α, β-DIAMINOBUTYRIC ACID OBTAINED FROM ASPARTOCIN

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α, β-Diaminobutyric acid, isolated from the acid hydrolysate of aspartocin, was resolved into two of the four possible isomers with a Technicon amino acid autoanalyzer. ORD and NMR studies of the isolated isomers indicated that one had the D-*erythro* and the other the L-*threo* configuration. The difference in NMR spectra of the two isomers was quite likely a consequence of restricted rotation about the C_α-C_β bonds due to electrostatic repulsion of charged amino groups. Whether the antibiotic contains both isomers or one was formed by racemization during hydrolysis was not determined.

As reported previously, seven amino acids and two fatty acids were isolated from the acid hydrolysate of the antibiotic aspartocin[1,2]. All the components were conclusively identified and characterized except α, β-diaminobutyric acid. Studies of the absolute configuration of this amino acid are reported herein.

Experimental

One gram of the sodium salt of aspartocin was hydrolyzed for 24 hrs under reflux in 100 ml of 6 N HCl in a nitrogen atmosphere. The product was extracted three times with 50 ml of chloroform to remove liberated fatty acids. The aqueous solution was then evaporated to a residue which was chromatographed on a column (1×40 cm) of strong acid ion-exchange resin (commercially available as Dowex 50×8, H^+ form, 200~400 mesh). The column was eluted with approximately 800 ml of 0.5 N HCl, 200 ml of 1 N HCl, and 400 ml of 2 N HCl. The α, β-diaminobutyric acid emerged from the column at 1,085~1,335 ml of effluent and was detected by a ninhydrin-spot test. Evaporation of this fraction gave a solid which yielded 107 mg of colorless platelets after crystallization from acetone and pyridine.

The product was identified by comparison to synthetic material[1]. Investigation of the isolated crystalline acid with a Technicon amino acid autoanalyzer with a pH 3.8 to pH 5.0 gradient revealed the presence of two isomers, termed components 1 and 2, with retention times of 684 and 737 minutes, respectively. A linear gradient was obtained from pH 3.8 and pH 5.0 citrate buffers described by Technicon.[3]. The autoanalyzer column, 0.6 cm×130 cm, was packed with Technicon Chromobead Type A resin and maintained at 60°C by a water jacket. Milligram quantities of each isomer were obtained for further study by scale-up experiments with the autoanalyzer. The buffer salts were removed from the resolved isomers by an ion-exchange desalting procedure described by DREZE *et al.*[4]

The isomers were resolved also, apparently through adsorption effects, by thin-layer electrophoresis on silica gel at 1,500 volts in a buffer system consisting of 2.44 g of sodium borate per liter of water adjusted to pH 10 with sodium hydroxide.

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Optical rotatory dispersion curves obtained on a Cary Model 60 spectropolarimeter with 1-dm cells at a concentration of 0.2 % except component 1 which was at an unknown concentration. Proton NMR spectra were obtained in approximately 2 N DCl-D_2O solutions on both Varian A-60 and DP-60 instruments. To prevent sample contamination it was found convenient to use an aqueous solution of sodium 2, 2-dimethyl-2-silapentane-5-sulfonate as an external reference.

Results and Discussion

Aspartocin was hydrolyzed with acid and the product was chromatographed on a strong acid ion-exchange resin which readily separated α, β-diaminobutyric acid from the other fragments. When the crystalline amino acid was investigated further with the Technicon amino acid autoanalyzer, it was resolved into two isomers termed components 1 and 2. In general, the isomers were difficult to resolve by other techniques; however, high voltage thin-layer electrophoresis also proved effective.

Proton NMR spectra of the two components were consistent for diastereoisomers of α, β-diaminobutyric acid (Figs. 1 and 2). The chemical shifts observed were typically pH-dependent as for amino acids and slightly different for the two components. More significant was the difference in the H_α-H_β coupling constants (3.6 and 6.6 Hz) that were constant over the temperature range −40°C to +80°C. This difference was quite likely a consequence of restricted rotations about the C_α-C_β bond

Fig. 1. NMR spectrum of L-*threo*-α, β-diaminobutyric acid (component 1)

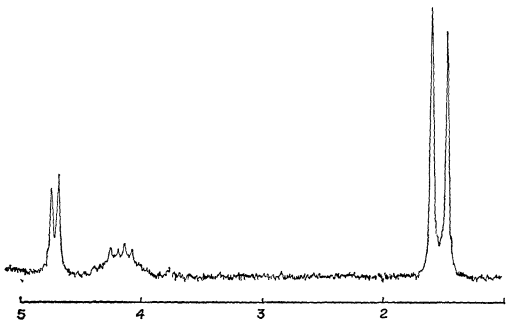
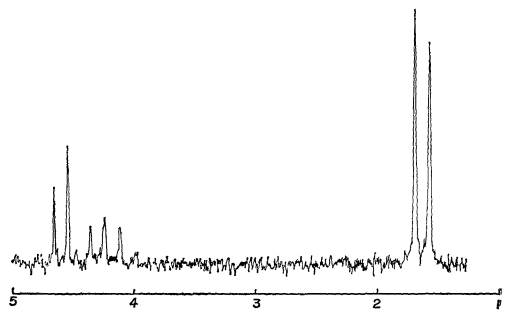
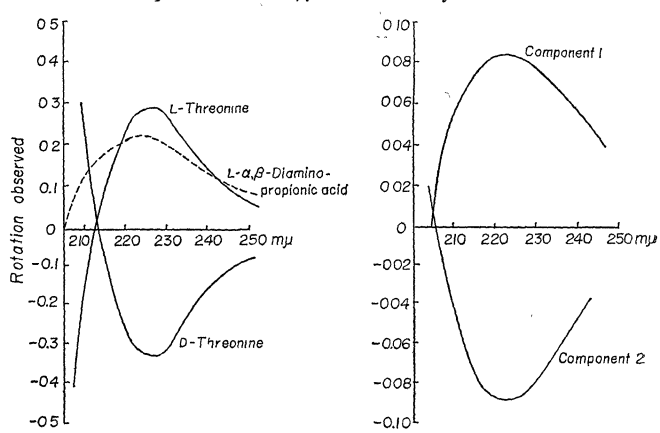


Fig. 2. NMR spectrum of D-*erythro*-α-β-diaminobutyric acid (component 2)



due to the electrostatic repulsion of the two positively charged amino groups. If these groups were *trans* to each other as expected, the vicinal hydrogens would be *gauche* and *trans* to each other for the *threo* and *erythro* forms, respectively. Component 2, which had the larger coupling constant, would have a *trans* arrangement for H_α and H_β, and

Fig. 3. Optical rotatory Dispersion curves for model compounds and α, β-diaminobutyric acid isomers

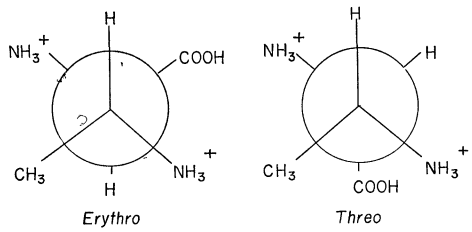


therefore the *erythro* configuration. The J-values were less than one would expect based on the KARPLUS curve[5], but it is well known that vicinal couplings are affected in this manner by electronegative substituents[7].

From optical rotatory dispersion studies[6], it was concluded that component 1 had the L-configuration and component 2 had the D-configuration at the α-carbon atom (Fig. 3).

Combining the conclusions from the ORD and NMR studies, we assign the L-*threo* configuration to component 1, and D-*erythro* to component 2.

A comparison of NMR and amino acid autoanalyzer results of the crystalline mixture of α, β-diaminobutyric acid isomers provided a rather surprising result. Integration of the C-CH_3 protons in the NMR spectrum indicated an approximate 1 : 1 ratio of isomers whereas the autoanalyzer study of the same mixture indicated the L-*threo* to D-*erythro* forms were in the proportion of 14 : 86 by ninhydrin color yield. The most plausible explanation of this discrepancy is that the L-*threo* isomer gave approximately 1/6 the color yield of the *erythro* form. Unfortunately a direct measurement of color yield on a weight basis was not possible due to limited supplies of the pure isomer.



Subjecting the D-*erythro* isomer to the same hydrolysis conditions used for the antibiotic resulted in some racemization about the α-carbon atom. Therefore, it is possible that the antibiotic contains only D-*erythro* or L-*threo* α, β-diaminobutyric acid and that the other isomer is formed by racemization during hydrolytic cleavage of the antibiotic. Attempts to clarify which isomer or if both are present in the antibiotic by varying the hydrolysis conditions, have been unsuccessful thus far.

The hydrolysate of glumamycin, a closely related antibiotic, has been reported to contain D-*erythro*-α, β-diaminobutyric acid[8]. It is interesting to note that no mention was made of the D-*threo* isomer.

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References

1) MARTIN, J. H. & W. K. HAUSMANN : Isolation and identification of D-α-pipecolic acid, α, L, β-methylaspartic acid and α, β-diaminobutyric acid from the polypeptide antibiotic aspartocin. J. Am. Chem. Soc. 82 : 2079, 1960.
2) HAUSMANN, W. K.; A. H. STRUCK, J. H. MARTIN, R. H. BARRITT & N. BOHONOS : Structure determination of fatty acids from the antibiotic aspartocin. Antimicr. Agents & Chemoth.-1963 : 352 ~359, 1963.
3) Buffers for micro column amino acid analysis. Technicon Research Bulletin, No. 10, Technicon Instruments Corp. Ardsley, N. Y. 10502, 1962.
4) DREZE, A.; S. MOORE & E. J. BIGWOOD : On the desalting of solutions of amino acids by ion exchange. Analytica Chemica Acta 11 : 554~558, 1954.

5) KARPLUS, M. : Contact electron-spin coupling of nuclear magnetic moments. J. Chem. Phys. 30 : 11～15, 1959.
6) JENNINGS, J. P.; W. KLYNE & P. M. SCOPES : Optical rotatory dispersion. X. Amino-acids. J. Chem. Soc. 294～296, 1965.
CRAIG, J. C. & S. K. ROY : Optical rotatory dispersion and absolute configuration. I. α-Amino acids. Tetrahedron 21 : 391～394, 1965.
7) KARPLUS, M. : Vicinal proton coupling in nuclear magnetic resonance. J. Am. Chem. Soc. 85 : 2870～2871, 1963.
ABRAHAM, R. J. & K. G. R. PACHLER : Proton magnetic resonance spectra of some substituted ethanes. Influence of substituents on CH-CH coupling. Mol. Phys. 7 : 165～182, 1963/1964.
8) FUJINO, M.; M. INOUE, J. UEYANAGI & A. MIYAKE : On glumamycin, a new antibiotic. V. The steric configuration of α, β-diaminobutyric acid. Bull. Chem. Soc. Jap. 38 : 515～517, 1965.