16 minutes) always afforded benzoylurea and 3,5-dimethyl-4-iodopyrazole.

Hydrolysis of F, with excess 10% hydrochloric acid, resulted in a quantitative yield of benzoic acid and 3,5-dimethylpyrazole hydrochloride.

Hydrazine hydrate and phenylhydrazine did not react with (F) and it displayed a similar negligible electrophilic activity with sodium acetate after eight hours refluxing, and also with one molar quantities of sodium and alcohol at room temperatures. In large excess (20 molar quantities of sodium) these latter reagents under reflux reduced (F) to a pyrazoline without however any observed formation of benzoylurea.

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CORK, IRELAND

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

The Isolation and Identification of Quercetin and Isoquercitrin from Black Currants (*Ribes nigrum*)[1]

BY BYRON L. WILLIAMS, CLARK H. ICE AND SIMON H. WENDER

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Black currants, *Ribes nigrum*, have been reported by both the guinea pig test and clinical assay method to possess a relatively high "vitamin P" activity, which is generally attributed to the flavonoid compounds present. This paper reports the isolation and identification of quercetin (3,3′,4′,5,7-pentahydroxyflavone) and isoquercitrin (quercetin-3-glucoside) from dried black currants.

Introduction

Bacharach, *et al.*,[2,3] have reported that a concentrate from black currants, *Ribes nigrum*, possesses a relatively high "vitamin P" activity. Apparently the tests were made using a concentrate prepared from black currants in a manner described by Pollard.[4] In a later study, Pollard[5] reports the isolation of a crystalline substance from black currants. From the properties exhibited by this substance, Pollard postulates that it is a flavonol. No positive identification, however, was achieved.

To date, to our knowledge, no flavonoid compounds have been reported as having been identified from black currants or their concentrates. The present paper reports the isolation and identification of quercetin (3,3′,4′,5,7-pentahydroxyflavone) and isoquercitrin (quercetin-3-glucoside) from dried black currants, *Ribes nigrum*.

For the isolation of the flavonoid compounds the following unit processes were employed: boiling water extraction, ion exchange chromatography, concentration and drying *in vacuo*, extraction with hot anhydrous acetone, adsorption chromatography, precipitation as the lead salt, decomposition of the lead salt, neutralization of a filtrate by ion exchange, and recrystallization from water. In the identification procedure, use was made of paper partition chromatography, melting point determinations, ultraviolet absorption spectra and preparation of a derivative by methylation and hydrolysis.

Experimental

Twenty pounds of dried black currants, *Ribes nigrum*, was soaked in water at room temperature for three hours. This caused the currants to swell and increased the effectiveness of the wet grinder, through which they were processed as the next step.[6] The discharged extract from the wet grinder was diluted to 20 gal. with distilled water and digested for one hour at the boiling point. The extract was cooled to approximately 70° and filtered. The residue was discarded and the filtrate allowed to cool to room temperature. The cooled extract was then passed over ion exchange columns at the rate of one gal./hr. for each column. Four columns were used with 5 gal. of extract passed over each. A column consisted of a glass tube 6 × 100 cm. drawn to an outlet at one end filled to a depth of 80 cm. with Amberlite IRC-50(H) (Rohm and Haas, Philadelphia, Pa.). The columns containing the material adsorbed from the extract were each washed with 2 gal. of distilled water to remove the sugar, and the effluent and washings were discarded.[7] The adsorbed material, containing the flavonoids present, was then eluted from the columns with 500 ml. of 95% ethyl alcohol for each of the four columns. This eluate was then taken to dryness *in vacuo* using a resin pot immersed in a hot water-bath. The pulverized residue was then transferred to a Soxhlet extractor and extracted for 36 hr. with 500 ml. of anhydrous acetone. This acetone extract, after cooling to room temperature, was passed through a chromatographic column, 38 × 220 mm., containing a bed of magnesol (Food Machinery and Chemical Corp., Westvaco Chemical Division, New York), 100 mm. deep. One hundred ml. of the extract seemed to be the optimum load for a column. Material in the extract was adsorbed on the magnesol, giving a band approximately 10 mm. deep and yellow in visible light. The chromatogram was developed with ethyl acetate saturated with water.[8] A band, yellow in both visible and ultraviolet light, moved off the column first. The portions containing this band were combined and concentrated *in vacuo* to 5 ml., and 50 ml. of pentane was added to the cooled solution. The solid was removed by centrifugation and identified as quercetin. The yield at this point was 20 mg. Yields reported in this paper, however, do not represent the actual flavonoid content of the currants, as purity for qualitative analysis was the object of the research, and no effort was made to determine the exact quantity of each flavonoid as present in the black currants.

By paper partition chromatography, the solid showed R_f values of 0.06 in 15% acetic acid, 0.77 in butanol–acetic acid–water (40-10-50%, by volume), and 0.42 in 60% acetic acid, and no separation from authentic quercetin by mixed paper chromatography in each of the solvents mentioned.

(1) This research was supported in part by the Office of Naval Research (Project NR-059 226), and by the Atomic Energy Commission (AT-(40-1)-235).

(2) A. L. Bacharach and M. E. Coates, *J. Soc. Chem. Ind.*, **63**, 198 (1944).

(3) H. Scarborough and A. L. Bacharach in "Vitamins and Hormones," Vol. VII, Academic Press, Inc., New York, N. Y., 1949, pp. 46–49.

(4) A. Pollard, *Nature*, **150**, 491 (1942).

(5) A. Pollard, *Fruit Products J.*, **24**, 139 (1945).

(6) L. S. Ciereszko, T. B. Gage and S. H. Wender, *Anal. Chem.*, **24**, 767 (1952).

(7) T. B. Gage, Q. L. Morris, W. E. Detty and S. H. Wender, *Science*, **113**, 522 (1951).

(8) C. H. Ice and S. H. Wender, *Anal. Chem.*, in press.

Corresponding R_f values reported for authentic quercetin are 0.07, 0.78 and 0.40.[9]

After recrystallization from ethanol–water, the m.p. of the quercetin was 315°, uncor.

The next band eluted from the magnesol column was yellow in the visible and brown under ultraviolet light. The portions containing this band were combined and concentrated *in vacuo* to 5 ml., and 50 ml. of pentane was added. The solid removed by centrifugation was dissolved in dry acetone. The chromatographic procedure was repeated until only one zone could be detected on paper chromatograms processed in the three solvent systems mentioned above. The product at this point was reddish brown in color and amounted to 75 mg. from the 20 lb. of currants. The crude product was dissolved in 95% ethyl alcohol, and a saturated solution of basic lead acetate in water added dropwise until no more precipitation was observed. The mixture was filtered, yielding an orange-yellow lead salt. The filtrate was discarded, and the precipitate was washed with alcohol, then water, to remove the unreacted material and excess lead acetate. The washings were discarded, and the lead salt was suspended in 10 ml. of ethyl alcohol. Concentrated sulfuric acid was then added dropwise until the formation of lead sulfate ceased. The lead sulfate was removed by centrifugation and discarded. To remove the excess acid the alcoholic filtrate was passed over an ion exchange column, 20 × 120 mm., packed from ethyl alcohol to a depth of 100 mm. with Amberlite IRA-45 (Rohm and Haas, Philadelphia, Pa.).[10] The eluate from the ion exchange column was taken to dryness *in vacuo*. The resulting solid was dissolved in 5 ml. of boiling water and allowed to cool in the refrigerator overnight. The precipitate was removed by centrifugation and recrystallized again. A brown, oily-like impurity persists in the product to this point. This impurity apparently increases the water solubility of the flavonoid, since in going from one recrystallization to the other, increasing amounts of boiling water are required to dissolve the product. Several recrystallizations are necessary to remove the impurity and produce a yellow solid. The yield at this point was 5 mg. The recrystallized solid was dried *in vacuo* at 80° in the presence of P_2O_5.

Identification of the Isoquercitrin.—The purified solid had a m.p. of 227° (uncor.) and showed R_f values of 0.46 in 15% acetic acid, 0.76 in butanol–acetic acid–water, and 0.75 in 60% acetic acid. These values correspond to the values recorded for both isoquercitrin and quercimeritrin (quercetin-7-glucoside) in these solvents. The unknown substance showed separation from rutin (quercetin-3-rhamnoglucoside) and quercitrin (quercetin-3-rhamnoside) by mixed paper chromatography in 15% acetic acid, but showed no separation from authentic isoquercitrin or quercimeritrin. The ultraviolet absorption spectrum was identical with that for isoquercitrin and quercimeritrin. To differentiate between the two possibilities, the tetramethoxy derivative was prepared by methylation and subsequent hydrolysis, according to the method of Shimokoriyama.[11] Three mg. of the isolated quercetin glucoside was dissolved in 10 ml. of anhydrous acetone, and 0.5 g. of anhydrous potassium carbonate and 0.3 ml. of dimethyl sulfate was then added. The mixture was refluxed for three hours and the acetone was removed *in vacuo*. The resulting cake was suspended in 5 ml. of water and neutralized by dropwise addition of concentrated sulfuric acid. Approximately three drops excess acid was added and the mixture refluxed for two hours to break the glycosidic linkage by hydrolysis. The contents of the reaction vessel were transferred to a centrifuge tube and allowed to cool overnight in the refrigerator. Centrifugation yielded a brown-yellow solid. The method was modified at this point to remove a brown impurity present. The solid was dried and then dissolved in anhydrous acetone. Then it was passed over a column packed with magnesol. Elution with ethyl acetate saturated with water removed a band that was light yellow under ultraviolet light, but not detectable in visible light. A visible brown remained at the top of the column. The eluate containing the yellow band was concentrated to 0.5 ml. and 5 ml. of pentane added. The solid was removed and washed twice with cold benzene. The product was a light yellow solid, m.p. 191–193° (uncor.) which agrees well with the literature value of 193–195°[12] for 3′,4′,5,7-tetramethoxy-3-hydroxyflavone. By this series of reactions, quercimeritrin would have yielded 3,3′,4′,5-tetramethoxy-7-hydroxyflavone which melts at 284–285°.[12] Thus, a quercetin glycoside in black currants has been identified as isoquercitrin.

The dried currants used in this investigation were purchased from a local grocery store. Labels on the unopened boxes indicated that they were of the Black Zante variety (California).

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NORMAN, OKLAHOMA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, NORTHWESTERN UNIVERSITY]

Effect of Promoters on the Alkylation of Benzene by Secondary Butyl Methyl Ether in the Presence of Boron Trifluoride

BY ROBERT L. BURWELL, JR., LLOYD M. ELKIN AND ALFRED D. SHIELDS

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In mixtures of *s*-butyl methyl ether, benzene and boron trifluoride prepared under anhydrous conditions, alkylation occurs at negligible rate when the boron trifluoride–ether mole ratio is less than 0.9, slowly when it is 1.0 and rather rapidly when it is about 1.08. Addition of water or sulfuric or chlorosulfonic acids in small amounts greatly increases the rate of reaction. The relatively rapid reaction of secondary ethers ordinarily observed appears due to the presence of water and an excess of boron trifluoride beyond the stoichiometric. The reaction appears to involve the *s*-butylcarbonium ion but the ion seems not, in general, to result from mere dissociation of the boron trifluoride–ether complex. In a mixture of ethyl ether and benzene, five moles to one, which is saturated with boron trifluoride, no ethylbenzene can be detected in 10 days at 25°.

In accordance with the proposal of Price and Ciskowski[1] it has been generally assumed that alkylation reactions of alcohols and ethers caused by boron trifluoride proceed *via* the formation of an addition product which is unstable under the reaction conditions and decomposes into a carbonium ion and a hydroxy- or alkoxytrifluoroborate anion. The carbonium ion effects alkylation in the customary fashion.

(1) C. C. Price and J. M. Ciskowski, THIS JOURNAL, **60**, 2499 (1938).

$$ROR' + BF_3 \rightleftarrows RR'O{:}BF_3 \longrightarrow R'OBF_3^- + R^+ \quad (1)$$

This paper reports results of the alkylation of benzene and toluene by *s*-butyl methyl ether which necessitate some modification of this mechanism, at least for secondary ethers. With mole ratios of boron trifluoride to *s*-butyl methyl ether of about 0.9 and aromatic hydrocarbon–ether ratios of about six, no alkylation can be observed in periods of months at room temperature. If one rejects the assumption that one mole of ether can in some