THE USE OF A BACTERIAL CULTURE FLUID AS A SOURCE OF αε-DIAMINOPIMELIC ACID

by

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αε-Diaminopimelic acid (DAP) is an amino acid widely distributed in bacteria, but absent from all other micro-organisms[1,2]. Study of the metabolism of DAP should yield information of considerable value to microbiological and comparative biochemistry, but has been limited by difficulties in preparation of the amino-acid. The observation by DAVIS[3] that certain lysine-requiring mutants of *Escherichia coli* accumulate DAP in their culture fluids, has suggested that the amino acid might be more easily extracted from such culture fluids than from the bacterial hydrolysate hitherto used as a source[2]. Accordingly, with the co-operation of Dr DAVIS, who kindly provided us with mutant strains of *E. coli* and with details of cultivation, we have worked out a simple procedure for the isolation of DAP from the mutant culture filtrate.

Using untreated culture filtrates, amino acids other than DAP could not be revealed by paper chromatography owing to interference by salts and non-amino-acid metabolic products. When the filtrate was passed through a column of Zeocarb 215 and the amino acids were displaced with ammonia[4], the main components of the eluate were DAP, alanine and glutamic acid, but valine, aspartic acid, glycine and lysine were also present. DAP could not be separated from the other amino acids on this type of column, but separation was achieved on a sulphonated polystyrene resin (Zeocarb 225 or Dowex 50) with HCl of differing concentrations as eluting agent[5,6]. 1.5 *N* HCl eluted all the amino acids except DAP and lysine, DAP was eluted by 2.5 *N* HCl, while lysine required 4 *N* HCl for elution.

When a hydrolysate of whole *Corynebacterium diphtheriae* cells was fractionated on the Zeocarb 225 column by HCl elution, DAP could not be separated completely from the other amino acids. It was eluted in 2.5 *N* HCl at the same time as leucine and tyrosine.

Details of the preparation of DAP are as follows. Lysine-requiring *E. coli* mutant (Strain no. 26–26) was grown at 37° for 44 h under full aeration, on the minimal medium of DAVIS AND MINGIOLI[7] with glucose concentration raised to 0.5% and with 0.001% L-lysine hydrochloride added. The cells were removed by centrifugation, and the supernatant, adjusted to pH 3 with HCl, was deproteinized by boiling and filtration through a celite pad. The filtrate, concentrated 10-fold *in vacuo* was treated with a large excess of charcoal to remove carbohydrates, and was then mixed with HCl to 1.5 *N*. An aliquot of this solution (equivalent to 2.25 l of original culture fluid and containing 24 g solids, 147 mg ammonia N and 80 mg combined N) was run on to a column (35.7 × 4.8 cm) of Zeocarb 225 (50–100 u) which had been equilibrated with 1.5 *N* HCl after cycling with 5 *N* NaOH and HCl. Aspartic acid, glutamic acid, glycine, alanine and valine were eluted in separate bands by 4.5 l of 1.5 *N* HCl; the acid strength was raised to 2.5 *N* HCl, and after 900 ml of effluent had been collected, DAP was eluted in a volume of 600 ml. The column was then washed with 8 l of 4 *N* HCl to remove absorbed substances, and after equilibration with 1.5 *N* HCl was ready for further use.

The DAP-containing effluent was concentrated *in vacuo* and decolourized with charcoal. The hydrochloride of DAP could be crystallized directly from the concentrate; but it was found more convenient to isolate the free amino acid because of the high water solubility of the hydrochloride. The concentrate was treated with Deacidite to remove chloride and then with ethanol to precipitate DAP. The precipitate was then crystallized either from water or ethanol-water. Yield 250 mg DAP.

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