localities and may prove to be a commercial source of the drug. Several samples of leaves collected at different stages of growth and at two different altitudes were examined. The leaves were collected in 1936 and 1937 near the Salina Experiment Station, Salina, Utah, in connection with studies on poisoning of livestock by cyanogenetic plants. They were air dried and had been stored in the laboratory for ten years. Crude rutin was obtained from a composite lot of these samples in 1937 but was not investigated further at that time. Analyses of the samples are in Table I.

TABLE I

Lot no.	Date collected, 1936	Approximate altitude, ft.	Moisture,[7] %	Rutin,[7] % mfb
47-7	June 15	8000	6.00	1.57
47-6	Aug. 17	8000	7.20	1.44
47-9	Aug. 20	8500	6.15	3.13
47-10	Composite	8000	7.60	1.63
	1937			
47-8	June 17	8500	6.40	3.88

The figures indicate a considerably greater content of rutin in leaves collected at the higher altitude but do not show much difference between young (June collections) and older (August) leaves.

Experimental

A quantity of the air-dried leaves (6,646 g.) was coarsely powdered, moistened with alcohol and extracted in a glass percolator with alcohol. The solvent was distilled from the percolate and the greenish residue was thoroughly extracted with boiling distilled water. The water solution was filtered from the fatty materials and allowed to cool. After standing several days a considerable quantity of brown flocculent material separated. This was filtered and redissolved in boiling water. The filtered solution deposited the substance on cooling. The crude rutin (24.0 g.) was greenish in color and contained 88% of the pure glucoside. This was bottled and preserved for several years. The rutin (2 g.) was purified by dissolving in alcohol (50 ml.), filtering from some amorphous matter, and diluting to 500 ml. with water containing 2 ml. of concentrated hydrochloric acid. The rutin which slowly precipitated during the ensuing twenty-four hours was of bright yellow color and crystallized in the characteristic microscopic fan-shaped bundles of needles. It was filtered, washed with water and dried at 110°. The plastic range ("melting point") was 172-174° raised by recrystallization from alcohol and from water to 190-192°. A spectrophotometric examination by W. L. Porter identified the substance as rutin by the following constants: The ultraviolet absorption spectrum of the anhydrous sample was characterized by absorption maxima near 3627 and 2577 Å., specific extinction coefficient, 32.6 liter g.$^{-1}$ cm.$^{-1}$ at 3627 Å., extinction ratio of 0.875 for wave lengths 3752 and 3627 Å. The corresponding figures for authentic rutin are: 3627 and 2577 Å., 32.5 at 3627 Å. and 0.875.

Anal.[8] Calcd. for $C_{27}H_{30}O_{16}$: C, 53.11; H, 4.95. Found: C, 52.92; H, 4.99.

Summary

Rutin has been isolated in significant quantities from black chokecherry, a species native to the mountain region of the western United States.

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(7) Analyses made by C. S. Fenske, Jr., of this Laboratory.

(8) Analysis by C. L. Ogg.

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[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Isolation of Rutin from a Citrus Hybrid

BY C. F. KREWSON AND J. F. COUCH

The discovery of Szent-Györgyi and co-workers[2] that citrus fruits contain a substance not identical with hesperidin or any other identified constituent of these fruits has led to much chemical and physiological research in an endeavor to isolate and characterize the active compound, which has been termed vitamin P.[3] Investigations begun in 1943 have established the fact that rutin, a flavonol glucoside present in some forty species of plants, has the physiological activity attributed to vitamin P.[4] The question whether the activity reported from citrus fruits which may be due to the presence of rutin has been investigated in this Laboratory. Valencia oranges, grapefruit and both green and ripe lemons, reported to be most active of the citrus fruits with respect to vitamin P activity, have been examined without revealing the presence of rutin. These have been subjected to three extraction procedures: the usual alcoholic technique,[5] a special process involving enzymatic hydrolysis of pectin, and an alkali extraction. In no case was rutin found by the alcoholic extraction. In the instance of a hybrid fruit, rutin was found by the other two processes, the alkali extraction described below being more effective.

Two years ago we obtained from the orchard of A. C. Krewson, Plymouth, Florida, specimens of a citrus fruit of the size and form of grapefruit but with a distinct odor and flavor of the lemon. The

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(1) One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture. Article not copyrighted.

(2) P. L. Armentano, *et al.*, *Deut. Med. Woch.*, **62**, 1326-1328 (1936).

(3) V. Bruckner and A. Szent-Györgyi, *Nature*, **138**, 1057 (1936).

(4) J. Q. Griffith, Jr., J. F. Couch and M. A. Lindauer, *Proc. Soc. Exptl. Biol. Med.*, **55**, 228-229 (1944); J. F. Couch, C. F. Krewson, J. Naghski and M. J. Copley, U. S. Department of Agriculture, Mimeograph Circular AIC-115, April, 1946.

(5) J. F. Couch and C. F. Krewson, U. S. Department of Agriculture Mimeograph Circular AIC-52, July, 1944.

juice of these fruits served to make excellent "lemonade." The hybrid has been tentatively identified by F. E. Gardner of the Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, as a cross between grapefruit and Satsuma orange, produced during experiments on cross-breeding citrus fruits and named Satsumelo.

Examination of the peel of these fruits finally resulted in the isolation of significant quantities of rutin after the usual alcoholic extraction had been unsuccessful. Exhaustive maceration with alkalies extracted the constituent slowly. Rind of green fruits picked January 3, 1946, gave 3.2% of rutin (moisture-free basis), whereas the rind of ripe fruit picked the previous year, February 10, 1945, gave approximately 0.9%, and that of over-ripe fruit picked April 28, 1945, yielded about 0.1% of rutin.

These data are consistent with the facts obtained with other rutiniferous plants[6] that immature tissues, plants or fruits contain more rutin than more mature tissues. Rutin appears to be associated with the most actively metabolizing portions of the plant, a fact in harmony with Szent-Györgyi's view that the flavonols form part of the oxidation–reduction chain in the plant cell.[7]

No trace of rutin was found, in repeated attempts both by alcoholic and alkali extraction techniques, in the peeled fruit, the pressed juice of the peeled fruit, the pulp of the peeled fruit, the fermented whole fruit, or in the air-dried leaves of the tree from which the fruits were obtained.

Experimental

The minced peel of the hybrid (258.5 g.) was mixed with 757 ml. of distilled water and sufficient 3 *N* sodium hydroxide solution to bring the mixture to a *p*H of 11, and stirred for one hour. The residue after filtration was again macerated with sodium hydroxide solution for one hour and filtered. This treatment was repeated twice more. The filtrates were separately adjusted to *p*H 4.5 with hydrochloric acid and allowed to stand in a refrigerator. The first two extracts yielded 1.50 and 0.36 g. of rutin, respectively, a total of 1.86 g. or 3.23% of the moisture-free material. The substance recrystallized from boiling water gave a plastic range ("melting point") of 185.5 to 187.5° (cor.). Spectrophotometric examination of the material after recrystallization from absolute ethanol revealed absorption maxima at wave lengths 2575 and 3625 Å. with a specific extinction coefficient of 32.74 at 3625 Å. Purified rutin from buckwheat, after recrystallization from absolute ethanol, shows maxima at the same wave lengths, with a coefficient of 32.55 at 3625 Å.[8]

A portion of the rutin was further purified by solution in alcohol and recrystallization from water.

Anal.[9] Calcd. for $C_{27}H_{30}O_{16}$: C, 53.11; H, 4.95. Found: C, 53.07; H, 5.11.

Hydrolysis of Rutin.—The purified glucoside (0.200 g.) was heated one hour with 8 ml. of 5% sulfuric acid and cooled overnight. The precipitated quercetin was filtered, washed and recrystallized from ethanol. When it was dried at 110° it melted at 313–313.5°.

Anal.[9] Calcd. for $C_{15}H_{10}O_7$: C, 59.61; H, 3.34. Found: C, 59.57; H, 3.45.

The pentaacetate melted at 194–196° and showed no depression when mixed with authentic quercetin pentaacetate. The carbohydrates produced by hydrolysis of the rutin were identified as rhamnose and glucose by the usual methods.

Summary

Rutin has been isolated from the rind of Satsumelo, a hybrid citrus fruit resulting from a cross between grapefruit and Satsuma orange.

PHILADELPHIA 18, PA. RECEIVED AUGUST 9, 1947

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(6) J. F. Couch, J. Naghski and C. F. Krewson, *Science*, **103**, 197–198 (1946).

(7) S. Szent-Györgyi, "Studies on Biological Oxidation and Some of Its Catalysts," Barth, Leipzig, 1937, pp. 67–98.

(8) The specific extinction coefficient of purified rutin from buckwheat previously reported (THIS JOURNAL, **69**, 572–573 (1947)), 31.9 liter g.$^{-1}$ cm.$^{-1}$, is raised to 33.22 by addition of a small quantity of acetic acid to the rutin solution prepared for spectrophotometric examination. The addition of this acid increases the coefficient but renders it insensitive to accidental traces of alkali present on the glassware or in the solvent. See W. L. Porter, B. A. Brice, M. J. Copley and J. F. Couch, U. S. Department of Agriculture Mimeograph Circular AIC-159, July, 1946, "Tentative Spectrophotometric Method for the Determination of Rutin in Various Preparations." Recrystallization of the purified buckwheat rutin from absolute ethanol, followed by application of the modified procedure, results in a specific extinction coefficient of 32.55 at 3625 Å., with an unchanged extinction ratio of 0.875 ± 0.004 for wave lengths 3750 and 3625 Å. We are indebted to W. L. Porter for the spectrophotometric work.

(9) Analyses by C. L. Ogg of this Laboratory.