
The Isolation of a Flavonoid Substance from Watermelon

BYRON L. WILLIAMS and SIMON H. WENDER,
University of Oklahoma, Norman

The current interest in the possible biological value of certain bio-flavonoids for animals has emphasized the need for a systematic study by valid chemical methods of various fruits for the purpose of acquiring knowledge of the flavonoid compounds present therein. The present investigation on the watermelon, *Citrullus vulgaris*, continues similar ones previously carried out by the authors on grapes and on black currants (1).

This paper reports the isolation of a flavonoid compound from the Florida Black Diamond Watermelon; studies on the properties of this compound; and preliminary investigation on its identity.

EXPERIMENTAL

Forty pounds of ripe Florida Black Diamond Watermelons, *Citrullus vulgaris*, were processed through a wet grinder. The ground product was

diluted to 20 gals; boiled for 2 hours, filtered while hot, and the residue discarded. The filtrate was allowed to cool, then passed over Amberlite IRC-50(H) ion exchange resin in a column at a rate of 1 gal./hr. The columns containing the adsorbed flavonoid material were then washed with distilled water until a clear eluate was obtained. The adsorbed material was next removed by elution with 95% ethyl alcohol. The solution, about 1 liter at this time, was concentrated to a volume of 100 ml. *in vacuo*, and then allowed to cool. The resulting concentrate was then extracted four times with 100 ml. portions of normal amyl alcohol. The amyl alcohol was dried and concentrated by reducing the volume *in vacuo* on a water bath to 10 ml. Then 50 ml. of commercial pentane was added, and the resulting precipitate removed by centrifugation. The solid became a brown, oily material on drying in the oven at 80°C. This oily matter was extracted with three 50 ml. portions of anhydrous acetone. Some resulting solid did not dissolve, and was discarded. The acetone solution was next chromatographed on a Magnesol column. The flavonoid material was among those adsorbed near the top of the column. The chromatogram, on being developed with ethyl acetate saturated with water, showed a forward band which was yellow in the visible and a darker yellow under the ultra-violet light. The second band was red to brown when viewed under ultra-violet light, but was not detectible in the visible. The second band was removed and concentrated. It was not studied further, however, after it failed to give the usual flavonoid type absorption spectrum curve or to behave on papergrams as do known flavonoids. The first band was also removed and concentrated. It was precipitated by adding pentane to the ethyl acetate concentrate. Then the resulting solid was recrystallized eight times from boiling ethyl alcohol, by adding boiling water. The absorption spectrum curve (Figure 1) of this recrystallized compound was obtained in concentration of 0.006 gm/l using a Beckman Model DU Spectrophotometer. This curve is a type characteristic of the flavonoids.

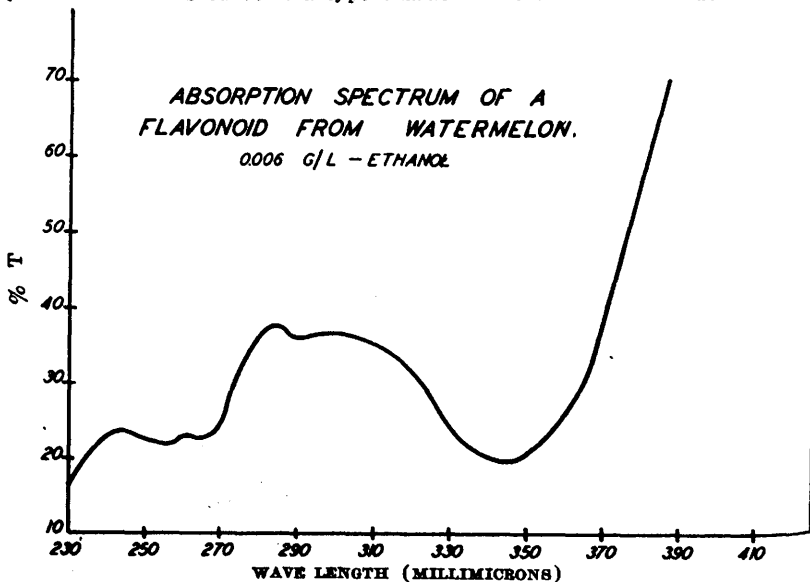


FIGURE 1.

The recrystallized solid gave a yellow intensification of color when treated with hydrochloric acid and magnesium in alcoholic solution. The ferric chloride test gave a greenish-purple color. The compound was yellow

in base. On Whatman No. 1 filter paper, its spot was yellow in the visible, and red-brown in ultraviolet light. After spraying with basic lead acetate solution, the spot was still yellow in the visible, but orange under ultraviolet light.

R_f values for the solid are as follows: with 15% acetic acid, 0.11; with 60% acetic acid, 0.50; with the butanol-acetic acid-water system (40-10-50%, by volume) 0.89; with isopropyl alcohol-water (60%) 0.65; and with chloroform saturated with water, 0.12.

The melting point on a Fisher-Johns block was 266-268°C, and 270-274°C using the Kofler Microscope stage type of block. The acetate was prepared, but no characteristic melting point could be detected. A crystal change, however, was noted on two samples at 138-140°C, using the Kofler apparatus, but no melting could be determined. Refluxing the flavonoid for 2 hours with 4% aqueous sulfuric acid solution did not produce perceptible change in melting point or R_f values in the solvents listed.

The yield of recrystallized flavonoid was 4 mg. from 40 pounds of starting material.

This investigation was supported in part by a contract from the Office of Naval Research.

LITERATURE CITED

1. WILLIAMS, BYRON L. AND SIMON H. WENDER. 1952. The isolation and identification of quercetin and isoquercitrin from black currants (*Ribes nigrum*). J. Am. Chem. Soc. 74:4566.
-