}$
<i>Staph. aureus</i>	0.25— 0.31
<i>E. coli</i>	12.5 — 25.0
<i>Strept. hemolyticus</i>	1.0 — 10.0
<i>S. typhi</i>	1.0 — 10.0
<i>Corynebact. sp.</i>	0.1 — 1.0
<i>B. subtilis</i>	0.1 — 1.0
<i>B. dysenteriae Shiga</i>	1.0 — 10.0
<i>Pseud. aeruginosa</i>	10 —100
<i>Alternaria circinans</i>	0.12— 0.25
<i>P. notatum</i>	0.25— 0.50
<i>A. niger</i>	1.0 — 10
<i>P. pullulans</i>	1.0 — 10
<i>Candida albicans</i>	12.5 — 25
<i>Trichoderma viride</i>	0.25— 0.50

The amino acid has R_F 0.15 (*n*-butanol: acetic acid:water, 4:1:5). A sugar test was made in the following way: The substance was heated in a solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid for a few hours. On cooling, a red precipitate of a tetranitrophenylosazone was formed. On adding a few drops of N sodium hydroxide to a solution of the precipitate in ethanol a blue colour was obtained (modified Bamberger reaction⁴).

The purest product obtained has been tested in broth-dilution assay against different microorganisms.

In mice LD 50 was about 1 200 mg/kg body weight when administered intravenously. In rats 50–500 mg/kg subcutaneously caused no acute toxic symptoms but anuria developed after 3 or 4 days. Examination of the kidneys showed severe tubular damage. Unlike streptothricin, a 1 % aqueous solution did not produce local irritation in rabbits' eyes.

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Correction to "Determination of Small Amounts of Total Cholesterol by the Tschugaeff Reaction with a Note on the Determination of Lathosterol" *

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In Table 3, p. 681 the following corrections are made:

Compound		Absorbance	
		mmoles/ml	
.....			
Cholesterol	for	9 593 read	9 067
Cholesterylacetate	for	8 349 read	6 348
.....			

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