On Glumamycin, a New Antibiotic. V.* The Steric Configuration of α, β-Diaminobutyric Acid

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The authors[1] previously isolated α, β-diaminobutyric acid (α, β-Dab) from the acid hydrolysate of glumamycin, an acidic peptide-type antibiotic. About the same time, Martin et al.[2] reported the separation of α, β-Dab from Aspartocin, an antibiotic resembling glumamycin. Although the two products seem to have the same configuration, judging from their optical rotation, this question has so far not been studied.

In a previous paper of this series[3] it was shown that glumamycin contains two moles of α, β-Dab and that only one β-amino group of the α, β-Dab is free. These facts were then utilized to determine the steric configuration of α, β-Dab.

The configuration of the α-carbon of α, β-Dab has been studied by the conversion of the free β-amino group of glumamycin into a hydroxyl group and by subsequent hydrolysis to α-amino-β-hydroxybutyric acid, and that of the β-carbon of α, β-Dab, by the oxidation of α, β-Dab to alanine.

That is, glumamycin was treated with sodium nitrite in 80% acetic acid to convert the free amino group into hydroxyl, and then the resulting deamino-glumamycin was hydrolyzed with hydrochloric acid. The reaction mixture was developed on a column of Dowex 50 with an ammonium formate buffer (pH 3.4),[4] after which a crystalline substance was obtained from the threonine fraction. The product was found to be a mixture of threonine and allothreonine by paper chromatography with *n*-butanol - acetone - ammonia - water (50 : 6.25 : 6.25 : 37.5)[5] and by a study of its infrared spectrum (Fig. 1).

Fortunately, it is known that DNP-L-threonine and DNP-L-allothreonine exhibit the same optical rotation, $[M]_D^{25} = +305°$ (4% sodium bicarbonate).[6] The above product was converted into its DNP derivative by the method of Levy[7] and then submitted to column chromatography on Amberlite IRC-50, according to the method of Seki,[8] in order to remove the DNP-OH which was formed as a by-product. The DNP derivative showed an optical rotation of $[M]_D^{24} = -294° \pm 14$ (*c* 0.48, 4% sodium bicarbonate), and its yield was 92%. The results mentioned above show that the threonine and allothreonine obtained in the present work take the D-configuration; consequently, the α-carbon of α, β-Dab with the free β-amino group belongs to the D series. Further, as the α, β-Dab obtained from the hydrolysate of deamino-glumamycin and that obtained from the hydrolysate of glumamycin have the same optical rotation and physical properties, the two moles of α, β-Dab in glumamycin evidently have the same steric configuration.

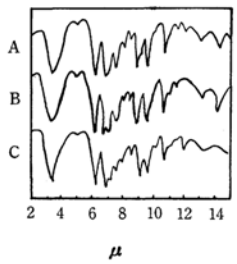

Fig. 1. Infrared-Spectra of α-amino-β-hydroxybutyric acid.
A: α-Amino-β-hydroxybutyric acid from deamino-glumamycin
B: L-Threonine
C: L-Allothreonine

Next, in order to establish the configuration at the β-carbon of α, β-Dab, it was oxidized with hydrogen peroxide according to the method of Dakin.[9] That is, the compound was allowed to react with 2 mol. of 3% hydrogen peroxide at 50°C for one hour in the presence of a small amount of ferrous sulfate. The reaction mixture was then passed

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1) M. Inoue, H. Hitomi, K. Mizuno, M. Fujino, A. Miyake, K. Nakazawa, M. Shibata and T. Kanzaki, This Bulletin, **33**, 1014 (1960).
2) J. H. Martin and W. K. Hausmann, *J. Am. Chem. Soc.*, **82**, 2079 (1960).
3) M. Inoue, This Bulletin, **35**, 1557 (1962).
4) C. H. W. Hirs, S. Moore and W. H. Stein, *J. Biol. Chem.*, **159**, 669 (1952).
5) K. N. F. Shaw and S. W. Fox, *J. Am. Chem. Soc.*, **75**, 3421 (1953).
6) K. R. Rao and H. A. Sober, ibid., **76**, 1328 (1954).
7) A. L. Levy, *Nature*, **174**, 126 (1954).
8) T. Seki, *Chemistry of Proteins (Tokyo)*, **4**, 209 (1956).
9) H. D. Dakin, *J. Biol. Chem.*, **1**, 171 (1905); **4**, 63 (1908).

through a column of Amberlite IR-120 (H^+), and the adsorbed substance was eluted with N ammonia, the effluent giving D-alanine. Thus, it was found that the configuration at the β-carbon of α,β-Dab belongs to the D series and that, consequently, α,β-Dab takes the D-erythro-configuration.

Experimental

Deamino-glumamycin.—Into a solution of 10 g. (ca. 7.7 mmol., mol. wt. taken as 1300*) of glumamycin in 100 ml. of 80% acetic acid, 1.06 g. (ca. 15.4 mmol.) of sodium nitrite and 11.6 ml. of N hydrochloric acid were stirred alternately over a period of 5 hr. at 0°C; the mixture was then left standing at room temperature for 12 hr.

The reaction mixture was concentrated to 20 ml. under reduced pressure, and water was added to separate the deamino-glumamycin. The precipitate was washed three times with water to remove the unchanged glumamycin, which is soluble in water; it was dissolved in acetone and reprecipitated by the addition of water. The product was washed twice with water and dried, giving 8 g. of deamino-glumamycin**.

The Hydrolysis of Deamino-glumamycin with Hydrochloric Acid.—Two grams of deamino-glumamycin was hydrolyzed by heating it with 40 ml. of 6 N hydrochloric acid at 110°C for 20 hr. in an oil bath. The hydrolysate was concentrated in vacuo, water was added to the residue, and the mixture was evaporated to dryness under reduced pressure.

The Isolation of the Threonine Faction.—The above residue was dissolved in 100 ml. of water, the solution was passed through a column (3×10 cm.) of Amberlite IR-4B (OH form) to remove acidic substances, and the effluent was evaporated to dryness in vacuo.

The residue was dissolved in 10 ml. of an ammonium acetate buffer (pH 5.0), and the solution was passed through a column (7×20 cm.) of Dowex-50×8 (200—400 mesh) treated beforehand with the same buffer.

The effluent was collected in 50-ml. fractions and examined by paper chromatography. All the neutral amino acids constituting deamino-glumamycin were then eluted out in 100—300 ml. of the effluent.

This portion of the effluent was evaporated to dryness under reduced pressure. The residue was dissolved in 10 ml. of an ammonium formate buffer (pH 3.4), and the solution was again passed through a column (6×60 cm.) of Dowex-50×4 (200—400 mesh) bufferized at pH 3.4. The fraction of the effluent showing the same R_f-value as threonine by paper chromatography with *n*-butanol - acetic acid - water (120 : 30 : 50) was collected and poured on a column of Amberlite IR-120 (H form). The adsorbed portion was eluted with N ammonia. The eluate was concentrated to give crude crystals, which were purified by recrystallization from aqueous ethanol, yielding 104 mg. of a mixture of threonine and allothreonine.

Found: C, 40.06; H, 7.81; N, 11.70. Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62; N, 11.76%.

The Isolation of α,β-Dab.—When the above column of Dowex-50×8 (pH 5.0) was further eluted with 0.25 N aqueous ammonia, it was found by paper chromatography that 230—300 ml. of the effluent contained α,β-Dab. This fraction was evaporated under reduced pressure. The residue was dissolved in a small amount of hydrochloric acid, and the ethanol was added to the solution, giving colorless needles which were purified by recrystallization from aqueous ethanol (yield, 130 mg.).

Found: C, 31.24; H, 6.88; N, 17.96; Cl, 22.41. Calcd. for $C_4H_{10}O_2N_2 \cdot HCl$: C, 31.06; H, 6.48; N, 18.11; Cl, 22.95%. $[\alpha]_D^{23} = +20$ (*c* 1, 6 N HCl).

The isolation of α,β-Dab from the hydrolysate of glumamycin was previously reported.[3]

The Dinitrophenylation and Optical Rotation of the DNP Derivative.—According to Levy's method, 10.42 mg. of the mixture of threonine and allothreonine obtained above was dissolved in 5 ml. of water; 200 mg. of sodium carbonate and 0.2 ml. of 2,4-dinitrofluorobenzene were added to the solution, and the mixture was kept at 40°C for 20 min. while being vigorously stirred. The reaction mixture was diluted with 10 ml. of water, shaken three times with each half volume of ether to remove the excess 2,4-dinitrofluorobenzene, and, after being made acidic, again shaken with ether to extract DNP-threonine and DNP-allothreonine. The extract was evaporated to dryness and developed on a column (4×30 cm.) of Amberlite CG-50 (type II) with 1% hydrochloric acid-ethyl methyl ketone (75 : 25). In this case, the simultaneously-produced DNP-OH was developed half as slow as DNP-threonine and DNP-allothreonine, so the former was completely separated from the latter. The fraction containing DNP-threonine and DNP-allothreonine was evaporated under reduced pressure, and the residue (yield, 92%*) was dissolved in 2 ml. of a 4% sodium bicarbonate solution. The optical rotation of the solution is $[\alpha]_D^{24} = -102° \pm 5$, and from this the molecular rotation is calculated to be $[M]_D^{24} = -294° \pm 14$.

Oxidation with Hydrogen Peroxide.—To a solution of 1 g. of α,β-Dab·HCl in 14.5 ml. of 3% hydrogen peroxide, about 5 mg. of ferrous sulfate was added; the mixture was then kept at 50°C for one hour with stirring. The reaction mixture was diluted with 2 vol. of water and passed through a column of Amberlite IR-120 (H^+ form). The column was eluted with N ammonia, the eluate was evaporated to dryness, and the residue was recrystallized from aqueous ethanol to give 120 mg. of D-alanine, $[\alpha]_D^{20} = -16°$ (*c* 1, 6N HCl).

Found: C, 40.51; H, 7.88; N, 15.44. Calcd. for $C_3H_7O_2N$: C, 40.44; H, 7.92; N, 15.72%.

* The mol. wt. of the glumamycin determined by the method of the Vapor Pressure Osmometer is 1285 (sample, 13.400 mg.; solvent, 1 ml. of water; mole concentration, 0.01043), which is in good agreement with the min. mol. wt. (1, 300) obtained by means of amino acid analysis.[3]

** The DNP derivative was not obtained by Sanger's method.

* This yield was calculated from the ultraviolet-absorption at 360 mμ.

Summary

Glumamycin has been treated with nitrous acid to convert the free amino group into hydroxyl, and the resulting deamino-glumamycin has been hydrolyzed with hydrochloric acid. A mixture of threonine and allothreonine has then been isolated from this hydrolysate. The DNP derivative of the product shows an optical rotation $[M]_D^{24} = -294° \pm 14$ (*c* 0.48, 4% sodium bicarbonate). This fact shows that the above product is a mixture of D-threonine and D-allothreonine.

Further, as the α, β-diaminobutyric acid obtained from the hydrolysate of deamino-glumamycin and that obtained from the hydrolysate of glumamycin have the same optical rotation, it may be concluded that the two moles of α, β-diaminobutyric acid in glumamycin have the same steric configuration.

On the other hand, the product obtained by the oxidation of α, β-diaminobutyric acid with hydrogen peroxide is in agreement with D-alanine. Thus, it has been found that the α- and β-carbons of α, β-diaminobutyric acid belong to the D series and that, consequently, α, β-diaminobutyric acid takes the D-erythro-configuration.

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