A New Method for the Isolation of Pure Quercitrin from Lemon Flavin[1]

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Introduction

Lemon flavin, a dry extract of the bark of *Quercus tinctoria* (North American dyers oak), has been used for many years as a source of quercitrin[2] (1). According to Morrow and Sandstrom (2), the lemon flavin is extracted with boiling water and the extract decolorized with vegetable charcoal. After standing 5–7 days the quercitrin crystals are filtered off. Other methods generally depend upon extraction with a suitable organic solvent, followed by recovery of quercitrin from the extract. Quercitrin prepared by these methods has been found to contain small amounts of quercetin.

Lemon flavin has also been used as a source for the preparation of rhamnose. Walton (3) has described a method whereby the flavin is hydrolyzed directly with dilute acid. After removal of the impure quercetin by filtration, the filtrate is neutralized with barium carbonate, decolorized with charcoal, and concentrated to a small volume to allow crystallization of the rhamnose.

The method reported here is marked by its simplicity and the high degree of purity of the product. The quercitrin is obtained free from quercetin, and may be used for the subsequent preparation of rhamnose without the aid of decolorizing agents.

This method should prove to be a valuable student exercise in biochemistry laboratory courses since it offers experience in chromatography as well as in the preparation of a sugar (rhamnose) from a glycoside.

Experimental

Isolation of the Flavonoid Mixture

Four g. of lemon flavin was extracted with 100 ml. of boiling acetone for 1 hr. The extract was then filtered through sintered glass, and passed through a 20-mm.

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[2] Quercetin-3-rhamnoside.

glass column containing 2.5 g. of a hydrous magnesium silicate (Magnesol).[3] The column was washed with 20 ml. acetone, and the combined effluents evaporated to an approximate volume of 15 ml. One hundred and fifty ml. of distilled water was added and the solution was stored overnight in the refrigerator to permit crystallization. The precipitate was filtered off, washed with cold water, dried, and weighed. Yield: 0.9 g.

The presence of a small quantity of quercetin in this precipitate was demonstrated by the use of paper-partition chromatography.

For the preparation of quercetin and rhamnose, a portion of the crystallized flavonoid mixture was hydrolyzed by refluxing with 0.5% sulfuric acid for 1 hr. The mixture was stored overnight in the refrigerator and the quercetin removed by filtration. The filtrate was colorless, and was suitable for the isolation of rhamnose without decolorization.

Purification of Quercitrin

A 100-mg. sample of the mixed flavonoid precipitate was dissolved in 25 ml. of dry acetone, and the solution was passed through a 33 × 200 mm. glass column packed to a depth of 75 mm. with Magnesol. (The column is prepared from a Magnesol–acetone slurry in such a manner as to obtain a uniform bed of adsorbent. A layer of solvent is maintained above the surface of the adsorbent, and care must be exercised in the addition of solution in order to avoid disturbance of the bed.) Observation under ultraviolet light revealed that the flavonoids had been adsorbed in a narrow band at the top of the column. Upon elution with a solution of ethyl acetate saturated with water, quercetin separated and appeared as a bright yellow band moving ahead of the quercitrin which moved as a brown band. The two bands were collected separately, and by paper chromatography were checked for contamination. The second band proved to be pure quercitrin showing no evidence of quercetin.

Summary

1. A simplified method for the isolation of pure quercitrin from lemon flavin has been described.

2. The method employs the technique of adsorption chromatography for the separation of flavonoid compounds.

References

1. Rigaud, L., *Ann. Chem. Pharm.* **90,** 283–97 (1854).
2. Morrow, C. A., and Sandstrom, W. M., Biochemical Laboratory Methods for Students of the Biological Sciences, 2nd Ed. John Wiley and Sons, Inc., New York, N. Y., 1935.
3. Walton, C. F., Jr., *J. Am. Chem. Soc.* **43,** 127–31 (1921).

[3] Industrial grade regular, kindly supplied by the Westvaco Chlorine Products Corp., South Charleston, W. Va.