CHROMATOGRAPHIC ADSORPTION STUDIES ON CERTAIN FLAVONES

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Chromatography affords a relatively simple and rapid technique for qualitative and quantitative separation of many biochemicals which may be present in extremely complex mixtures. The use of chromatography also has furnished a new criterion of purity. Many C. P. chemicals show impurities on chromatographic adsorption. Use of chromatography in preparations, then, makes possible a degree of purity in many products heretofore unobtainable.

Although chromatography has been widely used, there are relatively few chromatographic studies on flavonoid pigments. Mager (1942) and Robeznieks (1938) utilized chromatography to effect the separation and purification of certain flavonoid compounds. More recently, Schoulties and Wender (1947) applied chromatography to the separation of four flavonoid pigments in tobacco extracts.

Work is in progress at the University of Oklahoma on the problem of isolating and separating flavonoid pigments from plants native to Oklahoma. To aid in these studies, it was desired to know on what adsorbents the flavones could be isolated and in what order one might expect to find them on an adsorbent. The present investigation was therefore undertaken.

The present work was carried out with the first flavones that have become commercially available.

EXPERIMENTAL

The flavones used in this project were samples of quercetin, quercitrin, rhamnetin, xanthorhamnin, homoeriodictyol, and D-catechin purchased from S. B. Penick and Company, rutin (therapeutic preparation prepared by Abbott Laboratories), and naringin (Practical, Eastman).

The adsorbents used, were talc (U.S.P., J. T. Baker and Company), magnesol (industrial grade, regular, Westvaco Chlorine Products Corporation), florisil (60-100 mesh, Floridin Company), silica gel (28-200 mesh, anhydrous, Davison Chemical Corporation), Fisher adsorption alumina (80-200 mesh, Fisher Scientific Company), and barium sulphate (C. P., powder, Eimer and Amend Company). With the exception of silica gel, the adsorbents were mixed with celite analytical filter aid (Johns-Manville) prior to use. Two parts of the adsorbent to one part of the filter aid by weight was the most successful ratio for magnesol, talc and florisil. A 5-1 ratio was used for alumina-celite. The mixtures were ground in a ball mill for one to two hours to insure thorough mixing of the materials. 11 mm × 130 mm columns were used for all the adsorption studies reported in this paper. Columns prepared by both the "dry" and the "slurry" method were used in this study. In general the "slurry" method was more successful in the cases where a large proportion of filter aid was used, as there was less tendency for the adsorbent to settle and crack.

The columns were given a pre-wash treatment with a small portion of the particular solvent used for the flavonoid pigment prior to passage of the flavone solution through the column. The method of elution varied with the different adsorbents and pigments. Generally, a more polar solvent was required for elution than the solvent used for the chromatogram.

The presence of small quantities of impurities in some of the commercial samples of flavonoid pigments was noted following adsorption on certain of the adsorbents. Some of these were not detectable in ordinary light but were easily visible in ultraviolet light.

Adsorption on talc. None of the flavones studied was adsorbed on talc from acetone or ethyl alcohol solution.

Adsorption on magnesol. Xanthorhamnin was adsorbed onto magnesol from acetone forming a bright yellow band at the top of the column. When seen under ultraviolet light, the bright yellow band of xanthorhamnin was edged at the bottom with a narrow bluish-green fluorescent band. When the column was washed with ethyl acetate the fluorescent band was separated from the xanthorhamnin and finally eluted. The xanthorhamnin was eluted slowly by ethyl alcohol and more readily by alcohol-water mixtures.

Rhamnetin was adsorbed onto magnesol from acetone solution as a bright yellow band. The rhamnetin zone fluoresced greenish-yellow under ultraviolet light and was edged at the bottom by a bright, chartreuse, fluorescent band. On development with acetone a narrow pale coral zone appeared and moved down the column. This band was not visible under ultraviolet light. This fluorescent impurity could not be separated from rhamnetin by washing with ethyl acetate or alcohol. Rhamnetin was eluted by washing with alcohol-water (50-50 vol %).

Rutin in acetone was also adsorbed on magnesol. Elution was most successful by washing with 50% alcohol-water.

Catechin was adsorbed as a narrow pink band at the top of the column. Under ultraviolet light, the zone appeared more orange in color. No subsidiary zones were observed. Ninety-five per cent ethyl alcohol was an effective eluant.

S. B. Penick quercetin was adsorbed onto magnesol as a broad orange-yellow band. A narrow red-brown band composed of a red pigment was adsorbed above the quercetin zone. Ultraviolet light revealed a narrow lime green fluorescent zone below the quercetin band. Development with acetone easily removed the latter band. The quercetin was removed by elution with 95% ethyl alcohol. The red-brown band was not disturbed by ethyl alcohol or acetone but was easily eluted by dilute acetic or hydrochloric acid solutions.

Quercitrin behaved very similarly to quercetin. The same zones were observed and the same solvents effected their elution.

Homoeriodictyol was adsorbed onto magnesol from acetone as a dark orange zone at the top of the column. Elution of this pigment was accomplished by means of 50% ethyl alcohol-water.

A mixture of rhamnetin and xanthorhamnin in acetone was passed through a column of magnesol. On development with acetone the two pigment zones were slightly separated by a pale blue fluorescent band. The adsorbent was extruded from the column and the two main pigment zones separated and eluted with ethyl alcohol. From ultraviolet absorption spectra, obtained with the Beckman DU spectrophotometer, the upper band was identified as xanthorhamnin and the lower band as rhamnetin.

ADSORPTION ON FLORISIL. Acetone and alcohol solutions of quercetin, quercitrin, homoeriodictyol, and rutin were adsorbed on columns packed with florisil-celite. In each case the behavior appeared identical with that observed when magnesol was used as the adsorbent except that percolation through florisil was exceedingly slow.

ADSORPTION ON SILICA GEL. Ten grams of anhydrous silica gel was partially dehydrated by treating with 5 g of water and stirring until a dry powder was obtained. This material was used without mixing with a filter aid as an adsorbent for alcohol solutions of rutin. Rutin was adsorbed at the top of the column in a broad yellow band. Elution was effected with dilute alcohol or alcohol containing a small amount of HCl.

Anhydrous silica gel which had not been dehydrated adsorbed rutin strongly from alcohol solution, but difficulty was encountered in recovering the rutin from the column.

When the silica gel adsorbent was prepared with larger amounts of water, rutin was incompletely adsorbed and a diffuse yellow band was formed which extended the length of the column. Pre-washing with 95% alcohol was omitted in the case of silica gel because the water in the alcohol caused the silica gel to become too hydrated for adsorption to occur.

ADSORPTION ON ALUMINA. Rutin, naringin, and quercetin were each strongly adsorbed on alumina in narrow, yellow bands at the top of the column. Quercetin was overlayed with a thin red band. Very little development occurred even after long washing with alcohol or alcohol-water solutions. The pigments could be eluted with dilute HCl (0.1 N) and in the case of quercetin, the uppermost red band preceded the quercetin through the column. When the acid eluates were neutralized, flocculent yellow precipitates were formed which were tentatively identified as aluminum salts of the individual flavonoid pigments. Attempts to prevent the formation of these complexes by pre-washing the alumina with dilute HCl, followed by distilled water, were unsuccessful. Apparently, sufficient aluminum ion was available to combine with the minute quantities of flavonoid pigment present in the percolate.

ADSORPTION ON BARIUM SULFATE. Rutin was adsorbed on barium sulfate from alcohol in a small band at the top of the column. When the column was developed with alcohol the band spread somewhat and moved slightly down the column. The rutin was removed with alcohol-water solutions or with alcohol slightly acidified with HCl.

SUMMARY

The chromatographic behavior of quercetin, quercitrin, rhamnetin, xanthorhamnin, homoeriodictyol, D-catechin, rutin, and naringin on one or more adsorbents has been studied. Adsorbents used in the study were talc, magnesol, florisil, silica gel, alumina, and barium sulfate. Barium sulfate, magnesol, and silica gel were found to be the best adsorbents for the flavones studied. Talc did not adsorb any of the eight flavonoid pigments. Alumina adsorbed the flavones too strongly and apparently combined with them, possibly to form complex aluminum salts. Florisil was a good adsorbent for the flavones but packed too tightly in the column, which resulted in slower percolation rates. Evidences of impurities in most of the flavonoid pigments were noted.

This investigation was supported by a research grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

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