

Polyphenolic Constituents of Tobacco

Attempts to correlate the presence and level of the chemical constituents of tobacco with the quality of the leaves for smoking purposes have been made throughout the years with varying degrees of success. In almost all instances, the approach used in this work has involved determining broad chemical groups and relating the findings to quality. Tow and Earl (1940) and Phillips and Bacot (1953) have discussed the earlier efforts in this work and have included references to such "numbers and coefficients" as the methylene blue, lead, picrate, polyphenol, iodine, lead tetra-acetate, hydrogen sulfide and silver nitrate numbers, the Kovalenko coefficient, Shmuk coefficients, and the Pyriki quality number. Polyphenolic constituents are encountered as factors in many of these yardsticks of tobacco quality.

The importance of polyphenols in influencing tobacco quality was originally shown by Shmuk and Semenova (1927), who fractionated total reducing substances into polyphenols and carbohydrates by a lead precipitation procedure. Both fractions were shown to contribute to color and quality. Later Koenig and Dorr (1933) concluded that a component of the polyphenolic fraction containing chlorogenic acid was responsible for the aroma in tobacco. From this time on, group determinations of polyphenols became an integral part of many chemical analyses designed to delineate tobacco quality.

Recently, the complexity of the polyphenolic fraction of tobacco has become more fully appreciated, and at least one significant attempt to relate quality to specific polyphenolic compounds has appeared. Wilkinson *et al.* (1954) have observed a significant relationship between the levels of chlorogenic or caffeic acids and tobacco grade judged according to United States Government tobacco standards. Mikhailov (1956) also has recently emphasized the importance

of attempting to correlate specific polyphenols with quality rather than using general polyphenolic group determinations.

Whether or not such an approach will produce a striking contribution to the problem of relating constitution to quality remains to be seen. Undoubtedly, a myriad of factors are interrelated to produce leaf quality. The addition of an even greater variable—the effects of combustion—makes the problem of relating constitution to smoking quality still more complex. Much work of a basic nature remains to be done, and it would appear that a logical beginning would be a comprehensive study of tobacco polyphenolic composition. To this end, the present report is a compilation of current knowledge on this subject up to early 1957. No attempt will be made to discuss critically the methodology used for the detection, separation and structure determination of plant polyphenols; this subject is of a broad nature and has been extensively reviewed by Geissman (1955), Schmidt (1955) and Clarke and Nord (1955).

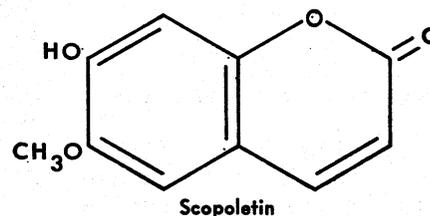
Although marked variations in composition among different types of tobacco exist, several of the major polyphenolic and related substances of pertinent *Nicotiana* species have been isolated and identified. Among these substances are flavonoids, cafetannins, coumarins, anthocyanins and hydroxylated cycloalkanes² occurring as free aglycones or combined with sugars as glycosides. The presence of simple hydroxylated benzene derivatives has also been claimed, but few of these compounds have been identified. An array of minor polyphenolic substances have been detected, but their conclusive identification has been lacking in most instances.

The published studies describing isolation of tobacco polyphenols are discussed below. Details on the chemical techniques and procedures used for isolating and identifying these substances have been presented by a

large number of workers, and this subject is to be reviewed by the present author at a later date. It should be noted that in many of the published studies, recovery of only micro quantities of compounds has necessitated identification by spectrophotometric and chromatographic means rather than by the classical procedure of making two derivatives and measuring their physical properties. Also, in many cases, exact descriptions of the tobacco employed in the studies were not detailed by authors, but when such information was provided, it has been included in the present review.

Coumarins

Thus far, scopoletin (6-methoxy-7-hydroxycoumarin) is the only coumarin which has been isolated from tobacco.



In 1948, Best described the pattern of scopoletin distribution in the tobacco plant, using histochemical techniques. Later, Mizukami (1951) isolated this coumarin from tobacco roots by extracting with aqueous alkali followed by precipitation with lead acetate. Three hundred mg of scopoletin were obtained from seven kg of roots. More recently, Reid (1956 *a* and *b*) has shown the presence of free scopoletin, scopoletin glycosides and unknown scopoletin-like substances in flue-cured South Carolina tobacco leaves. Surprisingly, these substances were found in the supernatant after lead acetate precipitation of the primary ethanol extract. Free scopoletin was identified in the ether extract of the acidified supernatant, and four substances giving the typical brilliant blue fluorescence of coumarins under ultraviolet light were recovered in vari-

¹ A laboratory of the Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

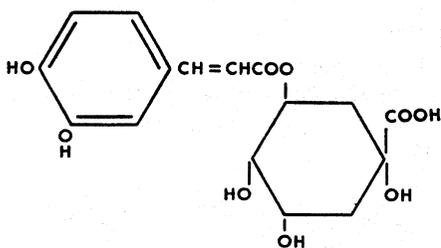
² Although not phenolic in nature, these compounds have been included here since at least one representative is closely related to the cafetannins.

ous other fractions of the acidified supernatant. One of these was shown to be a glucoside of scopoletin; the identities of the remaining three substances, which occur in varying small amounts, were not investigated. The quantity of isolated scopoletin glucoside was apparently greater than the amount of free coumarin obtained.

On the basis of available data, then, it appears that free scopoletin, a glycoside of scopoletin and at least three unidentified coumarin-like substances are present as minor polyphenolic substances in flue-cured tobacco.

Tannins and Their Hydrolytic Products

Of the diverse tannins found in nature, only certain caffetannins have been isolated from tobacco. In all but one instance, the isolated substances have been shown to be chlorogenic acid, its isomers, and its hydrolytic products, caffeic and quinic acids. Significant amounts of caffetannins are present in tobacco and these compounds must be considered to be major polyphenolic constituents.



Chlorogenic Acid: (caffeoylquinic acid).

As indicated by Phillips (1955), at least two studies related to chlorogenic acid in tobacco appeared before 1910. In one of these, Savery (1884) detected an unidentified substance which was named "tobaccotannic acid" and which might have been chlorogenic acid. Later, in a distribution survey of this caffetannin in plants, Gorter (1909) found that *Nicotiana tabacum* contained chlorogenic acid, although some doubt of the specificity of the color reaction employed was subsequently cited.

In 1930, Shmuk, and Shmuk and Piatnicki isolated a depside of caffeic and quinic acid, presumably chlorogenic acid, and free caffeic acid from fermented Samsun tobacco. Three years later, Koenig and Dorr (1933) isolated caffeic and quinic acids from a hydrolysis mixture and indicated that these compounds were derived from combinations of chlorogenic acid with terpene compounds. As noted above, these substances were recovered from a fraction believed to contribute significantly to

the aroma quality of the tobacco. More recently, three substances giving a positive Hoepfner test³ were separated on paper chromatograms of extracts of Pennsylvania Seed Filler tobacco (both fresh and dried) by Roberts and Wood (1951). One of these was identified as chlorogenic acid; the other two gave an atypical color reaction and were assumed to be similar to chlorogenic acid. Free caffeic acid could not be detected, and slight variations were obtained in the chemical composition of fresh and dried leaves. Subsequently a similar investigation was performed with South African tobacco by Pearse and Novellie (1953); the findings of Roberts and Wood were duplicated with the exception of one spot on a paper chromatogram, and the identity of chlorogenic acid was confirmed.

Within the last few years, two significant studies on the chlorogenic acid contents of tobacco have appeared from the United States Department of Agriculture. In 1954, Wilkinson, Phillips and Bacot related the chlorogenic and caffeic acid content of various grades of Type 12 tobacco to quality as judged by the Government grading system. However, no precise isolation or identification work was presented in this investigation. Contents of approximately 2.3-7.7 per cent chlorogenic acid and 0.2-1.0 per cent free caffeic acid were obtained. Possibly substances other than these acids were being measured by the method employed, e.g., isomers of chlorogenic acid and other compounds extracted with water, precipitated by lead acetate, and oxidized in the Slotta-Neisser procedure.⁴ Later, Phillips (1955) developed a new isolation method for obtaining chlorogenic acid from Eastern Carolina flue-cured tobacco (United States Type 12) after the procedure of Koenig and Dorr was found unsatisfactory. Approximately 0.15 per cent crystalline acid was obtained from samples of United States Grade H5L.

More recently, Dawson and Wada (1957) have studied the flavonoid and depside content of shade-grown and sun-grown green cigar tobacco and reported the qualitative and quantitative differences; two substances closely related to but not identical with chlorogenic acid were observed.

It should be emphasized that at least three isomers of chlorogenic acid are known. The most common

isomer, referred to as chlorogenic acid, has a depside linkage at the 3-hydroxyl group of quinic acid which is *cis* to the carboxyl. Isochlorogenic acid has the linkage at the 5-position and is *trans*; this isomer has not been crystallized (Barnes *et al.*, 1950). Neochlorogenic acid, more recently isolated, has been crystallized, but the position of the caffeoyl linkage is not known (Corse, 1953). Inversion of chlorogenic acid occurs very easily (sometimes during the development of paper chromatograms) and lactonization or hydrogen bonding may be encountered thus complicating isolation and identification studies (Williams and Roberts, 1956). Other isomers of chlorogenic acid probably exist.

In the study of Reid (1957) discussed above, chlorogenic acid and related substances were found in both the lead acetate-precipitated material ("major fraction") and in the supernatant of the extract after precipitation. The bulk of the compounds occurred in the major fraction. Approximately eight spots giving the color and fluorescent characteristics of chlorogenic acid were obtained from the material precipitated by the lead salt. Likewise, seven spots were found in the minor fraction, one of which was suspected of being neochlorogenic acid. Probably, some of these spots represented the products of the inversion of chlorogenic acid and its isomers which occurs during a chromatographic separation. Mikhailov (1956) has also observed several chlorogenic acid-like chromatographic spots from extracts of fermented Bulgarian tobacco, and has indicated that these are probably geometric isomers of the caffetannin.

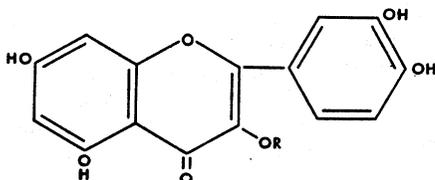
Although the presence of free caffeic acid in tobacco has been demonstrated frequently, the possible occurrence of free quinic acid has been of less interest to most workers. Shmuk and Piatnicki (1930) could not detect free quinic acid in fermented Turkish tobacco. Likewise, Dawson *et al.* (1955) reported the absence of this compound in cured Connecticut cigar wrapper samples. However, more recently, Palmer (1956) has demonstrated free quinic acid in mature, fresh cigar leaves and shown that quinic acid is lost during drying of the leaves at 80° C. Possibly this effect, either through laboratory manipulation or by "natural" drying (curing), may have contributed to reported failures to detect free quinic acid in certain tobaccos. On the other hand, the findings may have been simply a reflection of differences in polyphenolic contents among tobaccos.

³ A color reaction usually considered specific for chlorogenic acid.

⁴ Method is based on the quantitative oxidation of *o*-dihydroxyl groups.

Flavonoids

Rutin. Several flavonoids have been isolated from various organs of the tobacco plant. Among those detected thus far are glycosides or aglycones belonging to the flavonol (3-hydroxyflavone) type of structure. Perhaps the most commonly occurring flavonoid is rutin ("quercetin 3-rhamnosidoglucoside"; "quercetin 3-rutinoside"; "3-rutinosido-3', 4', 5, 7-tetrahydroxyflavone").



Rutin. [R=rutinose (L-rhamnosido-D-glucose).]

Although Charaux isolated rutin from tobacco flowers in 1924, the presence of this flavonoid in tobacco leaves was initially demonstrated in 1931 by Hasegawa who succeeded in extracting six gm of the compound from 30 kg of fresh leaves by precipitation with lead salts. Subsequently, Neuberg and Kobel (1935) duplicated the work of Hasegawa, with certain methodological changes, and showed that partial disappearance of rutin occurred during the drying of fresh Zichna cigarette tobacco. This disappearance was later found to be the result of an enzyme-catalyzed oxidative process in which the flavonoid was transformed into slightly soluble brown pigments (Neuberg and Kobel, 1936). In 1944, a report appeared which indicated that rutin was of value in the treatment of capillary fragility associated with hypertension (Griffith *et al.*, 1944). Since chemists at the Eastern Regional Research Laboratory of the United States Department of Agriculture had been working on isolation of polyphenols from tobacco at that time, this report served to stimulate further interest in studies in progress. As a result, a number of publications concerned with flavonoid contents of tobacco subsequently appeared from this laboratory. Details of the most effective isolation procedures were worked out (Couch and Krewson, 1944a), the rutin contents of numerous varieties of *Nicotiana rustica* and *N. glauca* were determined (Badgett *et al.*, 1949) and the distribution of the flavonoid in other species was examined (Krewson and Naghski, 1953). Although higher-yielding sources of rutin were subsequently

found that replaced tobacco for pharmaceutical purposes, the overall contribution of this program to the knowledge of rutin was extensive (Griffith *et al.*, 1955). In respect to rutin levels in tobacco, it was found that the quantity in flue-cured types varied considerably with leaf quality and age, ranging from 0.008-0.61 per cent with an average of 0.4 per cent for good quality leaf (Couch and Krewson, 1944a).⁵ Later work elaborated the fluctuations of rutin content during growth of *Nicotiana rustica* and showed that levels of flavonoid up to two per cent (moisture free basis) occurred in *N. glauca* (Badgett *et al.*, 1949). Of special significance was the observation that oxidation of rutin resulting in its disappearance (see above) occurs during air curing, but not during flue-curing (Couch and Krewson, 1944a, b); the inference that this pattern may be a reflection of heat inactivation of oxidative enzymes is obvious.

A few other reports concerned with the rutin contents of tobacco have appeared within the last decade. Dussy (1947) has examined ten species and varieties of *Nicotiana* for this flavonoid and on the basis of his findings has concluded that *N. tabacum* is actually a hybrid species. Nio and Wada (1951) isolated rutin from the flowers of a tobacco type designated "Bright Yellow" thus confirming the earlier observation of Charaux. In 1951, Roberts and Wood reported the presence of rutin as a major polyphenolic constituent in fresh dried cigar⁶ (Pennsylvania Seed Filler) and Indian (Harrison Special) tobacco, but no quantitative data were presented. Later, Shiroya *et al.* (1955) demonstrated the presence of rutin as well as chlorogenic and caffeic acids in Japanese cigarette tobacco. More recently, Reid (1956 a and b) identified rutin in South Carolina flue-cured tobacco as one of a considerable number of polyphenolic substances (see below). A similar, but less comprehensive, study by Mikhailov (1956) also showed rutin to be one of the important polyphenols in Bulgarian tobacco.

From the above, it is clear that rutin is a common polyphenolic substance in tobacco, occurring in significant amounts in leaves and flowers

and contributing, in some instances, to the brownish colors which develop during air-curing. Its role is apparently that of a substrate for enzymatic oxidative processes. The levels of rutin encountered in tobacco vary with species, variety, leaf position, age, handling (curing), and perhaps even experimental manipulations in the laboratory.

Isoquercitrin. Reports on flavonoids in tobacco other than rutin have appeared very infrequently. From the available information, perhaps the next most important flavonoid, from the standpoint of quantity and occurrence, is isoquercitrin. This compound is a glucoside of quercitrin (3, 3', 4', 5, 7-pentahydroxyflavone) having the sugar substituent at the same position as rutin.

Perhaps the initial report of the occurrence of isoquercitrin in tobacco was that of Kurilo (1935) in which the isolation of a glycoside giving quercetin and D-glucose on hydrolysis was described. However, the exact position of glucosidation was not established, and precise characterization could not be made. Two years later, in 1937, Kourilo isolated and identified isoquercitrin from unfermented Tyk-Koulak tobacco by differential extraction and crystallization. The flavonoid was found to be present in amounts varying from 0.25-1.7 per cent and the aglycone was characterized by the melting point of the methylated derivative in the usual manner. Subsequently, little interest in this tobacco glucoside was evident until 1950 when Howard, Gage and Wender succeeded in extracting the compound from low grade Kentucky Burley tobacco. This represented the first isolation of a flavonoid from air-cured tobacco and the level of isoquercitrin extracted was very small: five mg were obtained from 200 gm of sample. Of importance was the observation that the original Kourilo procedure for isolating the flavonoid was not successful in the study.

Since the Howard report, Roberts and Wood (1951) have failed to detect isoquercitrin in cigar filler and Indian tobaccos, Reid (1956b) has isolated the compound as a minor component from flue-cured samples, and Nio and Wada (1951) have reported a trace of it in flowers of the tobacco plant. The presence of isoquercitrin in four classes of fermented Bulgarian tobacco has also been observed (Mikhailov, 1956).

The above studies show that isoquercitrin is present in some types of tobacco and that this flavonoid represents a minor polyphenolic constituent.

⁵ This is intimately related to the initial observation of Hasegawa (1931) concerning a relationship between brightness of leaf color and flavonoid content, even though flavonoids are believed not to contribute directly to leaf color (Giovannozzi, 1955).

⁶ Since the relationship between the drying procedure employed here and standard air-curing practice cannot be determined from the published reports, it is not known whether this work and the above reports claiming loss of rutin during air-curing are in conflict.

Other Identified Flavonoids and Miscellaneous Phenolic or Related Substances. In addition to rutin and isoquercitrin, only one other flavonoid glycoside has been isolated and identified from the tobacco plant: Kaempferol-3-rhamnosidoglucoside obtained from flowers⁷ (Wada, 1952). However, Reid (1956b) has extracted a glycoside giving kaempferol, rhamnose and glucose on hydrolysis from flue-cured tobacco; perhaps this compound is the same as that reported by Wada. One report has also appeared on the detection of small amounts of quercetin in cured Burley tobacco (Howard *et al.*, 1950).

Among the miscellaneous polyphenolic and related substances identified thus far are simple phenolic derivatives, a hydroxylated cyclohexane compound and one anthocyanin, the last of these having been isolated from tobacco flowers (Yamafuji, 1933). Although Frankenburg (1950) has noted that simple phenols are present in cured tobacco to the extent of 0.01-0.04 per cent, little information is available on the individual compounds making up this component of plant tissue. Naghski *et al.* (1944) have conducted an investigation of the volatile phenols which react with the Folin-Denis reagent from various fire-cured and light air-cured tobaccos. Unfermented Pennsylvania cigar leaf and Maryland cigarette leaf tobaccos showed a phenolic content within the above range. Fire-cured types, including Latakia, yielded larger but variable quantities of phenols with one sample approaching one per cent of the total moisture-free leaf weight. It was felt that the higher phenolic content was a result of deposition of such substances on the leaf surface from the wood smoke evolved during curing. More recently, Onishi and co-workers (1955, 1956) have identified eugenol, phenol and guaiacol in a phenolic fraction obtained by steam distillation of leaves of Virginia tobacco. In general, workers have been more concerned with the phenolic constituents in tobacco smoke rather than in the intact leaf.

Among the other miscellaneous phenolic and related substances found in tobacco may be mentioned meso-inositol and melilotic acid. Meso-inositol (one of the geometric isomers of hexahydroxycyclohexane) does not actually possess a benzenoid structure, but may be involved in the biochemistry of polyphenolics in some way (Bruckner, 1936). The

⁷ This compound differs from rutin in that the 3'-hydroxyl substituent is absent.

compound is a growth accessory factor for certain microorganisms and is of historical interest as Wildier's "Bios I", a nutritional requirement for yeasts. The presence of inositol in tobacco has been known for almost thirty years (Shmuk, 1930a). Phillips and Bacot (1955) have recently studied the inositol content of flue-cured tobacco and isolated 0.13 gm of the meso-form from 200 gm of U. S. Grade B4GF. Melilotic acid (2-hydroxyhydrocinnamic acid) has been cited by Geismann (1952) as a constituent of *Nicotiana*, but no further information on this compound was given in the report.

Unidentified Polyphenolic Substances

In addition to the compounds cited above, a vast array of minor polyphenolic substances are known to exist in tobacco. This diversity of polyphenolic composition has become evident only within the last decade or so. Undoubtedly, the application of chromatographic technics has been the deciding factor in this progress. In the following section of the current report an attempt will be made to describe reported, unidentified polyphenolic substances in a correlative manner, although the diversity of isolation technics employed in past studies makes correlation quite difficult.

At least two rather unusual polyphenolic substances have been reported in the earlier literature. In 1930, Shmuk (1930b) isolated a material from unfermented Tyk-Kulak tobacco which yielded rhamnose on acid hydrolysis; another component of this substance was a depside of caffeic and quinic acids. From this description it appears now that the isolated fraction might not have been a single compound; the depside was almost certainly chlorogenic acid, which does not usually occur as a glycoside. Perhaps the rhamnose was derived from rutin or quercitrin, since these rhamnose-containing glycosides are also precipitated by the lead salt used in the extraction procedure of this study. However, the probability of quercitrin being present is slight since this compound has never been isolated from tobacco. One cannot be certain of the nature of this substance(s) by the published description. The other "unusual" polyphenol appearing in the older literature was described by Yamafuji in 1932. The isolated substance, "tobacinin," was a glycoside having certain properties of rutin, but yielding glucuronic acid on hydrolysis.

Approximately 15 years after these studies, Wender and co-workers initiated the use of adsorption technics

in separating the flavonoid substances of tobacco. Employing alumina, Celite and talc, alone and in combinations, four yellow pigments were separated from aqueous extracts of Burley lugs (Schoulties and Wender, 1947). Later three, and possibly four, of these pigments were tentatively classified as flavones on the basis of color reactions and absorption spectra, but chemical identification was not attempted (Naff and Wender, 1947).

Since publication of these findings several studies describing further isolations of polyphenolic substances have appeared. Two of these were brief investigations in which exhaustive attempts to identify the components were not made: Dussy (1947) isolated a glycoside differing from rutin and yielding rhamnose and a yellow, amorphous aglycone on hydrolysis (cf Shmuk's work above); and Wegner (1953) reported the appearance of four distinct spots not identical with chlorogenic acid and rutin on paper chromatograms of flue-cured tobacco extracts.

The remaining investigations have been more informative, and represent the beginning of exhaustive attempts to complete the picture of the polyphenols in tobacco. Roberts and Wood (1951) succeeded in isolating seven constituents on two-dimensional chromatograms of extracts of filler tobacco. Two of these were identified as rutin and chlorogenic acid, as noted above, two gave atypical Hoepfner reactions and were probably isomers of the caffetannin, and the remainder were unidentified, although it was established that isoquercitrin was absent. More recently, Mikhailov (1956) succeeded in detecting 11 flavonoid and caffetannin-like polyphenols in fermented Class II Bulgarian tobacco. Five of these were flavonols, including rutin and isoquercitrin. Quercetin was absent. Two of these five spots were classified as flavonol glycosides, but not further identified. No indication of flavone, isoflavone, or flavonone derivatives was noted. The remaining six substances were "substances associated with coumarins and coumarin-like derivatives," presumably chlorogenic acid-like substances.⁸ Since two of these six spots gave the Hoepfner reaction, they were assumed to be isomers of chlorogenic acid. The remaining spots were not identified.

Undoubtedly, the most comprehensive study of polyphenols in tobacco has been that of Reid. Mention has

⁸ In this paper (translated from Russian by EURDD personnel), the authors refer to chlorogenic acid as a "coumarin-like substance," rather than a caffetannin as is done in the present report.

been made above of some of his findings, and these will be briefly repeated here to describe more thoroughly details of the report. Initially, Reid extracted South Carolina flue-cured tobacco with ethanol and then precipitated the bulk of the polyphenols as their lead salts. The supernatant was then evaporated to an aqueous residue which was successively extracted with a number of solvents, including diethyl ether, ethyl acetate, *n*-butanol and acetone. All fractions were chromatographed on paper with a number of developing solvents. The major polyphenolic components were obtained from the precipitate of lead salts; in all, 18 components were found in this fraction. Of the 18, rutin, isoquercitrin, a kaempferol rhamnoglucoside, four chlorogenic acid-like spots and four spots closely related to chlorogenic acid were observed; the remainder showed a distinct fluorescence and one of these may have been caffeic acid. These components were considered the "major fraction." Surprisingly, some 15 substances were obtained in the supernatant after precipitation with lead ("minor fraction"), including the following: free scopoletin, four spots that might have been scopoletin glycosides, two spots with a flesh pink fluorescence and unknown structure, one spot probably a trace of chlorogenic acid, one spot resembling *p*-coumarylquinic acid but which failed to yield the proper hydrolysis products, and five unidentified spots, one of which showed a pale yellow color. On hydrolysis of certain of these substances, yellow aglycones suggestive of anthoxanthin-like compounds appeared.

As an extension of this isolation work, Reid also prepared an enzymatic fraction having significant polyphenoloxidase activity from the tobacco. Using chlorogenic acid and caffeic acid as substrates, products of the action of this enzyme were identified on chromatograms as being similar to certain of the spots obtained in the isolation studies. The enzyme had little oxidative activity with rutin and quercetin as substrates, but on simultaneous addition of chlorogenic or caffeic acids to these flavonoids, oxidation was accelerated and a number of other products appeared on chromatograms, most of which did not resemble those obtained from tobacco by direct isolation. The significance of the enzymological portion of the Reid paper is outstanding; such an approach is of value not only in biosynthetic studies but also in pure isolation work since knowledge of the precursors or products of com-

pounds frequently aids in the identification of the compound.

Summary

Current knowledge of the polyphenolic constituents in the tobacco plant is fragmentary. Polyphenols and related substances identified thus far belong to various chemical type structures: coumarins, flavonoids, caffetannins, simple phenolic derivatives, anthocyanins, and hydroxylated cyclohexanes. Free scopoletin and glycosidic derivatives thereof are the only coumarins observed thus far in the leaf and root. Flavonols are the only flavonoid type isolated to date, and of these, the glycosides, rutin, isoquercitrin, and a kaempferol rhamnoglucoside, as well as the flavonol aglycone, quercetin, have been identified with certainty in the flower or the leaf. Chlorogenic acid and perhaps its other geometric isomers are the caffetannins that have been detected in the leaf. Among the simple phenolic derivatives present in the leaf are eugenol, guaiacol, phenol, caffeic acid, and melilotic acid. Hydroxylated cyclohexanes are represented by quinic acid and meso-inositol. One anthocyanin of unknown structure has been isolated from flowers of the plant. At least 30 other polyphenolic substances have been detected in the leaves of one type of flue-cured tobacco and almost all of these remain to be identified. The qualitative and quantitative polyphenolic constitution varies with a number of factors, including species, age, leaf location, method of curing, and perhaps many others.

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