
The Use of Filter Paper Pulp in the Separation of Certain Flavonoid Compounds

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In an investigation of flavonoid compounds from plant sources, a mixture of two flavonol glycosides was isolated from the leaves of *Vaccinium vitis-idaea*. It was necessary to separate these two compounds in order to complete their final identification. The usual methods for separation of flavonoid compounds failed to resolve the mixture. Paper partition chromatography showed the compounds to have different R_f values in several solvent systems. The greatest difference was found in the system ethyl acetate-water. Chromatography using a paper column in conjunction

with a suitable solvent system would appear to offer possibilities as a method of separation.

EXPERIMENTAL

The adsorption columns were prepared by packing a glass column with S and S Analytical Filter Pulp No. 289. In some experiments the column was packed dry; in others it was packed with an acetone-paper slurry. Both methods were found to work equally well. Columns up to two inches in diameter were used. Better separations, however, were obtained with columns of smaller diameter.

In a typical experiment, a 100 mg. sample of the mixed glycosides was dissolved in 100 ml. of anhydrous acetone and the solution passed through a 1/2 x 18 inch adsorption column. The column was then washed with 300 ml. of acetone, the eluate being collected in three equal fractions. Finally, the column was eluted with 100 ml. of an 80 per cent acetone-water solution. Each of the four fractions was examined by paper chromatography, and each was found to contain different ratios of the two glycosides. By repeating the process on the separate fractions, it was possible to obtain each of the two in the pure form.

Series of experiments were carried out in which the mixture was adsorbed on the column from an ethyl acetate solution. The column was then eluted with a solution of ethyl acetate saturated with water, and the eluate collected in 4 equal fractions. The first fraction contained only one glycoside (R_f , butanol-acetic acid-water, 0.90); fractions two and three contained a mixture, while fraction four contained one glycoside (R_f , butanol-acetic acid-water, 0.73). Ethyl acetate was superior to acetone as a solvent for the separation.

SUMMARY

Chromatography on a paper pulp column has been used to separate a mixture of two flavonol glycosides. The method is suitable for the separation of small quantities of such mixtures but becomes cumbersome when applied to separations on a large scale.

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