CHROM. 3610

The formation of esters of glutamic acid and aspartic acid occasioned by the use of methanol in ion exchange chromatography*,**

In a previous communication[1] the progressive loss of glutamic and aspartic acids during ion exchange chromatography was reported. At that time there was no indication of the appearance of additional ninhydrin positive substances elsewhere in the chromatogram, when the usual standard amino acid mixtures were applied to the columns. Subsequent work has now established the nature of the products formed and the remedial action that can be taken to prevent the stated loss.

Reagents and procedures

The amino acids and amino acid esters used were obtained from Nutritional Biochemicals Corporation. The alcohols used were all Baker analyzed reagent grade. All buffers used contained versene. The method of PIEZ AND MORRIS[2] is the basis for our procedure, using 6 mm columns, each analysis requiring a little over six hours.

In all of this work the alcohol-containing buffer, if used, was present initially only in the first chamber of the varigrad and the line from the varigrad to the top of the resin bed. The alcohol concentration was 10 % (v/v).

Experimental

Glutamic acid. Two columns were loaded with 0.1 μmole of glutamic acid, the buffer above the resin bed contained methanol[3]. Column No. 1 was developed immediately; that is, buffer was started through the column within 30 min after the initiation of sample application. Development of column No. 2 was begun 19 1/2 h later. Column No. 1 produced two peaks, glutamic acid and a glycine elution time peak. The glycine elution time peak amounted to 17.5 % of the total area of the two peaks. For column No. 2 the glycine elution time peak amounted to 27.3 % of the total area.

A sample of glutamic acid γ-methyl ester was found to be eluted in the position of the glycine type peak. A sample of glutamic acid eluted without methyl alcohol in the buffers yielded a glutamic acid peak only.

Aspartic acid. The loss of aspartic acid is not as great as that of glutamic acid; however, it was found, that the β-methyl derivative occured in the serine area.

Other alcohols. With glutamic acid, ethanol produced a second peak in the alanine region while propanol produced a second peak eluted after valine and close enough to cystine to cause a shoulder on that peak.

Tertiary butanol. Tertiary butanol does not esterify readily. Experimentally, each of the amino acids under consideration were applied to columns and permitted to stand at 60° using tertiary butanol for 20 h.

In both cases, a single peak was obtained, and this peak eluted in the position predicted by elution of the same compound in the absence of any alcohol.

The threonine–serine resolution resulting from the use of tertiary butanol was

* Supported in part by the National Institutes of Health Grant No. GM10402.
** Publication No. 82 at the Institute of Arctic Biology.

at least equal to that provided by the use of methanol, with no apparent effect on the other portions of the chromatogram.

γ-Methyl glutamic acid. This compound is readily hydrolyzed to glutamic acid. Difficulty was encountered in gaining recovery factors for it. The procedure finally adopted was to make up a solution in distilled water, apply the sample to the column and begin the run; all within 15 min after making up the solution. Even with this rapid approach, a small glutamic acid peak amounting to 9 % did occur. Chromatography attempted in a similar fashion in the absence of methanol resulted in a single peak in the glutamic acid region. It seems then, that the resin catalyzes this conversion so that, in a very short item, the conversion is complete. Under such circumstances, only one peak would be observed and this would be attributed to the sample placed on the column. Our experience suggests that under our conditions, using methanol, the equilibrium for the γ-methylation is reached when 24 to 26 % of the glutamic acid exists as the γ-methyl ester. These figures are based on the results of chromatography.

Results and discussion

The situation with glutamic acid and aspartic acid appears clear cut. In the presence of methanol, some of the amino acid is converted to the γ-methyl or β-methyl ester. These compounds are then eluted in the areas of glycine and serine–asparagine respectively. This can be prevented by using tertiary butanol to replace methanol for the improvement of the resolution of threonine and serine.

HAMILTON[4] lists the position of γ-methyl glutamic acid in the region of glutamic acid. Our results indicate that this ester behaves more like glycine. In the absence of methanol, this ester is recovered quantitatively as glutamic acid. This probably explains the discrepancy in position, rather than invoking buffer schedule differences.

Tertiary butanol, used in place of methanol, gives no indication of any complications. It appears then, that tertiary butanol is the alcohol of choice for the improved resolution of threonine and serine.

The technical assistance of JILL CAMERON is gratefully acknowledged.

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Received May 20th, 1968.