

# PYROLYTIC STUDIES ON FRACTIONS SEQUENTIALLY EXTRACTED FROM TOBACCO

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Tobacco extracts, obtained by sequential solvent extraction of tobacco, were pyrolyzed, and the resulting products separated and identified and their relative yields determined. Pyrolysates were examined essentially for levels of neutrals, including benzo[a]pyrene, phenols and bases such as nicotinonitrile. Relationships between the composition of the various pyrolysates obtained and the leaf extracts, from which they arose, were determined to the extent that the data permitted.

## INTRODUCTION

Many of the identified constituents of tobacco smoke are either unreported in cured tobacco leaf or are present in the leaf in levels too minute to account for their presence in smoke. These compounds are generally assumed to arise from pyrolytic degradations of tobacco leaf constituents. Of particular interest have been the pathways for pyrosynthesis of aromatic hydrocarbons, especially polynuclear aromatic hydrocarbons (PAH) and the volatile phenols, two classes of compounds important to the physiological properties of tobacco smoke but either absent or present in leaf in minor amounts. Previous pyrolytic studies on flue-cured tobacco (11) have indicated that the hexane-extractable material in tobacco leaf is a major source for the pyrogenesis of aromatic hydrocarbons (6% of leaf weight contributing as much as 50% of certain simple aromatic hydrocarbons and up to 60% of the benzo[a]pyrene (BaP) in tobacco pyrolysates). On the other hand, the origin of the volatile phenols of tobacco smoke would appear to be attributable to leaf materials generally insoluble in hexane or similar non-polar solvents, e.g., the high molecular weight leaf constituents, pigment, lignin, pectin and cellulose (12), and other leaf carbohydrates (1). As a continuation of studies designed to determine precursor-product relationships in pyrolysis of flue-cured tobacco and to extrapolate these relationships to processes occurring in a burning cigarette, we extracted this tobacco with a series of solvents of increasing polarity, and examined the pyrolytic products of each fraction thus obtained. By comparing the composite pyrolysates (i.e. total from all fractions) with the pyrolysate obtained from the original tobacco sample, we sought to determine: 1) which fractions of the leaf, if any, give rise to a disproportionate amount of a certain class of pyrolytic product, e.g. PAH, phenols, and 2) whether specific products arise directly from individual leaf constituents irrespective of the presence of other leaf components, or whether, during pyrolysis, interactions or synergistic effects occur among the leaf constituents.

In addition to measuring levels of aromatic hydro-

carbons and volatile phenols in all pyrolysates, the fate of nicotine on pyrolysis was monitored by determining a specific and major pyrolytic product of nicotine, nicotinonitrile (5,6).

## EXPERIMENTAL

*Extraction of tobacco.* Dried, ground (Wiley Mill\*) flue-cured tobacco (1000 g) was extracted in a Soxhlet extractor successively with Skellysolve B, chloroform, acetone, ethanol and methanol, each extraction lasting 170 hours. A water extract was also obtained by macerating the tobacco (after methanol extraction) three times with distilled water in a Waring blender. All solutions of extracts were taken to dryness on a rotary evaporator prior to pyrolysis.

*Pyrolyses.* Pyrolyses were conducted in a horizontal quartz tube (4' x 1.25" O.D.), packed with quartz chips, at  $800 \pm 5^\circ\text{C}$ , the approximate burn temperature of a cigarette (16). The temperature was maintained with a Lindbergh Hevi-Duty\* furnace. A stream of dry nitrogen (30 ml/min) continually flushed the pyrolysis chamber and carried products into a series of cold-traps and gas scrubbing devices (11-14).

*Fractionation of pyrolysates.* The condensable pyrolysis products were recovered from the trapping system by successive washings with diethyl ether, 0.5% aqueous sodium hydroxide, and diethyl ether. The combined ethereal and aqueous (alkaline) solutions were intimately mixed and allowed to separate into an ether layer containing neutrals and bases, and alkaline layer containing acids. Bases were separated from neutrals with hydrochloric acid, and strong acids from weak acids by appropriate pH adjustments (11-14). Ultimately, 3 fractions (neutrals, bases, phenols) were obtained in concentrated dry ethereal solutions suitable for gas liquid chromatographic and other analyses (11-14).

### *Product analyses.*

#### *1. Gas liquid chromatography*

Compounds in the neutral, phenolic, and basic fractions from each pyrolysate were separated and determined by gas liquid chromatography on an Aerograph Model 200 gas chromatograph under conditions previously described (11). Individual phenols were additionally separated on a 12' x  $\frac{1}{4}$ " column of 15% SE-52 on Chromosorb W, programmed at  $1^\circ/\text{min}$  from  $100^\circ\text{C}$ , in a Perkin Elmer Model 800 Gas Chromatograph.

Gas chromatographic peaks were characterized from spectra (IR, UV, and/or mass) of their corresponding collected effluents, from retention data, and by co-chromatography with known compounds (11-14).

#### *2. Other analytical methods*

Total phenols were also determined by the 4-aminoantipyrene method (8), and BaP by a method utilizing thin layer chromatography and ultraviolet absorption spectrophotometry (11).

\*Mention of specific commercial products does not constitute an endorsement by the Department over others not named.

Table 1. Pyrolytic Products and Yields.

Relative Yields<sup>a</sup> of Major Products from Pyrolysis of

Products	Relative Yields <sup>a</sup> of Major Products from Pyrolysis of							Marc <sup>c</sup> (435.0 g)	Composite
	Tobacco (1000 g)	Skellysolve (71.9 g)	Chloroform (20.6 g)	Tobacco Acetone (175.0 g)	Fractions <sup>b</sup> Ethanol (120.0 g)	Methanol (70.5 g)	Water (107.0 g)		
Benzene	926	269	35	332	34	20	24	151	865
Toluene	897	347	36	266	34	20	27	173	903
Styrene	542	217	20	234	14	5	7	89	586
Naphthalene	1000	215	45	390	13	13	27	142	845
Alkyl naphthalene	589	302	41	144	13	10	17	109	636
Anthracene/Phenanthrene	261	55	14	125	<1	<1	7	34	235
Nicotinonitrile	259	26	22	5	78	18	<1	8	157
Phenol <sup>d</sup>	170	3	2	18	70	7	6	64	170
Cresols <sup>d</sup>	54	3	<1	<1	16	<1	<1	34	53

<sup>a</sup> As gas chromatographic peak areas relative to naphthalene (=1000). <sup>b</sup> Quantities in parentheses indicate weight of fraction per Kg tobacco; product yields have been adjusted to reflect the relative weight of each extract in a given weight of tobacco. <sup>c</sup> Residue after final extraction. <sup>d</sup> Data confirmed by colorimetric method for total phenols (8), although the method is not applicable to *p*-cresol.

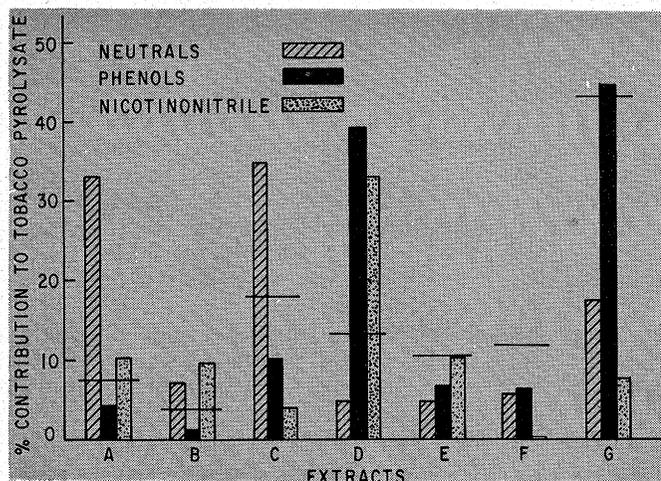


Fig. 1—Contributions to levels of neutrals, phenols and nicotinonitrile in tobacco pyrolysate from: A—Skellysolve extract, B—Chloroform extract, C—Acetone extract, D—Ethanol extract, E—Methanol extract, F—Water extract, G—Marc. Data have been adjusted to reflect the relative weight (horizontal bar) of each extract in a given weight of tobacco.

Table 2. Contributions of Leaf Extracts to Levels of Products in Tobacco Pyrolysate.

Extract	Percent Dry Leaf Weight	Percent Contribution to			
		Neutrals <sup>a,b</sup>	Phenols <sup>a</sup>	Nicotinonitrile <sup>a</sup>	BaP <sup>c</sup>
Skellysolve	7.2	33.33	2.68	10.03	26.80
Chloroform	2.1	4.53	0.89	8.49	7.22
Acetone	17.5	35.37	8.03	1.93	59.28
Ethanol	12.0	2.56	38.39	30.12	<1
Methanol	7.0	1.61	3.12	6.95	<1
Water	10.7	2.59	2.68	<1	<1
Marc	43.5	16.55	43.75	3.09	7.22
Total	100	96.54	99.54	60.61	100.52

<sup>a</sup> Determined gas chromatographically.

<sup>b</sup> Primarily aromatic hydrocarbons.

<sup>c</sup> Data are based on 195 mg of BaP obtained from pyrolysis of 1000 g of tobacco.

## RESULTS AND DISCUSSION

The relative yields in terms of peak areas of the major components of the various pyrolysates, as determined by glc, are presented in Table 1. The area of the peak corresponding to naphthalene, the largest single product of tobacco pyrolysis, has been adjusted to 1000 (per kilogram tobacco pyrolyzed) in order to facilitate interpretation of the data obtained; all other areas have been adjusted accordingly. Disproportionate contributions of various leaf fractions to the total tobacco pyrolysate are evident and are presented as percents of neutrals, phenols, and nicotinonitrile in Table 2, and graphically portrayed in Figure 1. The results are best understood by considering separately each class of compounds formed on pyrolysis.

**Neutrals.** Examination of the various pyrolysates indicated that the source of approximately 70% of the aromatic hydrocarbons in tobacco pyrolysates may be attributed to lose leaf components extractable by Skellysolve and acetone, and which amount to less than 25% of dry leaf weight (Tables 1, 2; Figure 1). The Skellysolve extractables (7.19% of leaf weight) account for approximately 33% of the neutrals (primarily aromatic hydrocarbons) obtained on pyrolysis. This data closely approximates the results obtained for hexane extractables (11), which are largely aliphatic and cyclic paraffins, fatty acids, phytosterols, polyenes, and steryl esters (2,15). The acetone soluble material (17.5% of dry leaf weight) may also contain oxygenated terpenes, sterols, fatty acids, etc. that contribute in a similar fashion to the neutrals in the tobacco pyrolysate. It was noted, however, that a higher proportion of aromatic hydrocarbons with alkyl side chains (e.g. toluene, alkyl naphthalenes) was found in the pyrolysate from the Skellysolve extract than in the pyrolysate from the acetone extract (Table 1). This observation suggests that the Skellysolve extractable material from leaf consists of greater amounts of unsaturated and branched chain hydrocarbons (preferred precursors of aromatic hydrocarbons with alkyl side chains) than does the acetone extract (7,13).

BaP was similarly obtained in largest yields from the two leaf fractions (Skellysolve and acetone) previously discussed, the total yield accounting for 84% of the BaP in tobacco pyrolysates (Table 2). Each of these fractions yielded approximately 700  $\mu$ g BaP per gram of material pyrolyzed. In contrast, the final tobacco residue (43.5% dry leaf weight), after extraction with all 6 solvents, yielded less than 34  $\mu$ g BaP/gram material pyrolyzed. It was observed that the cumulative ("composite") level of all aromatic hydrocarbons, including BaP, in the pyrolysates of the various fractions essentially equalled that obtained from the tobacco pyrolysate. It would appear that the various hydrocarbon precursors in leaf undergo pyrolytic degradation with few, if any, interactions or synergistic effects.

**Phenols.** The major volatile phenols obtained in this study (phenol and isomeric cresols) were predominantly localized in the pyrolysates from the ethanol extractables and the final leaf residue; the former (12% of dry leaf weight) accounted for 38% of these phenols and the latter (43.5% of dry leaf weight) for 44% (Tables 1 and 2). Ethanol extracts of tobacco leaf include, *inter alia*, polyphenols, the brown pigments, and, very likely, low molecular weight sugars. The brown pigments contain polyphenolic moieties, and, on

pyrolysis, produce relatively large amounts of volatile phenols (12). The final leaf residue would be expected to consist largely of cellulosic and other high molecular weight carbohydrates which, like other sugars, produce relatively low yields of phenols on pyrolysis in comparison with the brown pigments (1,12); some pigments, not removed previously, and lignin would also be present in the final residue. The data obtained in the present study confirm previous observations with regard to pigment pyrolysis and phenol production (12), and support the contention of Spears, *et al.* (1) that the leaf carbohydrates play a major role in the genesis of cigarette smoke phenols. This role is made significant only because so much of the bulk of tobacco leaf is carbohydrate in nature (up to 55% of dry leaf weight). On a unit weight basis, the brown pigments are much better phenol precursors than are carbohydrates, but the lower content of the pigments in leaf (4%) may decrease their significance in the total result.

More than twice as much of the cresols were found in the pyrolysate from the final residue than in the pyrolysate from the ethanol extract. Previous data (12) have shown that lignin, which one would expect to find in the final residue, is a highly efficient precursor of cresols. As in the case of the neutral products, the composite of the various fractions, essentially equalled that obtained from pyrolysis of untreated tobacco indicating that interactions or synergistic effects did not occur to any significant extent.

**Nicotine.** Compounds previously identified (5,6) as nicotine pyrolysis products at high temperatures (>800°), including nicotinonitrile, quinoline, isoquinoline, and pyridine bases, were found in all pyrolysates examined. Although only 60% of the nicotinonitrile (Tables 1, 2) found in the tobacco pyrolysate could be accounted for in the pyrolysates from all fractions, half of this amount was found in the pyrolysate from the ethanol extract. This would tend to confirm previous observations (3,4,14) that nicotine in the leaf is associated with the brown pigments. In addition, nicotine salts are generally extractable with ethanol. Although very little nicotinonitrile was isolated from the pyrolysate of the final tobacco residue, a significant yield of quinoline and isoquinoline was obtained. These products, the mononitrogen analogs of naphthalene, are among the major pyrolysis products of a variety of nitrogenous materials, not necessarily alkaloidal in nature, and could arise as well from those amino acids or proteins (9,10) remaining in processed tobacco leaf.

#### SUMMARY

Tobacco extracts, obtained by sequential solvent extraction of tobacco, were pyrolyzed, and the resulting products separated and identified and their relative yields determined. Pyrolysates were examined essentially

for levels of neutrals, including benzo[a]pyrene, phenols and bases such as nicotinonitrile. Relationships between the composition of the various pyrolysates obtained and the leaf extracts, from which they arose, were determined to the extent that the data permitted.

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