THE PURIFICATION AND QUANTITATIVE ESTIMATION OF QUERCETIN BY PAPER PARTITION CHROMATOGRAPHY

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The flavonoid pigments comprise a large group of yellow plant pigments which are widely distributed in nature. These pigments are quite difficult to separate and purify by crystallization due to their similar solubility relationships and their tendency to form mixed crystals. The method of paper partition chromatography has recently been successfully applied on a micro scale to the qualitative separation and identification of the individual flavones present in a mixture (Gage and Wender 1949). The present paper details preliminary results obtained in applying the paper partition method on a quantitative basis. This extension of the method involves the quantitative removal of the pigment from the filter paper strip used in the chromatographic separation and the determination of the amount of flavonoid by ultraviolet absorption spectral data.

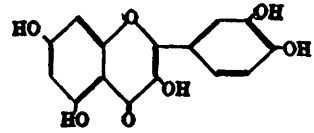

FIGURE 1. Quercetin

Since the ultraviolet absorption spectrum of quercetin (Figs. 1, 3) has been established beyond question, this pigment was chosen for the preliminary work.

EXPERIMENTAL

Materials. The butanol and acetic acid used as solvents for chromatographic separations were redistilled C. P. grade reagents. The Watman No. 1 filter paper used for chromatographic separations was extracted in an all glass Soxhlet extractor with ether for four hours followed by ethyl alcohol for eight hours. The strips were then air-dried and stored until required.

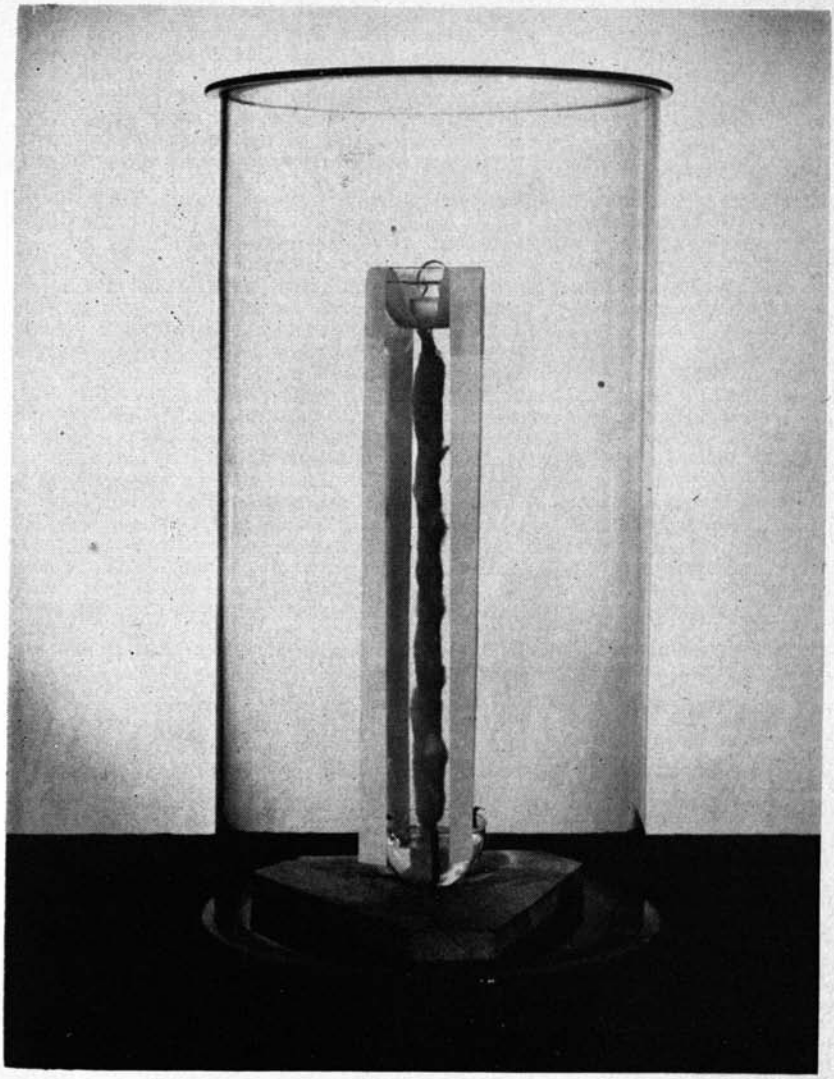

FIGURE 2. Chromatogram Chamber

Procedure. Crude quercetin was prepared from Lemon Flavin according to the general directions given by Morrow and Sandstrom (1935; 231, 294). In order to compare later calculations of the recovery of quercetin by chromatography, the quercetin content of the above crude product was determined by use of the Beckman DU spectrophotometer. Ten μl, containing 54.1 μg of the crude quercetin in 95% ethyl alcohol, were transferred to a 5 cc volumetric flask and diluted to volume with ethyl alcohol. The concentration of quercetin in this sample (0.00705 g/l) was calculated from the optical density (.545) at 375 mμ, divided by the extinction coefficient for quercetin (77.3) at the same wave length. One cm cells were used for all spectrophotometer readings reported in this paper. From the original weight of the sample, the per cent of quercetin was calculated to be 65.1.

For the chromatographic purification and estimation of quercetin, a Whatman No. 1 filter paper strip, 2.5 cm × 50 cm, was spotted 8 cm from one end with 35μl containing 42μg of crude quercetin in ethyl alcohol. The strips were air-dried and then placed in the chromatogram chamber (Fig. 2) and developed with the three component solvent, butanol-acetic acid-water (40-10-50 vol %). A blank strip was inserted in the chamber to provide a solvent blank in the determination of the ultraviolet spectrum. The strips were developed overnight (14 hours); after this period, they were removed and air-dried. The pigment zone, which was faintly visible in ordinary light, exhibited a strong yellow fluorescence in ultraviolet light. The pigment zone was cut out of the filter strip and a corresponding section was removed from the blank strip. The sections thus removed were leached with 95% ethyl alcohol in small chromatogram chambers by placing one end of each strip between two microscope slides which projected over the edge of the solvent trough and leading the other end of the strip between two square microscope slide cover glasses into a 5 cc beaker. The cover glasses facilitated the transfer of the leaching solution from the end of the strip into the beaker. The strips were leached 14 hours and the lower portion washed down with a few drops of ethyl alcohol. Removal of the last traces of quercetin from the strip could be checked by observation with the ultraviolet lamp.

The extract in each beaker, which amounted to approximately 2 cc, was made up to 5 cc volume with ethyl alcohol. The ultraviolet absorption spec-

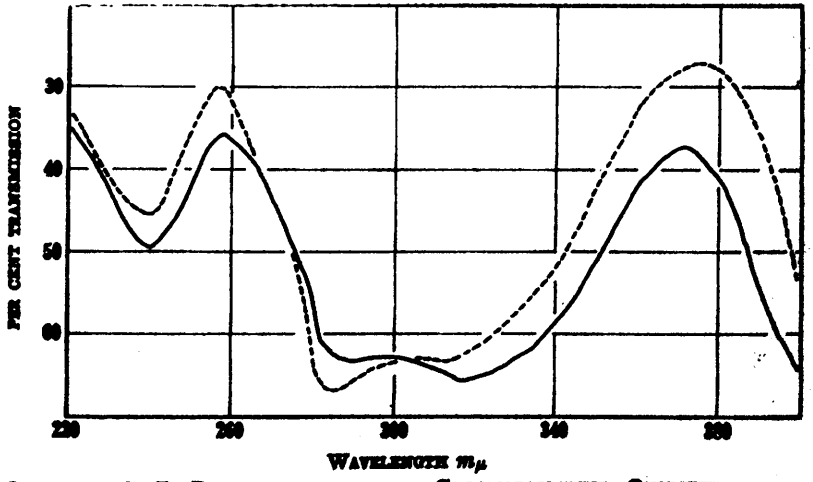

FIGURE 3. Absorption Spectrum of Quercetin

trum of the quercetin sample was then determined with the Beckman DU spectrophotometer. The extract of the blank filter paper strip was used for the solvent blank. In Fig. 3, the absorption spectrum of the chromatographed quercetin is compared with the absorption spectrum of some highly purified quercetin purchased from the S. B. Penick Company. The concentration of the S. B. Penick quercetin was 0.00736 g/l. From the optical density at 375 $m\mu$, the concentration of quercetin in the chromatographed sample was calculated to be 0.00655 g/l or 66.1%.

DISCUSSION

The 2-3% experimental error involved in this method is probably largely due to the micro pipette used for spotting the filter strips. The pipette was prepared from a medicine dropper by drawing out the tip to a fine capillary. It was calibrated by determining the number of drops of alcohol (200) required to fill a 1 ml volumetric flask. Triplicate determinations of the amount of material transferred by the pipette, made by use of the Beckman DU spectrophotometer, never varied more than 2%.

Another difficulty in the procedure, which at present has prevented the quantitative determination of mixtures, is that the concentration of pigment on the filter paper strip must be of the order of 30-50μg in order to determine the spectra in the 1 cm cells now on hand with the spectophotometer. When high concentrations of pigment are chromatographed on the filter strips, the pigment zone is diffuse and covers a much larger area than is the case when 7-12μg are used. When two or more pigments are present in high concentration on one filter paper strip one does not obtain clean separation into individual zones. Work is now in progress using better micro pipettes and also micro absorption cells for the spectrophotometer in attempts to remove these difficulties.

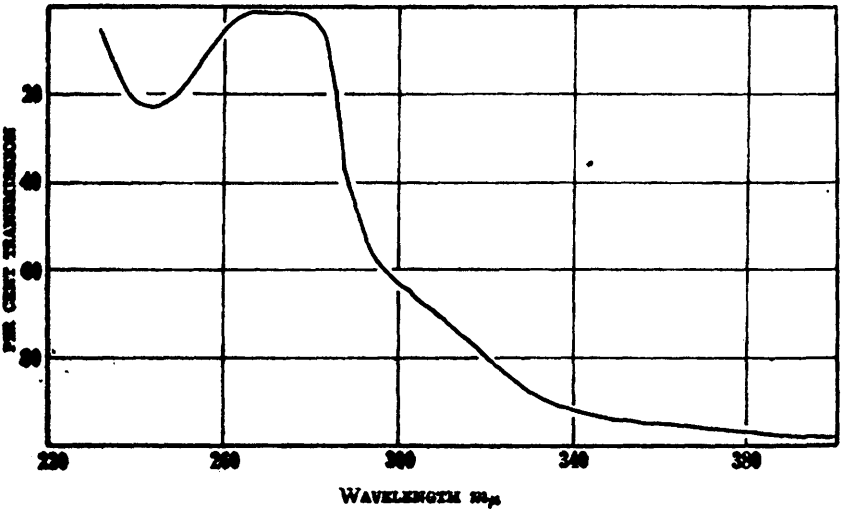

FIGURE 4. Absorption Spectrum of Interfering Substance

The difference in the shape of the absorption spectra curves for chromatographed quercetin and S. B. Penick quercetin in the region 220-310 $m\mu$ is due to an interfering substance, present in the paper or solvents, which

exhibits strong absorption in the ultraviolet region. Since the absorption of the interfering substance (Fig. 4) is only 3% at 375 $m\mu$, it is possible to determine the concentration of quercetin or other flavonoids with absorption maxima in this region. A more detailed discussion of this interfering material is presented in a paper by Douglass, Gage, and Wender (1948).

Further work is in progress on the quantitative estimation of flavonoid pigments by paper partition chromatography. Other solvents have been used for the chromatographic phase of the determination including ethyl acetate-water, 65% ethyl alcohol-water, and chloroform-water. The ultimate aim is to perfect a procedure whereby a few leaves or other small sample of plant material can be extracted, the flavonoid pigments separated and identified by paper partition chromatography, and quantitative determinations made by use of the Beckman DU Spectrophotometer.

SUMMARY

A micro method of quantitatively estimating the amount of flavonoid pigment in a plant extract has been proposed. The method involves the separation of the flavonoid pigment from other impurities by paper partition chromatography, the quantitative removal of the purified pigment from the filter paper by leaching with ethyl alcohol, and the determination of the amount of flavonoid pigment by means of the Beckman DU Spectrophotometer. The successful application of the procedure to quercetin has been described, and the presence of an interfering material has been noted.

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BIBLIOGRAPHY

Douglass, C. D., Gage, T. B. and Wender, S. H. 1948. The ultraviolet spectrum of naringin by use of the Beckman Model DU spectrophotometer. Presented before the Thirty-Seventh annual meeting of the Oklahoma Academy of Science on December 3, 1949 at Tulsa, Oklahoma.

Gage, Thomas B. and Wender, Simon H. 1948. Paper partition chromatography of flavonoid pigments. Science. *109*:287.

Morrow, C. A. and Sandstrom, W. M. 1935. Biochemical laboratory methods for students of the biological sciences. New York: John Wiley and Sons.