

Pyrolysis Products of Rutin, Quercetin, and Chlorogenic Acid

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Rutin (quercetin-3-rutinoside) and chlorogenic acid (3-caffeoylquinic acid) are among the principal polyphenols present in cigarette tobacco, but neither has been found in the smoke from cigarettes. It was, therefore, of interest to investigate the products of pyrolysis of these two compounds, as well as those of quercetin (3, 3', 4', 5, 7-pentahydroxyflavone), the aglycone of rutin.

Rapid, dry distillation of rutin produced catechol and smaller amounts of 4-methylcatechol, resorcinol, furfural, 5-hydroxymethylfurfural, and 5-methylfuran-2-aldehyde. Under similar conditions quercetin produced catechol, 4-methylcatechol, resorcinol, and phloroglucinol. No phloroglucinol, however, has been found among the products of pyrolysis of rutin. Chlorogenic acid distillation yielded catechol, 4-methylcatechol, 4-ethylcatechol, benzoic acid, and quinide (quinic acid- γ -lactone.) In addition, paper chromatographic analysis of the pyrolysis products of each compound indicated the presence of other, as yet unidentified, compounds.

Of the compounds listed above, catechol, resorcinol, furfural, and benzoic acid are included in a summary of compounds previously identified in cigarette smoke (Bentley and Berry, 1959 and 1960). Quinide (Nakagawa, Yang, and Wender, 1961) and 5-hydroxymethylfurfural (Yang and Wender, 1962) have also been found previously in cigarette smoke. The others have not previously been reported in cigarette smoke.

Experimental

Pyrolysis of quercetin. Quercetin (10 g.; Nutritional Biochemicals Corp., Cleveland, Ohio) was heated at atmospheric pressure in a 50 ml. flask

with a Bunsen burner in an all glass distillation unit (Metro Industries, ME 523), which had an 8 cm. distilling head and an 8 cm. water-cooled jacket. As quercetin melted, foamed, and charred, yellow fumes were evolved. After about 5 minutes, all of the original quercetin had blackened on decomposition, and the distillation had ceased. When a thermometer was inserted into the midst of the molten mixture in the flask, readings up to 600°C were registered before the heating was discontinued. The yellow distillate (2 ml.) was extracted with ether, and the extracts were dried over anhydrous sodium sulfate. On removal of the ether *in vacuo*, a semi-crystalline orange-colored residue resulted. Extraction of this residue with three 10 ml. portions of benzene gave, on slow evaporation in air, a brown crystalline solid (350 mg., called "Q-1"). The material that had not dissolved in benzene was now extracted with 20 ml. water. The aqueous yellow solution was then extracted five times with 15 ml. portions of ether. The combined ether extracts were dried over anhydrous sodium sulfate and taken to dryness *in vacuo* to yield 20 mg. of a yellow solid, called "Q-2."

Fraction "Q-1" was further fractionated on a 41 cm. \times 6 cm. diameter column of silicic acid (Mallinckrodt No. 2847) prepared from a slurry of 2:1 (w/w) silicic acid-water in benzene under 4 lb. pressure. 200 mg. "Q-1", dissolved in 4 ml. ether and diluted to 80 ml. with benzene, was added to the column and the resulting pale yellow band developed under pressure with benzene-ether (95:5 v/v) saturated with water. Two pale yellow bands moved down the column. The faster band gave a fraction (380 ml.) which on evaporation *in vacuo* yielded a small amount of an orange-red oil, called

"Q-1-A." The slower moving band (600 ml.) produced grayish-white crystals (130 mg., called "Q-1-B"), after removal of the solvent. Further elution of the column gave a very small amount of a white solid, called "Q-1-C."

"Q-1-A" was sublimed *in vacuo* to produce a white solid which has been identified as 4-methylcatechol. Its ultraviolet absorption spectrum showed a maximum at 283 m μ in ethyl alcohol, as did the reference 4-methylcatechol. The R_f values of "Q-1-A" and authentic 4-methylcatechol were identical when chromatographed together in the solvent systems studied (Table 1). The detecting spray reagent used in all these experiments was the stabilized diazo salt of p-nitroaniline (Fast Red Salt GG; Pearl and McCoy, 1960). The color produced was a reddish-orange with a bluish-purple rim.

"Q-1-B" was sublimed *in vacuo* to yield a white crystalline solid, m.p. 104-106°. A mixed melting point determination with authentic sublimed catechol gave no depression. All melting points listed in this paper are uncorrected. R_f values of "Q-1-B" and of authentic, reference catechol were identical (Table 1) on the same paper chromatograms.

The R_f values of "Q-1-C" (Table 1) and the yellow color produced with the spray reagent corresponded to those of reference resorcinol.

Fraction "Q-2" contained several compounds, including catechol and 4-methylcatechol. To this fraction was added 2.5 ml. ether, followed by 50 ml. benzene. The solution was concentrated to 40 ml., and the precipitate which formed was filtered off and washed with benzene. The precipitate was dissolved in acetone and streaked on eight sheets of Whatman No. 3 MM chromatography paper, and the chromatograms were de-

veloped in 2% acetic acid. The main band fluorescing blue with ammonia vapor as observed with ultraviolet light (3660 Å) was cut out and extracted with ether in a Soxhlet extractor. After removal of ether, the pale yellow residue was placed on a column packed with silicic acid-water (2:1 v/v) as before. After a blue fluorescent band was removed, the column was eluted with ether. A small amount of white solid, now pure "Q-2," was obtained from the ether eluate. Pure "Q-2" was identified as phloroglucinol. Its ultraviolet absorption spectrum in ethyl alcohol exhibited a maximum at 268 m μ , corresponding to that obtained with authentic, sublimed phloroglucinol. The R_f values of "Q-2" and reference phloroglucinol when chromatographed together were identical in the solvent systems tested (Table 1), as was also the orange color produced in each case by the spray reagent.

Pyrolysis of rutin. Rutin (10 g.; Penick and Co., New York) was pyrolyzed by the same procedure described for quercetin. The distillate (3 ml.) was diluted with water and extracted several times with ether. The ether extract was dried over anhydrous sodium sulfate. The ether was removed *in vacuo* to leave an orange oil (1 g., called "R-1").

Fraction "R-1" was dissolved in 20 ml. ethyl alcohol, and a freshly prepared 2,4-dinitrophenylhydrazine solution was added to give a brick-red precipitate immediately. After remaining 15 minutes at room temperature, the precipitate was filtered off, washed with aqueous ethyl alcohol, and dried to a brick-red powder (0.28 g., called "R-2").

The 2,4-dinitrophenylhydrazine fraction, "R-2", was fractionated on a 36 cm. x 6 cm. diameter column of 2:1 (w/w) silicic acid "Super Cel" (Fisher) in hexane under 5 lb. pressure. "R-2" was dissolved in 100 ml. warm benzene and diluted with 100 ml. hexane. A small amount of precipitate was filtered off, and the filtrate was added to the top of the adsorbent to form an orange band. On development with hexane — ether (96:4 v/v) under 5 lb. pressure, several colored bands appeared and were eluted separately. After removal of the solvent *in vacuo* from the eluate containing the fastest moving band (yellow), an orange powder was obtained (19 mg., called "R-2-1"). After evaporation of the solvent from the eluate containing the next eluted band (a broad orange band), dark red needles resulted (48 mg., "R-2-2"). When the "R-2-2" was chromatographed still another time on a fresh column, two compounds were

Table 1—Average R_f Values of Some Pyrolysis Products.

Compounds	Solvent Systems ^a			
	1	2	3	4
Catechol	0.38	0.79	0.50	0.89
4-Ethylcatechol	0.60	0.75	0.74	0.94
5-Hydroxymethylfurfural	0.34	0.85	0.74	
4-Methylcatechol	0.49	0.77	0.60	0.92
Phloroglucinol	0.03	0.65	0.00	0.75
Quinide	0.00	0.88	0.10	0.53
Resorcinol	0.15	0.76	0.19	0.90

^a Solvent systems: (1) benzene-acetic acid-water (6:7:3 v/v/v); (2) 2% acetic acid-water; (3) chloroform-acetic acid-water (2:1:1 v/v/v); and (4) n-butyl alcohol-acetic acid-water (6:1:2 v/v/v). Whatman No. 1 paper was used.

separated: The first one from the column produced a dark red, crystalline material called "R-2-2-1" (9 mg.) and the other gave 10 mg. of a red solid called "R-2-2-2".

Compound "R-2-1" was crystallized from ethyl alcohol-ethyl acetate to produce fine orange needles, m.p. 216-217°. No depression resulted on a mixed melting point determination with the 2,4-dinitrophenylhydrazone of 5-methylfuran-2-aldehyde.

Compound "R-2-2-1" was crystallized twice from ethyl alcohol-ethyl acetate to give fine, short, black needles (5 mg.) m.p. 199-200°. This 2,4-dinitrophenylhydrazone derivative has not yet been identified.

Compound "R-2-2-2" gave a red crystalline product (5 mg.), m.p. 225-227°. On mixed melting point determination with the synthetic 2,4-dinitrophenylhydrazone of furfural, no depression resulted.

When the original "R-1" was chromatographed two-dimensionally, first in benzene-acetic acid-water (6:7:3 v/v/v) and then in 2% acetic acid-water and subsequently sprayed with Fast Red Salt GG, no phloroglucinol was found.

Fraction "R-1" was streaked on chromatography paper and developed in chloroform-formic acid-water-methyl alcohol (500:2:48:50 v/v/v/v). The zone at R_f 0.74 was cut out and eluted first with acetone and then with methyl alcohol. The eluate was concentrated and analyzed. R_f values (Table 1) on the same paper chromatograms and color produced with 2,4-dinitrophenylhydrazine spray (Reio, 1958) corresponded to those exhibited by authentic 5-hydroxymethylfurfural.

An aliquot (1 g.) of fraction "R-1" was chromatographed on a silicic acid column by the procedure previously described for quercetin. Pale yellow bands were formed and eluted separately. Each eluate was studied

by paper chromatography, using the benzene-acetic acid-water solvent system. The zone at approximately R_f 0.50 yielded an orange oil (48 mg., called "R-1-A"). Sublimation of "R-1-A" gave a product identical with reference 4-methylcatechol in R_f values (Table 1), ultraviolet spectrum, and color developed with spray used. A zone with an R_f value 0.38 was chromatographed on another silicic acid column to give an eluate from which a semi-crystalline product (90 mg., called "R-1-B") was obtained. Sublimation at 50° *in vacuo* yielded a white solid, m.p. 104-106°. On mixed melting point determination with reference sublimed catechol, no depression was observed. R_f values and color produced on the same chromatograms also corresponded to those of authentic catechol.

The zone with R_f value of approximately 0.16 yielded a small amount of a yellow oil (called "R-1-C"). The R_f values (Table 1), and also the yellow color produced with the spray reagent agreed with those of reference resorcinol.

Pyrolysis of chlorogenic acid. Chlorogenic acid (4 g. CHR grade, Fluka, Switzerland) was pyrolyzed in a 20 cm. flask by the same procedure as described for quercetin. The orange distillate (1.3 ml.) was diluted with water and extracted five times with 15 ml. portions of ether. The ether solution was dried over anhydrous sodium sulfate and the solvent was removed *in vacuo* to produce a reddish-orange oil (called "C-1"). The aqueous fraction was allowed to evaporate in air to yield a pale yellow semi-crystalline solid (called "C-2").

Fraction "C-1" was chromatographed using silicic acid by the procedure described previously for the fraction "Q-1". The first fraction collected from the column, after

evaporation *in vacuo*, yielded an orange oil. "C-1-A". The next fraction produced a yellow solid "C-1-B". This was followed by two fractions which gave, after evaporation, yellow, oily compounds "C-1-C" and "C-1-D", respectively.

After rechromatography for greater purification, on another column containing silicic acid, "C-1-A" produced a pale yellow crystalline solid (19 mg., "C-1-A-1"). Sublimation of "C-1-A-1" *in vacuo* at 60° produced a white crystalline solid (15 mg.), m.p. 121-122.5°. On mixed melting point determination with authentic sublimed benzoic acid, no depression resulted.

Rechromatography of "C-1-B" on a silicic acid column gave a yellow oil, which on analysis by paper chromatography, gave an approximate R_f value of 0.64 in the benzene-acetic acid-water system. This oil was purified by streaking it on five sheets of Whatman 3MM paper and developing these chromatograms in 2% acetic acid—water. The dull purple band (R_f , 0.75) as seen under short wavelength ultraviolet light (2537 Å) was cut out and extracted with ether in a Soxhlet extractor. The pale yellow oil was further purified by column chromatography using silicic acid as described previously. The eluate with R_f 0.64 in the benzene-acetic acid-

water system was taken to dryness *in vacuo*, to produce a pale yellow oil, which proved to be identical with an authentic preparation of 4-ethylcatechol in R_f values (Table 1), color of spot (orange-red with bluish-purple rim) produced with the Fast Red Salt GG reagent, and in ultraviolet absorption spectrum (λ_{max} , 283 m μ and λ_{min} , 250 m μ).

Fraction "C-1-C" was further purified by silicic acid chromatography. The eluate was taken to dryness *in vacuo* to yield a yellowish orange oil (10 mg). Sublimation at 45° *in vacuo* gave a product identical with 4-methylcatechol in R_f values when chromatographed together, color, and ultraviolet absorption spectra.

After removal of solvents from fraction "C-1-D", a yellow crystalline product (117 mg.) resulted. Sublimation at 60° *in vacuo* gave white crystals, m.p. 104-106°. A mixed melting point with authentic catechol was not depressed. Also, R_f values (Table 1) on the same paper chromatograms and color reactions were identical to those of reference catechol.

R_f values (Table 1) of compound "C-2" were identical with those of authentic quinide when chromatographed together. Both gave a positive Cartwright-Roberts test (Cartwright and Roberts, 1955).

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