Constituents of the Antibiotic, Glumamycin

By Michitaka INOUE

(Received February 20, 1961)

In the previous paper[1] it was reported that glumamycin, an acidic peptide antibiotic, consists of six amino acids and an unsaturated fatty acid.

Subsequently another acidic amino acid was detected by paper chromatography in the acidic amino acid portion of the glumamycin hydrolyzate; this acidic portion was submitted to partition column chromatography and colorless needle-like crystals, m. p. 254~256°C, $[\alpha]_D^{20} = +13°$ (*c* 1, 5 N HCl) were obtained (Found: C, 40.54; H, 6.18; N, 9.27; C-CH_3, 10.33. Calcd. for $C_5H_9NO_4$: C, 40.82; H, 6.12; N, 9.52; C-CH_3, 10.20%). The infrared spectrum of this compound was in good agreement with that* of α(L), β-methylaspartic acid (β-Me-L-Asp), separated by Baker et al.[2]

Also in the previous paper[1] the basic amino acid component of glumamycin was presumed to be α, β-diaminobutyric acid (α, β-Dab). In the present work, the basic amino acid was converted with one mole of sodium nitrite

1) M. Inoue et al., This Bulletin, 33, 1014 (1960).

* The author wishes to express his thanks to Dr. N. Bohonos and Dr. J. H. Martin, Lederle Laboratories Division American Cyanamid Co., for their kind information on infrared spectrum of β-Me-L-Asp.

2) H. A. Baker et al., *Arch. Biochem. Biophys.*, 78, 468 (1958).

into α-hydroxy-β-aminobutyric acid monohydrate, m. p. 223°C (Found: C, 35.08; H, 7.98; N, 9.81; H_2O, 13.98%), and oxidation of the amino acid with periodic acid gave acetaldehyde. Thus, the structure of the basic amio acid was established to be α, β-diamino-*n*-butyric acid.

For further confirmation of this structure, an attempt** was made to synthesize α, β-diamino-*n*-butyric acid from ethyl α, β-dibromobutyrate via the azide compound II by the method used for the synthesis of β-alanine by Horner et al.[3] Hydrolysis with hydrobromic acid of the phosphin imine compound, obtained by the reaction between II and triphenyl phosphin, produced α, β-Dab, m. p. 178°C (decomp.) (IIIa), and reduction of II under high pressure yielded IIIa and hydrochloride of its isomer (IIIb), m. p. 199~201°C (decomp.). Benzoylation of IIIa by Schotten-Baumann's method gave IV, m. p. 216~218°C (Found: C, 66.14; H, 5.41; N, 8.73%). The ethyl α, β-dibenzoylaminobutyrate (V) from IV and from glumamycin exhibited the same infrared spectrum in chloroform.

The DNP-glumamycin obtained by Sanger's[4] method was hydrolyzed with 6 N hydrochloric acid and the hydrolyzate was extracted with ether, but no DNP-amino acid was found in the ether-soluble portion. Therefore the free amino group of glumamycin was assumed to be the α- or β-amino group of the α, β-Dab of glumamycin. The water soluble portion of the hydrolyzate was spotted on paper in the *n*-butanol, acetic acid, water (4 : 1 : 5) system, and gave a spot at R_f 0.68. On the other hand, in this system the β-mono-DNP-α, β-diaminobutyric acid had a R_f of 0.68 and α-mono-DNP-α, β-diaminobutyric acid had a R_f of 0.75. These two DNP-derivatives were prepared by partial dinitrophenylation of α, β-Dab from glumamycin hydrolyzate. Therefore the fact that a DNP-amino acid with R_f 0.68 was obtained from the DNP-glumamycin hydrolyzate shows that, in glumamycin, the β-amino group of α, β-Dab was dinitrophenylated. Consequently the β-amino group of the α, β-Dab is free, and the α-amino group takes part in the peptide linkage in glumamycin.

The amino acid components of glumamycin were determined quantitatively. Four moles of Asp, 2 mol. of Gly, 1 mol. of Pro, and 1 mol. of Val were detected by the method of Moore and Stein[5], but in this method, β-Me-L-Asp had the same elution curve with that of Asp, pipecolic acid gave an anomalous ninhydrin coloration, and α, β-Dab was not eluted respectively. For the determination of these three abnormal amino acids, the method of Levy[6] which comprises subjecting DNP-amino acids to paper chromatography was used. To obtain the analytical values, the determination was conducted after ascertaing that the dinitrophenylation rate and elution rate of each amino acid can be measured with an error within ±4%. Even with two dimensional paper chromatography (the first solvent; *n*-butanol saturated with 2 N aqueous ammonia, the second solvent; 1.5 M phosphate buffer)[7], Asp could not be separated from β-Me-L-Asp, but the analytical values of 4 mol. of Asp (including β-Me-L-Asp), 2 mol. of Gly, 1 mol. each of Pro, Val, and 2 mol. of α, β-Dab were in good agreement with the results by the method of Moore, Stein.

In the previous paper[1] the molecular weight of glumamycin was supposed to be about 1800 from its titration value, and about 1500 from the yield of unsaturated fatty acid. However, the molecular weight is calculated to be about 1300 from the result of the above amino acid determination; therefore about 1300 seems to represent the correct minimum molecular weight*** of glumamycin.

Aspartocin[8] in an antibiotic closely resembling glumamycin, but it is clearly different from the latter in the behavior in paper chromatography.

Research Laboratories
Takeda Chemical Industries, Ltd.
Higashiyodogawa-ku, Osaka

** Synthesis of α, β-Dab was carried out by Neuberg (*Biochem. Z.*, **1**, 282 (1906)), but since it could not be isolated in crystalline form, it was converted to the phenylisocyanate and picrate. Recently, Martin[8] attempted the synthesis but did not report the results in detail.

3) L. Horner and A. Gross, *Ann.*, **591**, 117 (1955).
4) F. Sanger, *Biochem. J.*, **39**, 507 (1945); **45**, 563 (1949).
5) S. Moore and W. H. Stein, *J. Biol. Chem.*, **192**, 663 (1951); **176**, 367 (1948).
6) A. L. Levy, ibid., **213**, 187 (1955); **213**, 487 (1955); *Nature*, **174**, 126 (1954).
7) K. Satake et al., Kagaku no Ryoiki Supplementum, **34**, 77 (1959).
*** The molecular weight of glumamycin is calculated to be 1350 from the adsorbance of DNP-glumamycin (M. Fujino, M. Inoue and A. Miyake; unpublished).
8) J. H. Martin et al., *J. Am. Chem. Soc.*, **60**, 2079 (1960).