

equiv., 100.1. Found: C, 59.9; H, 8.1; neut. equiv., 100.8.

Using the same procedure as in (a), 20 g. (0.1 mole) of *cis*-4,5-dimethylhexahydrophthalic acid and 24 g. (0.1 mole) of lead dioxide in 100 ml. of decalin afforded 1.71 g. (16%) of *cis*-4,5-dimethylcyclohexene; b.p. 127.8° (micro); n_D^{25} 1.4467.

Anal. Calcd. for C₈H₁₄: C, 87.2; H, 12.8. Found: C, 87.2; H, 13.0.

Oxidation with alkaline potassium permanganate yielded β , β' -dimethyladipic acid, m.p. 132–133, where m.p. 133° is reported for the higher melting isomer.¹⁵

(d) **Bicyclo[2,2,1]heptane-2,3-dicarboxylic Acid**.—Proceeding in the same manner and with the same molar quantities as above, this acid lost carbon dioxide and gave a mixture of product and decalin which could not be separated. A crude fraction (100 mg., b.p. 85–115°), was oxidized with potassium permanganate and gave 35 mg. of *cis*-cyclopentane-1,3-dicarboxylic acid, m.p. 120–120.5°. The reported m.p. is 120–121°.¹⁶

tane-1,3-dicarboxylic acid, m.p. 120–120.5°. The reported m.p. is 120–121°.¹⁶

Bisdecaboxylation of Bicyclo[2,2,2]octane-2,3-dicarboxylic Anhydride with Powdered Lead Dioxide.—A mixture of 2.0 g. of the anhydride, 10 g. of lead dioxide and 40 g. of powdered Pyrex was ground thoroughly in a mortar and placed in a 250-ml. flask with side-arm connected to a 100-ml. flask leading successively to an empty trap, one cooled in ice and containing 5% bromine in carbon tetrachloride and a third containing aqueous barium hydroxide. On heating slowly to 250°, a solid sublimed and barium carbonate precipitated. When sublimation ceased, the contents were cooled and extracted with *n*-pentane. Concentration of the *n*-pentane with a Vigreux column gave material which was sublimed at room temperature (0.2 g.). After crystallization from methanol and sublimation, the bicyclo[2,2,2]-octene melted at 141–144°; reported m.p. 143–144°.¹⁷

(16) S. F. Birch, W. J. Oldham and E. A. Johnson, *J. Chem. Soc.*, 818 (1947).

(17) R. Seka and O. Tramposch, *Ber.*, **75**, 1379 (1942).

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(15) F. Foltis and H. Wagner, *Ann.*, **433**, 103 (1923).

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

The Isolation and Identification of Quercetin and Isoquercitrin from Grapes (*Vitis vinifera*)¹

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RECEIVED MARCH 6, 1952

Grapes, *Vitis vinifera*, have been previously reported to possess a relatively high "vitamin P" activity, which is generally attributed to the flavonoid compounds present. This paper is the first to report the isolation and identification in pure form from grapes of quercetin (3,3',4',5,7-pentahydroxyflavone) and of isoquercitrin (quercetin-3-glucoside). The three types of grapes individually studied were Thompson white seedless, tokay and emperor. All three belong to *Vitis vinifera*.

Introduction

Scarborough² has reported that a concentrate from grapes, *Vitis vinifera*, possesses a relatively high "vitamin P" activity. This so-called "vitamin P" activity is generally attributed to the flavonoids present.³ To date, to our knowledge, isoquercitrin has not been reported as having been isolated and identified from grapes or grape concentrates. The present paper reports the isolation in pure form and identification of quercetin (3,3',4',5,7-pentahydroxyflavone) and isoquercitrin (quercetin-3-glucoside) from grapes, *Vitis vinifera*.

For the isolation of the flavonoid compounds, the method reported involves hot water extraction, ion exchange chromatography, concentration and drying *in vacuo*, extraction with hot anhydrous acetone, adsorption chromatography, and recrystallization from water. Use is made of paper partition chromatography, acetylation, hydrolysis, ultraviolet absorption spectra, and mixed melting points in the identification procedure.

The three types of grapes studied, individually, each in 50-lb. batches, were Thompson white seedless, tokay and emperor. All belong to *Vitis vinifera*.

Experimental

In a typical experiment, 50 lb. of one of the three types of grapes studied, were processed, with stems removed, through a wet grinder,⁴ and extracted in an aluminum pot with 20 gal. of distilled water at boiling temperature. The extract was filtered, the residue discarded, and the filtrate allowed to cool to room temperature. The cooled extract was then passed over ion exchange columns at the rate of 1 gal./hr. for each column. Four columns were used with 5 gal. of extract being passed over each. Each column consisted of a glass tube 6 × 100 cm. drawn to an outlet at one end. The resin bed was composed of Amberlite IRC-50(H), (Rohm and Haas, Philadelphia, Pa.). The columns containing the material adsorbed⁵ from the extract were each washed with 5 gal. of distilled water to get rid of the sugar. The effluent and washings were discarded. The adsorbed material, containing the flavonoids present, was then eluted from the columns with 500 ml. of 95% ethanol 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 extracted with five 100-ml. portions of hot, anhydrous acetone. These acetone extracts, after cooling to room temperature, were passed through a chromatographic column, 20 × 220 mm., containing a bed of magnesol (Food Machinery and Chemical Corp., Westvaco Chemical Division, New York) 10 mm. deep. This passage removed a considerable amount of dark, non-flavonoid material which remained adsorbed on the column. The effluent from the column was then chromatographed in a column, 20 × 220 mm., but with a fresh magnesol bed made 100 mm. deep. Material in the extract was adsorbed on the magnesol, giving a band about 5 mm. deep and yellow in visible light. The chromatogram was developed with

(1) This research was supported in part by the Office of Naval Research (Project NR-059-226).

(2) H. Scarborough, *Biochem. J.*, **39**, 276 (1945).

(3) H. Scarborough and A. L. Bacharach, "Vitamins and Hormones," Vol. VII, Academic Press, Inc., New York, N. Y., 1940, pp. 1–55.

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

(5) T. B. Gage, Q. L. Morris, W. E. Detty and S. H. Wender, *Science*, **112**, 522 (1951).

ethyl acetate saturated with water.⁶ A band, yellow in both visible and ultraviolet light, moved off the column first. This portion was taken to dryness *in vacuo*, and the solid identified as quercetin. It showed R_f values of 0.08 in 15% acetic acid and 0.80 in butanol-acetic acid-water (40-10-50%, by volume), and no separation from authentic quercetin by mixed paper chromatography. Corresponding R_f values⁷ reported for authentic quercetin are 0.07 and 0.78. The pentaacetate was prepared from the solid of the first eluate and recrystallized from ethyl acetate by adding pentane. Its m.p. was 195°, uncor. The recorded value for quercetin pentaacetate is 191-195°.⁸

The next band eluted from the magnesol with the ethyl acetate solution was a 15 mm. band, yellow in visible light, but brown under ultraviolet light. This portion was taken to dryness and dissolved in anhydrous acetone. The acetone solution was next rechromatographed on magnesol by the method described above until a sample of the eluate showed only one spot when analyzed by paper partition chromatography in 15% acetic acid and the butanol-acetic acid-water system, using basic lead acetate as a chromogenic spray.⁷ This pigment spot had a R_f value of 0.46 in 15% acetic acid and 0.74 in the butanol-solvent system. These correspond to the values recorded for both isoquercitrin and quercimeritrin (quercetin-7-glucoside) in these solvents. A typical yield, at this point, of crude product was 200 mg. from 50 lb. of grapes.

The crude product was now recrystallized by dissolving it in 5 ml. of boiling water, then centrifuging, and discarding the residue. The supernatant liquor was allowed to cool to room temperature, made slightly acidic with acetic acid, and then placed in the refrigerator overnight. Crystallization occurred. The solution was next centrifuged, and the mother liquor discarded. The residue of brown-yellow crystals was washed with ice-water to remove the traces of acid and thus prevent possible subsequent hydrolysis. This recrystallization procedure was repeated eight times, each time more water being required, and finally yielded a light yellow powder. This product was dried *in vacuo* in the presence of phosphorus pentoxide at 80° for 3 hr.; yield 30 mg.

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

(7) T. B. Cage, C. D. Douglass and S. H. Wender, *ibid.*, **23**, 1582 (1951).

(8) A. G. Perkin and A. E. Everest, "The Natural Organic Colouring Matters," Longmans, Green and Co., London, 1918, p. 188.

Identification of the Isoquercitrin.—Hydrolysis of a portion of the yellow powder with 1% sulfuric acid solution produced glucose, identified by its osazone and R_f , and quercetin, identified by the method already described above for quercetin. At this point, the known possibilities were only isoquercitrin and quercimeritrin.

The ultraviolet absorption spectrum of the recrystallized pigment before hydrolysis, was identical with that obtained with authentic isoquercitrin.

The m.p. of the isoquercitrin isolated from the grapes was 232°, uncor. No lowering of the m.p. occurred when the isolated product was mixed with available authentic isoquercitrin (m.p. 233°, uncor.).

A sample of the isoquercitrin from grapes was methylated with dimethyl sulfate and potassium carbonate in acetone solution, according to the method of Shimokoriyama.⁹ The resulting product was then hydrolyzed to yield 3',4',5,7-tetramethoxy-3-hydroxyflavone, which was recrystallized from benzene. The melting point was 193-195° (uncor.), which agrees with the literature value.¹⁰ By this same series of reactions, quercimeritrin would have yielded 3,3',4',5-tetramethoxy-7-hydroxyflavone, which melts at 284-285°.¹⁰ Thus, the quercetin glucoside from grapes has been identified as isoquercitrin.

The grapes used in this investigation were purchased from a local grocery store. Original labels on the un-opened crates indicated that they were California grapes, and of the type: Thompson white seedless, emperor or tokay.

Acknowledgments.—The authors express their appreciation to Dr. Carl D. Douglass, University of Arkansas Medical School, Little Rock, who, while at Oklahoma, made important experimental contributions on the preliminary studies of the flavonoids of grapes; to the Pharmacology Laboratory, Bureau of Agricultural and Industrial Chemistry, Albany, California, for the sample of authentic isoquercitrin and to Prof. T. R. Seshadri, Delhi, India, for the sample of authentic quercimeritrin.

(9) M. Shimokoriyama, *Acta Phytoclim. (Japan)*, **15**, 63 (1949).

(10) G. F. Attree and A. G. Perkin, *J. Chem. Soc.*, 234 (1927).

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[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, FEDERAL SECURITY AGENCY]

The Preparation of 1,6-Didesoxy-D-altritol, 1,6-Didesoxygalactitol and 1,6-Didesoxy-L-mannitol¹

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RECEIVED MARCH 13, 1952

Methyl α -D-altroside has been transformed by a series of reactions to 1-desoxy-D-altritol and 1,6-didesoxy-D-altritol. 1,6-Didesoxygalactitol has been prepared through the reductive desulfurization of L-fucose diethyl mercaptal, which can be obtained readily from the crude hydrolysis product of certain seaweeds. The reductive desulfurization of L-rhamnose diethyl mercaptal has produced 1,6-didesoxy-L-mannitol. Acetyl, methylene and benzylidene derivatives of these desoxyhexitols have been described.

In the continuation of our studies on the action of *Acetobacter suboxydans* on ω -desoxy sugar alcohols,² we have undertaken the synthesis of several 1,6-didesoxyhexitols that might serve either as substrates for that microorganism or as reference compounds for the subsequent identification of our products. The first of these substances, 1,6-didesoxy-L-glucitol (synonym, 1,6-didesoxy-D-guli-

tol), has already been described³ as a sirup that could, however, be characterized through a crystalline tetraacetate, a dibenzylidene derivative and a monobenzylidene derivative.

In the D-altritol series we have now converted methyl α -D-altroside, with ethyl mercaptan and concentrated hydrochloric acid, directly to D-altrose diethyl mercaptal, whose reductive desulfurization has furnished 1-desoxy-D-altritol.⁴ Trityla-

(1) Presented in part before the Division of Sugar Chemistry, Milwaukee Meeting of the American Chemical Society, March 31, 1952.

(2) For the biochemical oxidation of L-fucitol, see N. K. Richtmyer, L. C. Stewart and C. S. Hudson, *This Journal*, **72**, 4934 (1950).

(3) E. Zissis, N. K. Richtmyer and C. S. Hudson, *ibid.*, **73**, 4714 (1951).

(4) The 6-desoxy-D-altritol has been described previously, ref. 2.