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High Molecular Weight Pigment in Cigarette Smoke

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A high molecular weight pigment of probable polymeric structure has been isolated from cigarette smoke. The substance resembles certain polymeric tobacco leaf pigments which recently have been shown to contain polyphenols in at least two structural arrangements. Flue-cured tobacco has a relatively low molecular weight pigment consisting of equimolar amounts of scopolin, rutin and chlorogenic acid.[1] Air-cured[2–4] and sun-cured[5] tobaccos contain mixtures of pigments with molecular weights of about 4000 to more than 30,000 which are combinations of amino-acids and chlorogenic acid with or without rutin and iron. The pigment isolated from cigarette smoke more closely resembles the latter type but significant differences are noted. The occurrence of this pigment in cigarette smoke may be related to the observed tumourigenic activity of smoke in animals for two reasons: the physical properties of the leaf pigment parallel to some extent the properties of the unidentified cocarcinogenic substance(s) in tobacco leaf;[6] and the occurrence in smoke of non-characterised substances claimed to be high molecular weight polymers with cocarcinogenic activity has been superficially reported.[7]

Smoke condensate from domestic, commercial cigarettes was partitioned between ether and aqueous 1N sodium hydroxide as previously described.[8,9] The alkaline layer was immediately separated, diluted 1:8 or greater with water, and acidified to pH 3·2 by gradual addition of sulphuric acid with stirring. Under these conditions, a dark brown, flocculent precipitate was obtained; rapid acidification of the undiluted extract gave a black resinous tar which was difficult to handle. The flocculent material was reprecipitated from 1N sodium hydroxide by acidification and was filtered and washed thoroughly with water by several successive triturations and filtrations. After air-drying, the precipitate was extracted continuously with ether for 32 hours to remove relatively low molecular weight, ether-soluble contaminants. The precipitate was obtained in relatively high yields of about 4% of the condensate. The appearance, solubility, melting characteristics and infrared spectrum of the material were similar to those previously found for various fractions of the iron-polyphenol—amino-acid leaf pigment.

A comparative summary of the major properties of the leaf and smoke pigments is given in the Table. Data on several minor leaf pigments are not given in the Table since these were unavailable. The molecular weights of the leaf pigment were obtained by gel filtration and ultracentrifugal analysis. About 25% of the leaf pigment has a molecular weight of about 4000 and 75% consists of the other listed components with the 16,000-20,000 range predominating. Molecular weights of the smoke pigment were approximated by gel filtration on columns of polyacrylamide having different molecular exclusion ranges. By this method, the smoke pigment has a major fraction with molecular weights of ⩾100,000, a minor fraction of about 4000 and some unresolved material in the 10,000-100,000 range. There was no indication that significant adsorption or other responses independent of molecular sieve action[10] influenced the elution pattern when the separation was conducted using 0·01M phosphate buffer of pH 10·0. About 70% of the smoke pigment is non-dialysable against 0·13M phosphate buffer of pH 10·0 and the non-dialysable fraction gives essentially the same behaviour on gel filtration except for the lowest molecular weight component (m.w. 4000) which is missing.

Using the methods previously described,[2,3,5] the smoke pigment appears to have a much higher content of non-hydrolysable moiety than the leaf pigment as shown by the comparative yields of amino-acids, quinic acid and caffeic acid in the various hydrolyses. In fact, traces of caffeic acid could only be occasionally detected in the saponification of the smoke pigment. Of the 18 amino-acids found in the smoke pigment, all but two (β-alanine and γ-amino-butyric acid) have also been reported in the leaf pigment. No other products could be found in the ether-soluble fractions from the hydrolyses using thin layer chromatographic separations and a H_2SO_4-$K_2Cr_2O_7$ spray. In an attempt to determine the total hydrolysable and non-hydrolysable chlorogenic acid in the smoke pigment, a method was developed based on spectral determination of the protocatechuic acid released from the pigment and from authentic chlorogenic acid by potassium hydroxide fusion. Comparisons of the obtained value (see Table) with the amount of chlorogenic acid released by conventional potassium hydroxide hydrolysis showed that less than one-sixth of the polyphenol in the pigment is hydrolysable. Other differences between the leaf and smoke pigment are found in the hydrolytic products, and N and Fe contents. Rutin, which is present[2,3,5] in the leaf pigment fractions of molecular weight about 30,000, is absent in the smoke pigment. The iron content is much lower in the smoke pigment and accounts for at least 9·5% of the ash from the pigment.

Table

Comparative physical and chemical properties of tobacco leaf and cigarette smoke pigments

	Leaf pigment[a]	*Smoke pigment*
Molecular weights of components	4,000 10,000-15,000 16,000-20,000 (Major) 25,000-30,000 > 30,000	4,000 10,000-100,000 (Unresolved) $\geq$100,000 (Major)
Total nitrogen (%)	1·12-4·17	5·82
Chlorogenic acid (%)	8·2	< 1·0 6·1[b]
Iron (%)	0·3-0·9	0·02
Hydrolytic products		
HCl	16-20 amino-acids, quinic acid, caffeic acid, glucose, rhamnose[c]	18 amino-acids, quinic acid
H_2SO_4	Same as HCl except quercetin also found[c]	No quercetin
Acetic acid	Rutinose[c]	No sugars
KOH	Caffeic and quinic acids	Caffeic (traces) and quinic acid
KOH fusion products	Similar to those of chlorogenic acid and quercetin[c]	Similar to those of chlorogenic acid

[a]Composite of references 2, 3 and 5

[b]By determination of protocatechuic acid in potassium hydroxide fusion products. Other chlorogenic acid values in the Table were determined by quinic acid analysis of the potassium hydroxide hydrolysates using the thiobarbituric acid method.[5]

[c]Hydrolytic and potassium hydroxide fusion products of rutin found only in higher molecular weight fractions($\geq$10,000).

The presence of the high molecular weight pigment in smoke can be explained on the same basis as the other relatively non-volatile and/or heat-labile substances found therein, *e.g.* simple sugars and steroidal glycosides.[11,12] However, in addition to high temperature distillation and sublimation, another route may be operative: expulsion of cell contents by cellular eruption as a result of the sharp thermal gradient behind the cigarette coal.[13] Expelled cellular particles could serve as nuclei for aerosol formation or be adsorbed on preformed nuclei. In the case of the pigment, some thermal degradation and polymerisation are possible during the transition. Perhaps the less hydrolysable moieties in the pigment also possess greater thermal stability.

Although it would be desirable to obtain a "pure compound" from the mixture of pigment components, attempts to do so with either the leaf or smoke pigment have been unsuccessful. This is not unexpected since the melanins of plants have also defied separation, and the leaf and smoke pigments are similar in some respects to this group. However, the similarity may be superficial since the melanins are biosynthesised during plant growth and the leaf pigment is formed during the curing of harvested tobacco by enzymatic oxidation and condensation involving polyphenols and amino-acids.[2,3]

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