four times in this way, darkening at 209°, melting with decomposition at 216–217°; yield 60%.

Anal. Calcd. for $C_{23}H_{23}N_2O_5$: C, 67.71; H 5.92. Found: C, 67.48–67.75; H, 5.85, 6.06.[3]

Dextrose Schiff base of 4-(4-aminostyryl)quinoline. To a solution formed by heating 9.8 g. of I and 5 ml. of dimethylformamide to 130°, 13.8 g. of III was added slowly, with stirring, at 110°. The mixture was then heated to 120–130° for 30 min., until it solidified. The product was washed with benzene and with water and recrystallized four times from isopropyl alcohol, using a Soxhlet extractor, and three times from methanol; m.p. 189.7–191.7° (dec.).

Anal. Calcd. for $C_{23}H_{23}N_2O_5$: C 67.71; H, 5.92. Found: C, 67.48, 67.75; H, 5.85, 6.06.[3]

These compounds were not readily soluble in water but dissolved readily in hot propyleneglycol and in dimethylformamide.

CHEMISTRY DEPARTMENT
CARSON-NEWMAN COLLEGE
JEFFERSON CITY, TENN.

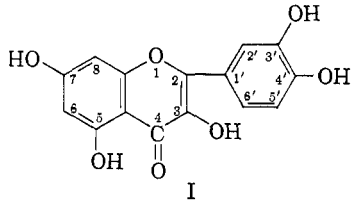
(3) Analyses by Galbraith Microanalytical Laboratories.

Methyl Ethers of Quercetin in Tobacco Flowers[1]

C. H. YANG, H. D. BRAYMER, E. L. MURPHY, W. CHORNEY, N. SCULLY, AND S. H. WENDER

Received February 26, 1960

Monomethyl and dimethyl ethers of quercetin (3,3′,4′,5,7-pentahydroxyflavone, I) having no methoxyl group at the 3-position, such as rhamnetin (quercetin-7-methyl ether), isorhamnetin (quercetin-3′-methyl ether), quercetin-4′-methyl ether, and rhamnazin (quercetin-3′,7-dimethyl ether) have been found previously in natural products, usually as glycosides. A 3,7,4′-trimethyl ether of quercetin, ayanin, has been isolated from the heartwood of the tree *Distemonanthus Benthamianus* by King, *et al.*[2] However, monomethyl and dimethyl ethers of quercetin that contain a methoxyl group at the 3-position have been obtained only by laboratory synthesis.[3–5] This note describes the isolation and identification of quercetin-3,3′-dimethyl ether from tobacco flowers. We have also found other related flavonol ethers in these flowers. One of these other compounds has been tentatively identified as quercetin-3-methyl ether.



I

EXPERIMENTAL

Separation of quercetin ethers. Samples each containing 100 g. of powdered, oven-dried flowers from tobacco plants, *Nicotiana tabacum*, one-sucker variety, grown in the greenhouse at Argonne National Laboratory during 1958, were extracted with 500 ml. of the following solvents in the order named: *n*-pentane, benzene, chloroform, ethyl acetate (anhydrous), and acetone. Each 500-ml. extract was concentrated *in vacuo* to 5 ml. and studied by paper chromatography. The flavonol ethers were mostly in the chloroform fraction, although at least two such compounds were present in small amounts in the ethyl acetate extract.

Each 5-ml. chloroform concentrate was streaked onto eight sheets of Whatman No. 3 MM chromatography paper (approx. 7″ × 22½″), and the chromatograms were developed by descending chromatography in 15% acetic acid–water for about 24 hr. The upper part of each chromatogram, containing the methylated flavonol compounds which moved only a relatively short distance in this solvent, was cut out and sewn onto a new sheet of S & S chromatography paper, No. 589, Red Ribbon. Each sheet was next developed in *n*-butyl alcohol–acetic acid–water (6:1:2 v./v.). After drying, the papers were viewed under long wave-length ultraviolet light (3660 Å). A dark brown zone was seen near the solvent front; it was poorly separated from some blue-fluorescing material. The broad, dark brown zone containing the mixture of methylated flavonols was cut from each chromatogram, eluted with methanol, and then subjected to further extended chromatography, first in 15% acetic acid for 36–48 hours, then on fresh sheets in 60% acetic acid–water. The latter effected separation of the quercetin dimethyl ether from a trace amount of another brown fluorescing substance which had the same mobility as authentic quercetin-3-methyl ether on chromatograms. The yield of this latter compound from the 1958 tobacco flowers was insufficient to confirm its identity. After elution of the brown fluorescing zone containing the quercetin dimethyl ether, the methanol eluates were subjected to further chromatography on S & S No. 589 paper, using four different solvent systems in the order: 15% acetic acid–water: ethyl acetate–formic acid–water (10:2:3 v./v., upper layer); *n*-butyl alcohol–acetic acid–water (6:1:2 v./v.); and finally 60% acetic acid–water. The quercetin dimethyl ether zone of each final chromatogram was then pure enough for identification studies.

Identification of quercetin-3,3′-dimethyl ether. On paper chromatograms, the quercetin dimethyl ether exhibited a dark brown fluorescence under ultraviolet light, but after the compound had been sprayed with a 1% solution of aluminum chloride in ethanol, it gave a yellow fluorescence. Flavone aglycones such as apigenin (4′,5,7-trihydroxyflavone); flavonol glycosides such as isoquercitrin (quercetin-3-glucoside); and certain 3-methyl ethers of flavonols, such as quercetin-3-methyl ether and quercetin-3,7-dimethyl ether exhibit this fluorescent behavior.

After the isolated tobacco quercetin dimethyl ether was refluxed with hydriodic acid, sp. gr. 1.7, for 4 hr., a product was obtained which proved to be quercetin. Identity was established by comparison of color tests, fluorescence, ultraviolet absorption spectra, and co-chromatography with authentic quercetin.

After the tobacco quercetin dimethyl ether was refluxed with dimethyl sulfate and sodium carbonate in acetone for 6 hr., the product showed a blue fluorescence under ultraviolet light and was identified as quercetin-3,3′,4′,5,7-pentamethyl ether by paper chromatographic comparison with an authentic sample. Thus, the unknown was definitely a methyl ether

(1) This work was performed in part under the auspices of the U. S. Atomic Energy Commission.

(2) F. E. King, T. J. King, and K. Sellars, *J. Chem. Soc.*, **155**, 92 (1952).

(3) R. Kuhn and I. Löw, *Ber.*, **77B**, 211 (1944).

(4) A. C. Jain, K. S. Pankajamani, and T. R. Seshadri, *J. Sci. Ind. Res. (India)*, **12B**, 127 (1953).

(5) T. R. Seshadri, *Tetrahedron*, **6**, 196 (1959).

of quercetin with at least one methoxy group at the 3-position.

An attempt was made to hydrolyze the quercetin dimethyl ether isolated from tobacco flowers by heating it in 7% sulfuric acid solution for 12 hr. on a steam bath. No sugar was found on paper chromatograms of the reaction mixture, nor was there any significant change observed in the unknown compound, although a trace of some nonflavonol material could be located on the chromatogram by observing the chromatogram under ultraviolet light. These tests indicated that the unknown compound was not a flavone nor a glycoside of quercetin.

When an ethanol solution of the tobacco quercetin dimethyl ether was shaken with sodium amalgam, and then acidified with hydrochloric acid, a salmon pink color was obtained. Thus, substitution of the 3-position of the quercetin was again indicated.[6]

Mixtures of quercetin-3-methyl ether and quercetin-3,7-dimethyl ether, which were synthesized and purified in our laboratory as described in later paragraphs, could be readily separated by paper chromatography, using the solvent system nitromethane-benzene-water (2:3:5 v./v., upper layer), with R_f values of 0.13 and 0.85, respectively. The naturally occurring compound had a R_f value of 0.83 in this solvent system, thus indicating the likelihood of its being a dimethyl, and not a monomethyl quercetin 3-methyl ether.

The fluorescence was quenched by the addition of acetic anhydride to the solid compound,[3] indicating a free 5-hydroxy group on the quercetin. The reaction of the isolated compound with alcoholic ferric chloride solution, and its behavior during methylation, likewise indicated a free phenolic group at the 5-position.

Addition of anhydrous sodium acetate to the solution of the tobacco quercetin dimethyl ether, by the method of Jurd and Horowitz,[7] caused a shift in the short wave-length band of its ultraviolet absorption spectrum from 254 to 275 mμ. This indicates that the 7-hydroxy position of the tobacco quercetin dimethyl ether is open.

The long wave-length band of the ultraviolet absorption spectrum of the tobacco unknown did not shift in absolute ethanol saturated with boric acid and anhydrous sodium acetate, by the spectral method of Jurd.[8] Thus, at least one hydroxyl of the *o*-dihydroxy group (3′,4′) of quercetin was blocked in the tobacco unknown in question.

Degradation of the isolated tobacco quercetin dimethyl ether was carried out by dissolving 1 mg. of the unknown in 30 ml. of a 2*N* solution of sodium hydroxide in a mixture of 50% ethanol and 50% water, and evaporating the solution to dryness in an oven at 120°. The residue was dissolved in water, acidified with hydrochloric acid to a *p*H of 2, and extracted four times with 20-ml. portions of ether. The ether solution was concentrated to 1 ml. and studied by paper chromatography. The acid obtained after degradation proved to be vanillic acid (4-hydroxy-3-methoxybenzoic acid) by the identification procedure of Hergert and Goldschmid.[9] Thus, the 4′-position of the tobacco quercetin dimethyl ether has a free phenolic group, whereas the 3′-position has a methoxy group on it. The structure of the isolated tobacco compound is, therefore, quercetin-3,3′-dimethyl ether.

Studies on both tobacco leaves and flowers obtained from a 1955 field-grown crop at Argonne indicated the presence in each of a quercetin dimethyl ether (which may have been quercetin-3,3′-dimethyl ether instead of the reported quercetin-3,7-dimethyl ether), plus a compound giving color tests similar to and co-chromatographing with authentic quercetin-3-methyl ether.[10] A third compound appeared to be kaempferol-3-methyl ether by preliminary tests. Kaempferol is 3,4′,5,7-tetrahydroxyflavone. The spectral tests of Jurd and Horowitz[7] and of Jurd[8] were not run on these 1955 samples, and their identifications were only tentative. On the 1958 greenhouse-grown tobacco flowers, the quercetin-3,3′-dimethyl ether was present in relatively larger amount, but the compounds which might have been flavonol monomethyl ethers were not present in sufficient amount to undertake the studies needed for unequivocal confirmation of their structures.

Preparation of pure quercetin-3-methyl ether and quercetin-3,7-dimethyl ether. Both of these compounds were synthesized by the method reported by Jain and co-workers[4] for quercetin-3,7-dimethyl ether. On paper chromatographic examination, the resulting methylated quercetin precipitate appeared to be a complicated mixture containing five or more different derivatives of quercetin. Using methanol as the suspending medium, the precipitate was adsorbed onto Magnesol (Food Machinery and Chemical Corp., New York). The column was developed with a solvent system containing two parts of water-saturated ethyl acetate and one part nitromethane. Brown-fluorescing material, with some traces of blue-fluorescing impurities, moved rapidly off the column, leaving the major portion of the blue-fluorescing substances on the column. The eluates containing the brown-fluorescing mixture were then further purified by extended paper chromatography, using in order the solvent systems 60% acetic acid–water, 15% acetic acid–water, and nitromethane–benzene–water (2:3:5 v./v. upper layer) for purification of the quercetin-3-methyl ether. For obtaining pure quercetin-3,7-dimethyl ether, the 60% acetic acid–water, nitromethane-benzene–water, and finally 60% acetic acid–water systems were used. R_f values in the 15% acetic acid and 60% acetic acid systems were respectively: quercetin-3-methyl ether, 0.17 and 0.63 and quercetin-3,7-dimethyl ether, 0.19 and 0.72. Each of these compounds was eluted from its final chromatogram with 50% methanol–water. The purified quercetin-3-methyl ether checked in every respect (fluorescence, R_f values, color tests, and spectral studies) with authentic quercetin-3-methyl ether kindly furnished by Dr. R. M. Horowitz, USDA Fruit and Vegetable Laboratory, Pasadena, Calif. The identity of the purified, synthetic quercetin-3,7-dimethyl quercetin was checked by procedures similar to those described above for the determination of the structure of the tobacco quercetin-3,3′-dimethyl ether isolated from tobacco.

CHEMISTRY DEPARTMENT
UNIVERSITY OF OKLAHOMA
NORMAN, OKLA.
AND
DIVISION OF BIOLOGICAL AND MEDICAL RESEARCH
ARGONNE NATIONAL LABORATORY
ARGONNE, ILL.

(6) L. H. Briggs and R. H. Locker, *J. Chem. Soc.*, **152**, 2158 (1949).

(7) L. Jurd and R. M. Horowitz, *J. Org. Chem.*, **22**, 1618 (1957).

(8) L. Jurd, *Arch. Biochem. Biophys.*, **63**, 376 (1956).

(9) H. L. Hergert and O. Goldschmid, *J. Org. Chem.*, **23**, 700 (1958).

(10) E. L. Murphy, Ph.D. dissertation, University of Oklahoma (1957).

Fluoro Analogs of Prostigmine

SEYMOUR L. SHAPIRO, THEODORE BAZGA, AND LOUIS FREEDMAN

Received March 17, 1960

The useful physiological properties of prostigmine,[1] I, and its analogs suggested exploration of

(1) A. Stempel and J. A. Aeschlimann, *Medicinal Chemistry*, Vol. III, John Wiley & Sons, New York, N. Y., 1956. p. 239 (see pp. 270–274).