Isoquercitrin and Quercetin in Concord Grapes

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Quercetin (3, 3', 4', 5, 7 pentahydroxy flavone) and isoquercitrin, its 3-glucoside, have been recently isolated and identified from the less highly colored grapes, Thompson white seedless, tokay, and emperor (1). Whether or not these two compounds are also present in the common, deeply colored American grown Concord grape could not be readily determined at the time by the method used, because of the presence of highly pigmented interfering matter. The present note describes a modified procedure which has been successfully used to indicate that the Concord grape—and some commercial products obtained from it—also contain isoquercitrin and quercetin.

Experimental

Four 6-fluid oz. cans of Minute Maid frozen Concord grape juice concentrate were diluted to 3 gal. with distilled water and then passed over an ion exchange column containing Amberlite IRC-50(H) (Rohm and Haas. Philadelphia, Pa.). The column consisted of a glass tube 8 x 120 cm. drawn to an outlet at one end. The column containing the material adsorbed from the diluted grape juice concentrate was washed with 3 gal. of distilled water to remove sugar still present. The effluent and washings were discarded. The adsorbed material was then removed from the column by elution with 1 l. of 95% ethanol. The alcoholic eluate was taken to dryness *in vacuo*, using a resin pot immersed in a hot water bath. The pulverized residue was then extracted with three 50 ml. portions of hot anhydrous acetone. These extracts were combined and, after cooling, were passed through a chromatographic column 6 x 100 cm. packed to a depth of 20 cm. with Magnesol (Food Machinery and Chemical Corp., Westvaco Chemical Division, New York). Flavonoid material in the extract was adsorbed at the top of the column. The chromatogram was developed with ethyl acetate saturated with water. Two flavonoid bands were eluted and collected in separate containers.

The first band (quercetin) was yellow in both the visible and ultraviolet light. For purification, the ethyl acetate was distilled off *in vacuo;* the still impure quercetin was dissolved in ethyl alcohol, put on four 20 x 60 cm. Whatman No. 1 filter paper sheets, and chromatographed in the 60% acetic acid-water solvent system. The yellow zone of each at about 0.40 was cut out and eluted with 95% ethyl alcohol. These eluates of quercetin were combined and the R_f values determined in the same chamber at the same time with an authentic sample of quercetin. These were identical. R_f values for both the authentic and the Concord grape quercetin were 0.08 in 15% acetic acid, 0.38 in 60% acetic acid, and 0.80 in butanol-acetic acid-water (40-10-50%, by vol.).

The second band (isoquercitrin) was yellow in visible light, but brown under ultraviolet light. For purification, the ethyl acetate was distilled off *in vacuo;* the still impure solid isoquercitrin was dissolved in ethyl alcohol, put on four 20 x 60 cm. Whatmen No. 1 filter paper sheets, and chromatographed in 15% acetic acid. The visible yellow zone of each at about R_f 0.45 was cut out and eluted with 95% ethyl alcohol. These eluates of isoquercitrin were combined and the R_f values determined in the same chamber at the same time with an authentic sample of isoquercitrin. They were identical. R_f values for both the authentic isoquercitrin and the Concord grape isoquercitrin were 0.45 in 15% acetic acid; 0.70 in 60% acetic acid, and 0.74 in the butanol-acetic acid-water system.

By such a procedure, samples of pure, bottled, Concord grape juice (Welch's), in addition to the frozen concentrate above, have been shown to contain both isoquercitrin and a relatively smaller amount of quercetin. Also, in a similar study of Concord grape jelly (Welch's), both of these compounds have been shown to be present. This time, however, only a relatively smaller amount of isoquercitrin plus a relatively larger amount of quercetin were indicated by the zones on the paper. This research was supported in part by 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 grapes (*Vitis vinifera*). J. Am. Chem. Soc. 74:4372.