[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF OKLAHOMA]

Quercetin and its Glycosides in Leaves of *Vaccinium myrtillus*

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This paper reports details of the isolation in pure form of quercetin (3,3',4',5,7-pentahydroxyflavone) and five of its glycosides from the leaves of the "huckleberry," *Vaccinium myrtillus*. The glycosides have been identified as quercetin-3-arabinoside, isoquercitrin (quercetin-3-glucoside); quercitrin (quercetin-3-rhamnoside); quercetin-3-gluco-glucoside; and apparently a new quercetin rhamnoside not identical with quercitrin.

Introduction

In a search for a source of relatively rare individual glycosides of quercetin, leaves of the blueberry or European whortleberry, *Vaccinium myrtillus*, commonly called "huckleberry" in this country, have been investigated. In this study, "huckleberry" leaves of the 1951 North Carolina crop have been found to contain not the usually expected one or two, but rather five glycosides of quercetin, in addition to quercetin itself. This paper reports details of the isolation in pure form of quercetin (3,3',4',5,7-pentahydroxyflavone) and of five of its glycosides from the leaves of *Vaccinium myrtillus*. The glycosides have been identified as quercetin-3-arabinoside; isoquercitrin (quercetin-3-glucoside); quercitrin (quercetin-3-rhamnoside); quercetin-3-gluco-glucoside; and apparently a new quercetin rhamnoside, not identical with quercitrin. No previous workers, to our knowledge, have successfully separated in pure form and identified so many quercetin glycosides from the same starting sample of natural product as the present paper describes for leaves of *Vaccinium myrtillus*.

Avicularin, a quercetin-3-arabinoside isolated from *Polygonum aviculare* by Ohta,[1] has been reported by him to be the first flavone pentoside to be found in nature. The quercetin-3-arabinoside isolated here from *Vaccinium myrtillus* probably is the same as Ohta's avicularin.

Isoquercitrin has previously been reported as being present in a number of plants, including foxberry leaves, *Vaccinium vitis-Idaea*.[2]

The more commonly known flavonol glycoside quercitrin is usually obtained from Lemon Flavin.[3]

Hayashi and Ouchi[4] isolated the quercetin glycoside "meratin," m.p. 179–180°, from the flowers of *Meratia praecox*, and reported its constitution to be 3-O-diglucosidyl quercetin. This diglucoside very probably is the same as the quercetin-3-gluco-glucoside reported here.

Experimental

Extraction of Flavonoids from the Leaves.—Two kg. of coarsely-ground, dry leaves was boiled for 2 hr. with 10 gal. of distilled water. The extract was decanted and the residue then re-extracted with 4 gal. of boiling water. The combined extracts were filtered through cloth and then allowed to cool overnight. Approximately 5 gal. of the extract was passed through a 2 × 40" glass column filled to a depth of 36" with Amberlite IRC-50 (H) resin (Rohm and Haas Co., Philadelphia, Pa.). Finally, the column was washed with 2 gal. of distilled water, and then eluted with 95% ethyl alcohol. This procedure was repeated on other columns with other 5-gal. portions of the extract. The flavonoid-rich alcoholic eluates were next combined and then concentrated by two passes through a flash evaporator. The resulting concentrate, now quite aqueous, was allowed to stand for several days and was then filtered to remove the bulk of the precipitated quercetin.

The filtrate was extracted twice with one-half its volume of *n*-amyl alcohol. The amyl alcohol fraction was then concentrated and dried by distillation at reduced pressure. It was next poured into 20 volumes of *n*-pentane, whereupon the flavonoids were precipitated as a light-colored flocculent mass. After standing in the refrigerator for several hours, the solution was filtered and the precipitate washed with *n*-pentane. The funnel containing the precipitate was stored for several days in the refrigerator, after which the solid was allowed to dry at room temperature. Finally, the product was dried for several days in the oven at 80°.

In this way, approximately 14 kg. of leaves was worked up, yielding 98 g. of flavonoid concentrate.

Separation of the Mixed Flavonoids.—The concentrate of mixed flavonoids was separated by adsorption chromatography on a column of untreated Magnesol, industrial grade, regular (Food Machinery and Chemical Corp., Westvaco Chemical Div., New York). A 0.5-g. sample of the mixture was dissolved in 50 ml. of anhydrous acetone and the solution was then passed through a 60-mm. diameter column packed to a depth of 160 mm. with Magnesol. When the column was eluted with ethyl acetate–water solution, six definite flavonoid bands could be seen when observed under ultraviolet light. Upon continued elution with ethyl acetate–water solution, five of the bands were removed from the column and were collected as separate fractions, although there was some overlapping of fractions on this first pass through the column. The fractions were labeled (I through V) in the order in which they were eluted from the column. The sixth band remained at the top of the column, which now appears to be rather indicative of flavonol glycosides containing more than 1 mole of sugar. Following removal of bands I–V by elution, the column was extruded, and the sixth band cut out. The sixth flavonoid was removed from this segment of the column by leaching with a 20% isopropyl alcohol solution.

Column separations were carried out with 0.5-g. samples until all of the starting flavonoid concentrate was exhausted. Similar fractions from each column were combined. Each combined fraction (I–VI) was next worked up separately to obtain the pure, individual flavonoid.

Purification and Identification of Quercetin (Fraction I).—The combined fraction I, consisting of quercetin not previously precipitated during concentration, was taken to dryness by distillation at reduced pressure. The residue was recrystallized three times from alcohol–water solution and was then dried in the oven at 120°. The product melted at 314–315°. All melting points listed in this article are uncorrected. The observed melting point corresponds to previously recorded values.[5]

The pentaacetyl derivative was prepared using acetic anhydride and sulfuric acid and was twice recrystallized from alcohol by addition of water; m.p. 195°. Quercetin pentaacetate has been previously reported as melting at 193–194°.[5] R_f values in the six solvent systems tried (15% acetic acid, 60% acetic acid, ethyl acetate–water, chloro-

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(3) L. Rigaud, *Ann. Chem. Pharm.*, **90**, 283 (1854); C. H. Ice and S. H. Wender, *Arch. Biochem. Biophys.*, **38**, 185 (1952).

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form–water, butyl alcohol–acetic acid–water (40–10–50% by volume), and isopropyl alcohol–water (1–3.5 parts by volume)) agreed with those of authentic quercetin.[6] The ultraviolet absorption spectrum was identical with that of known quercetin. Fraction I, therefore, is quercetin.

The major portion of the quercetin had precipitated on concentration before the Magnesol treatment. After extensive recrystallization, it was likewise proved to be quercetin by the methods described above. The approximate total yield of pure quercetin was 5 g.

Purification and Identification of Quercetin-3-arabinoside (Fraction II).—The combined no. II fractions were carefully evaporated to dryness; the residue dissolved in anhydrous acetone; and then the acetone solution added to a fresh Magnesol column. After thorough washing with anhydrous acetone of the column containing the adsorbed flavonol glycoside, fraction II was eluted with ethyl acetate–water. This product, on paper chromatographic study, still contained a trace of quercitrin. The fraction II was finally purified by chromatographing on S and S No. 597 paper, cut to a size approximately 20 × 58 cm. and using 15% acetic acid as the solvent system. The solution was put on the paper along the length of a line marked 8 cm. from one end of the strip. A margin of 3 cm. on one side and 1 cm. on the other was left in order to facilitate elution of the bands once they had been separated. After chromatographing, the strips were allowed to dry for three hours. On each of the papers, the fraction II band had a R_f value of approximately 0.46; whereas the trace of quercitrin was at a R_f of approximately 0.62. The fraction II band was cut out of each of the paper chromatograms. After cutting the other end of the strip (the 1-cm. margin) to a point, the end of the strip with no adsorbed material (the 3-cm. margin) was immersed in 95% alcohol as for descending elution. The immersed strips were then placed in air-tight chambers for 3 hr. with small containers in place to catch the alcohol solution dripping from each pointed end. The combined eluates from 20 strips were concentrated to a small volume. The resulting precipitate was recrystallized four times from alcohol–water solutions. The product was dried for 1 hr. at 110°, m.p. was 203°. Approximate yield of pure product was 20 mg.

R_f values determined in three solvent systems for fraction II were as follows: 0.46 in 15% acetic acid, 0.72 in 60% acetic acid, and 0.82 in butyl alcohol–acetic acid–water (40–10–50% by volume).

The ultraviolet absorption spectrum in 95% alcohol was measured and found to be identical with those of such known quercetin-3-monoglycosides as quercitrin and isoquercitrin.

Identification of the Sugar of Fraction II.—Ten mg. of the purified fraction II product was hydrolyzed by refluxing with 2% sulfuric acid. The hydrolysis was complete within a few minutes. After cooling for 24 hr., the quercetin was filtered off, dried and weighed (6.83 mg.). Calcd.: 1 mole of arabinose per 1 mole of quercetin, 6.96 mg.; 2 moles of arabinose per 1 mole of quercetin, 4.24 mg. The weight, therefore, indicated one mole of sugar per mole of aglycone. The sugar was identified by paper chromatography[7] in two-solvent systems: ethyl acetate–pyridine–water (2–1–2% by volume); and butyl alcohol–pyridine–water (2–1–1.5% by volume—the upper layer removed and 1 more part of pyridine added). Aniline hydrogen oxalate was used as the spray reagent. It gave the characteristic pink color with arabinose. Authentic samples of arabinose were used as checks.

Identification of the Aglycone of Fraction II.—The aglycone was recrystallized from alcohol–water solution and dried at 110° for 1 hr., m.p. 314°. The pentaacetyl derivative was prepared, m.p. 196°. R_f values agreed with those of authentic quercetin in every solvent system tried. These included 15% acetic acid, 60% acetic acid, chloroform saturated with water, and the butyl alcohol system.

Methylation and Position of Sugar of Fraction II.—A sample of the pure fraction II (the glycoside) was methylated with dimethyl sulfate and potassium carbonate in acetone solution, according to the method of Shimokoriyama.[8] The resulting product was then hydrolyzed to yield 3′,4′,5,7-tetramethoxy-3-hydroxy flavone. The crude product was dissolved in the minimum amount of boiling benzene and recrystallized by addition of *n*-pentane. After two recrystallizations, the product was thoroughly dried, m.p. 195–196°, which agrees well with the literature value.[9] Fraction II is, therefore, quercetin-3-arabinoside.

Purification and Identification of Quercitrin (Fraction III).—The combined fraction III eluates were taken to dryness, re-extracted with anhydrous acetone; and then as in the previously described manner, rechromatographed on fresh Magnesol columns two additional times to achieve complete separation from the overlapping small amounts of other quercetin glycosides. The final eluate was taken to dryness, and the product recrystallized from hot water. After drying at 110°, the yellow crystalline solid had a m.p. 183–184°, as did authentic quercitrin. No lowering of melting point occurred on admixture of the two. Approximate yield of pure quercitrin was 4 g.

R_f values in the three solvent systems tried agreed with those of known quercitrin. These were 0.61 in 15% acetic acid; 0.74 in 60% acetic acid; and 0.82 in butyl alcohol–acetic acid–water (40–10–50). No separation occurred on paper with a mixture of pure fraction III and authentic quercitrin. The ultraviolet absorption spectrum was identical with that of quercitrin.

Methylation, followed by acid hydrolysis of the product, then recrystallization, gave 3′,4′,5,7-tetramethoxy-3-hydroxy flavone, m.p. 195°. Absorption maxima of this derivative were at 250 and 361 mμ and minima at 233 and 285 mμ.

Hydrolysis with 1% sulfuric acid gave 1 mole of quercetin to 1 mole of rhamnose. The quercetin was identified by melting point, 314–315°, R_f values, and pentaacetate, m.p. 195°, as described in previous paragraphs. The rhamnose was identified by its osazone and by comparison with authentic rhamnose on paper chromatograms.

Fraction III, therefore, was identified as quercitrin.

Purification and Identification Studies of an Apparently New Quercetin-rhamnoside (Fraction IV).—The combined fractions IV were evaporated to dryness by the procedure described previously and two additional separations were carried out on fresh Magnesol columns. This gave a final product which showed only one zone on paper chromatograms run in three solvent systems. The R_f values of fraction IV in these solvent systems were: 0.62 in 15% acetic acid; 0.82 in 60% acetic acid; and 0.90 in butyl alcohol–acetic acid–water (40–10–50). Pure fraction IV separates from authentic quercitrin on paper chromatograms in the last two solvent systems. These separations from known quercitrin, together with the separation from quercitrin on Magnesol, would indicate that the compound is not quercitrin.

The ultraviolet absorption spectrum was identical with that of quercitrin, as were color reactions produced with chromogenic sprays. Approximate yield of pure fraction IV was 20 mg.

The glycoside was hydrolyzed by refluxing with 1% sulfuric acid for 1 hr. The aglycone was filtered off and weighed. After recrystallization, it was identified as quercetin by melting point 314–315°, R_f values, absorption spectrum and pentaacetate, m.p. 196–197°, as described in previous paragraphs. The rhamnose was identified by comparison with authentic rhamnose on paper chromatograms. The ratio of 1 mole of sugar per 1 mole of aglycone was confirmed by the method of Fisher, Parsons and Morrison.[10]

Fraction IV is, therefore, a quercetin-rhamnoside, but apparently not identical with quercitrin. Lack of additional sample prevented further structural studies.

Purification and Identification of Isoquercitrin (Fraction V).—The combined fraction V eluates were taken to dryness, re-extracted with anhydrous acetone, and then as in the previously described procedure chromatographed again on a fresh Magnesol column. The new fraction V was distilled to dryness *in vacuo*, and the product recrystallized three times from hot water. After drying at 100°, a yellow, crystalline solid, m.p. 241–242° was obtained. The ultraviolet spectrum agreed with that of authentic isoquercitrin. The R_f values in the three solvent systems tried agree with those of isoquercitrin. These were 0.46 in 15% acetic acid,

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(10) R. B. Fisher, D. S. Parsons and G. A. Morrison, *Nature*, **161**, 764 (1948).

0.74 in 60% acetic acid, and 0.72 in butyl alcohol–acetic acid–water (40–10–50), Approximate yield of pure isoquercitrin was 2.3 g.

Upon methylation, then hydrolysis and recrystallization, 3',4',5,7-tetramethoxy-3-hydroxyflavone, m.p. 195–196°, was obtained. The sugar is, therefore, attached to the 3-position of the aglycone.

Hydrolysis of the glycoside with 1% sulfuric acid yielded 1 mole of glucose per 1 mole of quercetin. The quercetin was identified by R_f values, melting point 313–314°, absorption spectrum and pentaacetate, m.p. 194°. The sugar was identified as glucose by its osazone and by paper chromatography.

Fraction V, therefore, is isoquercitrin.

Purification and Identification of Quercetin-3-glucoglucoside (Fraction VI).—Fraction VI was placed on a fresh Magnesol column from an acetone solution. The column was then eluted with ethyl acetate–water solution made slightly acid with acetic acid. This moved the flavonoid off the column as a sharp band. This band was collected and after evaporation to dryness, the solid was recrystallized from alcohol–water solution. The product was dried at 110 for 1 hr., m.p. 182–184°. Approximate yield of pure product was 55 mg.

R_f values were as follows: 0.56 in 15% acetic acid, 0.76 in 60% acetic acid, 0.86 in butyl alcohol–acetic acid–water (40–10–50), 0.71 in isopropyl alcohol–water (1:3.5), and 0.90 in 60% isopropyl alcohol.

The ultraviolet absorption spectrum was identical with one that was run on authentic rutin. Its behavior on Magnesol was also similar to that of rutin and further indicated the probable presence of 2 moles of sugar on the flavonol aglycone.

A sample of pure fraction VI was methylated, then hydrolyzed and recrystallized, giving 3',4',5,7-tetramethoxy-3-hydroxy flavone. This compound was identified by its melting point 196°, and by comparison of the absorption spectrum of this tetramethoxyquercetin with that of the known compound as prepared from authentic quercitrin. The sugar is, therefore, attached at position three.

A sample of fraction VI was hydrolyzed by refluxing in 2% sulfuric acid for 2 hr. After cooling for 24 hr., the aglycone was filtered off and weighed. The recrystallized quercetin was identified by its R_f values, melting point 314–315°, absorption spectrum and pentaacetate, m.p. 193°. The hydrolysis filtrate was neutralized with Amberlite IR-4B ion exchange resin, and the sugar identified as glucose by paper chromatography. The ratio of 2 moles of glucose per mole of quercetin was determined by the method of Fisher, Parsons and Morrison.[10] Fraction VI is, therefore, quercetin-3-gluco-glucoside.

Discussion.—The "huckleberry" leaves used were purchased from Meer Corporation, New York. They were authenticated by an official of that corporation as having been obtained from North Carolina, 1951 season, and classified as *Vaccinium myrtillis*.

A corresponding batch of "huckleberry" leaves from the 1950 crop was studied in detail and was found to contain quercetin, quercetin-3-arabinoside, isoquercitrin and quercitrin, but none of the quercetin-3-glucoglucoside and the new quercetin-rhamnoside.

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Infrared Spectra of Some Cholestanol Derivatives[1]

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The infrared absorption spectra from 2 to 16μ are presented for seven sterols differing only in double bond character. Many of the observed variations in absorption could be attributed to specific functional groups. The 6 and 3.3μ regions have been shown to be the most useful in providing information on double bond location. Some secondary effects of double bond location have also been noted.

Several general studies have indicated that infrared spectrophotometry may be useful in the determination of steroids[2–5] and such spectra were recently used in the identification of the new skin sterol, Δ^7-cholestenol.[6] In contrast to the steroids, data on the infrared spectra of the sterols are relatively meager.[4–10] Jones, *et al.*,[9,11] have studied effects of the location of certain ethylenic double bonds on the spectra of unsaturated steroids in solution. While others have observed the 6 μ C=C stretching band in the spectra of solid films of steroids,[5,8] Bladon, *et al.*, extended these studies to new compounds and included both solid state and solution spectra. In the present study complete solid spectra have been taken of a series of

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