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## Nitrogen-Containing Compounds in Tobacco and Tobacco Smoke

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### Contents

I. Introduction	295
II. Aliphatic Amines	295
III. Aromatic Amines	296
IV. N-Heterocyclic Compounds	297
A. Pyrroles	297
B. Pyridines	299
C. Pyrazines	300
D. Multiple Ring, Nonfused N-Heterocyclics (Alkaloids)	301
E. Fused Ring N-Heterocyclic Compounds	302
V. Amino Acids	303
VI. Nitriles	304
VII. Nitroalkanes, Nitroarenes, and Nitrophenols	305
VIII. N-Nitrosamines	306
IX. Amides	307
X. Amino Sugars	307
XI. Miscellaneous N-Containing Compounds	307
A. Nitrite Esters, Inorganics, and Others	307
B. Pigments	308
C. N-Containing Agricultural Chemicals	308
XII. Conclusion	309
XIII. Addendum	309
XIV. References	310

### I. Introduction

The isolation and identification of the chemical constituents that give tobacco and tobacco smoke their characteristic (organoleptic, pharmacological, and toxicological) properties<sup>3,12b,13,14</sup> have long been the goals of tobacco chemists. Obviously with increasingly better analytical techniques, periodic reviews<sup>1-5</sup> have become necessary to keep pace with the evergrowing number of newly identified compounds. At the present time this number has increased to such an extent<sup>6,7</sup> that a new summary of the more than 2200 compounds now known to be present in tobacco/tobacco smoke would be most appropriate. Since such a project would be awesome, a more manageable approach is undertaken in the present review by considering only one important class of constituents, namely, the nitrogen-containing compounds. These comprise nearly 30% of all compounds reported in tobacco and tobacco smoke. Such an approach is further justified because nitrogen-containing compounds (e.g., carbazoles, harmanes, hydrazines, indoles, naphthylamines, and pyrazines) with clearly defined biological activity have been recently identified in both leaf and smoke.<sup>9-11</sup> On the other hand, other nitrogen-containing compounds have

long been known to contribute to the biological activity of tobacco smoke, among the most important the alkaloid nicotine, hydrogen cyanide, and ammonia.<sup>9,12,13</sup> Thus, it is clear, that in inducing biological effects on man, tobacco smoke is not limited to nicotine, carbon monoxide, and polynuclear aromatic hydrocarbons (PAH)<sup>8,8a,8b</sup> but is also influenced by nitrogen-containing compounds (and others) present in various leaf and smoke fractions. Furthermore, their abundance in leaf (Figure 1) makes the N-containing compounds significant potential precursors of compounds that arise anew in the smoke stream as a result of combustion, or in the leaf as a result of tobacco processing (aging, curing, fermentation, etc.).

The last review of this kind was undertaken 7 years ago by Neurath.<sup>1</sup> Since then, the number of known nitrogen compounds in leaf and smoke has more than tripled. The purpose of the present review therefore is to update the previous publication by enumerating the nitrogen compounds now known to occur in both leaf and smoke, by discussing their mode of formation, by presenting quantitative data where available, and by examining the contribution of the nitrogenous compounds to the biological activity of leaf and smoke. What is presented here, especially as concerns smoke formation and biological activity, is based partially on our own data, as well as on our interpretation of the literature. Some of this interpretation may be hypothetical, but in our judgment best explains the presently available data.

### II. Aliphatic Amines

The aliphatic amines identified in tobacco and its smoke are given in Table I in order of increasing carbon number from C<sub>1</sub> to C<sub>8</sub>, and include primary, secondary, and tertiary amines among the 36 compounds listed. In practically all cases, these amines are found in both processed leaf<sup>19,24</sup> and smoke<sup>20,23</sup> indicating that a substantial number present in the smoke is due to transfer from the leaf. Their presence in leaf may also reflect a breakdown of proteinaceous material and/or amino acids during processing as a result of microbial or enzymatic action or when relatively high temperatures are encountered. Similarly, thermal degradation occurring during tobacco combustion may contribute to amines in the smoke.<sup>4</sup> Nicotine and other alkaloids may also be sources of the lower aliphatic amines.<sup>12</sup> Mild aeration of nicotine results in the formation of ammonia, methylamine, and other compounds.<sup>72</sup>

Analysis of the aliphatic amines presents certain difficulties because of their volatility and polar nature. Therefore, analytical

TABLE I. Aliphatic Amines

	Tobacco ref	Smoke ref
C <sub>1</sub>		
Methylamine	1, 19, 21, 54 (8.2) <sup>a</sup>	1, 20, 21 (4.6) <sup>b</sup>
C <sub>2</sub>		
Dimethylamine	1, 19, 21, 22 (6.6, 31)	1, 20, 21 (1.2)
Ethylamine	1, 19, 21, 54 (1.6)	1, 20, 21 (0.96)
Vinylamine		20
C <sub>3</sub>		
Allylamine		1, 20
Methylethylamine	1, 19, 22	1, 20
Isopropylamine	19, 21 (0.05)	1, 20, 21 (0.05)
<i>n</i> -Propylamine	19, 21 (0.12)	1, 20, 21 (0.03)
Trimethylamine	1, 21 (1.9)	1, 21 (0.71)
C <sub>4</sub>		
Butylamine		20
Isobutylamine	1, 19, 21 (0.83)	1, 20, 21 (1.0)
<i>n</i> -Butylamine	19	1, 20, 21 (0.09)
<i>sec</i> -Butylamine	21 (2.4)	1, 20, 21 (0.10)
<i>tert</i> -Butylamine	19	
Diethylamine	19, 21, 22 (8.0)	1, 20, 21 (0.10)
Methylisopropylamine	19	20
Methylpropylamine	22	20
C <sub>5</sub>		
Isoamylamine	1, 19, 21 (1.0)	1, 20, 21 (0.30)
<i>n</i> -Amylamine	19 (1.3)	1, 20, 21 (1.2)
<i>sec</i> -Amylamine	1	1, 20
2-Methylbutylamine		1, 20
Methylisobutylamine	19	1
Methyl- <i>n</i> -butylamine	19	1, 20
Methyl- <i>sec</i> -butylamine		20
Pentylamine		193
C <sub>6</sub>		
Di- <i>n</i> -propylamine	19	1
<i>n</i> -Hexylamine	19	1, 20
Methylisoamylamine	19	
<i>n</i> -Propylisopropylamine		1, 20
Triethylamine	21	
C <sub>7</sub>		
<i>tert</i> -Butylisopropylamine		20
<i>n</i> -Heptylamine	19	20
C <sub>8</sub>		
Di- <i>n</i> -butylamine	19	
Di- <i>sec</i> -butylamine	19	
<i>n</i> -Butylisobutylamine		20
<i>n</i> -Octylamine	19	20

<sup>a</sup> Values in parentheses are  $\mu\text{g}$  of amine/g of tobacco. <sup>b</sup> Values in parentheses are  $\mu\text{g}$  of amine (in smoke) per cigarette.

procedures generally involve a derivatization step, e.g., reaction with trifluoroacetic anhydride or other similar reagents prior to gas chromatography.<sup>19,20</sup> Most studies of the aliphatic amines in tobacco/tobacco smoke have been qualitative. Neurath et al.<sup>23,24</sup> and Kaburaki et al.,<sup>21</sup> however, determined the isolated quantities of a number of these in leaf and smoke as indicated in Table I.

The contribution of the aliphatic amines to tobacco toxicity has not yet been assessed, but it is doubtful that they play a significant role in this regard. The nicotine-free basic fraction of tobacco smoke particulates in which the less volatile, aliphatic amines would be found was shown to be slightly tumorigenic in dermal assays on mice but much less so than the neutral fraction rich in PAH.<sup>3,15</sup>

Nevertheless, the aliphatic amines, notably the secondary and tertiary amines, may play an indirect role in tobacco carcinogenesis if one considers the relative ease with which these can

LEVELS OF NITROGEN COMPOUNDS IN TOBACCO PRODUCTS

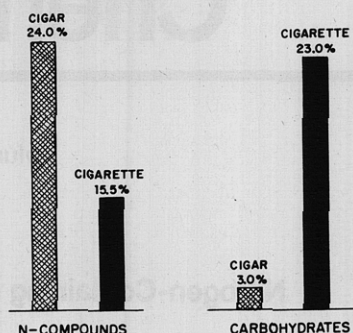


Figure 1. Percent, by weight, of N-containing compounds in tobacco.<sup>4</sup>

be converted to the corresponding nitrosamines in the presence of nitrite<sup>197</sup> or nitrogen oxides.<sup>132</sup> So far, only a limited number of aliphatic nitrosamines has been found in tobacco smoke.<sup>16,17</sup> However, careful analysis may indicate the presence of other nitrosamines, in both leaf and smoke, traceable to the corresponding secondary (and tertiary) amines found therein.<sup>18</sup>

### III. Aromatic Amines

Like the aliphatic amines, the aromatic amines (Table II) are fairly well distributed in both leaf<sup>19,24</sup> and smoke,<sup>20,23</sup> although a somewhat greater number is found in the latter. For the most part, these are aniline-related compounds, including those substituted on the ring and still others substituted on N. The substituents are usually alkyl groups, although methoxy- and phenyl-substituted anilines are also found. The diphenylamines are not as basic as the other aromatic amines and have been found in the neutral, nitromethane-soluble fraction of smoke condensate.<sup>25</sup> An aromatic diamine,<sup>20</sup> tyramine,<sup>26</sup> *N*-alkylaminophenols,<sup>27</sup> and 2-naphthylamine<sup>28,37</sup> have also been reported in tobacco or tobacco smoke. The latter compound is a bladder carcinogen in man,<sup>29</sup> but is present in cigarette smoke in amounts (22 ng/cigarette) too low to be considered a health hazard.<sup>37</sup> It is possible, however, that the concentration of 2-naphthylamine can serve as an indicator of the concentration of other carcinogenic amines in the smoke and as such has been correlated with the increased risk for cigarette smokers to develop cancer of the urinary bladder.<sup>195</sup> Tyramine (derived most likely from tyrosine by decarboxylation) is a vasoactive amine, causing a very marked increase in blood pressure in mammals when administered intravenously.<sup>30</sup> Although its concentrations in tobacco products are unknown, and certainly low when compared to nicotine, one can only speculate on its possible contribution to the reported cardiovascular effects attributed to cigarette smoke.<sup>31</sup>

The *N*-substituted aromatic amines listed are possible precursors of *N*-nitrosamines (Figure 2). Although these specific nitrosamines have not been reported to be present in leaf or smoke, careful analytical studies might be required to shed light on their occurrence or absence. It is rather surprising that this possibility has not yet been addressed. Whether such mixed (aryl + aliphatic) nitrosamines possess tumorigenic activity is interesting, inasmuch as *N*-nitrosodiphenylamine has been shown to be inactive.<sup>32</sup>

Similar to the aliphatic amines, the highly volatile aromatic amines are usually derivatized with trifluoroacetic anhydride, or similar acylating agent, prior to gas chromatographic analysis.<sup>19,20,23,24</sup> Again, although most studies of these compounds have been qualitative, some quantitative data have been reported.<sup>23,24</sup> These are indicated in Table II.

As for their origin, the aromatic amines likely arise from protein and/or amino acids via decarboxylation as a result of enzymic or microbial degradation (of phenylalanine or tyrosine,

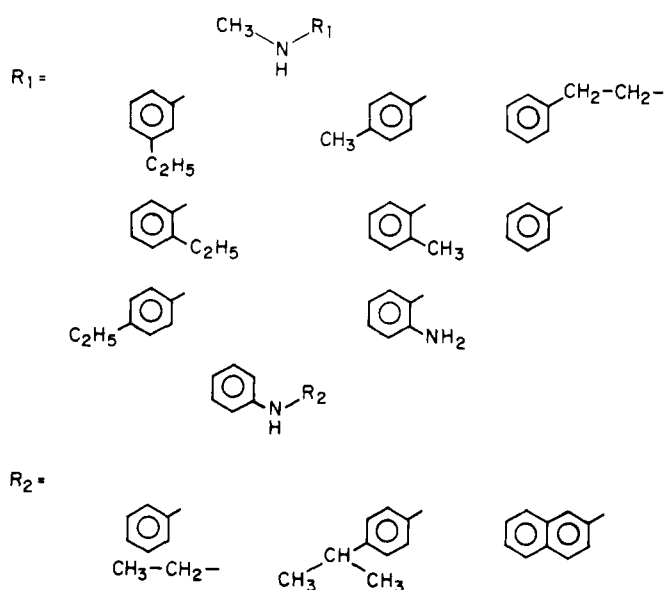


Figure 2. Secondary aromatic amines in tobacco and tobacco smoke that could serve as precursors for *N*-nitrosamines.

for example) in the leaf or through pyrolytic processes (e.g., formation of naphthylamines) during smoking.<sup>37</sup> Aromatic amines have not been shown to arise from pyrolysis of nicotine.<sup>33</sup>

#### IV. *N*-Heterocyclic Compounds

The *N*-heterocyclic compounds of tobacco and tobacco smoke constitute a large number of compounds of a variety of different types (Figures 3–9, I–LVII). Among these are five- and six-membered single ring compounds, e.g., pyrroles, pyridines, pyrazines, and their derivatives, two or more ring, nonfused compounds which include the alkaloids, and fused ring compounds, e.g., quinolines, indoles, carbazoles, acridines, and related substances. Although a substantial number of the *N*-heterocyclic (nonalkaloidal) compounds have been found in leaf as well as smoke, most of those occurring in the smoke, especially of the fused-ring variety, are believed to be pyrolytic products of amino acids, protein, or nicotine rather than simple transfer products.<sup>33,34</sup> The presence of these compounds in leaf, however, may reflect biological and other reactions (e.g., nonenzymatic) that occur during leaf processing.<sup>2,16</sup> Nicotine which contains a pyrrolidine as well as a pyridine ring is very likely one precursor of pyrroles and pyridines occurring in the smoke, and possibly in the leaf. As expected, the amino acid proline has been shown to generate pyrrole on pyrolysis; however, other amino acids behave similarly, and the pyrrolidine ring is evidently not a requisite for pyrolytic formation of pyrroles.<sup>35</sup> Similarly, although tryptophan is an ideal precursor of indole and skatole,<sup>36</sup> the indole nucleus is not necessary for high-temperature generation of indoles as other amino acids can also serve as precursors. In fact, the major product in pyrolyzates of tryptophan is quinoline. Nicotine is also a high-temperature precursor of quinoline and isoquinoline and possibly of indole and skatole.<sup>33,35</sup> Nicotine has also been shown to produce on pyrolysis the animal carcinogens, dibenz[*a,i*]acridine (XXXVII) and dibenz[*a,h*]acridine (XXXVIII), both of which are present in tobacco smoke.<sup>38</sup> Nornicotine-rich cigarettes have been observed to generate relatively high levels of myosmine and alkylpyridines.<sup>194</sup> It is clear that the generation of pyridines and pyrroles in single- or fused-ring systems is characteristic of most organic nitrogen compounds exposed to high temperatures. (Nitriles are also characteristic products of the pyrolysis of compounds that contain the >CH–N< grouping.<sup>33,35</sup>)

TABLE II. Aromatic Amines

	Tobacco ref	Smoke ref
Anilines		
Aniline	1, 19	1, 56 (1.2) <sup>a</sup>
Ring-Substituted (methyl)		
2-Methylaniline ( <i>o</i> -toluidine)	19	1, 20
3-Methylaniline ( <i>m</i> -toluidine)		1, 20
4-Methylaniline ( <i>p</i> -toluidine)		1, 20
Ring-Substituted (dimethyl)		
2,3-Dimethylaniline	19	1, 20
2,4-Dimethylaniline		1, 20
2,5-Dimethylaniline	19	1, 20
2,6-Dimethylaniline	19	1, 20
3,5-Dimethylaniline		20
3,4-Dimethylaniline	19	
Ring-Substituted (ethyl)		
2-Ethylaniline	19	1, 20
4-Ethylaniline		1, 20
Ring-Substituted (C <sub>3</sub> and C <sub>4</sub> )		
Alkyl (C <sub>3</sub> )-substituted anilines		20
Tetramethylaniline(s)		20
2,4,6-Trimethylaniline ( <i>Mesidine</i> )	19	1, 20
Ring-Substituted (methoxy)		
3-Methoxyaniline ( <i>m</i> -anisidine)	19	20
2-Methoxyaniline ( <i>o</i> -anisidine)		20
4-Methoxyaniline ( <i>p</i> -anisidine)		20
N-Substituted		
<i>N</i> -Alkylaminophenols		27
Diphenylamine		25
<i>N</i> -Ethylaniline		20
<i>N</i> -Methylaniline		1, 20
<i>N</i> -Methyl- <i>m</i> -ethylaniline		20
<i>N</i> -Methyl- <i>o</i> -ethylaniline		20
<i>N</i> -Methyl- <i>p</i> -ethylaniline		20
<i>N</i> -Methyl- $\beta$ -phenethylamine		1, 20
<i>N</i> -Methyl-phenylenediamine		20
<i>N</i> -Methyl- <i>o</i> -toluidine		20
<i>N</i> -Methyl- <i>p</i> -toluidine		20
<i>N</i> -Phenyl-4-isopropylphenylamine		25
<i>N</i> -Phenyl- $\beta$ -naphthylamine		25, 177
Naphthylamines		
1-Naphthylamine		28, 37, 193 (0.027)
2-Naphthylamine		28, 37 (0.022)
Others		
Benzylamine		20
<i>o</i> -Chloroaniline <sup>b</sup>		165
$\beta$ -Phenethylamine	1, 19	1, 20
(2-phenylethylamine)		
Tyramine	26	
Aminofluorene		193
<i>o</i> -Aminostilbene		193
Diaminotoluene		193

<sup>a</sup> Figures in parentheses represent amounts in smoke, in  $\mu\text{g}/\text{cigarette}$ .

<sup>b</sup> Degradation product of the fungicide, Dyrene.

#### A. Pyrroles

Twenty-seven pyrroles (Table III) have been reported in tobacco and tobacco smoke. These include pyrrole and *N*-substituted and *C*-substituted pyrroles. Substituents on the ring and on nitrogen consist of alkyl, acyl, cyano, furyl, and ester groups.<sup>43–53,56</sup> Hydropyrroles, including pyrrolidines and pyrrolines, number at least 15, and in addition to pyrrole and pyrrolidine include corresponding alkyl-substituted compounds.<sup>1,20,21</sup>

Other five-membered *N*-heterocyclic compounds reported in tobacco and tobacco smoke are dimethylpyrazole,<sup>56</sup> acetyl-imidazole,<sup>53</sup> and the imides, methylethylmaleimide, ethyl-methylsuccinimide,<sup>47,53</sup> and related compounds.<sup>184</sup> These imides may arise from the degradation (thermal) of the chemical

TABLE III. Single Ring N-Heterocyclic Compounds: Five-Membered Rings

	Tobacco ref	Smoke ref
Pyrroles		
Pyrrole		1, 56, 43
N-Substituted Pyrroles		
N-Isoamylpyrrole		43
N-n-Amylpyrrole		43
N-Isobutylpyrrole		43
N-n-Butylpyrrole		43
N-Ethylpyrrole		43
2-(2-Formylpyrrol-1-yl)-3-methylbutanoic acid	184, 199	
2-(2-Formylpyrrol-1-yl)-3-methylpentanoic acid	184, 199	
2-(2-Formylpyrrol-1-yl)-4-methylpentanoic acid	184, 199	
N-(Furfuryl-2')pyrrole		45
N-Methyl-2-acetylpyrrole	186	
N-Methyl-3-acetylpyrrole	53	
N-(3-Methylbutyl)-2-formylpyrrole	53	
N-(3-Methylbutyl)-1-pyrrole	53	
N-Methyl-2-formylpyrrole	46, 53, 199	
N-Methylpyrrole	48	1, 43, 49
N-Isopropylpyrrole		43
N-n-Propylpyrrole		43
C-Substituted Pyrroles		
2-Acetyl-5-methylpyrrole	46, 47, 53	
2-Acetylpyrrole	46-48, 50, 51, 53	44, 45
"Acyl" pyrrole(s)		44
2-Carbomethoxypyrrrole		44
2-Formyl-5-methylpyrrole	46, 47	
2-Formylpyrrole	46, 53, 199	44
Methylpyrrole-2-carbonitrile		44
2-Propionylpyrrole		44
Pyrrole-2-carbonitrile		44
Pyrrolidines and Pyrrolines		
2-Acetylpyrrolidine	184	
Alkylpyrrolidine(s)		20
Dimethylpyrrolidine(s)		20
2-Ethylpyrrolidine		20
Methylpyrroline, N-methylpyrroline <sup>a</sup>		20
2-Methylpyrrolidine		20
2-Methylpyrrolidine-3-carboxaldehyde	184	
N-Acetylpyrrolidine	184	
N-Formylpyrrolidine	184	
N-Methylpyrrolidine	21 (23.2) <sup>b</sup>	21 (3.0) <sup>c</sup>
N-Methyl-3-pyrroline	2	
N-(3-Methylbutanoyl)pyrrolidine	184	
Pyrrolidine	21 (15.9)	1, 20, 21 (0.16)
Pyrroline, $\Delta^3$ -pyrroline	19	1, 20
Alkylpyrrolines		193
Pyrrolidinones		
N-Methylpyrrolidin-2-one	184	
Pyrrolidin-2-one	184	
Other Five-Membered N-Heterocyclics		
1-Acetylimidazole	53	
Dimethylmaleimide	184	
Dimethylpyrazole		56
2-Ethylidene-3-methylsuccinimide	184	
2-Ethyl-3-methylmaleimide	184	
2-Ethyl-3-methylsuccinimide	184	
2-Ethyl-N-methylsuccinimide	184	
Maleimide	184	
Methylethylmaleimide	47, 53	
2-Methylsuccinimide	184	
N-Methylsuccinimide	184	
2-Propylmaleimide	184	
Succinimide	184	
trans-2-Ethyl-3-methylsuccinimide	53	

<sup>a</sup> In one study, the presence of this compound is inferred from the isolation of  $\gamma$ -methylaminobutyraldehyde in tobacco roots.<sup>210</sup> <sup>b</sup> Values in parentheses,  $\mu\text{g/g}$  of tobacco. <sup>c</sup> Values in parentheses,  $\mu\text{g}$  (in smoke)/cigarette.

suckering agent, maleic hydrazide, which finds widespread use on tobacco crops.

Other interesting five-membered ring compounds are the

pyrrolidinones, two of which have been reported in tobacco.<sup>184</sup> These are lactams analogous to the piperidones (Table IV) also reported in tobacco. Recent studies have suggested that many



TABLE V. Single-Ring N-Heterocyclic Compounds: Pyrazines

	Tobacco ref	Smoke ref
2-Acetyl-3-methylpyrazine	53	
2-Acetyl-6-methylpyrazine	46, 53	
Acetylpyrazine	53	
2,6-Diethylpyrazine	1	1, 45
2,5-Diethylpyrazine		45
Dimethylethylpyrazine	1	1
5,6-Dimethyl-2-(furyl-2')pyrazine <sup>a</sup>		45
2,3-Dimethylpyrazine	1, 53	1, 45
2,5-Dimethylpyrazine	1	1, 45
2,6-Dimethylpyrazine	1, 53	1, 45
2,3-Dimethyl-5-ethylpyrazine	184, 199	
2-Ethyl-3,6-dimethylpyrazine	53	
2-Ethyl-3,5-dimethylpyrazine	57	
2-Ethyl-5-methylpyrazine	46, 53	45
Ethylpyrazine		45
Furyl-2'-pyrazine <sup>a</sup>		45
2-Methyl-6-ethylpyrazine	1, 53, 199	1, 45
2-Methyl-5-(furyl-2')pyrazine <sup>a</sup>	1	1, 45
6-Methyl-2-(furyl-2')pyrazine <sup>a</sup>		45
Methylpropylpyrazine		45
Methylisopropylpyrazine		45
2-Methylpyrazine	1, 53	1, 45
Isopropenylpyrazine	53	
Pyrazine	1, 53	1, 45, 56
Quinoxaline <sup>b</sup>		1, 42
Tetramethylpyrazine	1, 199	1, 45
Trimethylpyrazine	1, 53, 199	1, 45
2-Vinyl-6-methylpyrazine	53	

<sup>a</sup> Bicyclic systems. <sup>b</sup> Fused ring system.

## C. Pyrazines

Twenty-eight pyrazines have been reported in tobacco and tobacco smoke (Table V). In addition to the parent compound, pyrazines present include di-, tri-, and tetraalkylpyrazines and others with furyl substituents.<sup>1,45,46,53,56</sup> A fused ring pyrazine

## MULTIPLE RINGS NON FUSED (TOBACCO ALKALOIDS)

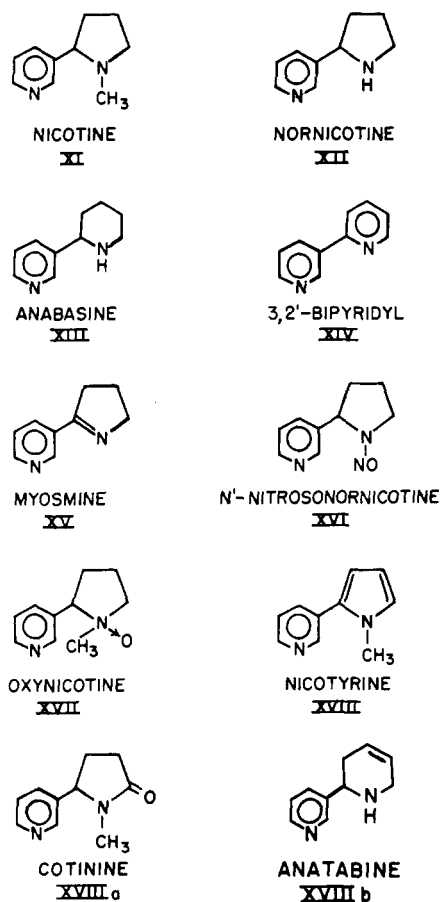


Figure 4. Tobacco alkaloids. The chiralities of some of these are discussed in the text.

TABLE VI. Multiple-Ring, Nonfused N-Heterocyclic Compounds (Alkaloids)

	Tobacco ref	Smoke ref
<i>N</i> -Acetylnornicotine	75	
Anabasine	1, 4, 12 (0.11) <sup>a</sup>	1, 4, 12 (3-12) <sup>b</sup>
Anatabine	1, 4 (0.54)	1, 4 (3.7-14)
Anatalline	76	
2,2'-Bipyridyl		87
2,3'-Bipyridyl (isonicotine)	1	1, 4 (7-27)
<i>N</i> -Carbomethoxyanabasine		67
<i>N</i> -Carbomethoxynornicotine		67
Cotinine	1, 4, 12	1, 4 (9-57)
2',3'-Dehydronicotine		1
<i>N</i> -Formylornicotine	75	
<i>N</i> -Hexanoylnornicotine	74	
<i>N</i> -Methylanabasine	88, 2	87
<i>N</i> -Methylanatabine	2	
5-Methyl-2,3'-bipyridyl	75	
<i>N</i> -Methylmyosmine (dihydronicotyrine) <sup>c</sup>		2, 194, 80
<i>N</i> -Methylnicotone <sup>d</sup>	4	89
Myosmine	1, 4	1, 4 (9)
Nicotelline	77-79	
Nicotine	1, 4, 12 (20)	1, 4, 12 (800-3000)
Nicotine <i>N</i> -oxide (oxynicotine) <sup>e</sup>	1, 2	1 (?)
Nicotyrine	1, 4	1, 4
Nornicotine	1, 4, 12 (0.64)	1, 4, 12 (26.8-88)
Nornicotyrine	1	1
<i>N</i> -Octanoylnornicotine	74	

<sup>a</sup> Values in parentheses, mg of alkaloid/g of tobacco. <sup>b</sup> Values in parentheses,  $\mu$ g/cigarette. <sup>c</sup> This compound exists in two forms, one with an intact pyrrolidine ring, and the other as an open structure, 4-*N*-methylamino-1-(3-pyridyl)-1-butanone, also known as pseudooxynicotine,<sup>80,194,214</sup> which forms on hydrolysis. <sup>d</sup> This compound is probably not present in tobacco or tobacco smoke; it is an oxidation product of the methyl iodide adduct of nicotine and was utilized to determine the absolute configuration of nicotine.<sup>12,80,217</sup> <sup>e</sup> Cis and trans isomers of nicotine 1'-*N*-oxide have been reported.<sup>207</sup>

TABLE VII. Fused Ring N-Heterocyclic Compounds

	Tobacco ref	Smoke ref		Tobacco ref	Smoke ref
Two-Fused Rings					
Indoles					
1,3-Dimethylindole		36 (282) <sup>a</sup>	Pyrrrolo[2,3- <i>b</i> ]pyridine		1, 42
1,4-Dimethylindole		36	Quinoxaline		1, 42
1,5-Dimethylindole		36	3,6,6-Trimethyl-5,6-dihydro- 7 <i>H</i> -2-pyridin-7-one	96	
1,6-Dimethylindole		36	1,3,6,6-Tetramethyl-5,6,7,8- tetrahydroisoquinolin-8-one	96	
1,7-Dimethylindole		36	Tetrahydroindolizin-8-one	215	
1,2-Dimethylindole		1	Three-Fused Rings		
2,3-Dimethylindole		1	Acridans		
Dimethylindole(s)		45	9,9-Dimethylacridan		25
1-Ethylindole		36 (126)	2-Isopropyl-9,9-dimethylacridan		25
3-Ethylindole		1, 44, 56, 92	Carbazoles		
Indole	46, 53, 98	1, 45, 56, 92 (13 900)	Alkylcarbazole(s)		2, 90, 91, 177
1-Methylindole		36 (422)	9-Alkylcarbazole(s)		177
2-Methylindole		56	Carbazole		1, 56 (1000)
3-Methylindole (skatole)		1, 44, 45, 56, 92 (14 000)	1,9-Dimethylcarbazole		94 (12)
5-Methylindole		56	2,9-Dimethylcarbazole		94
7-Methylindole		56	3,9-Dimethylcarbazole		94
Methylindole(s)		45, 56	4,9-Dimethylcarbazole		94 (6)
3-Phenylindole		93	Dimethylcarbazole		1, 90, 91
3-Propylindole		92	1-Methylcarbazole		1, 93
1,2,3-Trimethylindole		1	2-Methylcarbazole		93
Trimethylindole(s)		42	9-Methylcarbazole		94 (103)
Quinolines					
2,6-Dimethylquinoline		87	3-Methylcarbazole		93
Dimethylquinoline(s)		1, 45	4-Methylcarbazole		93
8-Hydroxyquinoline		89	Methylcarbazole(s)		2, 90, 91
Isoquinoline		1, 45	Others		
4-Methylquinoline	53		Azacarbazole		177
Methylquinoline(s)		1, 56	4-Azafluorene		177
Quinoline	53	1, 45, 56	7,8-Benzoquinoline		177
Others					
Adenine		2	Harmaine	84, 99, 100	84, 99, 100
Benzimidazole (methylbenzimidazole)		2, 42	Methylpyrocoll		177
Benzothiazole	53		Norharmaine	84, 99, 100	84, 99, 100
2-(2-Formyl-5-hydroxymethylpyrrol-1-yl)- propionic acid lactone	184, 199		Pyrido[2,3- <i>b</i> ]indole		1, 42
2-(2-Formyl-5-hydroxymethylpyrrol-1-yl)- 3-phenylpropionic acid lactone	184, 199		Pyrocoll		85, 86
Guanine	2		More than Three Fused Rings		
<sup>a</sup> Values in parentheses, ng/cigarette.					
			Dibenzi[ <i>a,h</i> ]acridine		38 (0.1)
			Dibenzi[ <i>a,j</i> ]acridine		38 (2.7)
			7 <i>H</i> -Dibenzo[ <i>c,g</i> ]carbazole		38 (0.7)

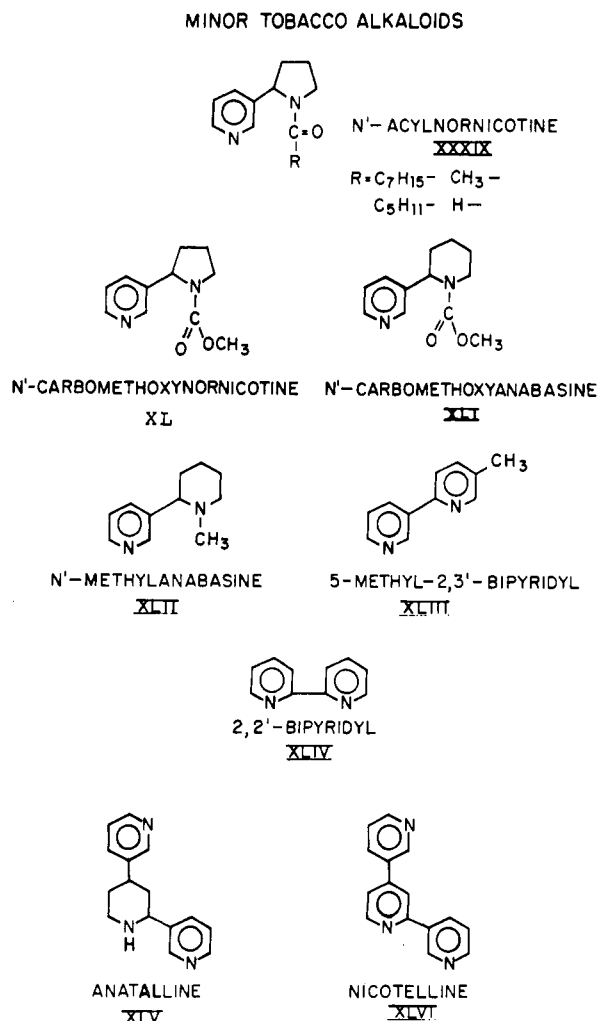
(quinoxaline, Figure 6, XXI) has also been reported.<sup>42</sup> The presence of so many pyrazines may be surprising. However, if one considers the elevated temperatures associated with leaf curing and the presence in leaf of the probable precursors, then the finding is rather to be expected. Pyrazines have typically been associated with foods that are toasted or roasted in their preparation, such as coffee and cocoa products and popcorn.<sup>58</sup> In fact, pyrazines are potent flavor compounds, and although they have been shown to be reaction products of sugars and amino acids (or ammonia) at elevated temperatures, they may also be added intentionally to food (and possibly tobacco) products because of their flavor characteristics. The tobacco literature cites at least one patent<sup>59</sup> describing the synthesis of acylpyrazines for use as tobacco flavorants ("popcorn" flavor). Nevertheless, as noted above, tobacco contains suitable precursors and at times experiences the appropriate high temperatures required for pyrazine formation. However, some of the pyrazines present in the smoke may be direct transfer products from the leaf. A recent study showed a correlation between levels of pyrazines in tobacco and the temperatures to which the tobacco was exposed.<sup>57</sup> Caution must be exercised in the isolation of pyrazines as they have been shown to be formed artifactually when overly drastic isolation conditions are used. Another mode of pyrazine

formation may be bacterial as *bacilli subtilis* have also been shown to give rise to tetramethylpyrazine during growth.<sup>58</sup>

## D. Multiple Ring, Nonfused N-Heterocyclics (Alkaloids)

Table VI lists multiple ring (nonfused) N-heterocyclics reported in tobacco and tobacco smoke, of which there are 25. Other alkaloids, consisting of fused rings such as the harmanes, are listed in Table VII. Those of the nonfused ring kind include the classical tobacco alkaloids (Figure 4) such as nicotine (XI), nornicotine (XII), anabasine (XIII), myosmine (XV), and others. Although nicotine is the principal alkaloid in commercial tobaccos (*N. tabacum* and *N. rustica*), nornicotine is the main alkaloid in most other species of *Nicotiana*; anabasine is the third most abundant tobacco alkaloid.<sup>4,12</sup> Anatabine (XVIIIb) is reported to be the most abundant of the minor alkaloids in the roots of these plants, although it is present also in the leaf and stem.<sup>208</sup> The level of nicotine in leaf is rather high (up to 5%), and although a considerable portion of it is transferred intact into mainstream smoke ( $\approx 15\%$ ), a portion ( $\approx 3-5\%$ ) of it is responsible for thermal-degradation products that are found in the mainstream particulates.<sup>39,60-62,84</sup> However, it is likely the





**Figure 5.** Recently identified tobacco alkaloids, except for nicotelline, which is shown for comparative purposes.

precursor of new compounds that arise in leaf during curing and other processing.<sup>4,12</sup>

Nicotine is present in tobacco in the *l* form and during tobacco combustion is transferred into the smoke without racemization.<sup>201</sup> Moreover, recent studies utilizing nuclear magnetic resonance have shown that the preferred configuration of the N-methyl group in nicotine is *trans* to the pyridine ring.<sup>202</sup> Except for nicotine, no reports are available on the chirality of the optically active alkaloids as they are present in tobacco smoke. In the plant, however, anatabine and anabasine have been reported to occur both as the racemic mixture and in the *l* form. Nornicotine occurs in the *d*, *l*, and *dl* forms.<sup>80,208</sup>

Over the years, much has been written about the chemistry and biological activity of nicotine. Its toxicity and metabolic pathways in mammalian and nonmammalian systems are well documented.<sup>12,12a,b</sup> As for its chemical aspects relevant to tobacco, its pyrolytic properties have been elucidated, indicating that it is the source of many, if not the majority, of the pyridines present in the smoke.<sup>62-65</sup> More recently its role in the formation of *N*'-nitrosornicotine (XVI) and other related nitrosamines (Figure 9, LVI, LVII) has received much attention (vide infra<sup>66-70</sup>). In addition, the oxidation-reduction properties of nicotine<sup>71</sup> may have a strong bearing on the dynamics of smoke chemistry. The autooxidation products of nicotine, for example, have been identified; these include nicotinic acid, oxynicotine (XVII), nicotyrine (XVIII), cotinine (XVIIIa), myosmine (XV), and others.<sup>72,73</sup> However, the redox properties of nicotine may affect smoke composition in a more subtle way as they may induce reactions

between nicotine and other easily reducible smoke components. This, of course, needs further study.

As for the health implications of the presence of nicotine in tobacco smoke, present evidence indicates that nicotine, as such, does not play a role in tobacco carcinogenesis; however, there is still room for study in this regard. Nicotine has been implicated, however, in the relationship between tobacco smoking and cardiovascular disease (although carbon monoxide is also under study here).<sup>13,14,14a,31</sup>

In considering the biological effects of nicotine, one must realize that the pH of tobacco smoke determines the ratio of protonated to unprotonated nicotine.<sup>203</sup> As demonstrated by Armitage and Turner,<sup>204</sup> unprotonated nicotine is more readily absorbed through mucous membranes than is protonated nicotine, a factor influencing the acute toxicity of this alkaloid.

Leaf and smoke composition studies have shown that, aside from nicotine and other well-known alkaloids, there are alkaloids characterized by substitution (other than methyl, usually acyl, XXXIX) on the nitrogen of the pyrrolidine ring (Figure 5). These include, for example, *N*'-hexanoylnornicotine,<sup>74</sup> *N*'-octanoylnornicotine,<sup>74</sup> *N*'-formylnornicotine,<sup>75</sup> *N*'-acetylnornicotine,<sup>75</sup> *N*'-carbomethoxynornicotine (XL),<sup>67</sup> and *N*'-carbomethoxyanabasine (XLI).<sup>67</sup> Relatively new alkaloids are 5-methyl-2,3'-bipyridyl (XLIII)<sup>75</sup> and anatalline (XLV)<sup>76</sup> which is analogous to nicotelline (XLVI),<sup>77-79</sup> a terpyridyl first isolated from tobacco 75 years ago. A review by Pailer summarizes the historical chemistry of the tobacco alkaloids.<sup>80</sup>

It should be noted that in cases where tobacco was treated with the fumigant and fermentation accelerator, ethylene oxide, hydroxyethylnicotine has been isolated from the treated tobacco but not from the mainstream smoke.<sup>81,196</sup>

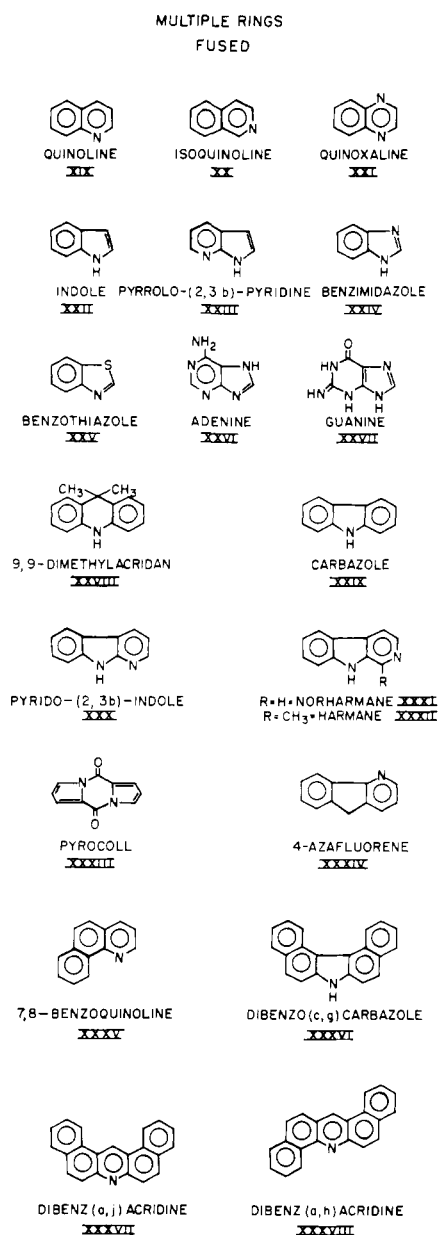
### 1. Alkaloid Biosynthesis

When one considers the alkaloids in tobacco, it is interesting that each type has specific amino acid precursors. Nicotine and nornicotine arise from nicotinic acid, methionine, choline, ornithine, and proline;<sup>4,12,82,83</sup> anabasine from nicotinic acid and lysine;<sup>4,12</sup> the harmanes from tryptophan;<sup>84</sup> and pyrocoll from proline.<sup>85,86</sup> Anatabine is derived from two molecules of nicotinic acid.<sup>206</sup> Similar precursors could be postulated for other tobacco alkaloids. In this regard, it is curious that thus far no specific plant function has yet been attributed to the alkaloids.<sup>4</sup> Some of our work indicates that nicotine is a reducing agent; in this respect we hypothesize that it may play a role in photosynthesis during the reduction of CO<sub>2</sub> to carbohydrate.

## E. Fused-Ring N-Heterocyclic Compounds

Among the more than 65 fused ring N-heterocyclic compounds reported in tobacco and tobacco smoke (Table VII) are those of ring systems containing two, three, and five fused rings (Figure 6). Nitrogen heterocyclics with four fused rings have not been reported. Most of these compounds are found in smoke rather than leaf. This is to be expected as many of them are believed to be pyrolysis products.<sup>33</sup> However, careful examination of leaf may reveal that they occur there also. Indoles and carbazoles with no substitution on nitrogen are less basic than the quinolines, for example. The former two groups are isolated, therefore, in the neutral fraction of the smoke.<sup>85,90,93</sup> N-Alkyl indoles<sup>36</sup> and N-alkyl carbazoles<sup>94</sup> have also been reported in the smoke; these possess different column and gas chromatographic properties from those of their C-alkyl isomers, and are likely more basic. The most prevalent of the fused ring N-heterocyclics in the smoke are the indoles and the carbazoles. Indole and skatole together (in smoke) comprise about 28 μg per cigarette (85 mm, nonfilter), more than 1000 times the amount of benzo[*a*]pyrene, for example.<sup>92</sup> In addition, the ratio of indole to skatole in unaged smoke is 1:1, whereas in aged smoke there is ten times more skatole than indole. This can be rationalized

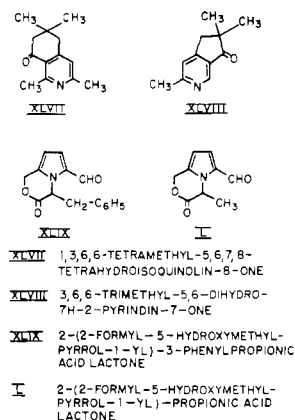




**Figure 6.** Fused-ring heterocyclic compounds in tobacco and tobacco smoke.

by the relatively low chemical reactivity of  $\beta$ -alkylated indoles such as skatole. Carbazoles as a group are present in the smoke to the extent of 3  $\mu\text{g}$  per cigarette<sup>93</sup> and *N*-alkyl indoles and *N*-alkyl carbazoles, 961 and 140 ng per cigarette,<sup>36,94</sup> respectively. As indicated, indoles and carbazoles have been shown to be pyrolytic products of amino acids, especially of tryptophan, although there is some indication that these may arise from thermal degradation of nicotine as well.<sup>33,36,95</sup> In this regard, the carbolines, harmane and norharmane, are present in the smoke and have been shown to originate from tryptophan by reaction with an aldehyde.<sup>64</sup> Harmane has previously found utility in the treatment of Parkinson's disease. Although harmane and norharmane (Figure 6, XXXI and XXXII) are alkaloids, they are included here among the fused ring compounds. Other fused ring compounds (Figure 7) recently isolated from Burley leaf, and considered to be terpenoid alkaloids, are 1,3,6,6-tetramethyl-5,6,7,8-tetrahydroisoquinolin-8-one (XLVII) and 3,6,6-trimethyl-5,6-dihydro-7*H*-2-pyridin-7-one (XLVIII).<sup>96</sup> These compounds are postulated to arise via a number of degradative enzymatic and chemical reactions, not very straightforward, during the aerobic treatment of the tobacco leaves (curing, aging), their precursors being "xanthophyll" terpenoids and

## NEW CLASSES OF NATURAL PRODUCTS



**Figure 7.** Terpenoid alkaloids and pyrrole lactones, new classes of natural products found in tobacco leaf.

ammonia. Besides quinolines, indoles, and carbazoles, one finds examples of benzimidazoles,<sup>2,42</sup> benzothiazoles,<sup>53</sup> bicyclic pyrazines (quinoxaline<sup>42</sup>), and others. Pyrocoll (Figure 6, XXXIII) has long been known to occur in leaf and smoke.<sup>85,86</sup> It, too, is considered an alkaloid, and it is suggested that it arises from the amino acid proline (condensation of 2 mol). Acridans have been reported in smoke, and these have been postulated to arise from appropriate *N*-aryl anilines.<sup>25</sup> Of course, subsequent dehydrogenation of the acridans could lead to acridines. The only examples of the latter reported in smoke are two dibenzacridines; these have been shown to form during pyrolysis from nicotine.<sup>38</sup> A dibenzocarbazole (XXXVI) has also been reported in tobacco smoke.<sup>38</sup> The last three fused ring compounds cited are tumorigenic in the experimental animal.<sup>97</sup> *N*-Alkyl indoles and *N*-alkyl carbazoles, on the other hand, display cocarcinogenic activity.<sup>8</sup>

Moreover, recent studies have shown that *N*-nitrosamines can be formed in vivo from secondary and tertiary amines.<sup>197</sup> Therefore, we should be concerned with the number of these amines (e.g., the fused ring heterocyclics) present in tobacco and in inhaled tobacco smoke.

The tobacco industry has been expending much effort in the search for tobacco flavorants.<sup>187</sup> Recently, one such effort has uncovered a new class of natural products, pyrrole lactones (Figure 7), namely, 2-(2-formyl-5-hydroxymethylpyrrol-1-yl)-3-phenylpropionic acid lactone (XLIX) and 2-(2-formyl-5-hydroxymethylpyrrol-1-yl)propionic acid lactone (L).<sup>184</sup> These are postulated to be products of the reaction between amino acids and hexoses.<sup>184</sup> Other formyl pyrrole derivatives, e.g., 2-(2-formylpyrrol-1-yl)-3-methylbutanoic acid (Table III), are postulated to arise in a similar way.<sup>184,199</sup>

## V. Amino Acids

In addition to the common 20 amino acids combined in the proteins of living organisms, a number of rather uncommon amino acids have been reported in tobacco and tobacco smoke (Table VIII). At least 48 compounds therein can be classified as amino acids. One would not expect such nonvolatile materials to survive tobacco combustion and find their way into the smoke; however, 16 amino acids have now been reported to be present in the smoke.  $\alpha$ -Alanine, the major amino acid identified in the smoke (from seven types of tobacco), is reported to be present in amounts ranging from 11 to 268  $\mu\text{g}$ /cigarette.<sup>103,104,113</sup>

The presence of free amino acids in cured leaf (and their subsequent mechanical transfer to smoke) can partially be explained by the hydrolysis of protein that occurs during tobacco processing, and although specific proteins have been identified in green leaf,<sup>114,116</sup> the question as to whether any proteins

TABLE VIII. Amino Acids

	Tobacco ref	Smoke ref
$\alpha$ -Alanine	101, 102	103, 104
$\beta$ -Alanine	102	103, 104
D-Alanyl-D-alanine	106	
$\alpha$ -Aminobutyric acid	26	
$\gamma$ -Aminobutyric acid	101, 102	103, 104
$\alpha$ -Aminoadipic acid	102	
Arginine	101, 102	
Asparagine	101, 102	
Aspartic acid	101, 102	103, 104
Azetidone-2-carboxylic acid	166	
Betaine	2	
Choline	2	
Citrulline	26	
Cystic Acid	102	
Cysteine	26	
Cystine	102	
Glutamic acid	101, 102	103, 104, 113
Glutamine	101, 102	103, 104, 113
$\alpha$ -L-Glutamyl-L-glutamic acid	105	
Glutathione	2	
Glycine	101, 102	103, 104
Histidine	101, 102	
Homocystine	102	
Homoserine	110	
6-Hydroxykynurenic acid	109	
Hydroxyproline	102	
Isoleucine	101, 102	
Leucine	101, 102	103, 104
Lysine	101, 102	
1-Methylhistidine	111, 4	
Methionine	26	
Methionine sulfone (methionine S-oxide)	102	
Nicotianamine	108, 209	
Nicotianine	107	
Nicotinamide	2	113
Nicotinic acid	2	113
Norleucine	26	
Ornithine	26	103, 104
Phenylalanine	101, 102	103, 104
Pipecolic acid	112	
Proline	101, 102	103, 104
Pyrrolidine-2-acetic acid	112	
Serine	101, 102	103, 104
Taurine	102	
Threonine	101, 102	103, 104
Tryptophan	101, 102	
Tyrosine	101, 102	
Valine	102	103, 104

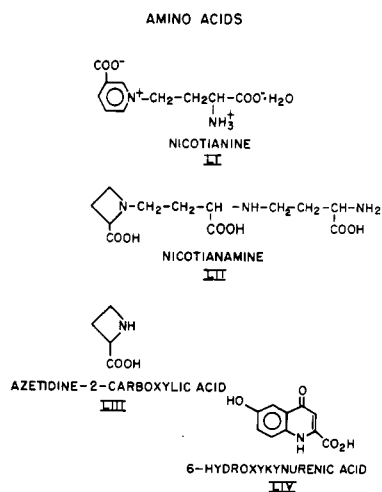


Figure 8. Recently identified amino acids.

(other than those with enzymic activity) survive curing has not yet been resolved satisfactorily. Peptides do occur in cured leaf, and possibly in the smoke, tied up in high molecular weight pigments<sup>116,117</sup> which contain other moieties as well. Amino acids and/or peptides liberated during curing undergo additional reactions including condensation with sugars to yield pyrazines<sup>57</sup> and brown pigments,<sup>118</sup> for example, and decarboxylations and oxidative deaminations to give rise to acetaldehyde, isobutyraldehyde, aliphatic and aryl amines, and ammonia. During fermentation, amino acids are believed to react with polyphenols to yield melanin-like compounds.<sup>2,4</sup>

The recent literature describes the isolation of several unusual amino acids (Figure 8, I–IV) from tobacco leaves. These include nicotianine [L-(+)-N-(3-amino-3-carboxypropyl)- $\beta$ -carboxypyridinium betaine],<sup>107</sup> nicotianamine [(2S),3'(S),3''S)-N-[N-(3-amino-3-carboxypropyl)-3-amino-3-carboxypropyl]-azetidone-2-carboxylic acid],<sup>108,209</sup> D-alanyl-D-alanine,<sup>106</sup> and  $\delta$ -L-glutamyl-L-glutamic acid.<sup>105</sup> The finding of D-alanyl-D-alanine is especially unusual because although free or bound D amino acids sometimes occur in varieties of microorganisms, they seldom occur in higher plants.<sup>106</sup> A rather unusual amino acid isolated from both green and cured tobacco leaves is 6-hydroxykynurenic acid (LIV);<sup>109</sup> if this finding is reconfirmed, it would suggest that the tryptophan catabolic pathways already known in mammals and microorganisms are also operative in plants. Azetidone-2-carboxylic acid (I), an amino acid with a four-membered ring related to nicotianamine (LII), has been reported in tobacco following biosynthetic studies with administered labeled methionine (1-<sup>14</sup>C). The latter gave rise to the carboxyl-labeled imino acid.<sup>166</sup>

Sugar compounds of certain amino acids have also been found in cured tobacco leaves. These will be discussed below as amino sugars (Table XIII).

At the 29th Tobacco Chemists' Conference, a method was described for the rapid gas chromatographic determination of the free amino acids in cured tobacco. It included ion-exchange chromatography followed by esterification of the carboxyl group and acylation of the amine function prior to gas chromatography.<sup>119</sup>

As indicated earlier, in addition to serving as precursors for a variety of compounds (via degradation) found in leaf and smoke, amino acids also serve as biosynthetic precursors for the various alkaloids associated with tobacco.<sup>82</sup>

## A. Protein

Much of the work on tobacco protein has been concerned with the green leaf, and methods for protein isolation have been described.<sup>114–116,175,176</sup> We know that as harvested tobacco ages, protein content decreases rapidly, and free amino acid content increases. Associated with these changes (in protein and amino acid levels) is an increase in protease activity and levels of amides.<sup>4,116</sup> The question, however, as to the presence of protein in cured leaf remains. One report cites a reduction in protein number (as studied by electrophoresis) with curing. Other reports note either the disappearance of protein ("Fraction 1") or the conversion of high to low molecular weight protein as a result of curing.<sup>116,120</sup> "Fraction 1" protein is considered to have potential as a food supplement and is proposed to be recoverable as a by-product of tobacco processing.<sup>213</sup> In our own experience, it is rather easy to demonstrate the presence of bound amino acids in cured tobacco leaf,<sup>33</sup> but the nature of their binding is still open to question. Whether they are bound as "true" protein or merely part of the complex brown pigment of tobacco (or both) are questions still awaiting answers.

## VI. Nitriles

So far the nitriles listed in Table IX have been reported only

TABLE IX. Nitriles

	Smoke ref
Aliphatic Nitriles	
Acetonitrile	1, 121, 123, 125
Propionitrile	1, 121, 123
Butyronitrile	1, 121
Isobutyronitrile	1, 121
Valeronitrile	1, 121
Isovaleronitrile	1, 122
Capronitrile	1, 121
Isocapronitrile	1, 122
Acrylonitrile	1, 121
Allyl cyanide	1, 124
Crotonitrile	1, 123
Methacrylonitrile	1, 121
Aromatic Nitriles	
Alkyl-naphthonitriles	177
Benzonitrile	1, 45, 56, 177
Benzyl cyanide (phenylacetoneitrile)	1, 45, 56
Cinnamionitrile, hydrocinnamionitrile	1, 45, 91, 126
2,5-Dimethylbenzonitrile	127
2,3-Dimethylbenzonitrile	127
2,6-Dimethylbenzonitrile	127
Dimethylbenzonitrile(s)	45
4-Ethylbenzonitrile	127
2-Ethylbenzonitrile	127
3-Ethylbenzonitrile	127
1-Naphthonitrile	177, 178
2-Naphthonitrile	177, 178
<i>m</i> -Tolunitrile	45, 177
<i>o</i> -Tolunitrile	45, 127, 177
<i>p</i> -Tolunitrile	45, 177
Others	
5-Methyl-2-cyanofuran	45
Methylpyrrole-2-carbonitrile	44
Pyrrole-2-carbonitrile	44

in tobacco smoke, suggesting that they are all pyrosynthetic products, most likely of amino acids, peptides, and alkaloids.<sup>33-35</sup> The most abundant nitrile present is hydrogen cyanide (Table XV), which has been reported in concentrations of 100–400  $\mu\text{g}/\text{cigarette}$ .<sup>9</sup> Hydrogen cyanide is known to be specifically toxic for the ciliated respiratory epithelium.<sup>220</sup>

A recent study has shown that cigarette smoke also contains cyanogen [(CN)<sub>2</sub>] and that most likely up to 5% of the reported HCN is artifactually formed from cyanogen during analysis.<sup>188</sup> Other volatile nitriles<sup>1,121-125</sup> are present in relatively high concentrations as well (acetonitrile, 44–140  $\mu\text{g}/\text{cigarette}$ ; propionitrile, 5.6–30  $\mu\text{g}/\text{cigarette}$ ). Johnson and Kang have demonstrated in model studies that upon pyrolysis in helium amino acids and protein form HCN as one major degradation product plus some acetonitrile.<sup>189</sup> Mechanisms for the formation of HCN and CH<sub>3</sub>CN are proposed by the authors. Another possible clue derives from the smoking of cigarettes containing labeled glucose or sucrose. The smoke of these cigarettes contains labeled acetonitrile (indicative of an interaction between sugars and nitrogen-containing materials during smoking).<sup>164</sup>

Many of the more recently identified nitriles, especially the aryl nitriles,<sup>91,127</sup> are found in the biologically active neutral fraction of cigarette smoke condensate. Similar to the more volatile nitriles, the question as to how these contribute to the toxic (or other biological) activity of the smoke remains to be answered. It should be emphasized, however, that the aryl nitriles comprise only a very small portion of the active fraction in question.

### VII. Nitroalkanes, Nitroarenes, and Nitrophenols

The concentrations of nitroalkanes and other nitro compounds (Table X) in the smoke are directly related to the nitrate content

TABLE X. Nitroalkanes, Nitroarenes, and Nitrophenols

	Smoke ref
Nitroalkanes	
Nitromethane	128 (523) <sup>a</sup>
Nitroethane	128 (1080)
2-Nitropropane	128 (1080)
1-Nitropropane	128 (728)
1-Nitro- <i>n</i> -butane	128 (713)
1-Nitro- <i>n</i> -pentane	128 (215)
Nitroarenes	
Nitrobenzene	129 (25)
4-Nitrocumene	129 (5)
2-Nitro-1,4-dimethylbenzene	129
4-Nitro-1,3-dimethylbenzene	129
4-Nitro-1,2-dimethylbenzene	129
2-Nitrotoluene	129 (21)
3-Nitrotoluene	129 (10)
4-Nitrotoluene	129
Nitrophenols	
2-Nitro-3-methylphenol	205 (30)
2-Nitrophenol	205 (35)
2-Nitro-dimethylphenol	205
2-Nitro-4-methylphenol	205 (90)
Nitro-methylphenol	205
Nitro-dihydroxybenzene	205 (80)
3-Nitrophenol	205
2-Nitro-5,6-dimethylphenol	205
4-Nitrophenol	205 (20)
Nitro-methyl-dihydroxybenzene	205
Nitro-ethylphenol	205
3-Methyl-4-nitrophenol	205
Nitro-methyl-ethylphenol	205
Nitro-methylnaphthol	205
Nitro-ethyl-dihydroxybenzene	205
4-Nitrocatechol	205, 131 (200)

<sup>a</sup> Values in parentheses, ng/cigarette.

of the tobacco.<sup>128,129</sup> Alkali nitrates (naturally present in tobacco) which decompose in the hot zones of a burning cigarette are most important in the generation of organic nitro compounds, such as nitroalkanes. These arise from the reaction of nitrogen oxides with organic radicals. The reported presence of 4-nitrocatechol in the smoke of cigarettes made from high-nitrate tobaccos could be rationalized in the same manner, although this compound is regarded by some to be an artifact produced by nitration of catechol in smoke collection traps.<sup>131</sup> However, careful analysis has confirmed its presence along with other nitrophenols (Table X) in freshly generated smoke,<sup>182,205</sup> albeit in small amounts.

The detection of nitroalkanes and nitroarenes in tobacco smoke requires careful analytical techniques because these compounds are especially volatile and present in rather low levels. The nitroalkanes range in concentration from 0.5 to 1.0  $\mu\text{g}$  and the nitroarenes from 5.0 to 25.0 ng per cigarette. They are highly sensitive to detection by electron-capture gas chromatography.<sup>128,129</sup> Similarly, the determination of the nitrophenols in tobacco smoke is accomplished by three derivatization steps, methylation of the phenolic functions, reduction of the nitro groups, and acylation of the resulting amines, followed by gas chromatography/mass spectrometry of the mixture.<sup>205</sup> Levels of nitrophenols in smoke range from below 1 to 200 ng per cigarette.<sup>205</sup>

Nitro compounds are especially prevalent in the smoke of "nitrate-rich" tobaccos.<sup>128,129</sup> Their formation competes with that of the polynuclear aromatic hydrocarbons (PAH), and as a result the smoke condensate from "nitrate-rich" tobaccos, in addition to containing lower levels of PAH, are also less tumorigenic to mouse skin than the smoke condensate from "nitrate-poor" tobacco.<sup>15</sup> Dontenwill et al. demonstrated similar

TABLE XI. N-Nitrosamines

	Tobacco ref	Smoke ref
<i>N</i> -Nitrosodi- <i>n</i> -butylamine		17
<i>N</i> -Nitrosodiethanolamine	139 (0.1–0.2) <sup>a</sup>	
<i>N</i> -Nitrosodiethylamine		1, 17, 135
<i>N</i> -Nitrosodimethylamine		1, 17, 141 (84) <sup>b</sup>
<i>N</i> -Nitrosodi- <i>n</i> -propylamine		17
<i>N</i> -Nitrosoethylmethylamine		17 (30)
<i>N</i> -Nitrosomethyl- <i>n</i> -butylamine		1, 136
<i>N</i> '-Nitrosornicotine	66–69 (0.3–90)	70, 137 (137)
<i>N</i> -Nitrosopiperidine		1, 17, 135, 167
<i>N</i> -Nitrosopyrrolidine		1, 17, 141
4-( <i>N</i> -Methyl- <i>N</i> -nitrosamino)- 1-(3-pyridyl)-1-butanone	192	
4-( <i>N</i> -Methyl- <i>N</i> -nitrosamino)- 4-(3-pyridyl)butanal	192	

<sup>a</sup> Values in parentheses, ppm in leaf. <sup>b</sup> Values in parentheses, ng/cigarette.

reduction in the tumorigenicity of whole smoke of nitrate-rich cigarettes when measured on the larynges of Syrian hamsters.<sup>130</sup> As with many other nitrogen compounds in tobacco and tobacco smoke, the biological activities of the aliphatic and aromatic nitro compounds remain to be evaluated. One has also to consider that aromatic nitro hydrocarbons can be reduced in vivo, at least partially, to aromatic amines.

### VIII. *N*-Nitrosamines

Interest in the presence of *N*-nitrosamines in tobacco and tobacco smoke (Table XI) has been keen because of the animal carcinogenicity exhibited by several of these compounds.<sup>32</sup> Tobacco smoke contains secondary amines and nitrogen oxides, and initially it was suggested that these react, possibly in the vapor phase of smoke, to produce *N*-nitrosamines,<sup>132</sup> which might help to explain the tumorigenic activity of the smoke. More recently, the formation of nitrosamines from tertiary amines has been reported,<sup>133,197</sup> and tobacco products have been shown to contain nitrosamines,<sup>66–70,134,135</sup> the levels of which are related to the nitrate content of the processed tobacco.<sup>67,134</sup>

Considering the smoke first, research workers initially concerned themselves with the volatile *N*-nitrosamines present therein. In early reports, methodology included the reduction of suspected nitrosamines (with LiAlH<sub>4</sub>, e.g.) to hydrazines, reaction of the latter with an appropriate aromatic aldehyde (e.g., 5-nitro-2-hydroxybenzaldehyde) to yield the corresponding hydrazones, separation of these by thin layer chromatography, and identification by ultraviolet absorption spectroscopy.<sup>136</sup> In subsequent methods, the resulting hydrazones (of 3,5-dinitrobenzaldehyde) were analyzed by gas chromatography.<sup>16,134</sup> Mass spectroscopy has also been utilized, and nitrosamine levels in the smoke have been calculated on the basis of the abundance of the molecular ions.<sup>17,167</sup> Recent studies in our laboratory with the "thermal energy analyzer" (TEA) have shown this detection system to be highly sensitive for the determination of nitrosamines in tobacco smoke (detection limit for dimethylnitrosamine: <10<sup>-10</sup> g).

Volatile nitrosamines in the smoke are present in parts per billion quantities, and their levels have been shown to be dependent on the levels of total volatile bases and of nitrate in the tobacco.<sup>17,181</sup>

Although the presence of *N*'-nitrosornicotine (Figure 9, LV) in the smoke has long been suspected,<sup>3</sup> only recently has its presence been actually demonstrated.<sup>70,137,173</sup> It has been shown to arise during processing either from nicotine or nornicotine in the leaf but because of the preponderance of the former alkaloid, nicotine is probably the major precursor.<sup>137,198</sup>

More surprising, however, are the relatively large quantities of *N*'-nitrosornicotine found in commercial tobacco (0.3–88.6

TABLE XII. Amides

	Tobacco ref	Smoke ref
Acetamide		142
1-Acetylimidazole	53	
<i>N</i> '-Acyl alkaloids <sup>a</sup>		
Asparagine	101, 102	
Caffeoylputrescine	4, 180	
<i>p</i> -Coumaroylputrescine	180	
Diethanolamides of fatty acids <sup>b</sup>		
<i>N,N</i> -Dimethylacetamide	184	
<i>trans</i> -2-Ethyl-3-methylsuccinimide <sup>d</sup>	53	
Feruloylputrescine	180	
Formamide		142
Glutamine	101, 102	103, 104, 113
<i>N</i> -(3-Methylbutyl)acetamide	53	
<i>N</i> -(3-Methylbutyl)formamide	53	
<i>N</i> -(2-Methylpropyl)acetamide	184	
Methylethylmaleimide <sup>d</sup>	53, 47	
<i>N</i> -Methyl-2,2-diphenylacetamide <sup>c</sup>	179	
<i>N</i> -Methylnicotinamide	2	
Nicotinamide	1	113
Propionamide		142

<sup>a</sup> See Table VI, this report. <sup>b</sup> Chemical suckering agents, under trial (143). <sup>c</sup> Degradation product of the herbicide, Diphenamide. <sup>d</sup> See Table III, this report, for other imides.

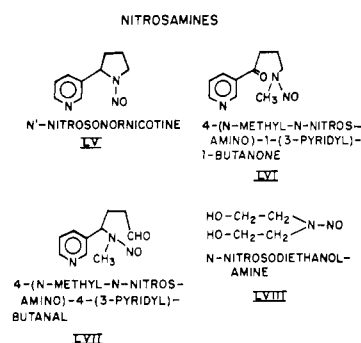


Figure 9. Recently identified tobacco-specific nitrosamines.

ppm),<sup>66,67</sup> and a portion of these quantities is transferred to the smoke as well.<sup>198</sup> It has been suggested that *N*'-nitrosornicotine in leaf is produced by bacterial or enzymatic nitrosation of nicotine (or nornicotine), probably during curing and fermentation.<sup>67</sup> Such a concept also suggests that other *N*-nitrosamines could be formed from various secondary (and tertiary) amines and amino acids (e.g., proline) present in leaf. The synthesis and identification in processed tobacco of 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (LVI) and 4-(*N*-methyl-*N*-nitrosamino)-4-(3-pyridyl)butanal (LVII) suggest that the tertiary amine nicotine serves as precursor for these nitrosamines via openings of the pyrrolidine ring.<sup>190–192</sup> A recent report demonstrated the utility of high-speed liquid chromatography, not only in determining levels of *N*'-nitrosornicotine in leaf, but also in resolving it into its syn and anti isomers, each of which may possess different biological activity.<sup>138</sup> The presence of *N*'-nitrosornicotine in leaf has been confirmed by workers in Japan<sup>69</sup> and Austria.<sup>200</sup> *N*-Nitrosornicotine administered in drinking water induces esophageal tumors in rats<sup>68</sup> and tracheal tumors in Syrian Golden hamsters.<sup>211</sup>

*N*-Nitrosodiethanolamine (LVIII) is another nonvolatile nitrosamine isolated from tobacco.<sup>139</sup> Its presence is related to the use on tobacco of certain commercial formulations of maleic hydrazide (MH-30) which contain diethanolamine. *N*-Nitrosodiethanolamine has been shown to be a hepatic carcinogen in rats.<sup>32</sup>

*N*-Nitrosopiperidine has been claimed to be present in Burley

TABLE XIII. Amino Sugars

	Tobacco ref
1-Deoxy-1-L-alanino-D-fructose	147, 148, 218
1-Deoxy-1-( <i>N</i> - $\gamma$ -aminobutyric acid)-D-fructose	147, 148, 218
1-Deoxy-1-asparaginofructose	150
1-Deoxy-1-L-phenylalaninofructose	150
1-Deoxy-1-L-prolino-D-fructose	147, 149, 218
1-Deoxy-1-tyrosinofructose	150
1-Deoxy-1-L-valino-D-fructose	150
Galactosamine	145
Glucosamine	146
1-Deoxy-1-L-threonino-D-fructose	216
1-( <i>N,N</i> - $\gamma$ -Aminobutyric acid)-di(1-deoxy-D-fructose)	218

TABLE XIV. Other Organic Nitrogen Compounds

	Tobacco ref	Smoke ref
Chlorophyll	2	
1,1-Dimethylhydrazine	139, 152 (0–150) <sup>a</sup>	
Methyl isocyanate		1
Methyl nitrite <sup>b</sup>		1, 151
Methyl thionitrite		1
Nucleic acids	2	
Phosphatides	2	
Porphyrin		177

<sup>a</sup> Value in parentheses, ng/g (ppb). <sup>b</sup> In aged smoke.

leaf, but this finding needs confirmation.<sup>140</sup> Certainly much more systematic studies are needed with regard to chemical assays of tobacco-specific N-nitrosamines.

### IX. Amides

Relatively few amides have been reported in tobacco and tobacco smoke, although if one considers certain amino acids, N-acyl nornicotines and various agricultural chemicals, the list (Table XII) might be somewhat longer. In Neurath's review<sup>1</sup> of nitrogen compounds, he suggests that 30% of all nitrogen compounds in the smoke are neutral nitrogen compounds. This would include amides, and on this basis, there are many more yet to be found. Indeed, at the 1975 Tobacco Chemists' Research Conference, numerous new amides were reported<sup>6,7</sup> (vide infra).

The C<sub>1</sub>–C<sub>3</sub> aliphatic amides (0.12–0.37% of particulate matter) identified in the smoke are believed to form during the smoking process, in as much as they are not found in unsmoked tobacco.<sup>142</sup> The secondary amides reported in tobacco leaf could likely degrade to simpler, primary amides during tobacco combustion. This remains to be demonstrated. However, in experiments in which nitrate-<sup>15</sup>N was incorporated into experimental cigarettes, and the latter were smoked, <sup>15</sup>N was recovered in the C<sub>1</sub>–C<sub>3</sub> aliphatic amides. Similar results were obtained when glycine-<sup>15</sup>N was utilized instead of nitrate-<sup>15</sup>N.<sup>144</sup> The amides were derived from intermediary ammonia which was generated by the nitrate and glycine during the smoking process. It may be possible, too, that during smoking the numerous nitriles present in the smoke are partially converted to amides (via hydrolysis), or perhaps the reverse may occur, that is, the dehydration of amides to nitriles. One can speculate also that the maleimide and succinimide reported in leaf are degradation products of the chemical suckering agent, maleic hydrazide, commonly used on tobacco crops, and that more such compounds (i.e., other alkyl maleimides and succinimides) are likely to be found.<sup>184</sup> So far nitrosamines have not been reported in tobacco products. Some of these compounds are converted in vivo to reactive species without intervening enzymes.

TABLE XV. Inorganic Nitrogen Compounds

	Tobacco ref	Smoke ref
Ammonia	1 (0.14) <sup>a</sup>	155 (67, 131) <sup>b</sup>
Cyanogen		1, 188
Hydrazine	154	154 (0.03)
Hydrogen cyanide		1 (100–400)
Nitric acid <sup>c</sup>		151
Nitric oxide		1 (0–600)
Nitrogen	1	1
Nitrogen dioxide <sup>c</sup>		1 (0–10)
Nitrous oxide		1
Thiocyanic acid		1
Thiocyanogen		1

<sup>a</sup> Value in parentheses, % of leaf. <sup>b</sup> Values in parentheses,  $\mu$ g/cigarette. <sup>c</sup> In aged smoke.

### X. Amino Sugars

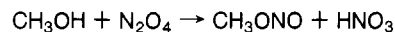
Amino acid (and amino) sugars found in tobacco<sup>218,219</sup> are listed in Table XIII. These are believed to be intermediates in the production of browning pigments in the Maillard reaction.<sup>199</sup> The total content of amino acid–sugar compounds in flue or sun-cured tobacco may be more than 2% of the dried leaf weight.<sup>2,4</sup>

In addition to forming via reactions between sugars and amino acids, amino sugars may be degradation products of glycoproteins reported to be present in green leaf.<sup>116</sup>

### XI. Miscellaneous N-Containing Compounds

#### A. Nitrite Esters, Inorganics, and Others

These include two nitrite esters, methyl nitrite, and methyl thionitrite (Table XIV). Methyl nitrite is not a product of fresh smoke, but arises as the smoke ages.<sup>151</sup> This has been shown by the use of rapid-scan Fourier-transform infrared spectroscopy which indicates that methyl nitrite forms by the following route:



Note that nitric acid is a product of the reaction as well. Oxygen is necessary for the reaction to proceed in tobacco smoke as it oxidizes the NO produced during smoking to NO<sub>2</sub> or N<sub>2</sub>O<sub>4</sub>.<sup>151</sup>

1,1-Dimethylhydrazine has been isolated from tobacco leaf. Its source has not yet been determined, but it may arise via bacterial or enzymatic action.<sup>152</sup> It has been shown to be tumorigenic in the experimental animal.<sup>153</sup>

Other N-containing compounds, inorganic in nature, are listed in Table XV. Of course, gaseous nitrogen makes up the bulk of the smoke (59%). [Nitrogen is present in the vapor phase of cigarette smoke. In one study this phase comprises about 92% of the smoke (500 mg) generated by a cigarette.<sup>3</sup> The particulate phase (or "tar") accounts only for 8% of the smoke, but contains the bulk of the compounds cited in this review.<sup>212</sup>] The oxides of nitrogen induce lesions in the lungs of experimental animals.<sup>9,14,14a</sup> However, a recent study questions the possibility that they are correlated with the induction of emphysema.<sup>14b</sup> Nitrogen oxides may play a role in nitrosamine formation in cigarette smoke.<sup>132</sup> As noted above, NO<sub>2</sub> is not present in fresh smoke but forms as the smoke ages.<sup>151</sup> Hydrogen cyanide, as well as cyanogen, was already discussed in the section on nitriles.

Hydrazine has been found in both leaf and smoke.<sup>154</sup> Its presence was initially believed to be due to the use of maleic hydrazide (MH-30) on tobacco.<sup>156</sup> However, recent studies indicate that it may arise from other sources,<sup>154</sup> such as amino acids and peptides. Like 1,1-dimethylhydrazine, it is tumorigenic in the experimental animal.<sup>157</sup>

**TABLE XVI. N-Containing Agricultural Chemicals in Use or under Test for Use on Tobacco<sup>4</sup>**

Acrylonitrile (vinyl cyanide)
Benefin ( <i>N</i> -butyl- <i>N</i> -ethyl- $\alpha,\alpha,\alpha$ -trifluoro-2,6-dinitro- <i>p</i> -toluidine)
Carbaryl (Sevin; 1-naphthyl <i>N</i> -methylcarbamate)
Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate)
Cotinine [1-methyl-2-(3'-pyridyl)-5-pyrrolidone]
Diazinon [ <i>O,O</i> -diethyl <i>O</i> -(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorothioate]
Dimethoate [ <i>O,O</i> -dimethyl <i>S</i> -( <i>N</i> -methylcarbamoyl)methyl phosphorodithioate]
Dinocap [crotonic acid 2-(1-methylheptyl)-4,6-dinitrophenyl ester]
Diphenamide ( <i>N,N</i> -dimethyl-2,2-diphenylacetamide)
Dyrene [2,4-dichloro-6-( <i>o</i> -chloroanilino)-5-triazine]
Fermate (Ferbam; ferric dimethyl dithiocarbamate)
Guthion [ <i>O,O</i> -dimethyl <i>S</i> -[4-oxo-1,2,3-benzotriazin-3(4 <i>H</i> )-yl] methylphosphorodithioate]
Hydrogen cyanide (HCN)
Maleic hydrazide [6-hydroxy-3(2 <i>H</i> )-pyridazinone]
Maneb [manganese ethylenebis(dithiocarbamate)]
Nicotine [1-methyl-2-(3'-pyridyl)pyrrolidine]
Parathion ( <i>O,O</i> -diethyl <i>O-p</i> -nitrophenol thiophosphate)
Pebulate (Tillan; <i>S</i> -propyl butylethylthiocarbamate)
Penar (7D-248; dimethyl dodecylamine acetate salt)
Phosphamidon (2-chloro- <i>N,N</i> -diethyl-3-hydroxycrotonamide)
Telone (Drw-M-2680; dichloropropene, 85%; chloropicrin, 15%)
Vernolate ( <i>S</i> -propyl dipropylthiocarbamate)
Vorlex (methyl isothiocyanate, 20%, chlorinated C <sub>3</sub> hydrocarbons, 80%)
Zineb [zinc ethylenebis(dithiocarbamate)]

## B. Pigments

The brown pigments formed during curing are polymeric polyphenols which provide the characteristic color to cured tobacco.<sup>2,116</sup> They comprise 0.3 to 4% of the weight of dry leaf depending on the method of extraction. They are included in this review inasmuch as they contain nitrogen in the form of protein (?), amino acids, alkaloids, and other pyridine derivatives. The presence of protein in the pigment structure is inferred from the finding of 18 to 20 amino acids upon hydrolysis.<sup>116</sup> Alkaloids and pyridines are released from pigment after alkaline fusion<sup>117</sup> or pyrolysis.<sup>33,64</sup>

Similar high molecular weight pigments have been isolated from smoke.<sup>2,117</sup> These contain the same moieties as the leaf pigment but have a much higher alkaloid content and a much higher molecular weight (100 000 vs. 30 000). They have been suggested by one group to comprise up to 4% of the smoke condensate<sup>2,116</sup> and have been shown to possess no tumorigenic activity.<sup>183</sup>

A postulation as to the origin of the smoke pigment has been made. A sharp thermal gradient is known to exist behind the coal of a burning cigarette; in the hot zone directly behind the coal, cellular eruption occurs with the resulting expulsion of the cellular contents, including leaf pigment, into the aerosol stream. These cellular particles serve as nuclei for further aerosol formation. Alternatively, they may be adsorbed on to preformed nuclei. During these processes, the leaf pigment also reacts with the volatile bases (and alkaloids) and other materials known to be present in the smoke, increasing in molecular weight and acquiring the other characteristics of smoke pigment.<sup>2,168</sup>

## C. N-Containing Agricultural Chemicals

N-Containing agricultural chemicals currently in use on tobacco, or being contemplated for such use, are given in Table XVI, a rather long list. These will certainly contribute N-containing compounds to tobacco and its smoke, not only by their mere presence, but by undergoing degradation (enzymatic or thermal) to new products. This is an area of research which requires more study. For the most part, we can only list these chemicals.

**TABLE XVII. Residues of Pesticides on Commercial Tobacco (ppm) 4,163,165**

	Residue	Tolerance
Carbaryl	<0.3, 1.5 <sup>b</sup>	10, 2.5 <sup>a</sup>
Guthion		2
Dimethoate	0-0.1	0.6 <sup>a</sup>
Phosphamidon	0.05	0.15 <sup>a</sup>
Dithiocarbamates	40-100 <sup>c</sup>	3.0 <sup>a</sup>

<sup>a</sup> Tentative, proposed by Federal Republic of Germany. <sup>b</sup> Flue-cured, Georgia Market, 1971. <sup>c</sup> Non-U.S. tobaccos (1969-1972).

However, maleic hydrazide (MH, IX) has undergone pyrolysis studies, and has been shown thereby to give rise to small amounts of hydrazine, ammonia, pyridines, aniline, toluidines, quinolines, and naphthylamines.<sup>154,158-161</sup> Penar (*N,N*-dimethyldodecylamine) has undergone similar studies and has been shown to generate similar products.<sup>158</sup> Whether these degradation products are generated during the actual processing or combustion of tobacco remains to be demonstrated.

Nitrosocarbaryl, a nitrosation product of the pesticide Carbaryl, has been shown to be a potent mutagen.<sup>162</sup> Although the nitroso product was formed under experimental conditions,<sup>162,172</sup> the possibility of its forming following application to crops (i.e., tobacco) should be considered. The same reasoning holds for other chemicals listed.

Besides the active principle contained in a pesticide (or herbicide) package, other substances, such as surfactants, solubilizing agents, and "inert" carriers make up the formulation. Maleic hydrazide (MH-30), for example, is partially formulated as the diethanolamine salt. This latter compound can itself give rise to new compounds during tobacco processing or combustion (e.g., *N*-nitrosodiethanolamine (LVIII)).<sup>152</sup>

Irrespective of the above observations, there is available relatively little information on the fate of N-containing agricultural chemicals after their application to tobacco. Studies have indicated that more than 75% of carbamates (e.g., Carbaryl) applied to tobacco could be lost during curing at temperatures of 150 °F (66 °C).<sup>174</sup> However, milder conditions prevail during the curing of Burley and cigar tobaccos. The problem of pesticide residues on tobacco is a difficult one because of the large surface area of the leaf and the appreciable loss of water during the curing. These factors are responsible for the still measurable residues on tobacco going into commercial sales. During the smoking of cigarettes, 95% of the Carbaryl and Guthion present in the tobacco is dissipated into the smoke. No information is available as to what form, however. Oxyguthion, an oxidation product of Guthion, appears during curing.<sup>165</sup> During combustion, carbamate insecticides are claimed to be essentially degraded at 800 °C.<sup>4,174</sup> The nature of the degradation products and the effects of the lower temperatures prevalent in a smoldering cigarette still need to be determined. Similar studies with carbofuran and its hydroxylated metabolite 3-hydroxycarbofuran indicate that less than 1% is transferred intact into the smoke.<sup>171</sup> According to studies with TDE and DDT (chlorinated insecticides), one may speculate that in the past, through the inhalation of smoke from one pack of cigarettes, one received an amount of pesticide equivalent to that consumed during the daily intake of food.<sup>4,174</sup> Some additional data on residues of N-containing pesticides on commercial tobaccos are summarized (Table XVII).<sup>4,163</sup>

The fungicide Dyrene, a chlorinated anilino-triazine, apparently does not reduce greatly in storage. In experimental cigars, 1% of Dyrene was reported transferred into mainstream smoke, and 2-chloroaniline was isolated from the smoke of such cigars containing Dyrene.<sup>165</sup> Maleic hydrazide was found in cured tobacco in levels ranging from 20 to 30 ppm, 4 to 10% of which was transferred into mainstream smoke. Earlier reports suggested that maleic hydrazide was tumorigenic, based on ex-

periments with male, new-born mice.<sup>169</sup> However, this has been disputed (because the technical product contained 0.4% of hydrazine), and confirmation of the finding still appears to be lacking.<sup>170</sup> The residue level of Penar on tobacco has not been firmly established because Penar may form a "complex" with plant constituents. However, it does appear in smoke following combustion of Penar-treated tobacco.<sup>4</sup>

It is clear from the above that the role of agricultural chemicals in influencing the chemical composition, biological activity and flavor of tobacco products will need continuous study, and all the more so as new formulations are introduced into use by farmers. Thus, the task is truly enormous, since we are still learning, albeit very little, about the effects of established formulations.

### XII. Conclusion

In the foregoing, we have presented a status report on the nitrogen-containing compounds known to be present in tobacco and tobacco smoke. By count alone, these came to over 400 compounds, several of which possess biological activity (Table XVIII). However, there are many more that we are aware of but cannot report on definitely because they constitute unpublished data. At the 1975 meeting of the Tobacco Chemists' Conference, for example, the group at R. J. Reynolds Tobacco Co. reported the presence of over 600 newly identified compounds in tobacco smoke. Of these, some 233 were N-containing compounds. So, if one assumes these have not been reported previously to occur in the leaf, then to the compounds described herein must be added 48 amides, 22 imides, 30 pyridine derivatives, 26 imidazoles, 31 lactams, and 27 miscellaneous N-heterocyclic compounds from the water-soluble portion of cigarette smoke particulate phase. Still more were identified in the ether-soluble portion of the particulate phase; these include 8 nitriles, 31 N-heterocycles, and classes of new compounds represented by the following: 2-ethylmaleimide, *N*,3-dimethylsuccinimide, 2-isopropylsuccinimide, phthalimide, 3,4-dimethylphthalimide, 3-methylphthalimide, 4-methylphthalimide, 2,4-dimethylbenzoxazole, 2-methylbenzoxazole, and *N*-methylimidazole. Therefore, the actual total of N-containing compounds (out of 2200 total) comes to over 600 (or 27% of the total).

Of course, as time passes, additional compounds will be identified as newer techniques and instrumentation come into use such as rapid scan Fourier-transform infrared spectroscopy, for example, and the thermal energy analyzer for analysis of N-nitrosamines. It is difficult at this time to speculate on what specific new compounds or classes of compounds remain to be identified. With the preponderance of secondary amines in tobacco and tobacco smoke, one would expect a variety of heretofore unexamined N-nitrosamines to come under close scrutiny, such as N-aryl-N-alkyl-N-nitrosamines. Other classes of compounds that deserve further attention are the nitroalkanes, nitroarenes, and nitrophenols and the numerous nitriles that seem to pervade tobacco smoke. In addition, the new classes of compounds (i.e., phthalimides, lactams, maleimides, imidazoles, and benzoxazoles) reported by the Reynolds' group to be in the smoke also deserve further attention. How these are formed and how they contribute to the biological activity of the smoke need to be considered. Certainly, one must not ignore the numerous N-containing agricultural chemicals that are applied to tobacco. How these are transferred to the smoke, if intact or degraded, and how in the final analysis they affect the smoker are still areas of needed study.

The present review has not attempted to cover flavorants and other additives that manufacturer's add to their tobacco products to make them more appealing to current or potential customers. Many of these materials may contain nitrogen (e.g., pyrazines); however, they are considered "trade" secrets and are not generally known. Obviously, they and their degradation products need examination as to their possible role in tobacco toxicity.

TABLE XVIII. Some Biologically Active N-Containing Compounds in Tobacco/Tobacco Smoke<sup>a</sup>

Compounds	Activity
Ammonia	Inflammatory agent to eye, upper respiratory tract, irritant
Dibenz[ <i>a,h</i> ]acridine	Tumor initiator (animal)
Dibenz[ <i>a,j</i> ]acridine	Tumor initiator (animal)
Dibenzo[ <i>c,g</i> ]carbazole	Tumor initiator (animal)
Hydrazines	Animal carcinogens
Hydrogen cyanide, cyanogen	Respiratory enzyme poison
N-Alkyl carbazoles	Tumor accelerators (animal)
N-Alkyl indoles	Tumor accelerators (animal)
2-Naphthylamine	Bladder carcinogen (in man)
Nicotine and other alkaloids	Act on central nervous system (CNS), LD <sub>50</sub> in man, orally: 30-60 mg
Nitrogen oxides	Inflammatory agents, irritants, affect respiratory tract
N-Nitrosamines	Animal carcinogens
Pyridine and related compounds	CNS depressant, skin and respiratory tract irritant
Quinoline	Animal carcinogen (rat liver)
Tyramine	Vasoactive agent

<sup>a</sup> Data obtained from various sources including the "Merck Index", 8th ed, Merck and Co., Inc., Rahway, N.J., 1968: "Toxicology, The Basic Science of Poisons", L. J. Casarett and J. Doull, Ed., Macmillan, New York, N.Y., 1975, and various references herein; ref 3, 9-14, 29, 30, 38, 68, 97, 153, and 185.

We hope to have shown that the nitrogen-containing compounds in tobacco products are not only of interest to the tobacco chemist and flavor specialist, but of more general interest because of their chemistry (e.g., modes of formation and degradation) and their contributions to the biological activity of tobacco products. The latter phenomenon alone should encourage scientists to continue the study of biologically active compounds in tobacco, tobacco smoke, and related materials and, if needed, their reduction in these major consumer products with public health implications.

### XIII. Addendum

This review stressed the role that pyrolysis and other thermal reactions play in the formation of various nitrogen-containing compounds found predominantly in the smoke and secondarily in the leaf. Two recent papers<sup>215,216</sup> and others already cited herein have, on the other hand, considered the importance of nonenzymatic browning reactions in the formation of nitrogen-containing flavor compounds, especially in the leaf. These reactions occur during various stages of processing, usually between sugars and amino compounds, and ultimately lead to such flavor compounds as pyrazines and pyrroles. These are then presumably transferred into the smoke as such. It should be stated, however, that the relationship between levels of nitrogen-containing compounds (e.g., amino acids) and sugars and tobacco leaf quality has long been known, and it is only recently that tobacco chemists have been studying their interaction in more detail. Flavor compounds are obviously becoming more important as new cigarettes are developed. These cigarettes are modified in some way, or contain less actual tobacco, or specially processed tobacco, and therefore require flavor enhancement.<sup>221</sup>

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