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The separation of isoasparagine from asparagine

The best available synthesis of L-isoasparagine involves the treatment of carbobenzoxy-L-asparagine with acetic anhydride followed by ammonia, and removal of the carbobenzoxy group[1]. Several recrystallizations of carbobenzoxy-L-isoasparagine are recommended[1] to remove carbobenzoxy-L-asparagine. Since such treatment cannot remove all traces of the unwanted material, we have devised an electrophoretic procedure (analytical scale) and a chromatographic procedure (analytical and preparative) to permit the preparation of L-isoasparagine free from L-asparagine.

Paper electrophoresis at pH 3.0 (0.1 M acetic acid plus formic acid to attain the proper pH) resulted in good separation of the isomers of asparagine (obtained by treatment of the carbobenzoxy-L-asparagines with 30 % HBr in acetic acid for 1 h), which, as indicated in Du Vigneaud's procedure[1] can be distinguished by the color of their reaction products with ninhydrin. In this system, asparagine moved as fast to the cathode as picric acid (used to follow the rate of migration) moved to the anode. Isoasparagine moved about twice as fast as asparagine. This is in accordance with the relative acidity of the two carboxyl groups[2] of aspartic acid ($pK = 1.995$ and 3.910).

Ion-exchange chromatography on an automatic amino acid analyzer by the accelerated system of Spackman[3] (buffer flow rate of 50 ml/h) provided excellent separation of isoasparagine from asparagine: Asparagine eluted after 107 min with pH 3.25 citrate buffer while isoasparagine eluted in the position of isoleucine when the pH 4.25 buffer was used for elution. The color yield with ninhydrin of isoasparagine was 57 % of that of norleucine.

These procedures indicated that the first crop of crystals from the Du Vigneaud synthesis[1] contained 8 % asparagine. Analysis of a second crop from the mother liquor showed that it contained only 9 % isoasparagine. With the ion-exchange system described it is possible to prepare large amounts of isoasparagine free from asparagine by removing the latter with pH 3.25 buffer and recovering the isoasparagine from the pH 4.25 eluate. Removal of the citrate buffer salts by standard methods yielded pure isoasparagine.

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