The synthetic method above described has many advantages; simplicity and good yield of the each steps, all intermediates were good crystallizable materials, and the esterification of the intermediary peptides by the TCAOPCP method was especially favorable compared with DCCD method, because there was no trouble such as acylurea formation or contamination of insoluble dicyclohexylurea.

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The Total Amino Acid Sequence of Substance A produced by *Streptomyces carzinostaticus*

Antibacterial polypeptide substance A[1] produced by *Streptomyces carzinostaticus* var. F-41 has a molecular weight of 8440, contains 87 amino acid residues but contains no histidine and methionine. It has an N-terminal amino acid alanine and C-terminal amino acid asparagine and a disulfide bridge[2] which concerned to antibacterial acitivity.

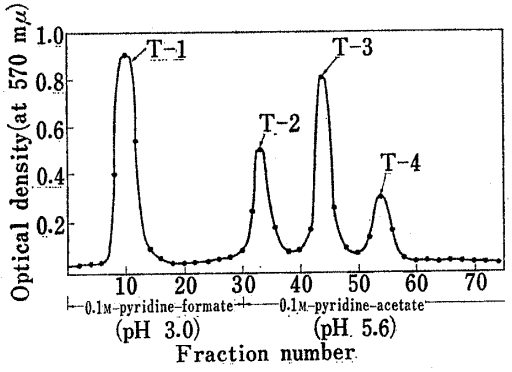

Fig. 1. Chromatogram of the Tryptic Peptides on Amberlite CG-120 Column

380 mg of the lyophilized powder was dissolved in 5 ml of 0.01N-HCl and absorbed on a column of Amberlite CG-120 (1.6×46 cm). The elution was made by the successive change of eluant from 150 ml of 0.1M-pyridine-formate buffer (pH 3.0) to 400 ml of 0.1M-pyridine acetate buffer (pH 5.6). Flow rate was set 0.5 ml/min, and the effluent was collected in 5 ml fraction. The peptide content of effluent was determined by ninhydrin reaction.

The present communication proposes the total amino acid sequence of substance A. In the previous paper,[2] partial acid hydrolysis was performed by the method of Partridge, *et al.*,[3] to obtained seven free amino acids (from A-1 to A-4 in Table I) and eighteen peptide fractions (from A-5 to A-22). These peptide fractions were further separated and purified by paper chromatography and paper electrophoresis, and the amino acid sequence of the each peptide fragment was determined as shown in Table I.

Tryptic digestion of the substance A was carried out by the following procedure. Fourhkndred and thirty mg (5.1×10^{-5} mole) of substance A was dissolved in 20 ml of 0.1M phosphate buffer (pH 7.6) containing 2.0M guanidinium chloride, and incubated with 4.3 mg of trypsin at 37° for 24 hr. After 24 hr

1) H. Sato, T. Tanimura and Z. Tamura, *J. Biochem.*, **65**, 901 (1969).
2) H. Sato, T. Tanimura, T. Nakajima and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **17**, 413 (1969).
3) S.M. Partridge, H.F. Davis and F.S. Adair, *Biochem. J.*, **61**, 11 (1969).

Table I. The Amino Acid Composition and Sequence of Peptides from Partial Acid Hydrolysis of Substance A

Fraction No.	Amino acid composition and sequence
A-1	Asp
A-2	Thr, Ser
A-3	Glu, Pro
A-4	Gly, Ala
A-5	Ala-Ala
A-6-1	Asp-Pro-Glu-Ser-Phe
A-6-2	Glu-Gly-Thr-Gly
A-7	Ala-Ala-Gly
A-8	Thr-Gly-Gly-Ala
A-9	Gly-Thr-Val-Lys-Val-Val-Ala-Thr-Gly-Gly-Ala
A-10	Ala-Pro-Pro-Glu-Thr-Ala-Ser-Leu-Ile-Phe-Ala-Val
A-11	Leu-Ser-Ala
A-12	Ala-Pro-Pro-Glu-Thr-Ala
A-13	Gly-Thr-Val-Lys-Val-Val-Ala
A-14	Ser-Leu-Ile-Phe-Ala-Val
A-15	Glu-Thr-Gly-Gly-Ala-Val-Ala
A-16	Thr-Gly-Gly-Ala-Val-Ala-Thr-Tyr
A-17	$CySO_3H$-Gly-Thr-Ala-Ala-Val-Leu-Glu-$CySO_3H$
A-18	Ser-Thr-Ala-Val-Gly-Ser-Phe-Leu
A-19	Thr-Tyr-Ser-Thr-Ala-Val-Gly-Ser-Phe-Leu
A-20	Ser-Phe-Leu
A-21	Phe-Leu
A-22-1	Gly-Thr-Arg-Ala-Gly
A-22-2	Ser-Arg-Val-Pro-Gly

Dashed arrows pointing to the right indicate sequences determined by the Edman–Dansyl procedure.[4] C-terminal amino acid (arrow to the left) was determined by DNS method after the hydrazinolysis of peptide. A–17 was degraded by the Edman–Dansyl procedure after oxidized with performic acid.

T-1 : Ala-Try-Gly-Asp-Leu-Ser-Ala-Asp-Gln-Gly-Thr-Gly-Glu-Asp-Ala-Pro-Pro-Glu-(Asp, Thr, Ser, Ala_2, Val, Leu, Ile, Phe)

T-2 : Ala-Ala-Gly-Asn-Pro-Ser-Glu-Thr-Gly-Gly-(Asp, Thr_3, Ser_2, Gly_2, Ala_3, Val_3, Leu, Tyr, Phe)-Lys

T-3 : Val-Pro-Gly-Asn-$CySO_3H$-Gly-Thr-Ala-Ala-Val-Leu-Glu-$CySO_3H$-Asp-Asn-Pro-Glu-Ser-Phe-Asp-(Gly, Thr,)-Arg

T-4 : Val-(Thr, Ser, Gly_2, Ala_2, Val)-Arg

Fig. 2. N- and C-Terminal Amino Acid of Each Tryptic Peptide and Partial Amino Acid Sequence of T-1, T-2 and T-3

N-terminal amino acid was detected by DNS method. C-terminal amino acid was detected by DNS method after the hydrazinolysis of peptide. Dashed arrows pointing to the right indicate sequences determined by the Edman–Dansyl procedure and, in case of tryptophan, aspartic acid or asparagine and glutamic acid or glutamine, by the direct identification as PTH-amino acid. T-3 was degraded by the Edman–Dansyl procedure after oxidized with performic acid.

4) W.R. Gray and B.S. Hartley, *Biochem. J.*, **89**, 379 (1963).

the hydrolysis was terminated by adjusting the mixture to pH 2.0 with 6N HCl. The bulk of the guanidinium chloride was removed by dialysis according to the procedure of Craig and King.[5] The tryptic hydrolysate was dialyzed for 40 minutes against 3 succesive portions of 10 volumes of 0.01N HCl in each time, and lyophilized. The lyophilized powder was dissolved in 0.01N-HCl and chromatographed on a column of Amberlite CG-120, and from the result of chromatography four major peptide fractions (T-1, T-2, T-3 and T-4) were obtained as shown in Fig. 1.

The amino acid composition of each peptide fragment are shown in Table II.

Table II. Amino Acid Composition of Tryptic Peptides

	T-1	T-2	T-3	T-4	Sum
Lys		1			1
Arg			1	1	2
Asp	4	2	4		10
Thr	2	4	2	1	9
Ser	2	3	1	1	7
Glu	3	1	2		6
Pro	2	1	2		5
Gly	3	5	3	2	13
Ala	5	5	2	2	14
Cys(½)			2		2
Val	1	3	2	2	8
Ile	1				1
Leu	2	1	1		4
Tyr		1			1
Phe	1	1	1		3
Try	1				1
Total	27	28	23	9	87

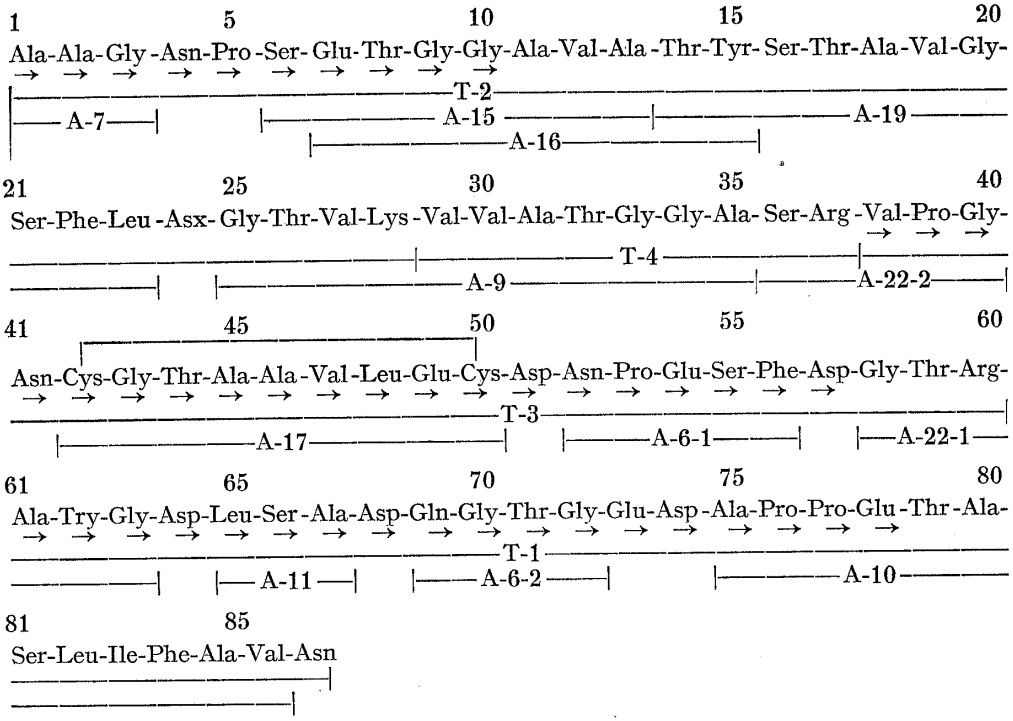

Fig. 3. Proposed Total Amino Acid Sequence of Substance A produced by *Streptomyces Carzinostaticus*

5) L.C. Craig and T.P. King, *J. Am. Chem. Soc.*, 77, 6620 (1955).

N- and C-terminal amino acid of each tryptic peptide and partial amino acid sequence of T-1, T-2 and T-3 were determined as shown in Fig. 2.

T-1 is assigned as the C-terminal portion of native substance A because of the absence of lysine and arginine, and consequently T-2 is assigned as N-terminal portion of substance A since the mother peptide has an alanine as the N-terminal amino acid.

By the combination of the results of the tryptic digestion and the partial acid hydrolysis, the total amino acid sequence of substance A was proposed as shown in Fig. 3.

Addendun (October 15, 1969)

Asx (No. 24) conformed to aspartic acid residue by the Edman degradation of Leu-Asx-Gly-Thr-Val-ε-DNS-Lys, which was obtained from chymotryptic digest of dansylated T-2.

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