Synthesis of Quercetin-2-C^{14}

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For use in biological tracer studies, the preparation of quercetin (3, 3′, 4′, 5, 7-pentahydroxyflavone) specifically labeled at the number 2 position has become of value. Although syntheses of quercetin have been previously described,[1] no published synthesis could be used unmodified for the authors' purpose. The usual starting materials cannot be readily obtained radioactive, and the methods were not designed to give the required purity of product. This paper describes a synthesis of chromatographically pure quercetin-2-C^{14} on a semimicro scale, using the readily available potassium cyanide-C^{14} as starting radioactive compound. Some steps of the synthesis have been adapted, though with significant improvements from Kostanecki *et al.*[2] All the steps of the labeled synthesis were first worked out in trial runs, using nonlabeled materials.

EXPERIMENTAL[3]

4-Iodoveratrole (I). This required intermediate was prepared by a method similar to that of Minnis.[4] A solution of 28 ml. (0.2 mole) of redistilled veratrole in 75 ml. of 95% ethanol was heated to 60° and, while stirring, was treated with 50 g. of iodine and 30 g. of mercuric oxide. The iodine, in 5-g. portions, and the mercuric oxide, in 3-g. portions, were added alternately over a period of 1 hr. After the addition was complete, the solution was filtered, and the alcohol was distilled from the filtrate. The residue, a dark red oil, was dissolved in ethyl ether and washed with solutions of sodium thiosulfate and sodium hydroxide, and finally with water.

After drying over anhydrous magnesium sulfate, the ether was evaporated and the residue distilled under reduced pressure. This gave 25 g. of a heavy pale yellow oil; b.p. 80–85° at 1 mm. A portion of this oil crystallized after a month in the ice box. Subsequent oils were seeded with these crystals.

Veratronitrile-(nitrile-C^{14}) (II). In an oil bath at 250°, with stirring, 2.4 g. (0.027 mole) of cuprous cyanide-C^{14}, specific activity 0.033 mc./mM obtained from commercially available, radioactive potassium cyanide by the method of Barber,[5] was heated for 2 hr. with 7.5 g. (0.028 mole) of I. The cooled, hard reaction mixture was extracted with several portions of anhydrous ether by rubbing, under ether, with a stirring rod.

A chromatographic column containing Magnesol brand magnesium silicate (Food Machinery and Chemical Corp., N. Y.) as adsorbent was prepared with anhydrous ethyl ether. After passage through the column of the ethereal solution of II, the eluant, light tan in color, was transferred to a flask of Corning No. 7280 glass. After removing the ether under reduced pressure, the residue, still containing some I, weighed 4.5 g.

Veratric acid-(carboxyl-C^{14}) (III). To the residue containing II were added 120 ml. of a 15% potassium hydroxide solution and 40 ml. of methanol. After boiling under reflux for a total of 30 hr., the methanol was distilled and the resulting aqueous solution was extracted twice with 15-ml. portions of ethyl ether to remove I and II. About 0.3 g. of these radioactive impurities were removed.

The veratric acid was precipitated by adding concentrated hydrochloric acid to the aqueous solution at 75°. Purification was accomplished by dissolving III in 25 ml. of dilute sodium hydroxide solution and heating with activated charcoal at 100°. After filtering, the filtrate was acidified with concentrated hydrochloric acid at 75°. The resulting white crystals were collected by filtration, washed with a small amount of dilute acid, and dried in a desiccator (yield, 4.0 g.; m.p. 181°; specific activity 0.034 mc./mM). Upon working up the mother liquors and washings, an additional 0.3 g. of III was obtained.

Decarboxylation of a small aliquot of the radioactive III yielded radioactive carbon dioxide and a residue with no detectable radioactivity.

Veratroyl chloride-(carbonyl-C^{14}) (IV). Four grams (0.022 mole) of III was heated with 25 ml. of purified thionyl chloride under reflux on a water bath for 2 hr. After removal, *in vacuo*, of excess thionyl chloride, the pale amber liquid solidified on cooling.

Veratraldehyde-(carbonyl-C^{14}) (V). The veratroyl chloride was reduced to the corresponding aldehyde by the Rosenmund reaction, using procedures similar to those of Hershberg and Cason.[6] Dry hydrogen, 0.022 mole of IV (based on the veratric acid), 30 ml. of purified xylene, 5 microliters of the quinoline-sulfur regulator, and 0.5 g. of the palladium–barium sulfate catalyst were used in the conversion. At the end of the reaction the solvents were removed under reduced pressure. The pale yellow residue, weighing 3.6 g., contained approximately 2.2 g. of the aldehyde. The product was carried on to the next step without purification.

2,4-Dimethylphloroacetophenone (VI). White crystals of VI, m.p. 81°, were obtained by a procedure involving the partial methylation of phloroacetophenone.[7]

In a dry flask, 12.6 g. of phloroacetophenone (dried at 120°) was dissolved in 45 ml. of anhydrous acetone. Then 225 ml. of anhydrous benzene, 45 g. of anhydrous potassium carbonate, and 14.5 ml. of dimethyl sulfate were added to this solution. The mixture was refluxed on a water bath for 12 hr. After filtration and washing of the residue with hot benzene, the filtrate and benzene solution were then washed with water and extracted with 5% aqueous sodium hydroxide. The extract was poured into cold, 25% hydrochloric acid which caused the dimethylphloroacetophenone to separate as white crystals. These crystals were filtered and washed with 5% aqueous sodium carbonate and then with water. After drying in a vacuum desiccator, the product weighed 12 g.; m.p. 81°. This product was purified by passage in anhydrous ether through a column containing Magnesol. The compound passed through the column with the solvent front; m.p. 83°.

Chalcone (VII) *of 3′,4′,5,7-tetramethyleriodictyol-2-C^{14}*. A mixture of 3 g. (0.015 mole) of VI, the residue containing V in 200 ml. of 95% ethanol, and 6 ml. of 50% aqueous potassium hydroxide was shaken at frequent intervals over a 30-min. period. After 48 hr. at 40°, the deep red solution was diluted with 400 ml. of distilled water. Concentrated hydrochloric acid was added dropwise to the solution until it showed an acid reaction to Congo Red paper. The floc-

(1) F. Mayer and A. H. Cook, *The Chemistry of Natural Coloring Matters*, Reinhold Publishing Corp., N. Y., 1943.

(2) St. Kostanecki and J. Tambor, *Ber.*, **37**, 792 (1904). St. Kostanecki, V. Lampe, and J. Tambor, *Ber.*, **37**, 1402 (1904).

(3) All melting points were determined on a Fisher-Johns melting point block.

(4) W. Minnis, *Org. Syntheses*, Coll. Vol II, 357 (1943).

(5) H. J. Barber, *J. Chem. Soc.*, 79 (1939).

(6) E. B. Hershberg and J. Cason, *Org. Syntheses*, **21**, 84 (1941).

(7) V. D. N. Sastri and T. R. Seshadri, *Proc. Ind. Acad. Sci.*, **23A**, 262 (1946).

culent yellow precipitate was filtered off. The filtrate was extracted with four 50-ml. portions of benzene until no more color was removed. The benzene extract was then used to dissolve the precipitate, and the resulting solution was dehydrated by azeotropic distillation.

The crude chalcone solution in dry benzene was passed onto a column of Magnesol, prewashed with anhydrous benzene. The chalcone developed as a bright yellow zone near the top of the column. Under ultraviolet light, this zone exhibited dark brown fluorescence. The first 125 ml. of eluant was colorless, and, after evaporation of the benzene, yielded 1.6 g. of unreacted VI. A sharp, distinct separation between this ketone and the unreacted V which followed in the next 75 ml. of eluant was not accomplished. The unreacted, radioactive veratraldehyde was recovered from the eluant.

The chalcone, by this time, had developed as a zone below the dark impurities at the top of the column and also below a narrow pale yellow zone just under the dark impurities. After washing with a total of two columns of anhydrous benzene, the Magnesol was extruded from the top of the column, and then cut with a stainless steel spatula into the three zones. The top zone contained unknown impurities. The second zone contained tetramethyleriodictyol. It was eluted with anhydrous acetone and combined with the crude tetramethyleriodictyol obtained in a later reaction. The third zone contained the purified chalcone and was eluted with the anhydrous acetone. After removal of the solvent, VII weighed 2.3 g. (30%).

3′,4′,5,7-Tetramethyleriodictyol-2-C^{14} (VIII). A mixture of 2.3 g. of VII dissolved in 300 ml. of 95% ethanol, and 11 ml. of concentrated hydrochloric acid in 30 ml. of distilled water was refluxed for 20 hr. Then 500 ml. of distilled water was added. The resulting bright yellow precipitate was filtered off without suction, and the filtrate was extracted three times with a total of 150 ml. of benzene. This benzene was then used to dissolve the precipitate. The solution was filtered to remove the drops of water present and dried by azeotropic distillation.

The flavanone solution was then passed onto a column of Magnesol, prewashed with anhydrous benzene. The flavanone was adsorbed tightly onto the adsorbent and appeared as an ivory-colored zone in visible light and as a dull gray in ultraviolet light. The chalcone developed just below VIII. About 250 ml. of anhydrous benzene was needed to wash the unreacted VII from the column. The chalcone was recovered from this eluate and twice recycled through the same ring-closure reaction and chromatographic separation. The Magnesol, which now contained only the desired flavanone and a small amount of impurities on the top surface, was washed with 300 ml. of anhydrous acetone. This removed VIII very readily. The flavanone solution was combined with additional fractions of VIII from subsequent runs. The solvent was removed from the combined solutions and VIII was obtained as a pale yellow solid which weighed 1.5 g., a 66% yield of the chalcone.

3′,4′,5,7-Tetramethylquercetin-2-C^{14} (IX). The methylated eriodictyol was converted by means of *n*-butyl nitrite and hydrolysis, adapted from a procedure by Row and Seshadri,[8] into the corresponding tetramethylquercetin. A total of 0.45 g. of IX was obtained.

Quercetin-2-C^{14} (X). The tetramethylquercetin was dried, made into a paste with acetic anhydride, and demethylated, using hydriodic acid, sp. gr. 1.70. The yield of X was 0.3 g. The over-all conversion of labeled potassium cyanide into quercetin was 3.5%. Previous runs with unlabeled material had given an 8% yield. In the C-14 synthesis, however, a 29% recovery of intermediates, based on the labeled potassium cyanide, was obtained.

The labeled quercetin was compared on paper chromatograms with authentic synthetic quercetin and with natural quercetin obtained by hydrolysis of buckwheat rutin. The labeled product showed only one spot and gave the same R_f value as the two standards in every solvent system tried. The R_f values obtained in *n*-butyl alcohol–acetic acid-water (6:1:2, by vol.) and in 60% aqueous acetic acid with S&S #589, red ribbon paper, were 0.72 and 0.34, respectively.

In order to show the position of the labeled carbon atom, 1.3 mg. of X was diluted with 68.5 mg. of unlabeled quercetin for various analyses. The diluted quercetin had a calculated specific activity of 0.00055 mc./mM. The diluted quercetin was completely methylated with dimethyl sulfate to produce white-needle crystals of pentamethylquercetin (XI), m.p. 147°. This melting point was not depressed when a mixed melting point was taken with an authentic sample of unlabeled pentamethylquercetin. The sample of XI had a specific activity of 0.0005 mc./mM.

Seventy milligrams of XI was degraded, using 15 ml. of a solution containing 4 g. of potassium hydroxide in 45 ml. of absolute ethanol, and refluxing for 8 hr. After the ethanol was distilled off, the resulting solid was dissolved in 10 ml. of water, and concentrated hydrochloric acid was added to make the solution acid. The acidic solution was extracted with three 10-ml. portions of ethyl ether. The ether solution was extracted with two 10-ml. portions of 5% sodium bicarbonate solution. The ketone fragment, which remained in the ether, was obtained as an oil which did not contain any detectable radioactivity. The bicarbonate solution, containing III, was acidified. About 10 mg. of III was collected; m.p. 179°; specific activity 0.0005 mc./mM.

The sample of veratric acid obtained by degradation was then decarboxylated to produce radioactive carbon dioxide and a residue containing no detectable radioactivity.

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(8) L. R. Row and T. R. Seshadri, *Proc. Indian Acad. Sci.*, 21A 130 (1945).

Two New Flavonol Glycosides in Commercial Xanthorhamnin

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Commercially available xanthorhamnin (rhamnetin-3-rhamninoside), supposedly pure, has been separated by mass paper chromatography into pure xanthorhamnin plus two other flavonol glycosides apparently new to the literature. All three of these glycosides contain 2 moles of rhamnose to 1 mole of galactose to 1 mole of flavonol aglycone. The carbohydrate attachment is through the number three position on each flavonol. The aglycones have been identified as rhamnazin (3′,7-dimethyl ether of quercetin), rhamnetin (7-methyl ether of