Quantitative Paper Chromatography of Certain Flavonol Aglycones

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Wender and Gage (2) have adapted the method of paper partition chromatography, coupled with the use of chromogenic sprays, to the *qualitative* separation and identification of mixtures of flavonoid pigments. Later, Gage and Wender (1) successfully extended the method to the *quantitative* separation and estimation of certain individual flavonol-3-glycosides, such as rutin, isoquercitrin, quercitrin, and xanthorhamnin, present in a mixture. The method, however, did not work successfully with the aglycones of the flavonol pigments, because the solvent system used failed to elute the aglycone. In fact, no solvent system had been found which would quantitatively elute the flavonol aglycone without also removing excessive amounts of an accompanying substance which interfered in the subsequent quantitative work. The present research was undertaken, therefore, with the purpose of finding a suitable solvent system for the quantitative elution of flavonol aglycones, and of finding a satisfactory technique for the subsequent quantitative estimation of the eluted flavonol.

A reasonably satisfactory scheme for quantitative paper chromatography of certain flavonol aglycones has been devised. Quercetin (3, 3′, 4′, 5, 7-pentahydroxy flavone) will be illustrated as a typical aglycone investigated.

Experimental

The most satisfactory method found for quantitative paper chromatography of quercetin involved the following steps:

The quercetin was separated from other substances by descending paper chromatography, using a butanol-acetic acid-water (40 : 10 : 50% by volume; top layer) solvent system for 18-25 hours (2). The strips containing the quercetin were shortened and then placed in a vapor tight eluting chamber

(Figure 1), and eluted with 30% ethyl acetate in 95% denatured ethyl alcohol. The eluant was collected off the strips into individual small beakers. The eluted solutions were then transferred into 5 ml. volumetric flasks. Two ml. of a dilute alcoholic aluminum chloride reagent (0.25 gm. aluminum

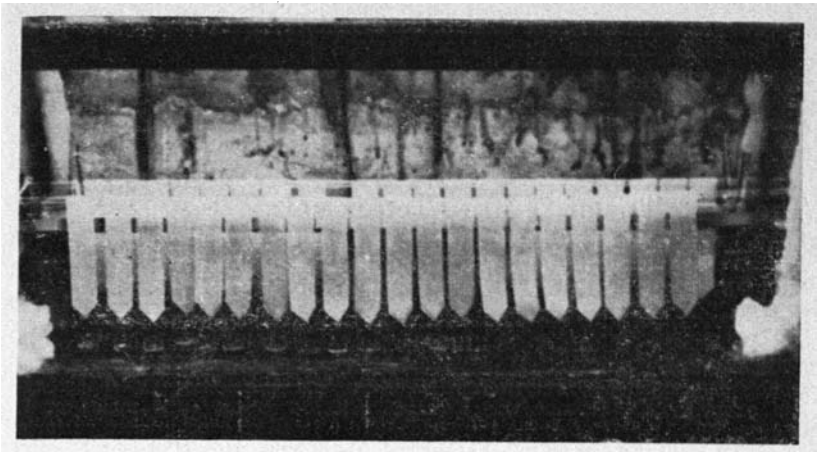

Figure 1.

chloride hexahydrate per liter 95% ethyl alcohol) was added to the flask. The flasks were next made to volume with additional eluting reagent. Three hours after the addition of the aluminum chloride reagent to the eluant, the optical densities of the solutions were taken at 428 millimicrons, using a Beckman spectrophotometer, model DU. Recoveries of 90% or more of chromatographed quercetin were obtained as compared to standard quercetin solutions treated in the same manner as the chromatographed quercetin with the exception that the quercetin was not placed on the paper. The recoveries are much higher when adjustment is made in the calculations to correct for the losses occurring during the chromatographic and leaching procedures.

Fluorimetric determinations of the solution obtained by treatment of the eluate with aluminum chloride reagent were quite sensitive to quantities of flavonol aglycone as low as 5 micrograms. Many conditions, however, such as *p*H, time of complexing, amount of water in the solution, and concentration of the aluminum ion were found to influence the intensity of the fluorescence. Thus, results varied considerably for different runs, and after many experiments, fluorimetric methods following paper chromatography were abandoned.

The use of Wilson's borocitric method (3) was also found to be unsatisfactory for determining quercetin eluted from paper chromatograms.

Summary

A satisfactory method for quantitative paper chromatography of certain flavonol aglycones has been reported. It consists of elution of the flavonol aglycone from filter paper with a solvent system consisting of 30% ethyl acetate in 95% denatured ethyl alcohol, treatment of the eluate with aluminum chloride reagent, and spectrophotometric determination of the resulting solution.

Acknowledgment

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