

Composition Studies on Tobacco. XXIII.
**Pyrolytic and Structural Investigations on the Polyphenol-amino
Acid Pigments of Leaf***

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INTRODUCTION

The presence of more than sixty polynuclear aromatic hydrocarbons (PAH) in tobacco smoke (1) is not unexpected since most, if not all, organic compounds generate PAH on pyrolysis at high temperatures. Although such generation is universal, the types and yields of PAH from various organic compounds have been found to vary markedly, depending on the structure of the compound and other factors (2, 3), when the pure compounds were pyrolyzed experimentally. Although extrapolation of such data to the events occurring during burning of a cigarette may be tenuous, there is some evidence that extrapolations may be made under certain conditions (4, 5). At any rate, it appears valid to presume that some tobacco constituents may contribute disproportionately to the PAH content of cigarette smoke, and that pyrolytic experiments may be revealing in this regard.

Among the tobacco leaf components implicated in PAH generation by past workers are the paraffins, sterols, isoprenoids and substances extractable from tobacco with n-hexane. Recently, Wynder and Hoffmann (6) have discussed published findings on the possible role of paraffinic hydrocarbons in PAH generation. Van Duuren (7) and Badger et al. (3) have presented and discussed data on the role of leaf sterols as possible PAH precursors. Wynder et al. (8) have investigated the pyrolytic products and tumorigenic properties of n-hexane extracts of tobacco, which are known to contain a large variety of lyophilic substances, including paraffins, sterols, and terpenes (9, 10). Pyrolysis of tobacco isoprenoids has been studied less intensively, although Grossman et al. (11) have investigated the pyrolytic products of solanesol. Postulations of the possible contributions of the acyclic terpenes of tobacco to the cycloalkanes and PAH contents of smoke have appeared (12, 13, 14) and could be extended easily to the several cyclic terpenes of leaf (15).

One group of leaf constituents has received little attention as a possible source of disproportionate PAH contribution: the polyphenols. These compounds consist of simple constituents, such as flavonols and caffeic acid esters (16), and of high molecular weight polyphenol complexes with or without amino acids and iron (17, 18, 19, 20). Zane and Wender (21) have pyrolyzed rutin and chlorogenic acid up to 600° C and isolated various low molecular weight oxygenated aromatic compounds, but no attempt was made to find PAH among the products. Likewise, no published studies

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have appeared on the pyrolytic products of the polyphenol-amino acid pigments. These compounds, which occur in tobacco in relatively large amounts (about 4% of leaf weight in Turkish tobacco), appear to be a potentially important source of aromatic compounds, including polycyclics, in smoke. The presence of thermodynamically stable aromatic rings in rutin, chlorogenic acid, phenylalanine, and related components make their potential contribution to PAH synthesis theoretically significant; also, the quinic acid moiety of chlorogenic acid may be an important contributor since it has been recently shown to be a precursor of phenol on pyrolysis (22). The present report describes the isolation and identification of several PAH among the pyrolytic products of the polyphenol-amino acid pigments of Turkish tobacco. Of secondary interest, some further information on the structure of these pigments is also presented.

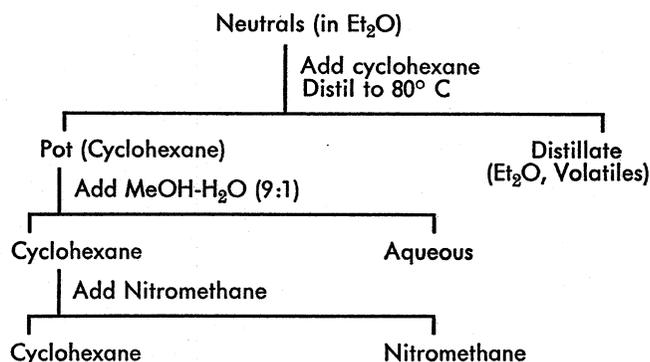
DISCUSSION

1. Pyrolysis

The pyrolysis of the pigment was conducted at 850° C, the approximate burn temperature of a cigarette. The more volatile products were condensed in Dry Ice-acetone traps and it was observed that most of the products were noncondensable gases. The condensate from the traps and the residue remaining in the pyrolysis chamber were fractionated into acids, bases, and neutrals by acidic and alkaline extraction in the usual manner. A phenolic fraction was also isolated, though not examined. Separation of the neutral components was carried out according to the scheme presented in Fig. 1, and the PAH were obtained in the nitromethane extract.

Methods of separation of PAH have been extensively reviewed (24), and several of the techniques mentioned therein were employed. The nitromethane extract was separated initially by column chromatography on activated silicic acid, a procedure that has been applied recently to the analysis of benzo(a)pyrene in cigarette smoke condensate (23). The silicic acid column was eluted with various proportions of n-hexane and benzene, to yield a series of fractions which were then separated by thin-layer chromatography (TLC)

FIGURE 1 Initial fractionation of neutral substance from pyrolysate



on acetylated cellulose. Of the several supports and solvent systems studied (24), acetylated cellulose plates developed with ethanol-toluene-water (17:4:4) proved to be the most effective system. The eluted zones were then separated by paper chromatography using acetylated paper and several solvent systems. The above solvent appeared to give the best separations, but N,N-dimethylformamide (DMF)-water and DMF-saturated hexane were also of value (24). With all solvent systems, R_f values were found to vary with different lots of paper and aging of solvent, the latter acquiring more polar properties which were better suited for the separation of higher PAH. Identification of the unknown PAH was accomplished by comparison to the chromatographic (R_f values and fluorescent color) and spectral (ultraviolet and fluorescence) characteristics of authentic polynuclears in the usual manner. Tables of spectral data, taken from the literature (25-32) were also consulted. In this manner, identities were assigned to the indicated PAH listed in Table 1; in addition, tentative evidence was obtained for the presence of the other PAH cited therein. Characterization of these latter PAH was based mostly upon fluorescence (SPF) (emission and excitation) spectra because of unavailability of authentic PAH or of sufficient isolated material for more detailed study. Of course, in some cases the SPF spectra do not exclude the possible presence of other compounds. Based upon chromatographic data and the intensity of the SPF emission bands, it was concluded that the major PAH were anthracene, pyrene, fluoranthene, chrysene, and benzo(a)pyrene. In addition to the PAH shown in Table 1, there were also many minor components that could not be identified. As a crude approximation it was estimated that the pyrolysis of the pigment had produced at least forty PAH.

TABLE 1

Polynuclear aromatic hydrocarbons (PAH) in pyrolytic products of the polyphenol-amino acid pigment of tobacco leaf

PAH	Criterion of identify*			PAH in cigarette smoke condensate**
	PC	UV	SPF	
Identified				
Anthracene	+	+	+	+
Phenanthrene	+	+	+	+
Pyrene	+	+	+	+
1-Methylpyrene	+	+	+	+
Fluoranthene	+	+	+	+
1,1'-Dinaphthyl	+	+	+	+
Chrysene	+	+	+	+
Benzo(b)fluoranthene	+	+	+	+
Benzo(a)pyrene	+	+	+	+
Benzo(e)pyrene	+	+	+	+
Anthanthrene	+	+	+	+
Perylene	+	+	+	+
Tentatively identified				
2-Methylanthracene			+	+
Benz(a)anthracene	+		+	+
2-Methylbenz(a)anthracene			+	
8,9-Dimethylfluoranthene	+		+	
Benzo(k)fluoranthene			+	+
11-H Benzo(b)fluorene			+	+
Dibenz(a,h)anthracene	+		+	+
Acenaphthene			+	
9,10-Benzphenanthrene	+		+	
Benzo(b)naphtho(2,3d)furan			+	
Picene			+	
Coronene	+		+	+
Benz(g,h,i)perylene	+		+	+
Dibenzo(a,i)pyrene	+		+	+
Dibenzo(a,l)pyrene	+		+	+

*PC = Paper chromatographic data (R_f values, fluorescent color)

UV = Ultraviolet spectra data

SPF = Spectrophotofluorometric data

**Source: reference (1)

two successive separations on standard plates.) Two successive TLC separations on long plates were usually sufficient to give a single, separate zone having the R_f value and fluorescence (violet-blue) of authentic BaP. The eluted spot was measured fluorometrically at 405 m μ using an excitation wavelength of 385 m μ , and the concentration obtained from a standard curve in the usual manner. A value of 0.42 ± 0.03 μ g of BaP per 25 cigarettes was obtained for the cigarettes with added pigment compared to 0.30 ± 0.03 μ g BaP per 25 cigarettes without added pigment. Thus, doubling the pigment content of cigarettes resulted in a 40% ($\pm 20\%$) increase in the total BaP content of the smoke condensate. These findings show that these pigments of tobacco may contribute to the formation of BaP under actual smoking conditions and may be a significant source of the polynuclears of tobacco smoke.

2. Pigment Characterization

From the work of Wright et al. (18) Runeckles (33) and Jacobson (19) it is apparent that a structural array of polymeric polyphenols with or without amino acids exists in tobacco. The pigments used in the present study more closely approximate those isolated by Wright et al. and Jacobson. Some further work has been done here on the properties of these pigments and, although of secondary interest to the main theme of this publication, these findings are summarized below.

Most of the PAH from the pyrolysis are also found in tobacco smoke condensate, as indicated in Table 1. Since the thermal decomposition and recombination processes of pyrolysis and smoking are superficially similar, it was of interest to compare the yields obtained during both processes. Using benzo(a)pyrene (BaP) as an index, the yield in the pyrolysis was determined and found to be relatively high: one gram of pigment yielded approximately 10^{-3} g. of BaP in the pyrolytic products. Although literature values are variable, the condensate from the smoke of 30 g. of cigarette tobacco, which contains about 1 g. of pigment, can be assumed to yield in the approximate range of 10^{-6} g. of BaP. Thus, on an equivalent basis, the pigment produces about a thousand times as much BaP as found in cigarette smoke. This suggests that the pigments could contribute disproportionately to the PAH in cigarette smoke.

To test this point, cigarettes containing sufficient added pigment to double the amount naturally present in the tobacco were prepared and smoked. The smoke condensate was fractionated in the same manner as above. The BaP was separated from the other polynuclears as described in one analytical procedure employing thin-layer chromatography (23) but with several modifications which greatly improved the method. The solvent system that was employed was methanol-toluene-water (7:1:1) instead of the pentane-ether solvent used in the original method. A second innovation was the use of long plates (23 \times 58 cm) as compared to the standard size (20 \times 20 cm) usually employed in TLC separations. (One separation on the long plates appeared equivalent to at least

The pigments were obtained from Turkish tobacco by extraction with aqueous alkali (pH 10) in higher yields (4% of leaf weight) than those reported by others. Elemental analyses showed the presence of C, H, N, S, and Fe. Hydrolyses under various conditions showed the presence of glucose, rhamnose, quercetin, caffeic acid, quinic acid, and twenty amino acids, including two not reported in the pigments studied by other workers (hydroxyproline and hydroxylysine). Ornithine was also detected, but is probably an artifact. (A special hydrolysis for tryptophane was not conducted.) Substantiations of the high molecular weight nature of the pigments were obtained by the pattern of elution from gel filtration columns. Ultracentrifugal analysis (UA) of fractions from the gel filtration of the ethanol-insoluble portion (about 60% of the total extractable pigment) showed four ranges of molecular weights in the following amounts: 7% of 10,000–15,000; 60% of 16,000–20,000; 24% of 25,000–30,000; and 5% of > 30,000. The missing 4% is due to a low molecular weight fraction that was not analyzed. These subfractions varied from 7–26% protein based on elemental analyses for nitrogen.

Attempts to determine quantitatively the rutin and chlorogenic acid contents of the pigment gave variable results. For rutin, determination of the rhamnose in acidic hydrolysates of both the alcohol-soluble and -insoluble fractions gave about 0.8 moles rutin per 1300 g. pigment. Chlorogenic acid gave values of about 0.3 moles (alkaline hydrolysis and determination of quinic acid by reaction with thiobarbituric acid) per 1300 g. pigment. Determinations of the two polyphenols (ethanol-soluble fraction) by spectral (UV and SPF) measurement of the aluminum chloride complexes were difficult to evaluate due to spectral interferences, (especially with SPF) but indications of a 1:1 proportion of rutin and chlorogenic acid were obtained.

Based on these data, some calculations can be made on possible proportions of the pigment moieties. For example, the ethanol-soluble portion of the pigment contains about 23% amino acid-like compounds, based on the nitrogen content (3.7%). If the pigment contains a 1:1 proportion of rutin and chlorogenic acid (total molecular weight 970) representing 77% of the pigment weight then the amino acids account for about 290 in molecular weight, giving a total of about 1300 for a "structural unit". Using an average molecular weight for an amino acid of 120, then the proportions would be approximately 1:1:2 rutin-chlorogenic acid-amino acids. Titration of the pigment showed three acidic hydrogens with an equivalent weight of about 440 (to pH 4.75), which correlates somewhat with the above data. Similar calculations on the ethanol-insoluble portion of the pigment gave a proportion of about 1:1:6 rutin-chlorogenic acid-amino acids with four acidic hydrogens. Determinations of free hydroxyl and amino groups by acetylation gave values which correlated with the theoretical based on the above data. Attempts to obtain further information by methylation of the pigment were unsuccessful. It should be emphasized that these data were obtained on mixtures and represent mean values.

METHODS

1. Extraction of Tobacco Pigments

Turkish tobacco leaf (500 g., 50 mesh) was mixed with 2 liters of aqueous sodium hydroxide (10^{-4} M) and the pH of the mixture was adjusted to pH 10 with aqueous 1.0 M sodium hydroxide. n-Hexane (1 liter) was added to the mixture, which was stirred frequently over a period of one to two days. The hexane was decanted and the mixture was filtered on a Buchner funnel using Filter Aid. The residue was washed with aqueous sodium hydroxide (10^{-4} M, 500 ml) and again stirred thoroughly with sodium hydroxide (10^{-4} M, 2 liters) and filtered. The aqueous portions were combined and extracted with n-hexane and chloroform, successively. Fresh chloroform (500 ml) was added to the brown, aqueous solution. The solution was acidified to pH 1.0 with 6 M sulfuric acid with vigorous stirring and the pigments were observed to precipitate at about pH 4.8. The pigments were filtered and washed with chloroform. The filtrate was saturated with salt to yield additional pigments. The filtered pigment was purified twice by redissolving with 0.5 M sodium hydroxide, extracting with chloroform, and reprecipitating with acid, as above. The purified pigment was extracted with ethanol *in vacuo* in a Soxhlet for about 6 hrs. and the ethanol-insoluble fraction was used in the pyrolysis work.

2. Pyrolysis

Pyrolysis was carried out in a cylindrical cylinder (1.2 m \times 10 cm) filled with quartz chips. The chamber temperature was determined with a chromel-alumel thermocouple, attached to a direct-

reading temperature potentiometer. The chamber was preheated to 850° C and flushed with nitrogen. The pigment (10 g.) was pyrolyzed in small portions (1.0–1.5 g.). The sample was placed in a boat and rapidly pushed into the concentric tunnel of the chamber. The entrance port was immediately sealed and the escaping vapors were condensed in a series of Dry Ice-acetone traps. Before opening, the chamber was flushed with nitrogen. The charred residue was extracted with ether and the extracts combined with the ether washings of the traps.

3. Fractionation of Pyrolysate

The ether extract of the residue and condensate was washed successively with 5% hydrochloric acid in salt-saturated water and 5% sodium hydroxide in salt-saturated water. To the ether layer were added 30 ml of cyclohexane, and the ether and volatiles removed by distillation (spinning band column) up to 80° C. The cyclohexane solution was shaken with 90% methanol-water (30 ml) (34). The methanol-water solution was then extracted with cyclohexane (2 × 30 ml), and all cyclohexane solutions were combined. The pooled solution was reduced in volume to 30 ml on a spinning band column. The concentrated solution was extracted with nitromethane (3 × 30 ml), and the nitromethane was removed on an evaporator.

4. Column Chromatography

The residue of nitromethane-solubles was mixed with benzene (5 ml), hexane (10 ml), and silicic acid (2 g.) and taken to dryness on an evaporator. The residue was then chromatographed on silicic acid by a previously described method (23). The column was eluted with n-hexane (400 ml) followed successively by these solvents containing the indicated concentrations of benzene in n-hexane (400 ml each): 0.5, 1.0, 3.0, 6.0, 10.0, 20 and 50%. Fractions of 50 ml were collected. Alternate fractions were examined for ultraviolet absorptive characteristics. Fractions up to 10% benzene in hexane contained the PAH of interest.

5. Thin Layer Chromatography

Several substrates and solvent systems were examined using resolution, streaking, intensity of fluorescence, and fluorescence color as the criteria of acceptability. The following adsorbent-solvent systems are listed in increasing order of preference: silica gel with pentane:ether (19:1); acetylated cellulose with N,N-dimethylformamide:water (1:1); and acetylated cellulose with ethanol:toluene:water (17:4:4). The last system was used more extensively. Plates were usually prepared in a thickness of 0.35 mm and activated at 120° C for 1 hr. Standard plates (20 × 20 cm) were used initially and developed in 1–2 hrs. Later it was found that long plates (23 × 58 cm) gave superior separations and these were used in large glass jars which were lined with sheets of Whatman #1 paper and preconditioned with solvent for 4–5 hrs. The plates were permitted to develop overnight. Fractions from the original silicic acid column having similar ultraviolet spectra were pooled and applied to the plates as streaks. Authentic benzo(a)pyrene (BaP) was spotted on the side as a reference. After development, the plates were air-dried, and the fluorescent color and positions of zones were marked. For narrow zones or micro separations on thin layers of cellulose (100 μ thickness), removal of adsorbent was best accomplished by means of the following technique: the narrow end of a disposable pipette was plugged with cotton and inserted into a vacuum line. The other end was attached with rubber tubing to a short glass tip cut from the constricted end of another pipette. The tip was scraped along the plate, while the vacuum sucked the adsorbent into the cotton-plugged pipette. The pipette was then inverted into a test tube, the tip was removed, the PAH were eluted with methanol and the solution was filtered through the cotton. For macro separations, the cellulose was scraped off with a spatula and removed by centrifugation.

6. Paper Chromatography

Polynuclears were separated on acetylated paper (54 × 57 cm), using the solvent system, ethanol-toluene-water (17:4:4), by the ascending method. The chromatograms were developed (18–20 hrs.) and BaP was used as a reference. Fluorescent colors and band positions were determined as above, and the bands were cut out and eluted with methanol. Ultraviolet spectra were taken in methanol or cyclohexane, and fluorescence spectra were determined in cyclohexane, which gave better definition of peaks.

7. Cigarettes with Added Pigment

Cigarettes were prepared containing 0.90 g. of a typical American blend of tobaccos with or without 0.04 g. of added pigment. Control and test cigarettes (25 each) were smoked mechanically (Phipps and Bird machine) using a 35 ml puff volume of 2 sec. duration once a min. to a butt length of 20 mm. The condensate was trapped on Cambridge filters and was fractionated as discussed above. The nitromethane-solubles were placed on an activated silicic acid column (50 g.) and eluted with n-hexane (200 ml) followed successively by 1% benzene, 5% benzene, and 10% benzene in hexane (400 ml each). The BaP was concentrated mostly in the 5% benzene eluate; however, the 1, 5, and 10% benzene eluates were combined and evaporated to dryness. The residue was subjected to TLC on long plates containing acetylated cellulose as the substrate and methanol-toluene-water (7:1:1) as the solvent. The samples were applied as a streak, with a spot of BaP on the side as reference. After development, the plates were dried in air. The zone corresponding to known BaP was scraped off into a centrifuge tube and extracted with methanol (3×10 ml) after which the solution was centrifuged. The solution was rechromatographed to give a single discrete zone, the R_f value and fluorescent color (violet-blue) of which matched that of authentic BaP. The zone was extracted and its emission fluorescence spectrum determined in cyclo-hexane as above. Pure sublimed BaP was used to prepare a standard curve of concentration versus emission intensity.

8. Pigment Structure

Elemental analysis showed the presence of C, H, N, S, and Fe. Iron was determined by the α, α' -dipyridyl method (35). The pigments were separated by an *in vacuo* Soxhlet extraction into an ethanol-soluble (I) and ethanol-insoluble fraction (II), with fraction (II) comprising about 60% of the total. Both fractions were subjected to acid hydrolysis with similar results. Hydrolyses in aqueous 1N sulfuric acid liberated some quercetin. Hydrolysis with 6N hydrochloric acid gave caffeic acid, quinic acid, and a series of amino acids. Separations and identifications of products were made by published methods (17), using ascending paper chromatography, with n-butanol-acetic acid-water (12:3:5) as the solvent. Ornithine, hydroxyproline and hydroxylysine were identified in addition to the amino acids found (17) by other workers in similar pigment material with the exception of tryptophane, for which a special hydrolysis is needed but was not conducted. Hydrolysis in ethanol-sulfuric acid (36) liberated glucose and rhamnose. The total sugars were determined by the usual gravimetric copper sulfate method and also by the more accurate arseno-molybdate color reagent (37). Glucose was determined colorimetrically by the specific, enzymatic "Glucostat"* reagent.

A 24-hour reflux of fractions I or II in 0.6N aqueous potassium hydroxide liberated quinic acid. The reaction mixture was extracted, acidified, and again extracted with ether. The aqueous solution was clarified by charcoal and analyzed for quinic acid by the thiobarbituric acid method (38). The absorbance of the colored product in isoamyl alcohol was measured at 549 m μ and the concentration of the quinic acid was obtained from a standard curve. Repeated methylation of the pigment with dimethyl sulfoxide, barium oxide, and methyl iodide followed by subsequent hydrolysis failed to yield any identifiable products.

The ethanol-soluble pigment was examined for its rutin and chlorogenic acid content by complex formation with aluminium chloride. Methanolic solutions of pigment or authentic samples of rutin and chlorogenic acid were prepared, and mixed with a 1% anhydrous aluminium chloride solution in methanol to achieve complexing. After 30 minutes, the absorbances were recorded for the rutin complex at 436 m μ and for the chlorogenic acid complex at 360 m μ . Rutin was also determined spectrophotofluorimetrically (SPF) by measuring the emission intensity of the complex at 510 m μ , when excited at 425 m μ . Chlorogenic acid could not be determined accurately in this way due to the spectral interference of rutin. The chlorogenic acid complex emits at 490 m μ , on excitation at 390 m μ .

The pigments were acetylated by refluxing with acetic anhydride, then neutralizing with standard aqueous sodium hydroxide and back titrating with standard acid. The chloroform-

* Mention of commercial products does not constitute endorsement by the U. S. Department of Agriculture over other items of a similar nature not mentioned.

soluble acetylation products were examined by infrared and ultraviolet spectral methods. The above analytical procedures were also applied to some subfractions of fractions (I) and (II) separated by gel filtration on Sephadex 25 and 100. This was achieved by passing aqueous solutions of the pigment carefully adjusted to pH 5.5 through Sephadex (3 m × 2.5 cm columns) which was previously conditioned at pH 5.5.

SUMMARY

The complex polyphenolic pigments of Turkish tobacco have been pyrolyzed to determine their possible contribution to the formation of aromatic compounds, especially polynuclear aromatic hydrocarbons (PAH), of smoke. The dark brown pigments were initially obtained by a basic aqueous extraction of tobacco. Various hydrolytic procedures showed the presence of rutin, chlorogenic acid, and a series of amino acids; some information on the structure of these pigments is presented. The pyrolysis of the pigments was carried out at 850° C and the products were fractionated to reveal the presence of more than a dozen PAH. Addition of pigments to cigarettes gave an increase in the level of benzo(a)pyrene in the smoke. The possible role of the polyphenolic pigments as a source of PAH in smoke is discussed.

ZUSAMMENFASSUNG

Die Autoren pyrolysierten die komplexen polyphenolischen Farbstoffe türkischen Tabaks, um festzustellen, wie diese Pigmente möglicherweise zur Bildung aromatischer Verbindungen des Rauches, insbesondere polycyclischer aromatischer Kohlenwasserstoffe (PAH), beitragen. Als Grundlage wurden die dunkelbraunen Pigmente durch eine erste Extraktion des Tabaks mit Wasser gewonnen. Durch verschiedene Hydrolyse-Verfahren wurde die Anwesenheit von Rutin, Chlorogensäure und einer Reihe von Aminosäuren nachgewiesen. Die Struktur der Stoffe wird beschrieben. Die Pyrolyse der Pigmente wurde bei 850° C durchgeführt. Die Fraktionierung der Pyrolyseprodukte führte zum Nachweis von mehr als 12 polycyclischen aromatischen Kohlenwasserstoffen. Die Zugabe von Pigmenten zu Zigaretten ergab eine Vermehrung des 3,4-Benzopyrens im Rauch. Die Funktion der polyphenolischen Farbstoffe als mögliche Ausgangsprodukte der polycyclischen Kohlenwasserstoffe des Rauches wird diskutiert.

RÉSUMÉ

On a fait une étude pyrolytique sur les complexes pigments polyphénoliques du tabac turc afin de déterminer de quelle manière ceux-ci contribuent à la formation des composés aromatiques de la fumée, en particulier des hydrocarbures aromatiques polynucléaires (PAH). Les pigments brun foncé servant de base à l'étude étaient initialement isolés du tabac par extraction aqueuse. Divers procédés hydrolytiques démontraient la présence de la rutine, de l'acide chlorogénique et d'une série d'acides aminés. La structure de ces pigments est décrite. La pyrolyse a été effectuée à 850° C. Le fractionnement des produits résultants révélait la présence de plus d'une douzaine d'hydrocarbures aromatiques polynucléaires. L'addition de pigments à des cigarettes provoquait une augmentation de la teneur en benzo-a-pyrène de la fumée. Le rôle possiblement joué par les pigments polyphénoliques comme origine des hydrocarbures aromatiques polynucléaires de la fumée est discuté.

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