

Pyrogenesis of Aromatic Hydrocarbons Present in Cigarette Smoke I

Role of the Hexane Soluble Fraction of Tobacco*

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The hexane soluble fraction of flue-cured tobacco leaf has been investigated in detail by *Stedman* and coworkers (1) who noted that this fraction comprises approximately 6% of dry leaf weight and consists largely of aliphatic and cyclic paraffins, solanesol, phyto-sterols, fatty acids, esters, and unidentified neutral "resins". A further characterization of those previously unidentified "resins" as including glycerides and steryl-fatty acid esters has recently been reported by *Cook* et al. (2). That various of these constituents can give rise to polynuclear aromatic hydrocarbons (PAH) upon pyrolysis has been demonstrated by a number of investigators. *Lam* noted the production of benzo(a)pyrene from tobacco leaf paraffins at temperatures ranging from 700 to 970° C and, in addition, observed its preferential formation from some common aromatic hydrocarbons with alkyl side chains (3, 4) not known to be present in the hexane extract. *Badger* et al. (5) demonstrated the pyrolytic production of PAH, including benzo(a)pyrene (BaP), from dotriacontane and stigmasterol, both of which have been identified as components of tobacco leaf (6). These workers (5) also discussed possible modes of formation of these PAH from suitable precursors. Although much attention has been devoted to the influence of the hexane soluble leaf constituents on the pyrogenesis of polycyclic aromatic hydrocarbons present in tobacco smoke (7, 8, 9, 10, 11), conclusions have varied widely. On the one hand, *Campbell* and *Lindsey* (7) reported that prior extraction of cigarettes with cyclohexane resulted in a greater than 50% reduction of PAH in smoke from such cigarettes while, on the other hand, *Rayburn* et al. (9) found no significant differences in total amounts of polycyclic aromatic hydrocarbons (absorbing at 385 m μ) in the smoke of cigarettes containing varying amounts of hexane-solubles.

Because of the previous work cited above, one may question the feasibility of further inquiry into the role of the hexane soluble fraction of tobacco in pyrosynthesis of polynuclear aromatic hydrocarbons. At this point it seems obvious that, with rare exception, anything organic (containing carbon and hydrogen) will give rise to polynuclear aromatic hydrocarbons on exposure to high temperature. As far as the hexane soluble fraction of tobacco is concerned, however, controversy centers around the degree of its contribution to the levels of polynuclear aromatic hydrocarbons present in cigarette smoke.

In view of the continuing controversy, and in light of recent observations in this laboratory (12) concerning reaction pathways for the high temperature formation of PAH, we felt that further investigations were in order. The present study was, therefore, undertaken to reexamine the major pyrolytic products of the hexane-extractables of flue-cured tobacco, and to estimate the

* Received for publication: 7th February, 1968.

contribution of these extractables to the levels of aromatic hydrocarbons present in tobacco smoke, with emphasis on the production of benzo(a)pyrene. The present study differs from previous ones in that, with the exception of BaP, products were separated and estimated by gas chromatography. This technique enabled us to get a fairly accurate representation of product distribution following pyrolysis. BaP, not amenable to gas chromatography in this instance, was determined by ultraviolet (UV) absorption spectroscopy following thin-layer chromatography; as a result of the latter procedure, BaP samples for UV measurement were relatively "clean" for studies of this nature. Furthermore, we pyrolyzed unextracted and extracted tobacco in addition to the hexane extract in order to determine whether the "whole was equal to the sum of its parts", and to evaluate, as well, the role of non-extractable material in the pyrosynthesis of PAH.

EXPERIMENTAL

Leaf Extract

The starting material utilized in this study was flue-cured, type 12 tobacco which had been previously dried to a moisture content of $< 5\%$ and ground to 60/80 mesh (Wiley mill). A total of 642 g. of tobacco was extracted with a total volume of 4 liters of hot n-hexane (Soxhlet, 16 hrs.). The hexane solution was reduced to a volume of 300 ml by distillation at 67–69° C and the remaining solvent removed under vacuum at 0° C. The recovered extracted material amounted to 36 g. (5.6% of dry leaf weight). Carbon-hydrogen analysis of this material gave values of 78.70% (C) and 10.89% (H). The infrared spectrum showed absorption bands generally characteristic of long chain aliphatic hydrocarbons, but included weaker bands indicative of carbonyl and hydroxyl functions.

Pyrolysis

Unextracted tobacco, the hexane extract, and the tobacco minus hexane-solubles, were each pyrolyzed in quartz tubes, under nitrogen (30 ml/min) at a temperature of $860 \pm 5^\circ$ C, utilizing apparatus and product collection techniques previously described (13). The selection of temperature was based on the measurements of *Touey* and *Mumpower* (14) and on the previous observation of *Lam* (4) that the maximum production of benzo(a)pyrene from tobacco leaf hydrocarbons occurs in the vicinity of 850° C.

Fractionation of Pyrolysate

In each case, the traps containing pyrolysate were successively washed with diethyl ether, 0.5% NaOH (aqueous) and diethyl ether. The ethereal solutions were combined and further extracted with the original alkali and 1M HCl to remove any acids or bases present. For gas chromatographic studies, the resulting ethereal solution of neutrals was dried over anhydrous Na_2SO_4 and concentrated under vacuum (at 0° C) to a volume of 10 ml.

After appropriate adjustments of pH, the aqueous solutions of acids and bases were extracted with ether; the resulting ether solutions were dried and concentrated, and also examined by gas chromatography.

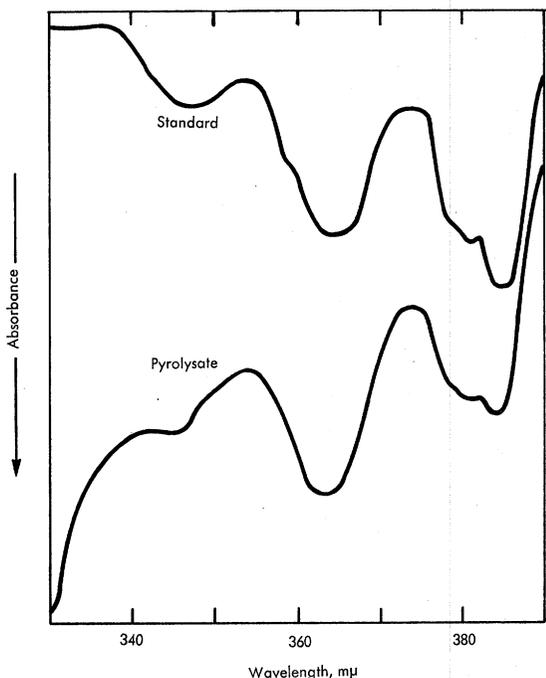
Gas Chromatographic Analysis

Gas-liquid chromatography was conducted on a Loenco* Model 70 Hi-Flex Gas Chromatograph, equipped with thermal conductivity and flame ionization detectors, and fitted with dual $5' \times 0.25''$ o. d. stainless steel columns containing 20% SE-30 on 60/80 mesh chromosorb W. Helium flow through the columns was maintained at 70 ml/min. Column temperature was programmed from 70° C to 250° C at 6° C/min. The order of elution is shown in Table 1. Effluents corresponding to various gas chromatographic peaks were collected and characterized by suitable spectral techniques, retention times and cochromatography. Relative yields in pyrolysates were determined from measurements of gas chromatographic peak areas; equal aliquots of comparable solutions representing 25 g. of tobacco were injected into the gas chromatograph for this purpose (*vide infra*).

* Mention of commercial item does not imply endorsement by the Department over similar products not named.

FIGURE 1

UV absorption spectra of BaP



bration curves. The ultraviolet spectrum of known BaP in the spectral range 340 to 390 mμ is compared with the spectrum of BaP isolated from a pyrolysate of tobacco leaf in Figure 1.

Benzo(a)pyrene Determination

Levels of benzo(a)pyrene in pyrolysates were estimated by a previously described thin-layer chromatographic method (15) with slight modifications. Glass plates (8" × 8") were coated with Silica gel "G" to a thickness of 0.5 mm. Known aliquots of the neutral tars from pyrolysates were streaked on the plate, and run against a known quantity (20 μg) of BaP. A solvent system of *n*-hexane-diethyl ether (19:1) served as the developing medium. In each analysis, the bands corresponding to the unknown and standard BaP were removed from the plate, and extracted successively with small portions of spectral-grade cyclohexane until no increase in absorbance at 363 or 385 mμ could be detected spectrophotometrically. Benzo(a)pyrene levels in pyrolysates and standards were determined by first measuring the absorbance in cyclohexane solution at 363 or 385 mμ and then reading the corresponding quantities of BaP from cali-

RESULTS AND DISCUSSION

Although this study was primarily concerned with the pyrolytic production of aromatic hydrocarbons from the hexane soluble residue of tobacco leaf, the phenolic and basic fractions of the pyrolysate were also screened. No evidence for the pyrolytic production of phenols from the hexane-solubles under the conditions used (860° C, N₂) was obtained; however, the basic fraction of the same pyrolysate contained minor quantities of nitrogen-containing substances characteristic of the pyrolytic products of tobacco alkaloids (16). It is likely that some portion of the nicotine in the leaf was removed with the hexane-extractables.

Table 1 summarizes the data obtained from analysis of the gas chromatographable neutral products of the pyrolysis (N₂, 860° C) of a) unextracted flue-cured tobacco, b) the hexane extract and c) the extracted tobacco. Relative yields of products are given in terms of peak areas which were obtained from comparable and equivalent chromatograms of the various pyrolysates. Such chromatograms corresponded to equal aliquots (injected into the gas chromatograph) of equal volumes

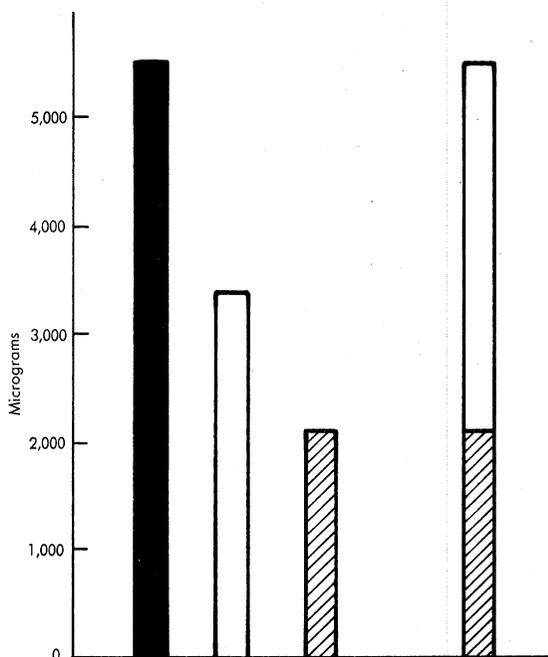


FIGURE 2
BaP levels in pyrolysates

- 25.0 gm flue-cured tobacco
- 1.4 gm hexane-extractables
- ▨ 23.6 gm extracted tobacco

TABLE 1

Products*	Relative yields of pyrolytic products**			% Product accounted for for (b + c) × 100	% Contribution to product levels in pyrolysate from a	
	a	b	c		b	c
				a		
Benzene	993	140	650	79	14	65
Toluene	477	170	301	99	35	63
Ethylbenzene	125	37	52	72	30	42
Styrene	291	55	182	81	19	62
Indene	257	35	154	74	14	60
Naphthalene	1211	282	625	75	23	52
Alkyl-naphthalenes	433	52	288	78	12	66
Biphenyl	38	16	18	89	42	47
Acenaphthylene	288	50	189	83	17	66
Fluorene	100	25	65	90	25	65
Anthracene	394	96	196	74	24	50
Phenanthrene						
Alkyl-anthracenes	59	26	29	93	44	49
Alkyl-phenanthrenes						
Fluoranthene	52	13	13	50	25	25
Pyrene	56	23	28	91	41	50
Total	4774	1020	2790	79	21	58

* In order of elution from gas chromatographic column. For BaP see Figure 2.

** Figures represent areas adjusted to an attenuation of 1.

a: flue-cured tobacco (25.0 grams)
b: hexane-extract (1.4 grams)
c: extracted tobacco (23.6 grams)

of pyrolysate solutions. Our interpretation of the data assumes that peak area is a measure of weight, and that equivalent weights produce equal areas. It is apparent from these results (Table 1) that the hexane-solubles contribute relatively greater proportions of the products found than would be predicted on the basis of dry leaf weight (5.6%) alone. The percent of neutral products attributable to the hexane soluble fraction ranged from a minimum of 12% (alkyl-naphthalenes) to a maximum of 44% (alkyl-anthracene/alkyl-phenanthrene), with an overall contribution of 21% of the total. The results of the benzo(a)pyrene analysis, presented in

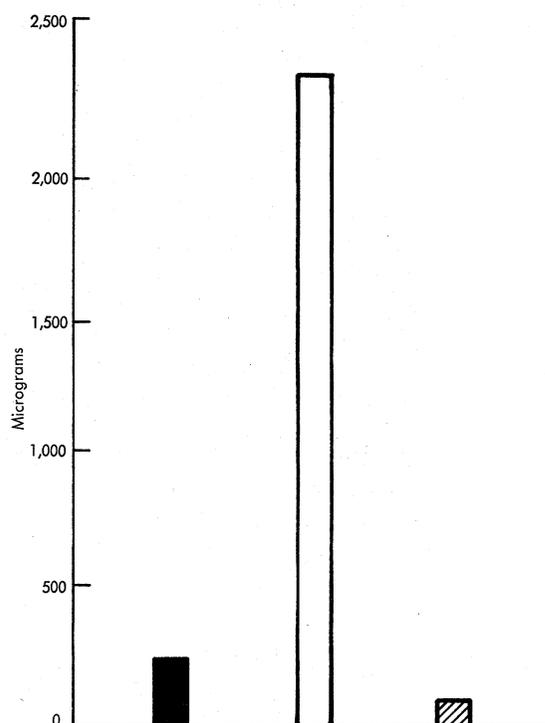


Figure 2, are even more striking. Unextracted flue-cured tobacco (25 g.) yielded an average of 5500 µg of BaP on pyrolysis. Amounts of hexane-extractables (1.4 g.) equivalent to 5.6% of the unextracted tobacco pyrolyzed yielded an average of 3350 µg of BaP or approximately 61% of the unextracted leaf yield. The remaining extracted leaf (23.6 g. or 94.4% leaf weight) yielded 2100 µg or approximately 38% of the total. Data presented in Figure 3, illustrating relative yields per g. of starting material, indicate that the hexane-extractables are approximately 10 times as effective in the pyrolytic production of BaP as the unextracted tobacco leaf, and 30 times as effective as the extracted tobacco leaf residue.

The production of the condensable products of tobacco smoke is generally assumed to involve

FIGURE 3
BaP levels in pyrolysates

■ 1.0 gm flue-cured tobacco
□ 1.0 gm hexane-extractables
▨ 1.0 gm extracted tobacco

a combination of processes including distillation and pyrolysis over a wide gradient of temperatures. However, the production of benzo(a)pyrene, and aromatic hydrocarbons in general, has been shown to occur most effectively at temperatures approaching the upper limit of this temperature range (4). As Wynder et al. have suggested (10) this observation, plus the observation that few significant qualitative variations in the PAH composition of tobacco smoke condensate and tobacco pyrolysates have been noted, strengthens the validity of pyrolysis experiments as means for examining the mode of PAH formation in the smoking process. The data presented here implicate, in no small way, the hexane soluble components of tobacco leaf in the pyrogenetic processes that lead to the formation of PAH in cigarette smoke.

SUMMARY

The hexane soluble fraction of flue-cured tobacco has been pyrolyzed to reevaluate the importance of its contribution to the formation of aromatic compounds, especially polynuclear aromatic hydrocarbons (PAH), present in cigarette smoke. The pyrolyses were performed at $860^{\circ} \pm 5^{\circ}$ C under nitrogen. In general, the studies indicate that the hexane-solubles contribute significantly more to the aromatic hydrocarbon levels of tobacco pyrolysate than would be predicted on the basis of percent of dry leaf weight alone. Estimates of benzo(a)pyrene levels in pyrolysates indicate that nearly two-thirds of the amount produced during tobacco pyrolysis may be attributed to the hexane soluble components of leaf.

ZUSAMMENFASSUNG

Der hexanlösliche Anteil aus flue-cured Virginiatabak wurde pyrolysiert, um erneut zu untersuchen, inwieweit diese Fraktion zur Bildung von aromatischen Verbindungen im Cigarettenrauch, insbesondere von polycyclischen aromatischen Kohlenwasserstoffen (PAH), beiträgt. Die Pyrolyse wurde bei $860^{\circ} \pm 5^{\circ}$ C unter Stickstoff durchgeführt. Die Ergebnisse zeigen, daß die hexanlöslichen Verbindungen in wesentlich stärkerem Maße zu dem Polycyclengehalt der Pyrolyseprodukte des Tabaks beitragen, als nach ihrem Anteil am Trockengewicht des Tabakblattes zu erwarten wäre. Untersuchungen der Pyrolyseprodukte auf ihren Gehalt an Benzo(a)pyren lassen erkennen, daß nahezu zwei Drittel der Benzopyrenmenge, die während der Pyrolyse entsteht, auf die hexanlöslichen Bestandteile des Tabakblattes zurückgeführt werden können.

RÉSUMÉ

On a pyrolysé la fraction soluble à l'hexane de tabac flue-cured afin de réévaluer sa contribution à la formation de composés aromatiques, en particulier d'hydrocarbures aromatiques polynucléaires (PAH), présents dans la fumée de cigarettes. Les pyrolyses ont été faites à $860^{\circ} \pm 5^{\circ}$ C sous courant d'azote. En général, la contribution des composés en question à la teneur en hydrocarbures aromatiques des pyrolysats du tabac s'est montrée beaucoup plus importante que l'on ne le prédirait sur la base du pourcentage du poids sec de la feuille. L'estimation de la teneur en benzo(a)pyrène des pyrolysats montre qu'environ deux tiers de la quantité du benzo(a)pyrène se formant au cours de la pyrolyse du tabac peuvent être attribués aux constituants solubles à l'hexane des feuilles de tabac.

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