

similar low-molecular-weight volatiles were produced but were not trapped on the system we used.

#### CONCLUSIONS

In previous reports of chemical analyses, 70% of ammoniated, nonradioactive aflatoxin B<sub>1</sub> was unaccounted for in a model system (Lee et al., 1974; Cucullu et al., 1976). When nonradioactive aflatoxin B<sub>1</sub> was added to peanut meal before ammoniation, the detection of conversion product was even lower, with 99% of the original aflatoxin B<sub>1</sub> unaccounted for after ammoniation (Lee et al., 1978). In the current report, in which radioactive aflatoxin was used, we accounted for 45–50% of the added toxin. Thus, use of labeled aflatoxin B<sub>1</sub> significantly improves the monitoring of its degradation by ammoniation over chemical assays alone. Our results offer sound evidence that the nonvolatile products of ammoniation of B<sub>1</sub> (which include B<sub>1</sub> unchanged by ammoniation) are associated with nonprotein particles. Degradation products are either loosely bound to meal particles and readily soluble in methanol or more tightly bound and not solubilized by methanol. Loss of activity due to volatilization of aflatoxin was not conclusively proven as only a small part of the radioactivity was detected in the volatiles trapped after ammoniation. This report, however, offers the first experimental evidence that some aflatoxin B<sub>1</sub> is reduced to volatiles during ammoniation. Since aflatoxin B<sub>1</sub> is a potent toxicant and carcinogen, further experiments are warranted to effectively trap and determine the nature of the volatiles produced by ammoniation of B<sub>1</sub>. More sophisticated studies will have to be designed to determine the nature of binding of aflatoxin or aflatoxin fragments to meal constituents.

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## Chemical Studies on Tobacco Smoke. 63. On the Fate of Nicotine during Pyrolysis and in a Burning Cigarette

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Products obtained from the thermal degradation of [<sup>14</sup>C]nicotine in a combustion tube (under pyrolytic conditions) and in a cigarette (undergoing machine smoking) were examined by gas-liquid chromatography (GLC), by GLC-mass spectrometry, and by radiochromatography. Under pyrolytic conditions in a combustion tube, nicotine underwent extensive degradation to pyridines, quinolines, aryl nitriles, and aromatic hydrocarbons. In contrast, in a burning cigarette, a substantial portion of nicotine remained intact (≈41%), 12.5% underwent oxidation to CO<sub>2</sub>, up to 11% was degraded to volatile pyridine bases, and negligible amounts were converted to neutral or acidic particulate components. A major portion of nicotine and its degradation products was also diverted to sidestream smoke. These results suggest to us that pyrolysis experiments may be of limited value for establishing the fate of nicotine and possibly other tobacco components in a burning cigarette.

Nicotine and compounds derived from it contribute significantly to the organoleptic nature and biological activity of cigarette smoke (Schmeltz, 1971). Therefore, it is important to determine the fate of this major tobacco alkaloid during smoking. Previous studies with endoge-

nous or exogenous nicotine in cigarettes have demonstrated that a substantial portion transfers intact into the smoke stream (Houseman, 1973; Jenkins et al., 1976; Schmeltz et al., 1978). The remaining portion of nicotine undergoes thermal degradation, the extent of which has been alluded to in pyrolysis studies with nicotine (Woodward et al., 1944; Jarboe and Rosene, 1961; Schmeltz et al., 1972; Schmeltz and Hoffmann, 1977) and in studies of smoke from cigarettes containing added nicotine (Bush et al., 1972) or nornicotine (Glock and Wright, 1962). Nevertheless, such studies did not fully describe the pathway of nicotine in a burning cigarette, where several complex processes occur

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simultaneously. Moreover, the recently described pyrolysis system that simulates smoking (Higman et al., 1977) has not demonstrated that nicotine will behave even in this setting in the same manner as in a burning cigarette. The present study was undertaken to elucidate the fate of nicotine in cigarettes undergoing actual smoking. The use of cigarettes containing  $^{14}\text{C}$ -labeled nicotine provides a more realistic representation of the fate of this alkaloid during cigarette smoking.

#### EXPERIMENTAL SECTION

**Apparatus.** The syringe-type applicator for impregnating cigarettes with radioactive materials was developed at Oak Ridge National Laboratories, Oak Ridge, TN. Its use has been previously described (Hoffmann et al., 1977). Cigarettes were smoked on a Phipps and Bird single port smoking machine. Both mainstream and sidestream smoke were collected utilizing a specially designed smoking chamber (Liu et al., 1974) and trapping system (Marmor and Minnemeyer, 1975).

Gas chromatography (GLC) was performed on a Hewlett-Packard Model 5710A with flame ionization detector. This was interfaced with a Packard gas proportional counter in order to monitor the radioactivity in GLC peak effluents. Radioactive zones on thin-layer chromatographic (TLC) plates were detected in a Packard radiochromatogram scanner, Model 1201. Other radioactive materials were counted in a Nuclear-Chicago Isocap 300 liquid scintillation counter. Gas liquid chromatography-mass spectrometry (GLC-MS) was run on a Hewlett-Packard System (Model 5980 MS in tandem with a Model 5710 GLC and a Model 5393A computer). High-pressure liquid chromatography (LC) was accomplished on a Waters Associates Model ALC/CTCP-202, equipped with a 6000A solvent delivery system, a U6K septumless injector, and a 440 UV/visible detector.

**Reagents.** Organic solvents were spectrograde; other chemicals were reagent grade. Counting solutions were Tolucent I (ICN Pharmaceuticals, Inc.) for radioactive materials soluble in organic solvents and Aquascent I (ICN Pharmaceuticals, Inc.) for water-soluble radioactive materials.  $[2\text{-}^{14}\text{C}]\text{Nicotine}$  (sp act., 54.01 mCi/mmol, >97% pure) was obtained from New England Nuclear, Inc.; and  $[\text{methyl-}^{14}\text{C}]\text{nicotine}$  (sp act., 25 mCi/mmol, 99% pure) from ICN Pharmaceuticals, Inc. Randomly labeled  $^{14}\text{C}$  nicotine was isolated from tobacco grown in a  $^{14}\text{CO}_2$  atmosphere (vide infra; Schmeltz et al., 1978). Purity of  $^{14}\text{C}$  materials was ascertained by GLC and TLC.  $^{14}\text{C}$ -Nicotine tartrate was prepared by reacting equivalent amounts of tartaric acid and  $^{14}\text{C}$  nicotine in aqueous solution.

**Counting Procedures.** These have been described elsewhere (Schmeltz et al., 1978). Additional procedures in the present study included the use of a gas proportional counter in tandem with a GLC. Effluents from the exit port of the latter were led into the counter via a heated transfer line. For purposes of quantification (or calibration of radioactive peaks),  $^{14}\text{C}$  reference materials (of known activity) were injected into the combined GLC counter, and the peaks attributable to radioactivity were triangulated.

**Chromatographic Procedures.** The basic fraction as well as other fractions from tobacco leaf, tobacco smoke, and nicotine pyrolyzates were monitored by various chromatographic techniques including GLC (3.05 m  $\times$  3.3 mm o.d. 4% Carbowax 20M, at 80  $^\circ\text{C}$  for 4 min, and then programmed at 8  $^\circ\text{C}/\text{min}$  to 200  $^\circ\text{C}$ ), LC (30 cm  $\times$  3.9 mm i.d.  $\mu$ -Porasil with a solvent system of methanol/ $\text{NH}_3/\text{CHCl}_3$ , 3:0.5:96.5, at a flow rate of 2 mL/min; nicotine

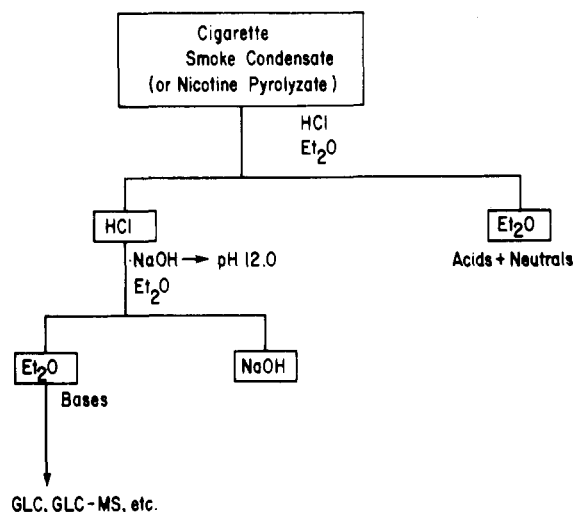


Figure 1. Fractionation scheme for  $^{14}\text{C}$ nicotine pyrolyzates and for smoke condensate from cigarettes containing  $^{14}\text{C}$ nicotine.

retention time: 6.2 min), and TLC (Schmeltz et al., 1978).

**Isolation of Randomly Labeled Nicotine.** Randomly labeled  $^{14}\text{C}$ nicotine was isolated from tobacco plants exposed to an atmosphere of  $^{14}\text{CO}_2$  for a period of 38 days. Following extraction as described (Schmeltz et al., 1978), it was purified by either TLC or LC.

**Application of  $^{14}\text{C}$ Nicotine to Cigarettes, Smoking, and Fractionation of Condensate.** Prior to being smoked, commercial 85 mm, nonfilter (U.S.) cigarettes were modified by the addition, via syringe (Liu et al., 1974; Hoffmann et al., 1977), of  $3 \times 10^6$  to  $4 \times 10^6$  dpm of  $^{14}\text{C}$ nicotine per cigarette as the tartrate in 10  $\mu\text{L}$  of methanol. The radioactivity was distributed throughout the cigarette from the tip (burning end) to a point 23 mm from the butt end of the cigarette. The treated cigarettes were kept at constant temperature-humidity (22  $^\circ\text{C}$ , 60% RH) for 24 h. They were then smoked one at a time under standard conditions. Mainstream and sidestream smoke were trapped in dry ice-acetone. The resulting condensates were washed with 2 N HCl and diethyl ether, and the two solutions were then extracted with each other (Figure 1). The acidic solution containing bases was made alkaline (1 N NaOH to pH 12.0) and was subsequently extracted with diethyl ether to remove liberated organic bases. This ether solution was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated (using a Vigreux column). Aliquots (1–3  $\mu\text{L}$ ) of the concentrated ether solution (100–250  $\mu\text{L}$ ) were then monitored by injection into a GLC interfaced with a gas proportional counter.

**Pyrolysis.** Pyrolysis was accomplished by first adsorbing an appropriate amount ( $8 \times 10^6$  to  $10 \times 10^6$  dpm) of  $^{14}\text{C}$ nicotine onto 1 g of silica gel or by combining the nicotine as the tartrate with 1 g of tobacco and then inserting either the silica gel or tobacco matrix into a combustion tube preset to the desired temperature. The pyrolysis products carried along in  $\text{N}_2$  (44 mL/min), passed through a series of four traps, the first cooled in ice-water, and the second and third in dry ice-acetone. The last trap contained aqueous 2 N HCl through which the product stream was bubbled to insure collection of relatively volatile organic bases. Fractionation of pyrolytic products was identical with that used for smoke condensate.

#### RESULTS AND DISCUSSION

In this study, we compare the fate of nicotine in an experimental pyrolysis system and in a cigarette undergoing machine smoking. In the former situation, when the nicotine is inserted into the pyrolysis tube, it experiences

Table I. Pyrolysis of [<sup>14</sup>C]Nicotine from a Silica Gel Matrix

products	% of initial radioactivity <sup>a</sup> from:					
	[methyl- <sup>14</sup> C]-nicotine			[2'- <sup>14</sup> C]-nicotine		
	600 °C	750 °C	900 °C	600 °C	750 °C	900 °C
pyridine	0.2	1.4	0.4	0.7	2.0	0.6
2-picoline			0.1			0.1
3-picoline	0.7	0.7		6.1	1.2	0.2
4-picoline						
2-vinylpyridine <sup>b</sup>		0.3	0.4		0.4	0.3
3-vinylpyridine	2.6	0.8		7.9	0.4	0.7
3-cyanopyridine	0.7			6.4		
2-phenylpyridine <sup>b</sup>		0.2	0.2	0.3	0.1	0.2
2,2'-bipyridyl <sup>b</sup>	2.8		0.1	1.3		
nicotine	7.7					
quinoline	0.8	0.7	2.1	4.7	1.4	1.0
isoquinoline	0.3	0.3	0.1	0.8	1.0	0.4
8-methylquinoline <sup>b</sup>						
6-methylquinoline <sup>b</sup>			0.2		0.1	0.4
4-methylquinoline <sup>b</sup>						
dimethylquinoline <sup>b</sup>						
cyanoquinoline		0.1				0.2
benzo[h]-quinoline		0.1	0.2			0.2
benzo[f]-quinoline <sup>b</sup>			0.1			
acridine <sup>b</sup>			0.3			0.1
azaacridine <sup>b</sup>		0.1	0.1		0.1	
benzonitrile	0.1	3.4	6.1	2.0	2.2	12.8
tolunitrile <sup>b</sup>		0.7	1.1	0.1	0.3	1.6
naphthonitrile		0.7	0.9		0.7	
benzene				4.5	15.0	4.0
toluene	0.2					
styrene			1.2	0.7	2.1	1.0
phenylacetylene <sup>b</sup>					0.9	0.5
benzylacetylene <sup>b</sup>			0.2			
methylphenylacetylene <sup>b</sup>			1.0		12.8	0.6
naphthalene		1.6	7.5	0.5	0.8	7.1
methyl-naphthalene <sup>b</sup>	0.7		0.2			0.3
biphenyl		0.4				0.2
ace-naphthylene						0.4
acenaphthene		0.2	1.0	0.3		0.4
fluorene <sup>b,c</sup>					0.2	
anthracene			0.6			
indole		0.1	0.9		0.2	
azaindole				0.3		

<sup>a</sup> Where no value is given, percent radioactivity is <0.1. Other products (percent radioactivity, <0.1) characterized in nicotine pyrolyzates in this study include: 3-ethylpyridine, methylvinylpyridine, methylcyanopyridine,<sup>b</sup> azaquinoline, methylazaquinoline,<sup>b</sup> and dimethylbenzonitrile.<sup>b</sup> The degree of variability in determinations of radioactivity in triplicate analyses is maximally ±10% (relative standard deviation). <sup>b</sup> Newly identified pyrolysis products of nicotine. <sup>c</sup> Elutes with phthalonitrile.

a very rapidly increasing temperature gradient. The effects observed are due essentially to the upper temperature, which is reached very nearly instantaneously and maintained throughout the tube. This is evident by noting the differences in product composition (Tables I and II; Jarboe

Table II. Pyrolysis of [2'-<sup>14</sup>C]Nicotine<sup>a</sup> from a Tobacco Matrix at 600 °C

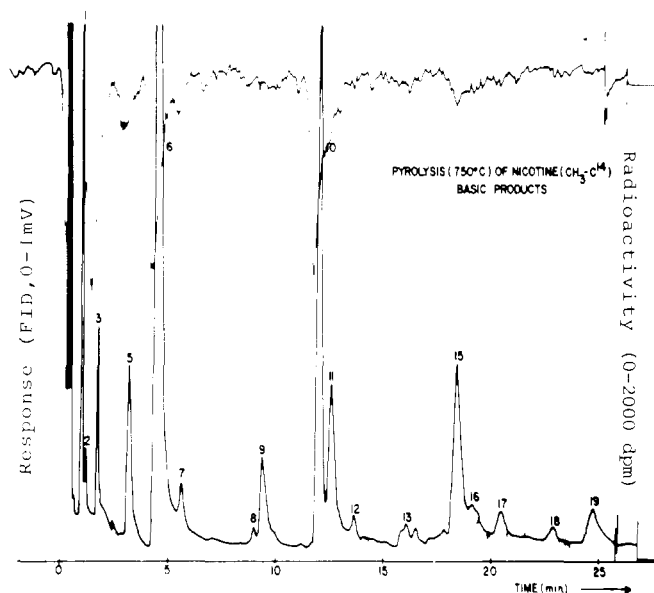
products	radioactivity, <sup>b</sup> %
pyridine	<0.1
2-picoline	0.1
3-picoline }	
4-picoline }	0.3
2-vinylpyridine	0.1
3-vinylpyridine	17.4
3-cyanopyridine	<0.1
3,2'-bipyridyl	0.3
nicotine	2.4
myosmine	0.3
quinoline	0.6
isoquinoline	<0.1
8-methylquinoline	<0.1
6-methylquinoline	0.3
toluene }	
benzene }	2.9
styrene	0.3
methylphenylacetylene	0.3
naphthalene	2.6
methylnaphthalene	0.4
biphenyl	1.8
methylfluorene	0.5
benzonitrile	2.4

<sup>a</sup> As the tartrate. <sup>b</sup> Percent of initial radioactivity.

and Rosene, 1963) after nicotine is introduced into combustion tubes preset at different temperatures. Were lower temperatures of prime importance, then one would observe little difference, no matter what upper temperatures were reached.

In the case of a cigarette undergoing smoking, the temperature gradient extends over the entire length of the cigarette, from the burning end (combustion zone) to the butt. This temperature gradient is maintained, varying of course with cigarette length and during puffing, until the cigarette is extinguished. In or near the burning cone, nicotine will experience the effects of elevated combustion temperatures, and down the rod it will be exposed to progressively lower temperatures. Thus the resulting smoke stream is expected to contain nicotine as a distillation or "direct transfer" product and other compounds as thermal-degradation products of nicotine. Oxidative reactions will also occur to some extent in a burning cigarette, and so some of the nicotine should be combusted to CO<sub>2</sub> and other oxygenated compounds. Even though pyrolysis studies have traditionally been done in an inert atmosphere, they have nevertheless provided insight into the nature of the thermal degradation products to be expected from a given precursor. In this sense, such studies may be said to approximate the reducing atmosphere prevalent just behind the combustion zone of a cigarette (Keith, 1972).

**Pyrolysis Studies.** Pyrolysis of [2'-<sup>14</sup>C]nicotine and of [methyl-<sup>14</sup>C]nicotine was undertaken at various elevated temperatures in a stream of N<sub>2</sub>. Products collected in the traps were fractionated (Figure 1) and resolved by GLC and GLC-MS. Radioactivity in GLC effluents was monitored by passage of the latter directly through a gas proportional counter (Figures 2 and 3). Products identified included simple pyridines, quinolines, aryl nitriles, and aromatic hydrocarbons. More than 38 compounds were identified in the nicotine pyrolyzates (Tables I and II). Among the pyrolysis products, aryl nitriles and aromatic hydrocarbons were present in fairly substantial amounts and contained relatively high levels of <sup>14</sup>C. As seen in earlier studies (Jarboe and Rosene, 1961), most extensive degradation of nicotine occurs at temperatures above 600 °C. At 600 °C, some nicotine still survives

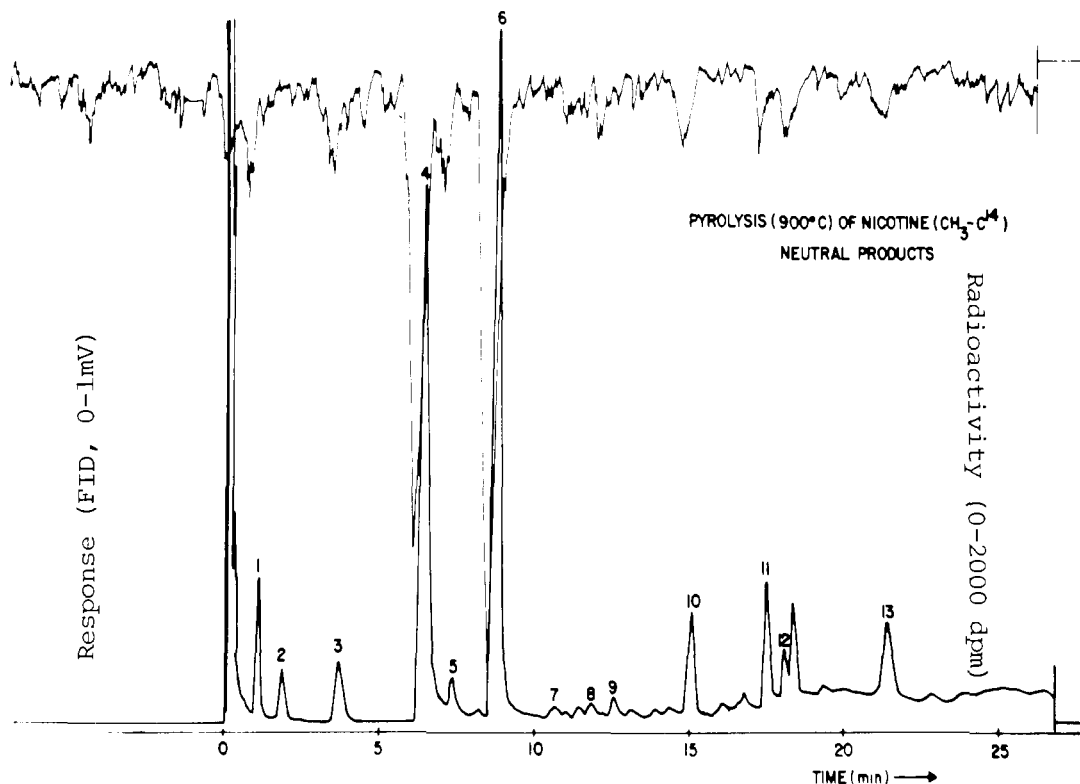


**Figure 2.** Gas-radiochromatogram of basic products from pyrolysis of [ $^{14}\text{C}$ ]nicotine. Peak identities are: (1) pyridine, (2) 2-picoline, (3) 3- and 4-picoline, (4) unidentified, (5) 2-vinylpyridine, (6) 3-vinylpyridine, (7) methylnicotinonitrile, (8) unidentified, (9) benzonitrile, (10) quinoline, (11) isoquinoline, (12) methylquinoline, (13) phenylpyridine, (14) unidentified, (15) indole + azaindole, (16) bipyridyl, (17) cyanoquinoline, (18) benzoquinoline, (19) azaacridine.

pyrolysis, and, in our study, nicotine accounting for up to 8% of the initial radioactivity was found in 600 °C pyrolyzates. This is somewhat lower than that reported previously and was likely due to the pyrolysis of nicotine from silica gel, rather than in the free form. For practical reasons, it was found necessary to adsorb the small amounts of radioactive nicotine available on silica gel prior

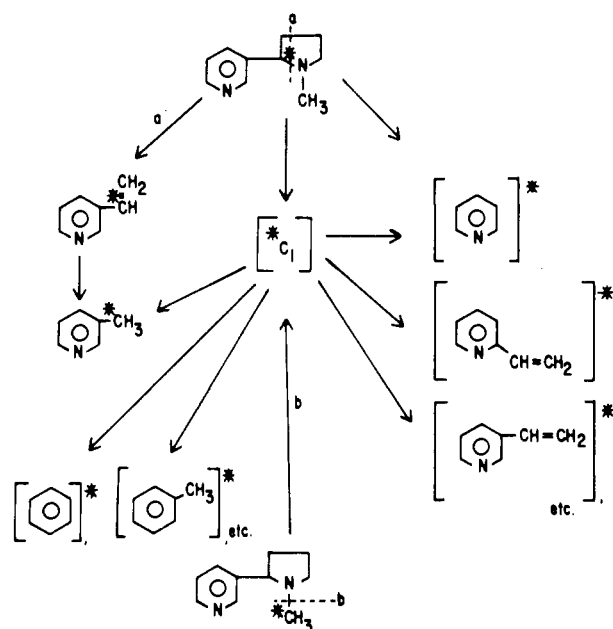
to pyrolysis, and this appears to have influenced the product composition. In addition, at 600 °C from a silica gel matrix, [ $2\text{'-}^{14}\text{C}$ ]nicotine yielded 3-vinylpyridine, 3-picoline, and 3-cyanopyridine with higher levels of incorporated radioactivity than did [*methyl- $^{14}\text{C}$* ]nicotine (Table I), a finding that was not unexpected in view of the possible pyrolytic pathways. Even when pyrolyzing [ $2\text{'-}^{14}\text{C}$ ]nicotine as the tartrate in a tobacco matrix, we noted that extensive degradation still took place (Table II). It is apparent from an examination of the pyrolysis products and the label contained therein that there is no simple pathway from nicotine to products as a result of thermal degradation. In addition, there were relatively high levels of label in the aryl nitriles (especially at the higher temperatures) and in a number of the aromatic hydrocarbons produced. Under pyrolytic conditions there appears to be extensive rupture of the pyrrolidine ring and possibly of the pyridine ring into small fragments containing either carbon and hydrogen or carbon, hydrogen, and nitrogen; these fragments then recombine to form the pyridine and quinoline nuclei, in addition to aryl nitriles and aromatic hydrocarbons.

The pyridine ring of nicotine is apparently not the only source of pyridine formed during the pyrolysis of nicotine. The N- $\text{CH}_3$  carbon and the carbon in the 2' position (pyrrolidine ring) also contribute to the high-temperature formation of pyridine. 1-Methylpyrrolidine, for example, could give rise to the pyridine nucleus via a ring enlargement mechanism by which the N- $\text{CH}_3$  group is incorporated into the ring. In this regard, it is rather interesting that both species of labeled nicotine ( $\text{CH}_3\text{-}^{14}\text{C}$ ,  $2\text{'-}^{14}\text{C}$ ) gave rise to 3- $^{14}\text{C}$ vinylpyridine, attesting to the multiplicity of pathways that operate in the generation of new compounds. The formation of labeled 3-vinylpyridine from [ $2\text{'-}^{14}\text{C}$ ]nicotine likely involves rupture of the pyrrolidine ring in two places, the bond between 1'-N and 2'-C and that between 3'-C and 4'-C. Such bond ruptures could



**Figure 3.** Gas-radiochromatogram of neutral products from pyrolysis of [ $^{14}\text{C}$ ]nicotine. Peak identities are: (1) styrene, (2) benzylacetylene, (3) methylphenylacetylene, (4) benzonitrile, (5) tolunitrile, (6) naphthalene, (7) methylnaphthalene, (8) unidentified, (9) biphenyl, (10) acenaphthylene.

## PYROLYTIC FORMATION OF PYRIDINES AND OTHER COMPOUNDS



**Figure 4.** Postulated pathway for the formation of products and location of label from the pyrolysis of [<sup>14</sup>C]nicotine, via a pool of C<sub>1</sub> fragments. Location of label is assumed, as no degradative studies have been done.

eventually also lead to 3-picoline (Figure 4).

More difficult to rationalize is the pathway by which the labeled methyl group attached to the N of the pyrrolidine ring incorporates into 3-vinylpyridine. A possible explanation would involve the establishment of a pool of labeled C<sub>1</sub> fragments following extensive bond rupture in the [<sup>14</sup>C]nicotine molecule. The labeled C<sub>1</sub> fragments would then be incorporated into the various products. The establishment of a pool of C<sub>1</sub> fragments would also explain the incorporation of label into the aromatic hydrocarbons and nitriles produced (Figure 4). This is analogous to the reported pyrolytic formation of aromatic hydrocarbons from methane (Oro and Han, 1965).

The formation of nitriles from nicotine at higher temperatures suggests that HCN is split out to some extent during thermal degradation, and this scission would likely involve the rupture of bonds on either side of the 2'-C and 1'-N positions (Johnson and Kang, 1971). The HCN generated could then recombine with other fragments to generate nitriles. This is shown by the relatively high levels of label in the benzonitrile generated by [2'-<sup>14</sup>C]nicotine.

Although the pyrrolidine ring appears to rupture extensively, it is difficult at this time to assess the fragmentation in the adjacent pyridine ring. Its aromatic character would argue for its stability. Previous studies by Hurd et al. (1962) and Hurd and Simon (1962) comparing the pyrolysis of pyridine and alkylpyridines demonstrated the higher stability of the unsubstituted pyridine. However, pyridine itself was shown to undergo thermal degradation to bipyridyls, nitriles, quinoline, and benzene. Interestingly, pyrolysis of methylpyridines yielded HCN in addition to other products. The fact that in our study some pyridine forms anew from nicotine labeled in the CH<sub>3</sub>-<sup>14</sup>C or 2'-<sup>14</sup>C carbon attests to the stability of the pyridine ring system. Additional studies with nicotine labeled only in the pyridine ring are necessary to elucidate this question.

The above pyrolysis data then are indicative of the extent of degradation of the nicotine moiety under static high-temperature conditions. Although such data are

**Table III.** Distribution of Radioactivity in Smoke Condensate from Cigarettes Containing Randomly Labeled [<sup>14</sup>C]Nicotine<sup>a</sup>

	percent initial activity	
	main-stream (MS)	side-stream (SS)
water-soluble compounds	2.6	3.7
ether-soluble neutrals and acids	2.0	1.1
ether-soluble bases	20.0	32.0
nicotine	16.8	24.0
other bases	3.0	8.0
pyridine	0.1	0.2
2-picoline	0.2	
2,6-lutidine	0.1	
3- and 4-picoline <sup>c</sup>	0.8	0.6
2,4-lutidine	b	0.2
3-vinylpyridine	1.2	2.6
myosmine		0.7
bipyridyl	0.1	0.2

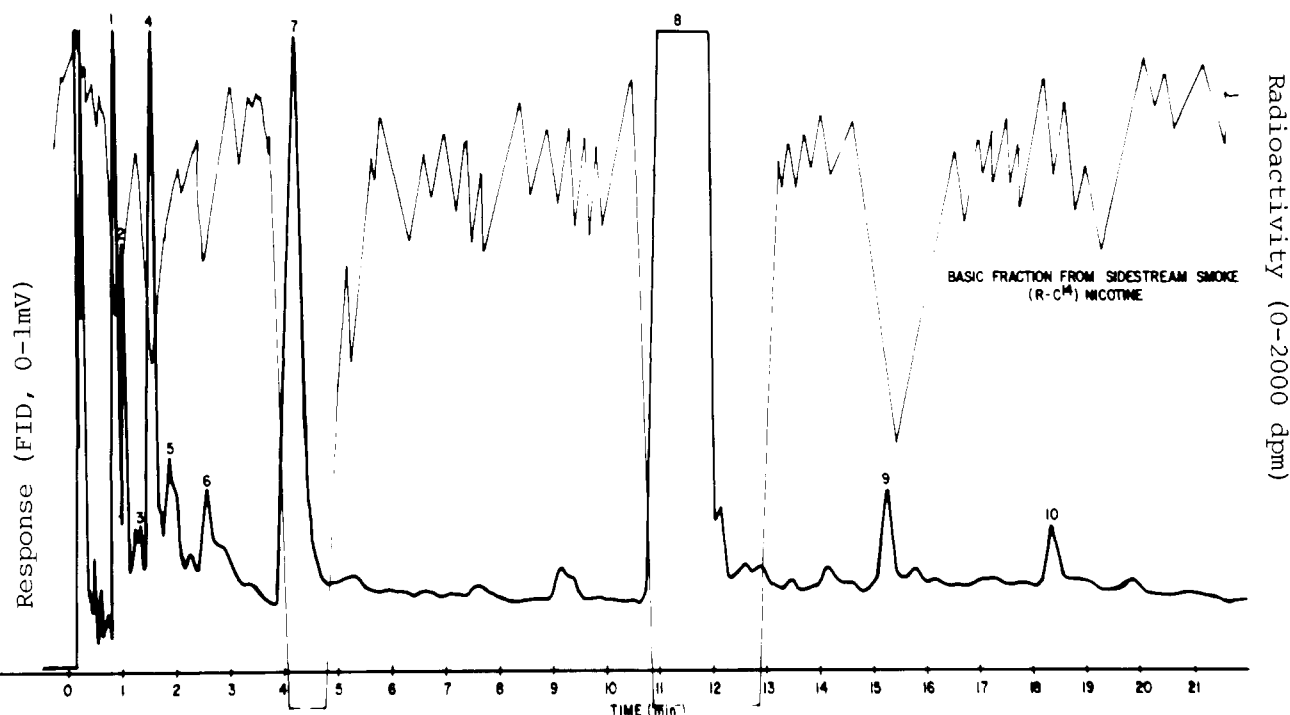
<sup>a</sup> Nicotine from tobacco plants grown in <sup>14</sup>CO<sub>2</sub>.

<sup>b</sup> Where no value is given, level of measured radioactivity is <0.1% of initial radioactivity. <sup>c</sup> 3- and 4-picoline elute together.

useful for identifying the products formed from nicotine under high-temperature conditions, they reflect only partially the events that occur to this alkaloid in a burning cigarette, as we show in the subsequent section.

**Smoke Formation Studies.** Cigarettes containing [<sup>14</sup>C]nicotine were smoked under standard conditions, and smoke condensate was collected and fractionated as shown (Figure 1). The bulk of the recovered radioactivity resided in the basic fraction, and more than 84% of this amount was attributable to nicotine. Levels of radioactivity in nicotine and other components of the basic fraction were monitored by gas radiochromatography. When cigarettes containing randomly labeled [<sup>14</sup>C]nicotine were smoked, radioactivity distributed in the condensate bases as illustrated in Figure 5. In addition to nicotine which contained the highest level of radioactivity, it can also be seen that 3-vinylpyridine, 3- (and 4-) picoline and myosmine contained sufficient label to postulate their formation from nicotine during smoking, likely by pyrolytic reactions. However, as their activity was markedly lower than that observed for nicotine (Table III), it is apparent that their formation from nicotine during smoking constitutes a minor process.

This concept is supported when one considers the labeling pattern observed among the components in similar basic fractions of the smoke from cigarettes containing either [methyl-<sup>14</sup>C]- or [2'-<sup>14</sup>C]nicotine (Table IV). Again, in these fractions nicotine bears the major portion of the label. In cigarettes containing nicotine labeled in the N-methyl group, relatively little <sup>14</sup>C carbon was found transferred to compounds other than nicotine in the basic fraction from condensate. This is quite different from the fate of the <sup>14</sup>C-methyl group in pyrolysis experiments, in which a variety of new compounds was observed to incorporate the labeled carbon atom. The data obtained from cigarettes containing nicotine labeled in the N-methyl group do indicate, however, that upon smoking nicotine gives rise to myosmine and possibly 3-vinylpyridine partially by a mechanism other than the simple loss of the N-methyl group. This concept is currently under investigation in our laboratory. Besides incorporation into myosmine and 3-vinylpyridine (in sidestream smoke), another major pathway for the N-CH<sub>3</sub> group may be to methane or other highly volatile smoke constituents. As



**Figure 5.** Gas-radiochromatogram of basic fraction of condensate derived from cigarettes containing randomly labeled  $[^{14}\text{C}]$ nicotine. Peak identities are: (1) pyridine, (2) 2-picoline, (3) 2,6-lutidine, (4) 3- (and 4-) picoline, (5) 2,4-lutidine, (6) unidentified, (7) 3-vinylpyridine, (8) nicotine, (9) myosmine, (10) bipyridyl.

**Table IV.**  $^{14}\text{C}$ -Labeled Constituents of Cigarette Smoke

	derived from: % of initial radioactivity			
	$[\text{methyl-}^{14}\text{C}]$ -nicotine		$[\text{2'}\text{-}^{14}\text{C}]$ -nicotine	
	MS <sup>a</sup>	SS <sup>b</sup>	MS	SS
pyridine	0.1	0.1	0.2	0.3
2-picoline	<sup>c</sup>	0.2	0.1	0.3
2,6-lutidine	0.1			0.1
3- and 4-picoline <sup>d</sup>	0.1	0.1	1.1	0.5
2,4-lutidine	0.1		0.1	0.2
3-vinylpyridine	0.1	0.4	1.1	2.9
nicotine	14.0	21.0	19.1	23.3
myosmine	0.4	0.6	1.3	0.7
bipyridyl	0.1	0.1		0.2
	15.0	22.5	23.0	28.5

<sup>a</sup> MS, mainstream cigarette smoke. <sup>b</sup> SS, sidestream cigarette smoke. <sup>c</sup> Where no value is given, level of measured radioactivity is <0.1% of initial radioactivity. <sup>d</sup> 3- and 4-picoline elute together.

for 3-vinylpyridine, the high level of activity in the sidestream portion of this compound is not unexpected. Previous studies have indicated that in the burning cigarette, pyrolytic products are more prevalent in sidestream than in mainstream smoke (Schmeltz et al., 1975; Brunemann et al., 1978) and 3-vinylpyridine is apparently a common pyrolytic product of organic nitrogen compounds (Schmeltz et al., 1972).

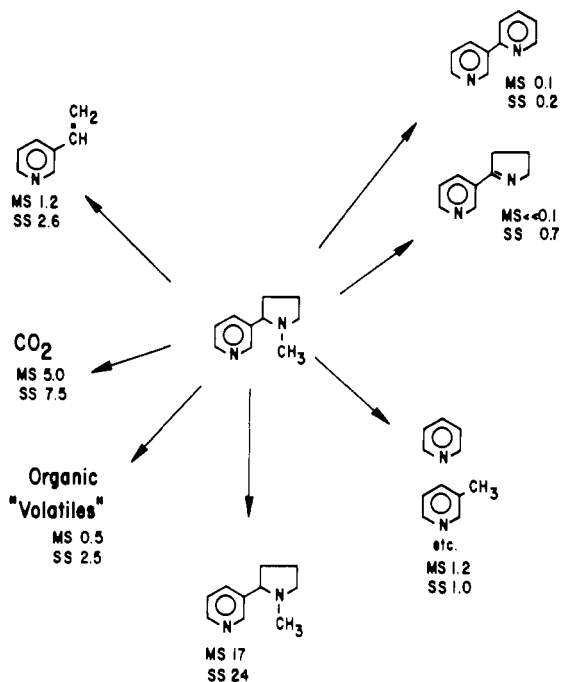
When one considers the fate of  $[\text{2'}\text{-}^{14}\text{C}]$ nicotine in a burning cigarette, one gets a better picture of the relatively simple pyrolytic reactions that nicotine undergoes in this case—as opposed to the more extensive fragmentation in a pyrolysis tube. In the basic condensate fraction, one now finds substantial label in 3-vinylpyridine, in myosmine, and in 3-picoline (Table III). The formation of these compounds from nicotine, of course, involves “simple” bond cleavage; no major rearrangements need be postulated. For example, in this case, myosmine evolves from nicotine via loss of the methyl group, and label is apparently retained

**Table V.** Relative Contribution of Selected Nicotine Positions to Some Cigarette Smoke Constituents

	ratios			
	mainstream $\text{2'}\text{-}^{14}\text{C}/\text{CH}_3\text{-}^{14}\text{C}$		sidestream $\text{2'}\text{-}^{14}\text{C}/\text{CH}_3\text{-}^{14}\text{C}$	
pyridine	1.9	(1.0) <sup>a</sup>	2.0	(1.5)
2-picoline			1.4	(1.1)
2,6-lutidine		(1.1)		
3- and 4-picoline	12.2	(8.9)	4.3	(5.0)
2,4-lutidine				
3-vinylpyridine	2.3	(8.8)	7.4	(6.5)
nicotine	1.3	(1.2)	1.1	(1.1)
myosmine	3.0		1.1	(1.2)
bipyridyl		(0.9)	1.7	(1.9)

<sup>a</sup> Values in parentheses are relative contributions of randomly labeled and  $\text{CH}_3\text{-}^{14}\text{C}$ -labeled nicotine ( $[\text{R-}^{14}\text{C}]$ nicotine/ $[\text{methyl-}^{14}\text{C}]$ nicotine) to labeled constituents in the smoke.

in the 2' position. 3-Vinylpyridine forms from cleavage of the bonds between 1' and 2', and 3' and 4'. 3-Picoline would form via a similar pathway (Figure 4). The formation of 3,2'-bipyridyl would imply a ring expansion as a result of the incorporation of the *N*-methyl group into the pyrrolidine ring. The degree to which each of the two atoms ( $\text{CH}_3$  carbon and 2'-C) contribute to the common pyridine bases encountered in smoke condensate can be estimated from the data in Table V. As can be seen, the 2' carbon (as compared to the  $\text{CH}_3$  carbon) makes a disproportionate contribution to 3-picoline, to 3-vinylpyridine, and to myosmine, which is expected from simple thermal decomposition (bond cleavage). The data indicate, however, that bond formation also occurs by the fact that both 2'-C and  $\text{CH}_3$  carbons contribute to pyridine, bipyridyl, and 3-picoline. It should be emphasized, however, that pyrolysis makes a minor contribution and that in the burning cigarette, transfer of intact nicotine into the smoke stream, and formation of  $\text{CO}_2$  and other as yet unidentified smoke gases are much more prevalent pathways. The formation of aromatic hydrocarbons, of nitriles, and of



**Figure 6.** Summary of contribution of "leaf" nicotine to smoke constituents. Data represent percent of initial  $^{14}\text{C}$  randomly labeled nicotine distributed into components of mainstream and sidestream smoke.

quinolines from nicotine that we observe in model pyrolysis studies of this alkaloid does not occur to any significant extent in a burning cigarette.

In summary, in a burning cigarette, about 41% of the nicotine transfers intact into both smoke streams, higher quantities going to sidestream. About 3–11% is converted to other volatile bases which are also found in the particulate phase, again more in sidestream than in mainstream smoke. Twelve and one-half percent are converted to  $\text{CO}_2$  (Figure 6). The remainder likely consists of highly volatile organic compounds, most of which are not readily trapped. Very little nicotine, if any, is converted to ether-soluble neutral or acidic compounds extractable from condensate. In addition, most of the products arising from nicotine appear in higher amounts in sidestream smoke; however, the simple pyridines unexpectedly do not. This indicates that there are sources other than leaf nicotine for these smoke constituents. This tentative conclusion is supported by another study which showed that the proteinaceous material in tobacco is the major precursor for quinolines in the smoke. (Dong et al., 1978). In ongoing studies we are now identifying those compounds that are formed from nicotine only as minor compounds (<0.1%) which nevertheless can contribute to the toxicity of the smoke. To this group of minor smoke constituents having nicotine as a precursor belong the dibenzacridines (Van Duuren et al., 1960) and *N*-nitrosornicotine and other tobacco specific *N*-nitrosamines (Hecht et al., 1977).

This study compares the thermal degradation of nicotine in an experimental pyrolysis system and in a cigarette

undergoing machine smoking. Certain differences are noted which are mainly due to extensive degradation of nicotine in the pyrolysis tube as contrasted to substantial distillation of nicotine in the burning cigarette. The complex degradative pathways characteristic of high-temperature pyrolysis do not occur to any great extent in a burning cigarette, although they may occur to a minor extent. Also, even in pyrolysis systems, it would appear that the products obtained are partially a function of the form nicotine is in (i.e., salt vs. free form). Obviously, caution must be exercised in interpreting pyrolysis data and in their practical application.

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