On Glumamycin, a New Antibiotic. II[*1]. *Isolation and Identification of Amino Acids Constituting Glumamycin*

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Glumamycin[1-3], an antibiotic isolated from the culture broth of a soil streptomyces *Streptomyces zaomyceticus* No. 7548, shows a strong activity against Gram positive bacteria, particularly penicillin- and tetracycline-resistant bacteria such as *M. aureus* and *B. subtilis*. It is interesting to note that glumamycin is more active in vivo than in vitro[4]. The author has long been engaged in the investigation of glumamycin, and as described in previous communications[2,3] the antibiotic was found to consist of the following amino acids; L-aspartic acid, α(L), β-methylaspartic acid, L-proline, L-valine, glycine, D-pipecolic acid, and α, β-diaminobutyric acid. This paper deals with the identification of these amino acids.

As already reported[1,2] glumamycin was extracted from a filtered broth with isoamylalcohol or *n*-butanol at pH 2.0, transferred to water at pH 7.0~8.0, and separated from brown pigments by column chromatography on active carbon. Further purification was effected by Craig's countercurrent distribution (300 transfers).

Pure glumamycin, a white crystalline powder, is soluble in water and methanol, and when dissolves in a sodium hydrogen carbonate solution, produces carbon dioxide. But it is insoluble in chloroform, benzene and ethyl acetate. Analysis of glumamycin gives a value consistent with the molecular formula of $C_{59}H_{91}O_{20}N_{13}$. The antibiotic is an optically

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1) M. Inoue, K. Nakazawa, A. Miyake et al., *J. Antibiotics*, A15, 1 (1962).
2) M. Inoue et al., This Bulletin, 33, 1014 (1960).
3) M. Inoue, ibid., 34, 885 (1960).
4) Y. Oka, M. Matsui and T. Araki, *Ann. Rept. Takeda Research Lab.*, 20, 207 (1961).

active substance and gives biuret reaction but no ninhydrin reaction. Infrared spectrum of glumamycin shows the presence of the linkage characteristic of peptide, but its ultraviolet spectrum exhibits no specifuity. From these results glumamycin was presumed to be an acidic peptide, so its amino acids were investigated by hydrolytic degradation. Hydrolysis of glumamycin with 6 N hydrochloric acid separated an oily substance, which was removed by extraction with ether. In the water layer seven amino acids were detected by paper chromatography and paper electrophoresis, of which two were acidic, one was basic, and the remaining four were neutral. In general, peptide-type antibiotics so far known belonged to D-series or specific amino acids, so the seven amino acids were isolated by a combination of ion-exchange chromatography and partition chromatography, and their properties were investigated.

One of the two acidic amino acids was L-aspartic acid and three of the four neutral amino acids were L-proline, L-valine and glycine, and they were identified by their infrared spectrum, optical activity and behavior in paper chromatography.

One of the remaining three amino acids was obtained as fine needles, $C_5H_9NO_4$, and though acidic, it was not identical with glutamic acid on paper chromatography. The compound was presumed to be methylaspartic acid from detection of one C–CH_3 group by the Kuhn-Roth method, and identified as α(L), β-methylaspartic acid[5] from its optical rotation, melting point and infrared spectrum*3.

Another compound of the three unidentified amino acids was isolated as a neutral amino acid, fine needles, $C_6H_{11}NO_2 \cdot HCl$, and containing no *N*-methyl group and no amino-nitrogen, and giving a specific color with ninhydrin, it was presumed to be a piperidine carboxylic acid. The compound was compared with synthetic DL-pipecolic acid (piperidine-α-carboxylic acid) and its isomers by paper chromatography, and it was assumed to be pipecolic acid. Further, as the optical rotation of the compound was in reverse relation with that of L-pipecolic acid monohydrochloride obtained from a plant[6], it was presumed to be D-pipecolic acid. Therefore, its (+)tartrate[7] was compared with D-pipecolic acid (+)tartrate obtained by optical resolution of synthetic DL-pipecolic acid, and they were observed to be in good agreement in the melting point, optical rotation and infrared spectrum. These findings establish conclusively that the neutral amino acid is D-pipecolic acid.

TABLE I. R_f VALUES OF THREE ISOMERS OF PIPERIDINE CARBOXYLIC ACID ON P. P. C. BY VARIOUS SOLVENT SYSTEMS

	Solvent systems		
	A	B	C
DL-Pipecolic acid	0.45 (0.44)	0.48 (0.45)	0.92 (0.92)
D-Pipecolic acid (from glumamycin)	0.45	0.48	0.92
L-Proline	0.34 (0.34)	0.36 (0.34)	0.90 (0.90)
Nipecotic acid	(0.34)	(0.33)	(0.95)
Isonipecotic acid	(0.34)	(0.28)	(0.95)

R_f Values in bracket are from "Chromatographic Technique" ed. by I. Smith, William Heinemann Medical Books, Ltd. London (1958).

The remaining one of the three unidentified amino acids, which was basic on paper electrophoresis, was isolated as hydrochloride, $C_4H_{10}N_2O_2 \cdot HCl$. Although the molecular formula of this amino acid was in accord with that of α,γ-diaminobutyric acid obtained from

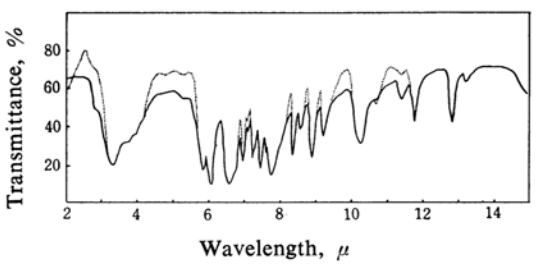


Fig. 1. Infrared absorption spectrum of α(L), β-methylaspartic acid.
—— α(L), β-Methylaspartic acid (from glumamycin), KBr
····· α(L), β-Methylaspartic acid (H. A. Barker[5]), KCl

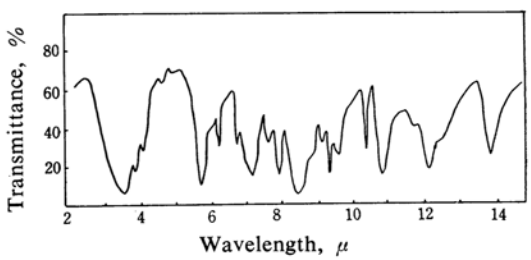


Fig. 2. Infrared absorption spectrum of D-pipecolic acid, KBr.

5) H. A. Barker et al., *Arch. Biochem. Biophys.*, **78**, 468 (1958).

*3 The author thanks Dr. N. Bohonos and Dr. J. Martin, Lederle Laboratories Division of American Cyanamid Co., for their kind information on IR spectrum of α(l), β-methylaspartic acid.

6) R. M. Zacharins, J. F. Tompson and F. C. Steward, *J. Am. Chem. Soc.*, **76**, 2908 (1954).

7) R. L. Morrison, *Biochem. J.*, **50**, 474 (1953).

TABLE II. COMPARISON WITH α, β-DIAMINOBUTYRIC ACID AND α, γ-DIAMINOBUTYRIC ACID

	M. p., °C		R_f Value Solvent systems	
	Monohydrochloride	Monopicrate	A	B
α, β-Diaminobutyric acid (from glumamycin)	202	209	0.15	0.32
α, γ-Diaminobutyric acid (synthetic)	228~230	184~185	0.13	0.20

such antibiotics as Polymixin B[8], it seems different from α, γ-diaminobutyric acid because it gave a values of nearly one mole of C-CH_3 by the Kuhn-Roth method, and the melting points of its hydrochloride and picrate and its R_f-value were all different from those of α, γ-diaminobutyric acid. So this basic amino acid was presumed to be α, β-diamino-*n*-butyric acid or α, β-diaminoisobutyric acid. To clarify its identity the amino acid was deaminated with one mole of sodium nitrate carefully and resulting ninhydrin positive product $C_4H_9O_3N \cdot H_2O$, which gave acetaldehyde on oxidation with periodic acid, was assumed to be α-hydroxy-β-aminobutyric acid, and thus the abovementioned basic amino acid was established to be α, β-diamino-*n*-butyric acid.

To confirm the result more positively, attempt was made on the synthesis of α, β-diaminobutyric acid. As to the synthesis there is found no other report than that of Neuberg[9], who effected it with α, β-dibromobutyric acid and aqueous ammonia under pressure, but as the product was a syrupy substance, it was isolated as phenylisocyanate or picrate. However, since the α-bromine seems readily to be split off in this method as pointed out by Neuberg[9], the present author employed the method used by Horner[10] for the synthesis of β-alanine. Namely, ethyl α, β-dibromobutyrate (I) was allowed to react with sodium azide, the resulting ethyl α, β-diazidobutyrate (II) was further reacted with triphenylphosphine, and the phosphinimide thus produced was hydrolyzed with hydrobromic acid to give α, β-diaminobutyric acid (IIIa). The diamino acid was also obtained by reducing the diazide compound under high pressure and hydrolyzing the reaction mixture directly, but in this case, besides IIIa, its isomer IIIb crystallizable as hydrochloride was obtained.

Benzoylation of IIIa and IIIb by Schotten-Baumann's method afforded IVa, m. p. 216°C and IVb, m. p. 106~108°C, respectively. Dehydration of IVa with benzoyl chloride in pyridine yielded a substance assumable as the corresponding azlactone, which was converted to ethyl α, β-dibenzoylaminobutyrate (Va) by boiling with ethanol. The infrared spectrum of the product was in accord with that of α, β-dibenzoylaminobutyrate prepared by the same method from the dibenzoyl compound derived from glumamycin.

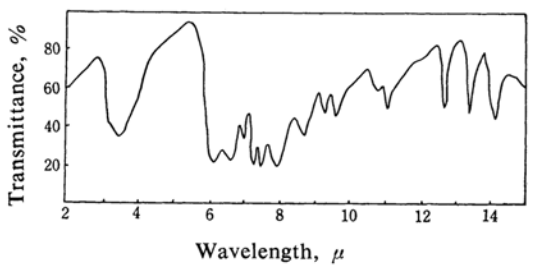


Fig. 3. Infrared spectrum of α, β-diaminobutyric acid monopicrate, KBr.

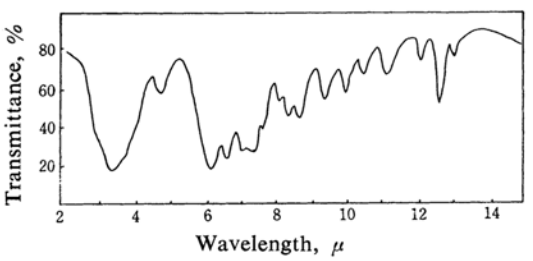


Fig. 4. Infrared absorption spectrum of α, β-diaminobutyric acid monohydrochloride, KBr.

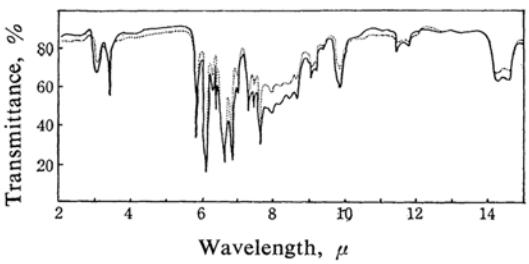


Fig. 5. Infrared absorption spectrum of ethyl dibenzoylaminobutyrate, KBr.
—— From glumamycin
····· Synthetic

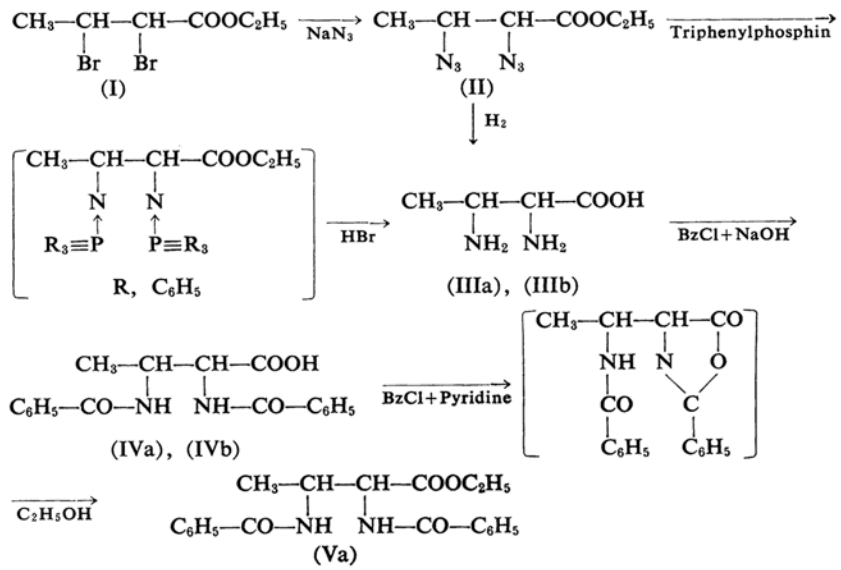
From the results mentioned above the plane structure of the basic amino acid of glumamycin was established to be α, β-diamino-*n*-butyric acid.

Of the seven amino acids constituting glumamycin, α(L), β-methylaspartic acid, D-pipecolic acid and α, β-diaminobutyric acid are the first to be isolated as components of peptide-type compounds. It was found that glumamycin

8) W. Hausmann, *J. Am. Chem. Soc.*, **78**, 3668 (1956).

9) C. Neuberg, *Biochem. Z.*, **1**, 282 (1960).

10) L. Horner and A. Gross, *Ann.*, **591**, 117 (1955).



also follows the rule that peptide-type antibiotics contain always D-series amino acids.

Experimental

Paper Chromatography.—Whatman No. 1 paper and Toyo Roshi No. 50 paper were employed with the following solvent systems: A, *n*-butanol-acetic acid water (4 : 1 : 5); B, *n*-butanol-pyridine-water (1 : 1 : 1); C, phenol-water (4 : 1).

Paper Electrophoresis. — Whatman No. 1 paper employed with the following conditions: Buffer; 2 N acetic acid. Voltage; 300 V. Time; 3 hr.

Glumamycin. — The crude antibiotic which was obtained as described in a previous paper[1] was purified by countercurrent distribution (300 transfer : 20 ml.) in the system chloroform-methanol-0.02 hydrochloric acid (2 : 2 : 1). The materials in the tubes (158~168), whose distribution corresponded closely to the theoretical $k=1.17$, were combined and recrystallized to yield the material for all degradative studies. Glumamycin was obtained as a white crystalline powder by recrystallization from a methanol-ethyl acetate mixture, m. p. 230°C (decomp.), $[\alpha]_D^{20}$ $+8.5\pm0.5$ (*c* 1, in ethanol).

Found: C, 54.58; H, 7.25; N, 13.60. Calcd. for $C_{59}H_{91}O_{20}N_{13}$: C, 54.25; H, 6.99; N, 13.99%.

Acid Hydrolysis.—Hydrolysis of glumamycin at 110°C for 12 hr. with 6 N hydrochloric acid and, after removing the ether soluble material and working up in the usual way, gave a mixture of amino acids which, when examined by paper chromatography (solvent system A, Toyo Roshi No. 50) and paper electrophoresis, was found to contain seven amino acids. Prolonged heating with hydrochloric acid did not change the composition of this mixture.

Separation and Identification of Hydrolyzate.—The acid hydrolyzate of the antibiotic (5 g.) was treated with ether and concentrated in vacuo in the usual way.

Aspartic Acid.—The above residue was dissolved in 200 ml. of water. The solution was put on a column (3 cm.×20 cm.) of Amberlite IR-4B (OH-form). The column was eluted with 1 N hydrochloric acid, the ninhydrin positive fraction was collected and concentrated under reduced pressure, and the residue was recrystallized from ethanol to give colorless granular crystals, m. p. 160°C $[\alpha]_D^{23}$ +23° (*c* 8, in 6 N hydrochloric acid).

Found: C, 36.10; H, 5.41; N, 10.30%.

α(L), β-Methylaspartic Acid.—The mother liquor from the aspartic acid was evaporated to dryness and the residue was subjected to partition chromatography, using a column (3 cm.×35 cm.) of cellulose powder and developing with *n*-butanol-acetic acid-water (120 : 30 : 50). Amino acids were detected in the eluate fractions (5 ml.) by subjecting them to paper chromatography (solvent system A). The fractions showing a single spot at about R_f 0.35, which colored purplish red with ninhydrin, were combined and evaporated to dryness. The residue was dissolved in water, the solution was passed through a column of Amberlite IR-120 (H-form), and the column, after washing with water and 0.2 N acetic acid, was eluted with 0.2 N aqueous ammonia. The eluate was concentrated under reduced pressure, the residue was dissolved in aqueous butanol, the acetone was added to the solution, giving colorless needles, which were purified by recrystallization from aqueous ethanol, m. p. 254~256°C (decomp.), $[\alpha]_D^{21}$ +13.0° (*c* 1, in 5 N hydrochloric acid).

Found: C, 40.52; H, 6.18; N, 9.27; C-CH_3, 10.33. Calcd. for $C_5H_9NO_4$: C, 40.80; H, 6.12; N, 9.52; C-CH_3, 10.20%.

Separation of Neutral Amino Acids.—The above effluent from Amberlite IR-4B was combined with the washing and evaporated to dryness in vacuo. The residue was dissolved in 20 ml. of ammonium acetate buffer (pH 5.0), the solution was passed through a column (8 cm.×40 cm.) of Dowex 50-X8 (100~200 mesh) treated beforehand with the same buffer, and the column was eluted with the same buffer. The effluent was collected in fractions of 200 ml. each and investigated by paper ionophoresis, finding that all neutral amino acids were eluted out in 200~800 ml. of the effluent.

This portion of the effluent was evaporated to dryness under reduced pressure and the residue was extracted several times with 30 ml. portions of methanol to divide it into methanol soluble and methanol insoluble parts.

D-Pipecolic Acid.—The solution of the methanol soluble part was evaporated to dryness and developed on a column (3 cm.×35 cm.) of cellulose powder with *n*-butanol-acetic acid-water (4 : 1 : 5). Of the effluent the part which flowed out first and gave a single spot coloring bluish purple with ninhydrin was evaporated under reduced pressure, and the residue was recrystallized from a mixture of methanol and ether, m. p. 240°C (decomp.), $[\alpha]_D^{21}$ +10.0° (*c* 1, in water).

Found: C, 43.82; H, 7.47; N, 8.76; Cl, 22.61. Calcd. for $C_6H_{11}NO_2 \cdot HCl$: C, 43.50; H, 7.30; N, 8.76; Cl, 21.40%.

D-Pipecolic Acid (+)Tartrate. — According to the method of Morrison[7], an aqueous solution of 250 mg. of the above pipecolic acid monohydrochloride was passed through a column (0.9 cm.× 6.0 cm.) of Amberlite IR-4B (OH-form) to remove the hydrochloric acid and evaporated to dryness. The residue and 232 mg. of (+)tartaric acid were dissolved in 2.5 ml. of hot methanol and the solution was allowed to cool, precipitating the desired product, which melted at 187°C after recrystallization from ethanol.

Found: C, 43.54; H, 6.06; N, 4.80. Calcd. for $C_{10}H_{17}O_8N$: C, 43.00; H, 6.14; N, 5.02%. $[\alpha]_{25}^{D}$ +20° (*c* 10, in water). D-Pipecolic acid (+)tartrate[7], m. p. 187°C, $[\alpha]_D^{25}$ +18° (*c* 10, in water).

Proline.—In the treatment of the methanol soluble part mentioned before, an effluent giving a yellow ninhydrin reaction flowed out after the eluate of D-pipecolic acid. This portion was evaporated and the residue was recrystallized from *n*-butanol to yield prisms, m. p. 218°C (decomp.) $[\alpha]_D^{21}$ −82° (*c* 1, in water).

Found: C, 52.13; H, 8.04; N, 11.87%.

Valine.—The methanol insoluble part mentioned above was developed on a column of cellulose powder with *n*-butanol-acetic acid-water (4 : 1 : 5). The effluent which flowed out first and gave a single ninhydrin positive spot was evaporated and the residue was recrystallized from water, furnishing colorless prisms, m. p. 312°C, $[\alpha]_D^{23}$ +25° (*c* 3.4, in 6 N hydrochloric acid).

Found: C, 51.10; H, 9.46; N, 11.98%.

Glycine.—The eluate which flowed out after valine and which exhibited a single ninhydrin positive spot corresponding to glycine was evaporated and the residue was recrystallized from aqueous methanol yielding prisms, m. p. 233°C.

Found: C, 32.34; H, 7.20; N, 18.69%.

Synthesis of DL-Pipecolic Acid. — A mixture of 10 g. of α-bromo-ε-benzoylaminocapric acid, 100 ml. of water, 100 g. of acetic acid, 100 g. of concentrated hydrochloric acid was boiled under reflux for 6 hr. The reaction mixture was evaporated under reduced pressure to remove the hydrochloric acid and acetic acid, and an aqueous solution of the residue was passed through a column of Amberlite IR-120 (H-form), the effluent being discarded. The column was washed with water and eluted with 0.5 N aqueous ammonia, and the eluate was evaporated to dryness under reduced pressure. The residue was extracted with ethanol, the extract, after addition of a small amount of hydrochloric acid, was evaporated again to dryness, and the residue was recrystallized from a mixture of ethanol and acetone to yield colorless needles, m. p. 240°C (decomp.), $[\alpha]_D^{21}$ 0 (*c* 1, in water).

Found: C, 43.62; H, 7.42; N, 8.80; Cl, 22.34%.

Optical Resolution of DL-Pipecolic Acid. — D-Pipecolic acid (+)tartrate was prepared from DL-pipecolic acid monohydrochloride by the method of Morrison[7] as in the case of D-pipecolic acid described before. The melting point and optical rotation were 187∼188°C and $[\alpha]_D^{25}$ +20° (*c* 1, in water), respectively.

Found: C, 43.80; H, 6.06; N, 4.08. Calcd. for $C_{10}H_{17}O_8N$: C, 43.00; H, 6.14; N, 5.02%.

α,β-Diaminobutyric Acid Monohydrochloride.— The column Dowex 50-X8, which was eluted with 1000 ml. of ammonium acetate buffer (pH 5.0) as mentioned before, was further eluted with 0.25 N aqueous ammonia, and it was found by paper ionophoresis that 400∼800 ml. of the effluent contained a basic amino acid. This fraction was evaporated under reduced pressure, the residue was dissolved in a small amount of hydrochloric acid, and the solution, after addition of mathanol and ether, was allowed to stand in a cool place, whereupon colorless needles melting at 202°C (decomp.) after recrystallization from aqueous ethanol precipitated.

Found: C, 31.39; H, 7.30; N, 17.64; Cl, 21.99. Calcd. for $C_4H_{10}O_2N_2 \cdot HCl$: C, 31.06; H, 6.48; N, 18.11; Cl, 22.95%. $[\alpha]_D^{22}$ +19° (*c* 1, in 6 N hydrochloric acid), $[\alpha]_D^{22}$ +16° (*c* 1, in water), $pk_a' < 2$, 6.6, 9.6. Treated with picric acid it gave α,β-diaminobutyric acid monopicrate, m. p. 209°C (decomp.).

Found: C, 34.72; H, 3.84; N, 19.95. Calcd. for $C_4H_{10}N_2O_2 \cdot C_6H_3O_7N_3$: C, 34.59; H, 3.74; N, 20.10%.

Benzoylated by the Schotten-Bauman's method it gave α,β-dibenzoylaminobutyric acid, m. p. 210°C.

Found: C, 66.14; H, 5.59; N, 8.42. Calcd. for $C_{18}H_{18}O_4N_2$: C, 66.24; H, 5.56; N, 8.58%.

α-Hydroxy-β-aminobutyric Acid.—To a solution in 30% acetic acid of 1 g. of α,β-diaminobutyric acid from glumamycin was added dropwise a solution of 4.5 g. of sodium nitrite in 10 ml. of water over a period of about 1 hr. with stirring and cooling with ice water. The reaction mixture was left standing for 10 hr. The product, diluted with water, was absorbed on a column of Amberlite IR-120 (H-form) and the column was eluted with 1 N aqueous ammonia. The eluate was evaporated in vacuo, and the residue was recrystallized from water, m. p. 123°C (decomp.).

Found: C, 35.08; H, 7.98; N, 9.81; H_2O, 13.98. Calcd. for $C_4H_9O_3N \cdot H_2O$: C, 35.03; H, 8.03; N, 10.21; H_2O, 13.00%.

Periodic Acid Oxidation of α-Hydroxy-β-aminobutyric Acid. — To a solution of 113 mg. of α-hydroxy-β-aminobutyric acid in 10 ml. water was added dropwise a solution of 230 mg. periodic acid in 5 ml. of water with stirring and cooling with ice water. The reaction mixture was left standing

for 2 hr. at room temperature and the product was distilled with 20 ml. of water. To the distillate was added a solution of 180 mg. of dimedon in aqueous alcohol and the resulting colorless substance was recrystallized from aqueous alcohol, m. p. 140°C. Yield, 220 mg. A mixed melting point with authentic acetaldodimedon (m. p. 140°C) showed no depression and infrared spectrum of the two were quite identical.

Ethyl α, β-Diazidobutyrate (II).—To a solution of 27.4 g. of ethyl α, β-dibromobutyrate (I) in 137 ml. of ethanol was added dropwise a solution of 19.5 g. of sodium azide in 78 ml. of water over a period of about 3 hr. with stirring and heating on a water-bath. After the reaction was completed by heating about one hour more, the ethanol was distilled off under reduced presssure, and the aqueous solution was extracted with benzene. The benzene solution, after washing with water, diluted hydrochloric acid and sodium hydrogen carbonate solution successively and drying over anhydrous sodium sulfate, was evaporated under reduced pressure, leaving 16.8 g. of a residue. The residue was distilled in vacuo and the portion b. p. 70~85°C/2 mmHg. (11.2 g. or 56.6%) was redistilled to give the diazide compound, b. p. 80~83°C/2 mmHg.

DL-α, β-Dibenzoylaminobutyric Acid (IVa), (IVb).—i) To a solution of 5 g. of the diazide compound II in 100 ml. of benzene was added dropwise a benzene solution of 13 g. of triphenylphosphin with stirring, when a reaction set in with evolution of heat and nitrogen gas. The reaction temperature was kept at 30°C. After addition of triphenylphosphin the reaction mixture was heated on the water-bath for 2 hr. and then the benzene was distilled off. The crude phosphinimine compound thus obtained was heated with 50 ml. of 40% hydrobromic acid, the reaction mixture was concentrated under reduced pressure and after dilution with water, the precipitate was filtered off. The filtrate was passed through a column of Amberlite IR-120 (H-form), the column was eluted with 4 N aqueous ammonia, and the eluate was evaporated to dryness under reduced pressure, leaving a residue, which gave crystals by addition of ethanol, m. p. 178°C (decomp.).

This compound IIIa was benzoylated by the method of Schotten-Baumann and the resulting crude product was recrystallized from ethanol after treating it with ligroin, m. p. 216~218°C.

Found: C, 66.14; H, 5.41; N, 8.73. Calcd. for $C_{18}H_{18}O_4N_2$: C, 66.24; H, 5.56; N, 8.58%.

ii) A solution of 20 g. of the azide compound in 80 ml. of ethanol was reduced on Adams platinum oxide at room temperature under 100 atmospheric pressure. The catalyst was filtered and the filtrate was evaporated under reduced pressure to give a light yellow syrupy substance. The substance was boiled with 175 ml. of 20% hydrochloric acid for 3 hr., the hydrolyzate was evaporated in vacuo, and the residue was allowed to stand with ethanol-ether, separating 5.5 g. (37.6%) of white prisms (IIIb), m. p. 199~201°C (decomp.). The mother liquor was concentrated in vacuo, the residue was dissolved in water, and the solution was treated by the same method in IIIa, giving 3 g. of IIIa, m. p. 178°C.

Compound IIIb was benzoylated by the same method and the resulting product was recrystallized from aqueus ethanol, yielding white needles, m. p. 106~108°C.

Found: C, 62.82; H, 5.81; N, 8.20. Calcd. for $C_{18}H_{18}O_4N_2 \cdot H_2O$: C, 62.78; H, 5.85; N, 8.14%.

DL-Ethyl α, β-Dibenzoylaminobutyrate (Va). To a solution of 700 mg. of the dibenzoyl compound (IVa) in 7 ml. of pyridine was added 0.2 ml. of benzoyl chloride under cooling with ice water. After one hour, the reaction mixture was poured onto chipped ice, neutralized with hydrochloric acid, and extracted with ethyl acetate. The extract, after washing with water, sodium hydrogen carbonate solution and water successively and drying over anhydrous sodium sulfate, was evaporated under reduced pressure and the residue was treated with ether, leaving a substance, m. p. 187°C, which was assumed to be an azlactone. The substance, was dissolved in ethanol, the solution, after being boiled for one hour, evaporated and the residue was recrystallized from aqueous ethanol to given colorless needles, m. p. 187°C.

Found: C, 67.00; H, 6.20; N, 7.68. Calcd. for $C_{20}H_{24}O_4N_2$: C, 67.78; H, 6.26; N, 7.91%.

Summary

Isolation of the amino acids constituting glumamycin, a new antibiotic, was effected. As a result, α(L), β-methylaspartic acid, besides L-aspartic acid was obtained as an acidic amino acid. In addition to L-valine, glycine, and L-proline, D-pipecolic acid was yielded as a neutral amino acid. Further, α, β-diaminobutyric acid was obtained as a basic amino acid and its structure was established to be α, β-diamino-*n*-butyric acid. For positive confirmation of the structure the acid was synthesized, and it was found that the *N*, *N*-dibenzoyl ethyl ester of the synthetic product and that of the basic amino acid derived from glumamycin were in complete agreement.

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