468. *Amino-acid Separations by Use of a Strong-base Resin.*

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The possible value of an ion-exchange resin of strong-base type in the separation of amino-acid mixtures has been studied. Good separations of leucine–glutamic acid and leucine–methionine mixtures have been effected

Considerable success has been attained in the fractionation of amino-acid mixtures by means of ion-exchange resins of the strong-acid type (Partridge and Westall, *Biochem. J.*, 1949, **44**, 418; Partridge, *ibid.*, 1949, **44**, 521). The mechanisms operative in these separations have been discussed in the papers quoted and elsewhere (Davies, *ibid.*, 1949, **45**, 38). They depend on (*a*) the extent of ionisation of the individual amino-acids in their passage down the resin column, (*b*) the charges carried by the ions present, and (*c*) the van der Waals forces between the resin and the various species present. The relative importance of these effects can be controlled in a number of ways, by variation of the resin structure, of the nature of the liquid phase, and of the temperature, so that the versatility of the method is considerable. Improved separations resulting from a change of the developing solution (Partridge, *ibid.*, 1949, **45**, 459) and from an alteration of the temperature (Partridge, *ibid.*, in the press) have already been demonstrated; and a further possibility that is being explored is to utilise the strong tendency of the amino-acid anions to associate with bivalent metal cations.

The use of basic resins has, until recently, been severely restricted by the lack of strong-base exchangers. The earlier basic resins, which owed their exchange properties to amino- and imino-groups, have been successfully applied to the separation of the acidic from the neutral amino-acids, and to the separation of the acidic acids from one another (Consden, Gordon, and Martin, *ibid.*, 1948, **42**, 443; Partridge and Brimley, *ibid.*, 1949, **44**, 513); but they were quite unsuitable for use with the neutral amino-acids. The position has now been changed by the advent of strong-base resins of the quaternary-ammonium type, and the object of this short paper is to report preliminary measurements made by using one of these resins, Dowex 2. The resin was first characterised by the methods described by Partridge and Westall (*loc. cit.*). Next, a mixture of glutamic acid and leucine was separated on the regenerated column and an excellent separation was obtained. This pair of acids could, of course, be separated by the earlier methods to which reference has been made; but the use of a strong-base resin gives an equally efficient technique. Finally, the separation of a mixture of leucine and methionine was tested. These two acids have closely similar dissociation constants (leucine $pK = 2{\cdot}36$, methionine $pK = 2{\cdot}28$) for the process $A^+ \rightleftharpoons H^+ + A^\pm$, and in the fractionation of a mixture of amino-acids on a negatively-charged resin they appear in the same band. For the process $A^\pm \rightleftharpoons H^+ + A^-$, however, the pK values are: leucine, 9·60, and methionine, 9·21, so that the separation of the anions on a strong-base resin should be relatively simple. This was found to be the case.

Experimental.

Materials.—The resin was a sample of Dowex 2 graded to 250—500 mesh. It was in the form of cream-coloured micro-beads, and was supplied as the chloride. It was regenerated with carbonate-free sodium hydroxide until chloride-free, and washed with carbonate-free distilled water until the alkalinity of the eluate fell to below pH 8. The importance of having all solutions carbonate-free cannot be over-emphasised. If any carbonate becomes adsorbed on the resin, carbon dioxide will be released by the eluting acid and will seriously disturb the chromatogram.

AnalaR hydrochloric acid and acetic acid were used, being diluted as required with carbonate-free distilled water. The amino-acids were laboratory reagents.

Characterisation of the Resin.—By utilising the method described by Partridge and Westall (*loc. cit.*) values for λ_{90} (the boundary width in cm.) and ε (the capacity in millimols. per g. of dry resin) were obtained. Four runs were carried out with hydrochloric and acetic acids. The concentrations of the acids and the rate of progression of the boundary (S) were varied as indicated. For these experiments a column 10·7 cm. high and 1·5 cm. in diameter was used, containing 8·4 g. of the resin. The results obtained are shown in the Table.

Acid.	*Concn. (g.-equiv./l.).*	*S (cm./hr.).*	λ_{90}.	ε.
Hydrochloric	0·108	2	0·96	3·73
Hydrochloric	0·205	4	1·38	3·63
Acetic	0·106	4	2·13	3·67
Acetic	0·249	2	1·92	3·71

The boundaries are wider than those obtained with Zeo-Karb 215 (Partridge and Westall, *loc. cit.*) in spite of a lower boundary progression rate. The wider boundaries for acetic acid, compared with hydrochloric acid, may be due to van der Waals adsorption of the acid molecules. The values obtained for ε show that the resin behaves unifunctionally over the pH range studied.

The Separation of Leucine and Glutamic Acid.—For the experiments on the separation of amino-acids a second, smaller column of resin was fitted beneath and in series with the column used in the characterisation experiments. This smaller column contained 2·9 g. of resin, and was 8 cm. high and 1 cm. in diameter. The two columns were operated as a single unit.

5 Millimols. each of leucine and glutamic acid were dissolved in 100 ml. of carbonate-free distilled water and run on to the regenerated and washed column. The adsorbed band occupied about one-third of the length of the column. The amino-acids were displaced with 0·1M-hydrochloric acid, the rate of flow being adjusted so that the amino-acid boundary, which was plainly visible, travelled down the larger column at a rate of 1·5 cm./hr., and down the smaller column at a rate of 2 cm./hr. As the boundary approached the bottom of the lower column, 5-ml. fractions of the eluate were collected; this was continued until the eluate gave a positive reaction for chloride. A spot from each fraction was analysed by the paper chromatographic method described by Consden, Gordon, and Martin (*Biochem. J.*, 1944, **38**, 224). Glutamic acid could only be detected in the last of the fourteen leucine fractions, and only the first fraction of the glutamic acid band contained leucine.

The Separation of Leucine and Methionine.—The experimental procedure was identical with that described for the separation of leucine and glutamic acid, and similar quantities of the two acids were used. As the displacement proceeded, a band of insoluble material was seen to form immediately in front of the hydrochloric acid boundary. To prevent this blocking up the column the rate of displacement had to be increased considerably above that of the previous run. In spite of this a good separation was obtained, and only four out of the twenty-four amino-acid fractions contained mixed acids. The paper chromatogram indicated that the insoluble material was cystine, which frequently occurs as an impurity

in commercial methionine. As the cystine became concentrated into a band its low solubility was exceeded, and crystals separated out in the column.

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