The Polyphenols of Flue-Cured Tobacco Separation and Identification of the Major Polyphenols

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Introduction and Summary of Previous Knowledge

Polyphenolic substance have been known to occur in tobacco for about 50 years, and for a long time it has been known that when the cells of the tobacco leaf die, the polyphenols produce an oxidation product which acquires a red-brown color and which is mainly responsible for the color changes taking place in the leaf during curing (Low, 1900). It is known that the enzyme, polyphenolase, which occurs in green tobacco is able to oxidize certain polyphenols (see Mason 1955, for review), and it has been suggested that the development of brown pigments in the tobacco leaf during curing is due to the enzymic oxidation of rutin (Neuberg and Kobel, 1935, 1936). Other reactions between oxidized polyphenols and amino-acids are also thought to occur during curing (Frankenburg, 1950). The color of the cured tobacco is important as it is used in subjective grading of the leaf. In flue-cured tobacco the most desirable color is a clear, bright lemon, the next most desirable color is orange and the third rank color is taken by red or mahogany. Although there may be a relationship between color and smoking quality or manufacturing quality, no work as yet has been carried out on this problem. The polyphenols may also be of great importance since it is believed that the presence of these compounds contributes towards the flavor of tobacco during smoking (Frankenburg, 1950; Koenig and Dorr, 1933). Thus the polyphenolic component of tobacco leaf is closely related not only with the coloring, but also with the quality of the tobacco. A number of studies in this connection have been made by Bruckner (1936) who states that the amount of the polyphenols present is directly proportional to the quality of the tobacco, and also by Schmuk (1931) who proposed a polyphenol index for use in the chemical evaluation of tobacco qualities. Furthermore, in a recent paper by Wilkinson, Phillips and Bacot (1954) it has been shown that the chlorogenic acid content of flue-cured tobacco is directly related to quality as shown by U.S.D.A. grades.

The first polyphenol to be identified in tobacco was chlorogenic acid (3-Caffeoyl-quinic acid) (Schmuk, 1930) and this compound was later isolated from tobacco by Koenig and Dorr (1933). They reported that the chlorogenic acid contents of German tobaccos averaged from 2.5 to 4.5 per cent. By means of two-dimensional paper chromatography Roberts and Wood (1951) were able to show that chlorogenic acid was present in tobacco and that possible isomers of chlorogenic acid were also present. The identification of chlorogenic acid in tobacco using paper chromatography has also been reported by several other workers (Wegner, 1953; Pearse and Novellie, 1953; Shiroya, Shiroya and Hattori, 1955) while Phillips (1955) has recently isolated chlorogenic acid from American flue-cured tobacco.

The presence of flavones has been reported in various tobaccos, but only rutin (quercetin-3-rhamnoglucoside) has been studied in any detail. Hasegawa (1931) obtained crude preparations of rutin from fresh tobacco leaves and Neuberg and Kobel (1935, 1936) also isolated it from green tobacco leaf. The extraction of rutin from flue-cured tobacco was first carried out by Couch and Krewson (1946) who obtained a patent for their method. Several workers (Roberts and Wood, 1951; Wegner, 1953; Pearse and Novellie, 1953; Shiroya *et al*, 1955) have recently confirmed the presence of rutin in tobacco by paper chromatography while Akaike (1955) has isolated this compound from the ethanol extract of tobacco. A second flavone, isoquercitrin (quercetin-3-glucoside) has been isolated from the leaves of unfermented tobacco by Kurilo (1937) and from air dried tobacco by Howard, Gage and Wender (1950). These latter workers also obtained some evidence for the presence of quercetin. Four, as yet unknown, flavones have been isolated by column chromatography from tobacco (Naff & Wender, 1947; Schoulties and Wender, 1947). Scopoletin (6-methoxy-7-hydroxycoumarin) was extracted by Best (1944) from tobacco plants which had been infected with tomato spotted wilt virus while the same substance has been isolated from tobacco roots (Mizukami, 1951) and has also been reported to be present in cultured tobacco tissue (Tryon, 1956). Recently, Akaike (1955) isolated scopoletin from the ethanol extract of tobacco leaf, obtaining about 0.15 g. of the coumarin from two Kg. of tobacco.

The most detailed account of the polyphenols of tobacco is that recently reported by Reid (1956) who identified the following polyphenols in tobacco; chlorogenic acid, rutin, scopoletin, scopolin, isoquercitrin, kaempferol-3-rhamnoglucoside and caffeic acid. This is the first time that scopolin, which is the 7-glucoside of scopoletin, has been reported in tobacco although it has been found in oat roots (Eberhardt, 1955). The presence of free caffeic acid in to-

bacco has been reported by other workers (Wilkinson *et al*, 1954; Shiroya *et al*, 1955; Akaike, 1955), but Roberts & Wood (1951) could find no evidence for the presence of this acid, and some doubt exists as to whether it may have been formed by the slight hydrolysis of chlorogenic acid during extraction used in the published studies.

It would appear that a great interest is being shown at the moment in the polyphenols of tobacco, since the latest paper on this subject has appeared in Russian. Mikhailov (1956) examined the ethanol extract of fermented Bulgarian tobacco using two-dimensional paper chromatography with the conventional solvent systems (Cartwright & Roberts, 1954). By comparison with authentic specimens he was able to confirm the presence of rutin, isoquercitrin and chlorogenic acid. From the results of hydrolysis experiments he concluded that three unknown flavonol glycosides were also present. Using the method of Schmuk he found that the polyphenol content varied in dependence on the place of origin of the tobacco plant, from 2.9 to 5.7 per cent.

The importance of fluorescent compounds in determining the quality of tobacco leaf was shown by Johanson (1953). He found that normal tobacco leaves, cured or uncured fluoresced with a brilliant bluish white light tinged with green when exposed to ultra-violet radiation. On the other hand, fully trashy leaves, whether cured or uncured remained dull showing only a reddish brown color. It was found that the absence of fluorescence was associated with the chemical composition typical of trashy leaf (Johanson, 1951). That is, low reducing sugar content and a high total nitrogen. By means of chromatographic separation of extracts he showed that the fluorescence of tobacco leaf was the resultant of four major components, one of which was shown to be scopoletin. The amount of fluorescent material present was found to decrease with increasing trashiness and there were indications that the relative amounts of the four components changed as the fluorescence diminished. Johanson states that further details of the work would be published, but so far nothing else has appeared.

This investigation was therefore undertaken in order to identify and estimate the *major* individual constituents of the polyphenol fraction in the hope that these studies might lead to a much clearer understanding of the relationship between composition of the tobacco leaf and quality than has been possible so far.

Apparatus and Methods

Material. Flue-cured tobacco was used in this study and the grade of American tobacco (Georgia Belt) was equivalent to U.S.D.A. grade B3L-B4L. The flue-cured tobaccos obtained from other countries were chosen to be comparable with this grade.

Extraction of Polyphenols. The tobacco leaf (two g.) was ground up with distilled water (six ml.) and the resulting mixture squeezed by hand through a cloth. The juice obtained was then centrifuged at 3,000 r.p.m. for 30 minutes and the resultant clear, brown liquid used for the experiments.

Two-Dimensional Chromatography. Apparatus: The apparatus consisted of an all glass tank (12" square) with a close-fitting glass top and a dural chromatographic frame modified in size from that used by Datta, Dent and Harris (1950) to take paper 10" square. The frame accommodated five papers allowing the solvent front to run about 20 cm. from the starting line in each direction. *Solvent Systems:* Two solvent systems were used in the chromatographic work on polyphenols:

(a) The upper layer of a mixture of n-butanol-acetic acid-water (4:1:5).

A very similar solvent system has been used by other workers (Roberts & Wood, 1951; Reid, 1956; Roberts and Wood, 1953; Forsyth, 1955).

(b) The upper layer of a mixture of ethyl acetate-pyridine-water (2:1:2).

Jermyn and Isherwood (1944) have used this system for the chromatographic separation of carbohydrates but it does not appear to have been used for polyphenols. The chromatograms were first run upwards in the ethyl acetate-pyridine-water (2:1:2) mixture and then at right angles to the previous direction of solvent movement with the n-butanol-acetic

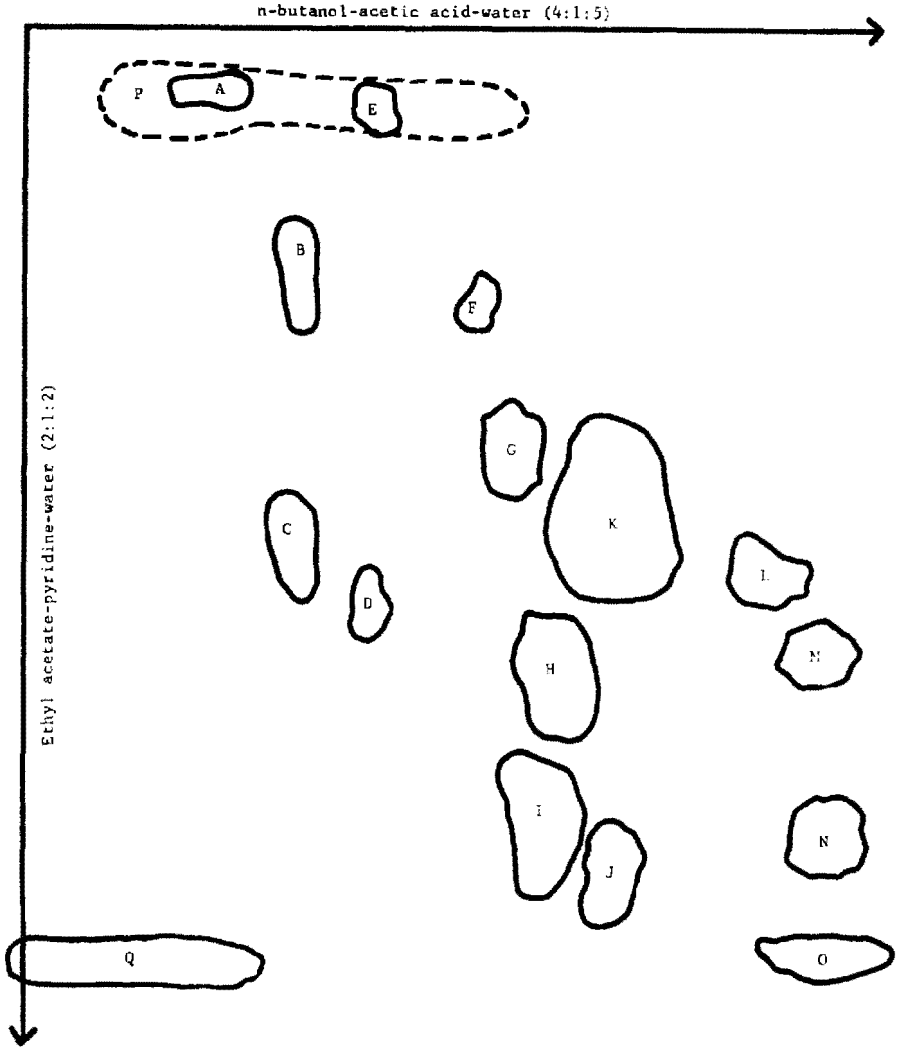


Figure 1. Two-dimensional paper chromatogram showing the relative positions of the polyphenols present in flue-cured tobacco. (Rhodesian)

acid-water (4:1:5) mixture. The two-dimensional chromatograms were prepared on Whatman No. 1 paper sheets using 15μl. of the extract prepared as on page 2.

In this case the papers were allowed to equilibrate overnight (16 hours) and between each run were dried in a current of warm air.

Location of the Polyphenols. Most of the polyphenols were located on the paper chromatograms by means of their fluorescence in ultra-violet light and by the change in their fluorescence in ultra-violet light in the presence of ammonia vapor. In ordinary light chlorogenic acid and anthoxanthins showed up as yellow spots with ammonia vapor.

Hydrolysis of Aqueous Extract. Two ml. of the aqueous extract were heated under reflux with two ml. of 2*N* sulphuric acid solution for two hours. The solution was then centrifuged to remove a brownish-black solid which had separated during the reaction. After adjusting the *p*H of the solution to 6.0 with 10 per cent sodium hydroxide solution the volume was reduced to one ml. under reduced pressure. 15μl. of this hydrolysate were then examined on two-dimensional chromatograms.

Column Chromatography. In an effort to isolate individual polyphenols some attempt was made to separate them on cellulose columns. The dimensions of the column used were 50 cm. long x three cm. diameter. It was filled with ethyl acetate-pyri-

Table 1. Appearance of spots on two-dimensional paper chromatograms of aqueous extracts of flue-cured tobacco.

Spot	R_f(1)	R_f(2)	Fluorescent color		A		B	C	D
			U-V	U-V + Ammonia	Hoepfner Reagent	1% $FeCl_3$	NH_3—$AgNO_3$	$FeCl_3$—K_4FeCN_6	Bisdiazotised Benzidine
A	0.06	0.20	Greenish blue	Yellowish green	Pink	Grey	+ve	+ve	Brown
B	0.26	0.28	Pale greenish blue	Pale greenish blue	—	—	—	—	—
C	0.52	0.27	Pale greenish blue	Pale greenish blue	—	—	—	—	—
D	0.58	0.34	—	Yellow	—	—	—	—	—
E	0.08	0.36	—	Blue	—	—	—	—	—
F	0.29	0.45	Pale pink	Pale pink	—	—	—	—	—
G	0.43	0.49	Blue	Apple green	Pink	Grey green	+ve	+ve	Pale brown
H	0.67	0.54	Blue	Blue	—	—	—	—	—
I	0.82	0.53	Dark brown	Yellow brown	—	Olive green	+ve	+ve	Reddish brown
J	0.87	0.60	Pale green blue	Yellow green	Pink	—	—	—	—
K	0.49	0.59	Blue	Apple green	Pink	Grey green	+ve	+ve	Pale brown
L	0.55	0.74	—	Blue	—	Pale yellow	—	—	—
M	0.65	0.80	—	Blue	—	—	—	—	—
N	0.84	0.82	Pale green blue	Pale green blue	—	—	—	—	—
O	0.95	0.85	Blue	Greenish blue	—	—	—	—	—
P	0.06	0.11	—	—	—	Dark grey	+ve	+ve	Pale brown
Q	0.98	0.09	—	—	—	Orange brown	+ve	—	—

Solvent (1) Ethyl acetate pyridine-water (2:1:2)

Solvent (2) n-butanol-acetic acid-water (4:1:5)

a Hoepfner, 1932 modified by Roberts and Wood, 1951.
b Roberts and Wood, 1955.
c Barton, Evans and Gardner, 1952.
d Linstedt, 1950.

dine-water (2:1:2) solvent mixture and Whatman cellulose powder (standard grade) added slowly in a fine stream so that any air bubbles which were trapped in the powder could escape. From time to time the column was packed down with a glass rod and then finally well washed with the solvent mixture to remove impurities. Ten grams of tobacco were extracted with water as before, the extract concentrated to two ml. under reduced pressure and then applied to the top of the column. Elution was carried out with the above solvent mixture and fractions of 10 ml. collected. These fractions were concentrated and examined on one-dimensional chromatograms.

Fractionation of Aqueous Extract. This was carried out using the scheme shown on page 5.

Results

From one-dimensional chromatograms it was seen that large numbers of fluorescent compounds were present in the aqueous extract and it was obvious that two-dimensional chromatography would have to be employed in order to ensure that all the polyphenols were separated. A trace obtained from a typical two-dimensional chromatogram obtained from the aqueous extract is shown in figure 1. At least 17 polyphenolic substances are present, nearly all of them being fluorescent in ultra-violet light. Their characteristics are given in table 1.

Identification of Polyphenols

Spots I and K. From their R_f values and color reactions, spots I and K appeared to be rutin and chlorogenic acid respectively. Addition of authentic specimens of rutin and chlorogenic acid to the extract before chromatography considerably enhanced the intensity of spots I and K thus establishing the provisional identity of these two spots. Two-dimensional chromatograms of rutin and chlorogenic acid alone also confirmed the R_f values of I and K.

Spot G. This spot had an R_f value of 0.49 in the n-butanol-acetic acid-water system and from its color reactions is obviously very closely related to chlorogenic acid. This compound is most likely to be neo-chlorogenic acid, an isomer of chlorogenic acid, which was first isolated by Corse (1953) from peaches and shown to be formed from quinic acid and caffeic acid. The position of the attachment of the caffeic acid to the quinic acid has not yet been determined. Cartwright *et al* (1955) have recently reported the presence of a compound similar to chlorogenic acid (R_f 0.49) in the aqueous extracts of tea, apple and pear. In the case of tea, Roberts (1956) has proved that this compound was neo-chlorogenic by comparison with an authentic specimen. Unfortunately an authentic specimen of neo-chlorogenic acid could not be obtained for comparison purposes but Dr. Roberts agrees with us that spot G is due to neo-chlorogenic acid.

Spots L and M. These spots only gave a blue fluorescence in ultra-violet light in the presence of ammonia vapor and were only present in trace amounts compared with chlorogenic acid.

L. R_f in n-BuOH-HOAc-H_2O = 0.74
M. R_f in n-BuOH-HOAc-H_2O = 0.80

Cartwright *et al* (1955) have reported the presence in the aqueous extracts of the tea, apple and pear leaf of two very similar compounds to the above. They found that these compounds only gave a blue fluorescence in ultra-violet light in the presence of ammonia vapor and had R_f values of 0.72 and 0.79 in the n-butane-lacetic acid-water system. When they hydrolyzed small amounts of these compounds (prepared by paper chromatography) with 6.*N*HCl, substances identified chromatographically as p-coumaric and quinic acids were produced. On this evidence these substances were tentatively identified as p-coumaryl quinic acids.

Spots L and M are therefore most likely to be due to these p-coumaryl quinic acids, but as they were only minor constituents no further work was carried out on them.

Spot A. This spot was obviously related in some way to chlorogenic acid and since it had very low R_f values may be an intermediate in the oxidation of this acid by enzymes. It is possible that this spot is identical with Spot A obtained by Roberts and Wood (1951). Pearse and Novellie (1953), however, could find no evidence of Spot A as found by Roberts and Wood.

Spot J. This spot which is also related to chlorogenic acid is probably identical with Spot G obtained by Roberts and Wood (1951). Since it has a fairly large R_f value it may be an isomer of chlorogenic acid. The only other isomer of chlorogenic acid so far known is iso-chlorogenic acid (Barnes, Feldman and White, 1950). Its R_f value of 0.62 in the n-butanol-acetic acid-water system is however much lower than that obtained for an authentic specimen of iso-chlorogenic acid in the same solvent system (R_f = 0.76) (Roberts, 1956).

Spot P. This was not so much a spot as a streak of yellowish brown material. It gave very strong reactions with all phenolic reagents and is most likely polymeric material formed by the oxidation of the polyphenols of tobacco with polyphenol oxidase during the process of curing.

There were three compounds F, H and O which appeared to be present in fairly large quantities, but it was not possible to identify them at this stage.

Free Caffeic Acid. It was at first thought that Spot O was due to caffeic acid, but its R_f values were slightly different and it did not give any color reactions. The comparison of one-dimensional and two-dimensional chromatograms of the aqueous extract with chromatograms of authentic caffeic acid, however, showed that free caffeic acid was not present.

Hydrolysis of the Aqueous Extract. As an aid in the identification of the unknown compounds F, H and O the aqueous extract was hydrolyzed with 2*N* sulphuric acid. After hydrolysis compound O was still present, but F and H had disappeared indicating that they were either glycosides or esters. Two new compounds appeared and were shown by comparison with authentic specimens to be caffeic acid and quercetin which had been formed by the breakdown of chlorogenic acid and rutin respectively.

Attempted separation of Polyphenols by Column Chromatography. In order to find out more about compounds F, H and O an attempt was made to isolate them by chromatography on a column of cellulose. The results were not very promising, as, owing to the large number of compounds present, clear cut separations could not be achieved. Fractions 10 to 18 were combined and taken to dryness under reduced pressure. The residue was then worked up using a combination of the methods of Phillips (1955) and Hulme (1953). Finally a small amount of a white solid m.pt. 208°C was obtained which did not depress the melting point of an authentic sample of chlorogenic acid. Further proof that this compound was chlorogenic acid was obtained when it co-chromatographed with chlorogenic acid in a number of solvent systems (Swain, 1953) and also gave the same color reactions.

Fraction 1 contained a small amount of the unknown compound O, but removal of the solvent only gave a trace of oily material. Its R_f values in the two standard solvent systems were 0.96 and 0.87. At this stage, from its R_f values and change of fluorescence with ammonia this

compound was thought to be scopoletin (7-hydroxy-methoxy coumarin).

Fractionation of the Aqueous Extract. In a further attempt to identify compounds F, H and O it was decided to carry out a fractionation of the aqueous extract using a modification of the method of Reid (1956).

Fraction A. One-dimensional and two-dimensional chromatograms of this fraction showed that it contained as the major components rutin, chlorogenic acid, neo-chlorogenic acid and an unknown polyphenol similar to chlorogenic acid (Spot A). Compounds F, H and O were not present.

Fraction A1. Two-way chromatograms of this solid showed one large and one very small yellow spot. The major component was identified as rutin by comparison with an authentic specimen. The smaller component had R_f values agreeing with isoquercitrin (Howard, Gage and Wender, 1950), but it was only there in a trace amount. An attempt was made to separate these two compounds on a column of magnesium trisilicate using ethyl acetate saturated with water (Ice and Wender, 1952), but only one yellow band separated. Elution of this material and crystallization from 50% ethanol gave rutin as yellow needles m.p. 185-187°C undepressed when mixed with an authentic specimen, it also had the spectrum and R_f values identical with an authentic specimen. No evidence for the presence of a kaempferol rhamnoglucoside reported by Reid (1956) could be found.

Fraction C. According to Reid (1956) this fraction should contain scopoletin. One-dimensional chromatograms on this material showed the presence of one blue fluorescent compound which from its R_f values was the same as compound O obtained on a two-dimensional chromatogram of the aqueous extract. The sample had the correct R_f values for scopoletin in several solvent systems (Eberhardt, 1955; Swain, 1953). It had a very low R_f value in ethyl acetate-2*N*. NH_3 (1:1), which according to Swain (1953) is indicative of coumarins having a free phenolic hydroxyl group. Final proof was obtained when the compound co-chromatographed with an authentic specimen of acopoletin (7-hydroxy-6-methoxycoumarin) in several solvent systems.

Spot O is therefore scopoletin, but when two-dimensional chromatograms were sprayed with the benzidene solution this spot did not give a grey color or a violet color when oversprayed with sodium carbonate solution (Swain, 1953). This is due to the sensitivity of the reagent, since the amount of scopoletin present in the tobacco is small. The amount present on the two-dimensional chromatograms was estimated to be $<1\mu$g. However, when one-dimensional chromatograms of the concentrated fraction C were sprayed with the benzidene reagent the characteristic color reactions of scopoletin were obtained.

Fraction B. One-dimensional chromatograms showed the presence of several compounds, but the major one was a blue fluorescent compound whose fluorescence did not change in the presence of ammonia vapor.

R_f in n-butanol-acetic acid-water (4:1:5) = 0.53.

R_f in ethyl acetate-pyridine-water (2:1:2) = 0.64.

This compound which was obviously compound H was isolated free from the other compounds by means of band chromatograms. An ethanol solution was obtained which was taken to dryness and the residue then hydrolyzed with 2*N* sulphuric acid. Chromatograms run on this solution showed that the original compound had disappeared and that a fast running blue fluorescent compound had been formed.

R_f in n-butanol-acetic acid-water (4:1:5) = *0.83*.

R_f in ethyl acetate-pyridine-water (2:1:2) = *0.95*.

From its R_f values in other solvent

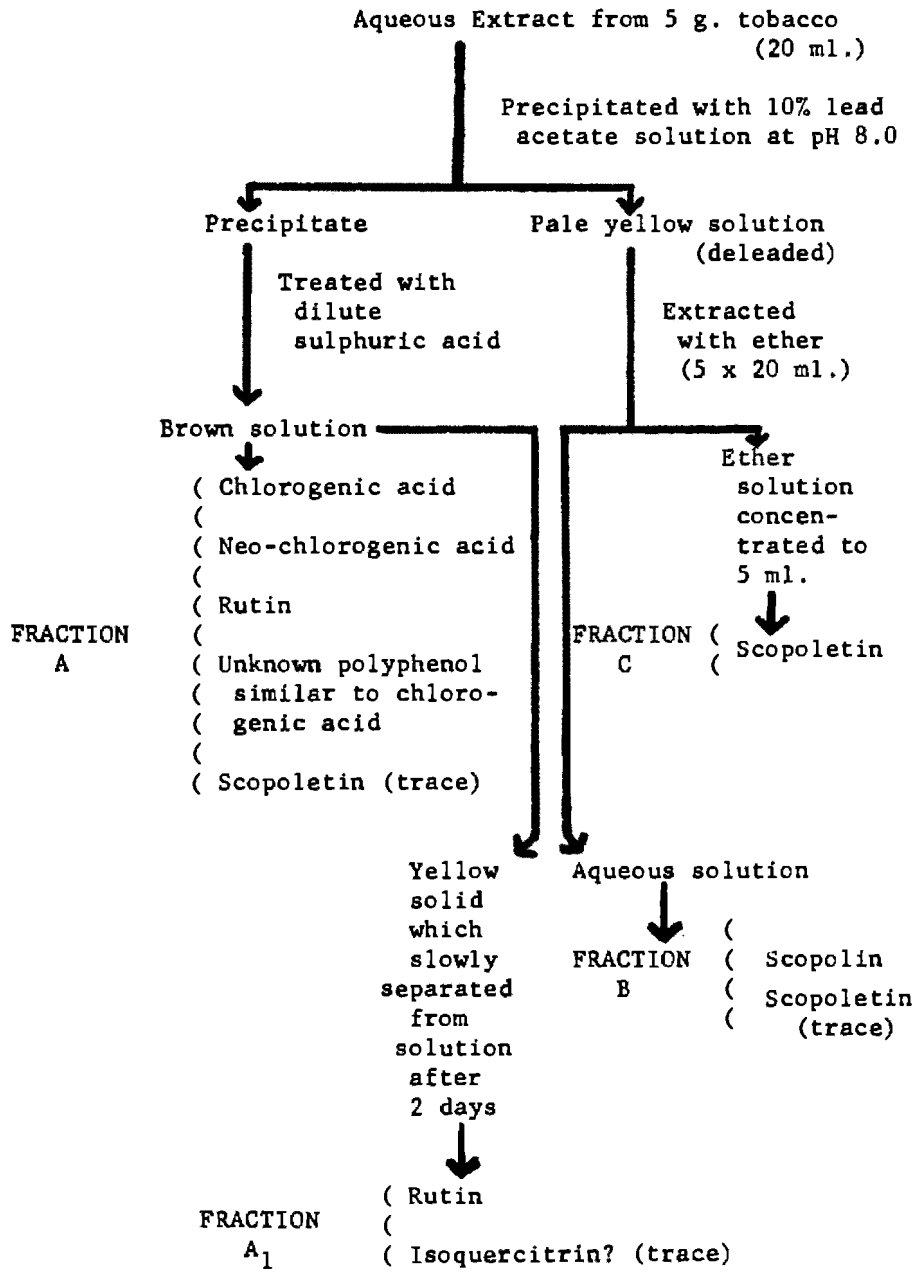
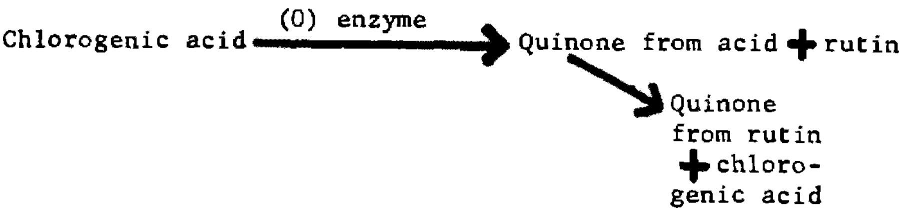


Figure 2.

in the presence of chlorogenic acid. This may be due to the fact that the acid is easily converted into a quinone, which, owing to its reactivity can oxidize rutin, itself being converted back into chlorogenic acid.



The regenerated chlorogenic acid may then take part in further oxidations while the rutin quinone being very reactive can then take part in other reactions, such as hydroxylation followed by polymerization or coupling with amino acids (James, Roberts, Beevers and de Kock, 1948). This would explain why the chlorogenic acid content of flue-cured tobacco is high compared with that of the other polyphenols, since as long as other oxidizable compounds are present chlorogenic acid itself will not be destroyed. Reid (1956) has found that chlorogenic acid alone is oxidized by the tobacco enzyme to give finally, orange brown products with low R_f values. Rutin is a flavonol glycoside and was the only compound of this type found in tobacco although there was some evidence for a trace of isoquercitrin. It is quite likely that other flavonol glycosides are in the green leaf, but being present in small quantities they are destroyed during curing. Rutin is probably only present in the flue-cured leaf owing to the fact that its initial content is fairly high. The enzymic oxidation of the polyphonols does not proceed indefinitely during curing since the oxidase is finally inactivated by the heat. In this connection the results obtained from a brief study of the polyphenols of fire-cured and air-cured tobaccos are interesting. In these methods of curing the enzyme remains active and as would be expected nearly all of the polyphenols were found to be destroyed. Only two compounds, scopolin and scopoletin were found to be present in any large quantity.

Scopoletin was present in fire-cured tobacco to the same extent as in flue-cured tobacco although there was only a trace of scopolin. In air-cured tobacco, scopolin was absent and there appeared to be a decrease in the amount of scopoletin. This may indicate that during the curing the enzymes convert scopolin into scopoletin and glucose. Scopoletin which contains only one free hydroxyl group in contrast to the diorthohydroxyl groups of rutin and chlorogenic acid may then only be slowly oxidized by the polyphenol oxidase. No work has been carried out on the action of the oxidase on scopoletin although some work has been done on aesculetin (6:7-dihydroxy coumarin) which is a derivative of scopoletin (James, Roberts, Beevers and de Kock, 1941). Aesculetin was found to be oxidized by the polyphenol-oxidase at a readily measurable rate, but did not form a colored compound either alone or with addition of amino acid.

Frankenburg (1950) had suggested that tobacco might contain tannins or polyphenols resembling those occurring in tea leaf. The chief polyphenols of tea are catechins and leucoanthocyanins (Roberts and Wood, 1953) which contain pyrogallol groupings, but no spots giving a dark blue-black color with ferric chloride which is characteristic of these polyphenols were found in the present study. Nor were any polyphenols detected in positions corresponding with those taken up by the tea catechins and leucoanthocyanins. This is in agreement with the results obtained by Roberts and Wood (1951).

Several workers claimed to have found free caffeic acid in tobacco (Wilkinson *et al*, 1954; Shiroya *et al*, 1955; Akaike, 1955; Reid, 1956), but in these studies no exidence for the presence of this compound could be obtained. This result is in agreement with that of Roberts and Wood (1951) who, however, worked with fresh cigar tobacco. Reid in a private communication has stated that there was only a trace of caffeic acid present and that it could only be detected in very strong concentrates of the aqueous extract. These differences with regard to the presence of caffeic acid may be a reflection of tobacco types.

Conclusions

1. The polyphenols of flue-cured tobacco have been found to comprise numerous compounds.

2. The major constituents are rutin (3-rhamno-glucoside of 5:7:3′:4′—tetrahydroxy flavonol), the chlorogenic acids, scopoletin (7-hydroxy-6-methoxy coumarin), scopolin (7-glucoside of scopoletin) and an unidentified compound which has a pink fluorescence in ultra-violet light.

3. The polyphenols of tobacco do not contain catechins or leucoanthocyanins which have been found however to be the major constituents of the polyphenols of tea and cacao.

4. No evidence for free caffeic acid could be found in the samples of flue-cured tobacco examined.

5. No qualitative differences in the major polyphenols have been found in several samples of flue-cured tobacco. There are however quantitative differences in the polyphenols.

6. The method of curing employed has a large bearing on the polyphenols present, since in fire-cured and air-cured tobaccos it was found that most of the major polyphenols except scopolin and scopoletin had been destroyed.

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