

# Characteristic Products from Pyrolysis of Nitrogenous Organic Substances\*

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Nitrogenous organic substances comprise a significant portion of tobacco leaf (Table 1 and references 1 and 2), and the products to which they give rise at elevated temperatures undoubtedly influence the chemical and biological properties of cigarette smoke (3). However, with the possible exception of nicotine (4, 5), none of the nitrogenous constituents of tobacco leaf has been subjected to extensive pyrolytic studies. With this in mind, we undertook to characterize the pyrolytic products of such materials, including protein and amino acids, tobacco leaf pigment, and nicotine. Where we encountered difficulty in isolating specific nitrogenous substances from leaf, e.g. protein, we pyrolyzed model compounds from other sources. The present report summarizes the data obtained, and in addition attempts to survey, in general, the characteristic products generated from nitrogenous substances exposed to high temperatures.

**Table 1. Nitrogenous components of flue-cured cigarette tobacco (1, 2).**

	% of dry weight
Total volatile bases, as ammonia	0.282
Nicotine	1.93
Ammonia	0.119
Glutamine, as ammonia	0.033
Asparagine, as ammonia	0.025
$\alpha$ -Amino nitrogen, as ammonia	0.665
Protein nitrogen, as ammonia	0.91
Nitrate nitrogen, as $\text{NO}_3^-$	Trace
Total nitrogen, as ammonia	1.97

## EXPERIMENTAL

### Materials

Substances pyrolyzed were generally reagent-grade unless otherwise specified. A possible proteinaceous fraction was extracted from tobacco leaf by the method outlined in Figure 1. Flue-cured (Maryland) tobacco was pyrolyzed, and its pyrolytic products characterized for comparative purposes.

### Hydrolysis of "Proteinaceous" Fraction

Three hundred mg of the precipitate (Figure 1) obtained in the dialysate were hydrolyzed (under reflux conditions) in 125 ml aqueous 6N HCl for 20 hr. The HCl was removed carefully over a steam bath, and the residue was taken up in 4 ml 0.1N aqueous HCl. Insoluble materials were removed by centrifugation, and the solution of "amino acids" in dilute HCl was saved for further analysis including resolution by thin-layer chromatography (ref. 7, page 399), and "auto-analysis" on a Phoenix Amino Acid Analyzer\*\* model VG-6000 B (Table 4) using a modification of the method of *Piez and Morris* (16). Thin-layer chromatography of the hydrolyzate indicated the presence of at least seven ninhydrin-positive components including leucine, methionine, glycine and aspartic acid. The column-chromatographic method provided better resolution (Table 4), however.

### Pyrolyses

Pyrolyses were conducted in horizontally positioned quartz (or Vycor) tubes, either 2.6 cm o.d.  $\times$  35 cm length, or 1.25 in. o.d.  $\times$  4 ft. length, continually flushed by a stream of nitrogen. Temperatures were maintained, usually in the 800° to 860° C range, by a Lindberg Hevi-Duty furnace, within  $\pm$  5° C. Samples were introduced into the pyrolysis tubes in ceramic boats in quantities of 0.5 to 2.0 grams. Pyrolytic products were collected in glass traps cooled with mixtures of ice-water and dry ice-acetone.

### Fractionation of Products

Products were fractionated, where required, into acids, bases and neutrals (6). The various fractions were then resolved by chromatographic techniques including gas and thin-layer chromatography.

### Chromatographic Analyses

Gas chromatography was routinely carried out on a Varian-Aerograph Model 1200 single-column instrument equipped with a flame ionization detector. Bases were resolved on a 5 ft.  $\times$  1/8 in. o.d. stainless steel column containing 15% carbowax 20 M on 60/80 mesh

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\*\* Mention of commercial items does not imply their endorsement by the Department over similar products not mentioned.

chromosorb W programmed from 60° C to 250° C at 6° C/min. Neutral materials were separated on a similar column packed with 5% SE-30 on 80/100 mesh gas chrom Q programmed from 60° C to 270° C at 6° C/min. In both cases, helium flow was set at 32 ml/min., and injector and detector temperatures were set at 300° C.

Thin-layer chromatography of aromatic nitrogen containing compounds was carried out on 8 in. × 8 in. glass plates coated with silica gel G to a depth of 50 mm. Solvent systems included ethyl acetate/methyl alcohol/formic acid (80:10:10) and benzene/methyl alcohol (95:5) (7). Utilization of thin-layer chromatography for the determination of benzo(a)pyrene has been described (8), and this method was used where applicable.

#### Characterization of Products

Products were tentatively identified by their chromatographic retention characteristics, and in addition, in most cases, by their spectral (IR, UV and/or mass) properties (including comparisons with known compounds). When spectra of pyrolytic products were required, gas chromatographic separations were performed on an instrument equipped with hot wire detectors (Varian-Aerograph Model 202 B), and the corresponding peak effluents were trapped in U-shaped glass tubes, cooled in dry ice-acetone, from which they (the effluents) were transferred to appropriate receptacles (e.g. IR cells, salt discs).

In those cases where pyrolytic yields were determined, triangulation of gas chromatographic peaks was the method used.

## RESULTS AND DISCUSSION

### Tobacco

Tobacco was pyrolyzed at 850° C in an atmosphere of nitrogen, and a substantial portion of the bases generated was characterized (Table 2). The products were found to be similar to those present in cigarette smoke condensate (Table 2), although a fewer number were actually identified. As a first approximation, then, one can regard pyrolysis studies as reflecting (albeit to a limited degree) processes occurring in a burning cigarette. Moreover, pyrolysis of model compounds and identification of products thus generated can be helpful in establishing precursor-product relationships. As will be evident from the additional data assembled in this report, the nitrogenous compounds pyrolyzed generated products very similar, if not identical, to those generated from tobacco itself.

### Nicotine

In addition to confirming the results of Woodward et al. (4), and Jarboe and Rosene (5), we identified a

**Table 2. Comparison of bases found in various pyrolyzates and tobacco smoke condensate.**

	Pyrolyzates			Tobacco smoke condensate
	To-bacco	Pigment	Nico-tine	
Pyridine	+	+	+	+
2-Methylpyridine	+	+	+	+
2,6-Lutidine				+
3- and/or 4-Methylpyridine	+	+	+	+
2,4- and/or 2,5-Lutidine		+		+
2,3-Lutidine and/or collidine				+
3-Ethylpyridine		+		+
3,5-Lutidine		+		+
3,4-Lutidine				+
3-Vinylpyridine	+	+	+	+
Pyrrole			+	+
Pyridine-3-aldehyde			+	+
Nicotinonitrile	+	+	+	+
2- and/or 4-Cyanopyridine			+	
Methyl-3-pyridyl ketone				+
Nicotine	+		+	+
Nornicotine/myosmine	+			+
Anabasine	+			+
Metanicotine				+
2,3'-Bipyridyl				+
Quinoline	+	+	+	+
Isoquinoline	+	+	+	+
Aminoquinoline			+	
7,8-Benzoquinoline	+			
Other benzoquinolines	+		+	
Alkylquinolines	+			
Benzacridines	+		+	+
Benzcarbazoles				+

**Table 3. Products from nicotine pyrolysis (860° C, N<sub>2</sub>).**

	% of pyrolyzate
<b>Basic fraction</b>	
Pyridine <sup>d</sup>	5.3
2-Methylpyridine	0.51
3- and/or 4-Methylpyridine <sup>d</sup>	8.1
3-Vinylpyridine <sup>d</sup>	1.0
3-Cyanopyridine <sup>d</sup>	25.3
2- and/or 4-Cyanopyridine <sup>b, d</sup>	6.2
Quinoline <sup>d</sup>	7.2
Isoquinoline <sup>d</sup>	1.3
Nicotine <sup>d</sup>	16.4
Benzacridines <sup>a</sup>	
Benzquinolines <sup>a</sup>	
<b>Neutral fraction</b>	
Benzene	0.40
Pyrrole	1.2
Toluene	0.3
Benzonitrile <sup>d</sup>	0.69
2- and/or 4-Cyanopyridine <sup>d</sup>	0.89
Indole	1.2
Skatole	0.25
Styrene <sup>c</sup>	
Indene <sup>c</sup>	
Naphthalene <sup>c, d</sup>	
Acenaphthene <sup>c</sup>	
Anthracene/phenanthrene <sup>c</sup>	

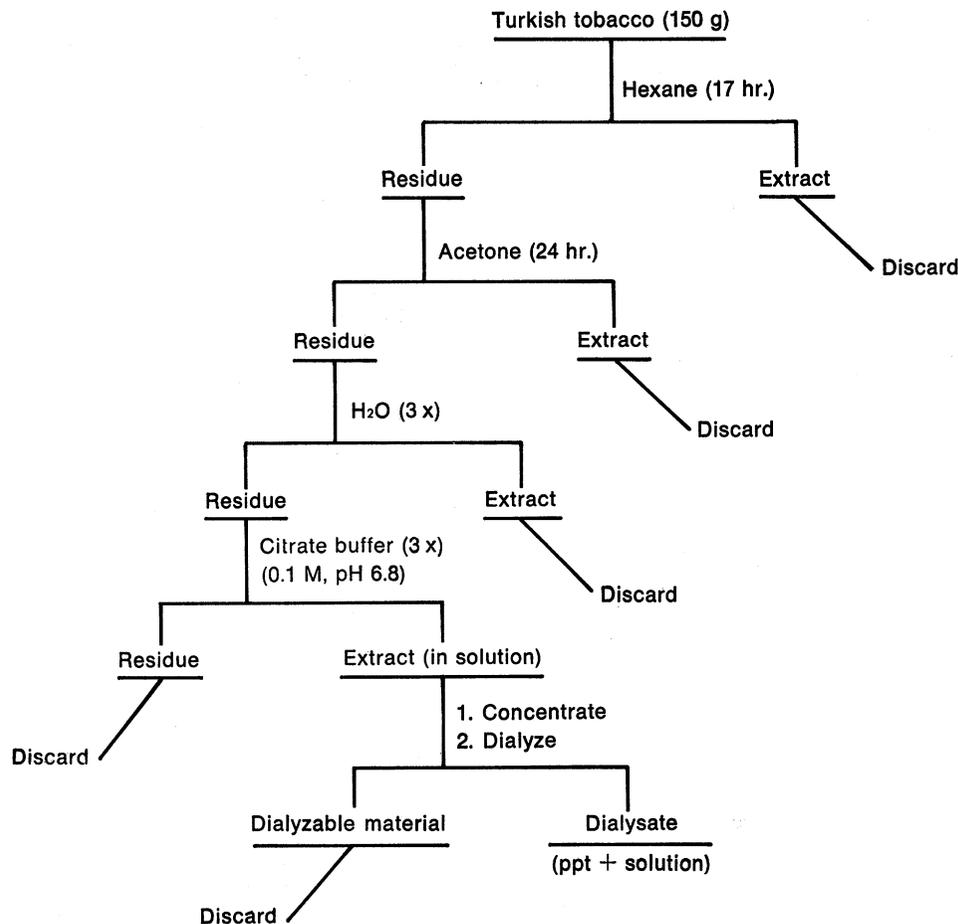
(a) Possibly present (TLC).

(b) In both neutral and basic fractions.

(c) In trace amounts.

(d) Reported also by Woodward et al. (4), and/or Jarboe and Rosene (5).

Figure 1. Attempt to extract proteinaceous material from cured tobacco (Turkish).



number of previously unreported compounds in nicotine pyrolyzates (Table 3). Most of these newly identified compounds were in the neutral fraction and included simple aromatics, pyrrole, acenaphthene, anthracene (phenanthrene), indole and skatole. In the basic fraction we noted the presence, *inter alia*, of 2-methylpyridine, benzacridines and benzoquinolines. We could not detect benzo(a)pyrene in nicotine pyrolyzates, nor could we confirm the presence of the physiologically active dibenzacridines and dibenzcarbazole reported in tobacco smoke and in nicotine and pyridine pyrolyzates by Van Duuren (3).

At 860° C, under nitrogen, the product generated from nicotine in highest yield was nicotinonitrile (3-cyanopyridine) — amounting to 25% of the total. Nicotine itself was recovered in the pyrolyzate and amounted to 16% of the total. Other products generated in lower, but relatively high yields were 3- and/or 4-methylpyridine (8%), quinoline (7%), 2- and/or 4-cyanopyridine (7%) and pyridine (5%). The neutral products were generally present in the pyrolyzate at much lower concentration levels than were the bases.

Possible pathways from nicotine to the products generated from nicotine at high temperatures have been discussed by Jarboe and Rosene (5). The formation of 3-substituted pyridines from nicotine can be rationalized on the basis of preferential rupture of the pyrrolidine ring (vs. the pyridine ring). However, formation of many of the other products is much more difficult to rationalize at the present time.

#### Proteins and Amino Acids

Our interest in the pyrolytic properties of tobacco leaf protein (and amino acids) was hindered by the dearth of published reports on the amounts of protein present in cured leaf and on methods for its isolation from such leaf. With little to aid us in the way of precedence, we attempted to isolate protein from cured leaf according to the scheme shown in Figure 1.

The material (dialysate) we obtained after dialysis consisted of a flocculent precipitate in a dark colored solution. The precipitate amounted to 350 mg, and the residue (obtained after freeze-drying) from the dialysate solution weighed 4.4 g (from 150 g Turkish tobacco). The precipitate contained about 2% nitrogen, and on hydrolysis (300 mg with 50 ml 6N HCl for 20 hr.) yielded a large number of amino acids characteristic of protein (Table 4). Of course, such amino acids have been obtained from hydrolysis of pigment (9, 10). We next pyrolyzed the residue from the dialysate solution, and identified a number of pyridines, and possibly indoles and carbazole in the pyrolyzate (Table 5). However, on the basis of all our data, we could not conclude that we had indeed isolated proteinaceous material from cured leaf, especially since the pyrolytic products obtained were similar to those obtained from pigment (Table 5).

To get more meaningful results from protein pyrolysis, we pyrolyzed purified protein obtained from sources other than tobacco such as casein and  $\beta$ -lactoglobulin

**Table 4. Amino acids from dialysate (precipitate) after hydrolysis\* +.**

	Relative amount
Aspartic acid	2.59
Threonine	1.64
Serine	1.72
Glycine	3.00
Glutamic acid	2.20
Proline	0.95
Alanine	3.00
1/2 Cystine	Trace
Valine	2.06
Methionine**	Trace
Isoleucine	1.38
Leucine	1.94
Tyrosine	0.612
Phenylalanine	1.00
Lysine	1.24
Histidine	0.27
Arginine	0.91

\* 85 mg hydrolysate from 300 mg starting material,

glycine = alanine = 3 × 1.

+ Hydrolysis in 50 ml 6N HCl, 20 hr.

\*\* As sulfoxide.

**Table 5. Pyrolytic products.**

	From dialysate (residue)	From pigment
Indole	+	+
Skatole	+	+
2-Methylindole	+ (?)	+ (?)
Carbazole	+ (?)	+
Phenol		+
Cresols		+
Xylenols		+
Pyridine	+	+
2-Methylpyridine	+	+
3-Methylpyridine	+	+
3-Ethylpyridine	+	+
3-Vinylpyridine	+	+
Nicotinonitrile (3-cyanopyridine)	+	+
Quinoline	+	+
Isoquinoline	+	+

A from milk, and collagen from cow hide (6). In preliminary studies, we identified indole, methyl indoles, phenol and p-cresol in the pyrolyzate from  $\beta$ -lactoglobulin A. The pyrolysis of the other two proteins was more extensively studied. Most of the pyrolytic products were identified, and the data have been reported (6). They are presented here (Table 6) for comparative purposes. Among the products identified were simple aromatic hydrocarbons, pyridines, nitriles, indole, and quinoline. Mechanistic implications based on the products obtained have been discussed (6). In addition, we have pyrolyzed several amino acids. The products obtained from two of them are shown in Table 6. Pyrolyzate composition was found to be qualitatively similar to that from protein and nicotine (Tables 6 and 2, and reference 6). At this point, the

**Table 6. Pyrolytic products of proteins and amino acids.**

	Casein	Col-lagen	Proline	Glycine
Pyridine	+ <sup>a</sup>	+ <sup>a</sup>	+ <sup>a</sup>	+
2-Methylpyridine	+	+ <sup>a</sup>	+	+
3- and/or 4-Methylpyridine	+	+ <sup>a</sup>	+	
3-Vinylpyridine	+	+		
Aniline	+	+	+	
Quinoline	+ <sup>a</sup>	+	+	
Isoquinoline	+	+	+ <sup>a</sup>	
Benzene	+	+	+	+
Pyrrrole (toluene)	+ <sup>a</sup>	+ <sup>a</sup>	+ <sup>a</sup>	+
Styrene (xylenes)	+	+		
Benzonitrile	+	+	+	+
Indene	+	+		+
o-Tolunitrile	+	+	+	
m-Tolunitrile	+	+	+	
Naphthalene	+	+		
Indole	+	+	+ <sup>a</sup>	
Fluorene	+			
Phenol	+ <sup>a</sup>	+ <sup>a</sup>		
o-Cresol	+			
m- and/or p-Cresol	+ <sup>a</sup>	+		
Ethylphenol	+	+		
Xylenol	+	+		

(a) Principal products.

data suggest the operation of certain pyrolytic pathways leading to the formation of similar products from different nitrogenous organic compounds.

#### *Tobacco Leaf Pigment*

Tobacco leaf pigment has been shown to contain bound proteinaceous (or peptidal) material (9, 10) and nicotine (11). It was, therefore, not entirely unexpected to note the presence of characteristic degradation products of nicotine in pigment pyrolyzates (12). Some of the products may have been due to degradation of proteinaceous material in the pigment as well. The relevant data have been published (12), but are presented here (Table 2) for purposes of comparison (and continuity).

In passing, it should be noted, moreover, that pyrolysis of pigment results in the formation of phenols (13), and polynuclear aromatic hydrocarbons, including benzo(a)pyrene (9), in addition to the bases noted above.

#### CONCLUSION

We have attempted to survey the products resulting from the thermal degradation of nitrogenous substances, especially of those found in tobacco. Usually, we noted qualitative similarities among the pyrolyzates, indicating that at high temperatures, at least, there is some tendency for nitrogenous compounds, even though they differ structurally, to generate similar products — especially those products which can be considered nitrogen containing analogs of aromatic hydrocarbons.

At high temperatures, then, pyrolysis of nitrogenous materials can be expected to yield, among other things, pyridine and its derivatives, polycyclic aromatic hydrocarbons and their nitrogenous analogs, and possibly nitriles. In special cases, for example in the pyrolysis of tyrosine (6), and other amino acids, phenols can be generated as well, although a phenolic nucleus has been shown not to be essential for a phenol-precursor (14, 15).

One general rule that one is cautiously tempted to arrive at on the basis of high temperature pyrolysis studies is that although at relatively low temperatures the products generated from various materials (containing carbon, hydrogen and nitrogen, for example) differ, as the temperature of pyrolysis increases differences in product composition tend to be minimized (qualitatively), and at high enough temperatures these differences may disappear completely.

#### SUMMARY

The products resulting from the pyrolysis of tobacco and a number of nitrogenous organic compounds similar, if not identical, to those found in tobacco have been compared. Similarities have been noted in the composition of the various pyrolyzates, but essentially no physiologically active materials were found. Generalities with regard to pyrolysis of nitrogenous organic substances have been suggested by the data assembled.

#### ZUSAMMENFASSUNG

Die Pyrolyseprodukte des Tabaks und eine Reihe organischer Stickstoffverbindungen, die mit den im Tabak vorkommenden Verbindungen Ähnlichkeiten aufweisen oder sogar identisch sind, wurden miteinander verglichen. Die Zusammensetzung der verschiedenen Pyrolyseprodukte zeigte Ähnlichkeiten, im wesentlichen wurden aber keine physiologisch aktiven Stoffe gefunden. Aus den Untersuchungsergebnissen werden allgemeine Gesichtspunkte über die Pyrolyse organischer Stickstoffverbindungen abgeleitet.

#### RESUME

Nous avons comparé les produits provenant de la pyrolyse du tabac avec certaines substances organiques azotées semblables sinon identiques à celles trouvées dans le tabac. Nous avons noté une analogie dans la composition des différents pyrolysats, mais en substance aucun composé physiologiquement actif. Les

données rassemblées suggèrent un tableau général de composés organiques azotés.

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