

SHORT COMMUNICATIONS

*Glutamycin, A New Peptide-type Antibiotic**

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A *Streptomyces* strain isolated in March, 1954 from a sample collected in Osaka Prefecture resembled the *Zaomycin* producing strain, *S. zaomyceticus*¹⁾, in morphological and cultural characteristics and the utilization of carbon source, and therefore it was named *S. zaomyceticus* No. 7548 tentatively. The strain, when cultivated on a medium containing 3.0% of starch, 1.0% of polypepton, 0.2% of potassium phosphate 0.3% of soybean oil, and 2.0% of rice bran for 96 hr., produced an antibiotic, but the potency lowered abruptly thereafter.

The antibiotic inhibited the growth of Gram-positive bacteria such as *Micrococcus aureus* 209p and *B. subtilis* and was especially active against the bacteria resistant to known antibiotics. Although this antibiotic was similar to *Amphomycin*^{2,3)}, *Zaomycin*¹⁾ and *Crystallomycin*^{4,5)}, it differed from them clearly. The authors, having found it to be a new antibiotic, named it *Glutamycin* and investigated its constituents.

The antibiotic was isolated pure from the broth by Craig's counter current distribution method 300 plates; chloroform-methanol-0.02N hydrochloric acid (2:2:1), and it gave only one spot in paper chromatography and paper ionophoresis at various pH's. M. p. 230°C (decomp.). Found: C, 51.71; H, 7.20; N, 12.99%. From its dissociation constant, the molecular weight was estimated to be about 1800. The antibiotic had its isoelectric point at pH 3.4, and though it showed the typical infrared-spect-

rum characteristic of peptides, it exhibited no peculiar absorption in the ultraviolet-region.

The antibiotic was not hydrolyzed by any hydrolyrase such as pepsin, trypsin and carboxypeptidase, but it was completely hydrolyzed by heating with 6N hydrochloric acid at 110°C for 20 hr. When the hydrolysate was subjected to two dimensional paper chromatography *n*-butanol-acetic acid-water (4:1:5) or 80% phenol containing 0.5N ammonia, six amino acids were detected, four of which were aspartic acid, glycine, valine and proline and the other were unknown. Of the two unknown amino acids, one was a basic amino acid differing from lysine, histidine and arginine on paper chromatogram, and the other was a neutral amino acid which was characterized by a violet blue color with ninhydrin and a blue color with isatin. The six amino acids were separated from each other in crystalline form when chromatographed first on ion-exchange resin (IR-4B, Dowex 50×8) and then on cellulose powder.

After recrystallization from 75% methanol, the unknown basic amino acid showed m. p. 202°C (decomp.), $[\alpha]_D^{25} = +16^\circ$ (c, 1: H₂O), $P_k < 2$, 6.6, 9.6, and gave the values of 1.0 mol. of C-CH₃ (Kuhn-Roth) and 1.8 mol. of amino nitrogen (Van Slyke), and it was different from authentic α, γ -diaminobutyric acid on paper chromatogram. From these facts it was presumed to be α, β -diaminobutyric acid. On the other hand, the unknown neutral amino acid, when recrystallized from methanol-ether, melted at 240°C (decomp.), showed $[\alpha]_D^{25} = +10^\circ$ (c, 1: H₂O) and accorded with C₆H₁₁O₂N·HCl, but no amino nitrogen (Van Slyke) and C-CH₃ (Kuhn-Roth) were detected. This compound was in complete agreement with synthetic DL-pipecolic acid in paper chromatography with various solvent systems, and the optical rotations of its hydrochloride and tartarate were $[\alpha]_D^{25} = +10^\circ$ (c, 1: H₂O) and $[\alpha]_D^{25} = +20^\circ$ (c, 1: H₂O), respectively. As the sign of optical activity of the neutral amino acid was opposite to that of the natural pipecolic acid it belongs to the D-series; the aspartic acid, valine, and proline derived from the antibiotic belong to the L-series.

The hydrolysate mentioned before contained about 10% of a substance soluble in ether, which was found to be a monobasic unsaturated fatty acid, b. p. 139°C (1 mmHg), $[\alpha]_D^{25} = +4^\circ$

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1) Y. Hinuma, *J. Antibiotics*, ser. A 7, 134 (1954).

2) B. Heinemann, M. A. Kaplan, et al., *Antib. & Chemoth.*, 3, 1239 (1953).

3) G. Giolitti, G. Corti et al., *Giorn. Microbiol.*, 3, 70 (1957).

4) G. F. Game et al., *Antibiotiki*, 2, (6) 14 (1957).

5) N. N. Lomakina and M. G. Branzhnikova, *Biokhimiya* 24, 425 (1959).

(c, 1:C₂H₅OH), C₁₃H₂₄O₂. Oxidation of the substance with ozone afforded a compound C₉H₁₈O₂, b. p. 154°C (25 mmHg), $[\alpha]_D^{21} = +12^\circ$ (c. 1:C₂H₅OH), and from this and other results, a 4-isotridecenoic acid structure, C₁₃H₂₄O₂, was assigned to the unsaturated fatty acid.

Thus Glumamycin was assumed to be composed of 4-isotridecenoic acid, L-aspartic acid, glycine, L-valine, L-proline, D-pipecolic acid, and α, β -diaminobutyric acid combined through peptide linkages. Full details of this work will be reported shortly.

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