

# The Form of Nicotine in Tobacco. Thermal Transfer of Nicotine and Nicotine Acid Salts to Nicotine in the Gas Phase<sup>†</sup>

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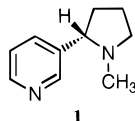
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Thermal transfer to nicotine in the gas phase from neat nicotine, from various nicotine carboxylic acid salts, and from endogenous nicotine in Burley, Bright, and Oriental tobacco samples has been examined by thermogravimetric/differential thermal analysis/mass spectroscopy and evolved gas analysis. Under the conditions used in these studies, the peak transfer temperatures of these substances to nicotine in the gas phase are nicotine and nicotine acetate, both ca. 110–125 °C; nicotine malates, ca. 110–210 °C for nicotine to malic acid ratios of 1:0.56 and 1:1 and ca. 160–210 °C for a nicotine to malic acid ratio of 1:2; (*S*)-nicotine bis[(2*R*,3*R*)-hydrogen tartrate] dihydrate, ca. 195–210 °C; and tobacco samples, a range of ca. 160–220 °C. These results suggest that nicotine is mostly protonated in tobacco leaf. In all cases, the temperature of the transfer of nicotine to the gas phase was found to be many hundreds of degrees below the temperatures observed around the coal of a burning cigarette (smolder, ca. 500–775 °C; dynamic smoking, 600 to over 950 °C). Within the narrow zone of a puffing cigarette that encompasses an intermediate temperature range (125–250 °C), kinetic data suggest that these temperatures are not sufficient to volatilize significant amounts of nonprotonated nicotine, assuming any exists at all, during the short puff duration (2 s). It is concluded that nonprotonated nicotine and protonated nicotine (salts of nicotine with natural tobacco carboxylic acids) will transfer nicotine to smoke with comparable yields and efficiencies during the smoking process.

**Keywords:** Nicotine; nicotine carboxylic acid salts; thermal transfer; gas phase; tobacco; thermogravimetric/differential thermal analysis; mass spectroscopy analysis; TGA/DTA/MS; evolved gas analysis; EGA; "bound" nicotine

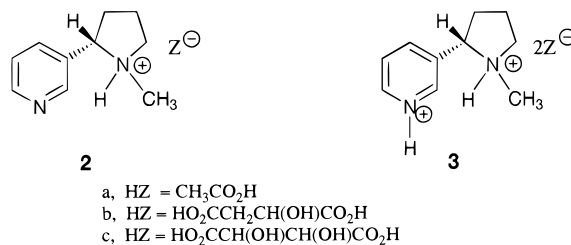
## INTRODUCTION

(*S*)-(-)-Nicotine (**1**), hereafter "nicotine", is the principal alkaloid in tobacco



(*Nicotiana tabacum*), the Bright, Burley, and Oriental varieties of which are the principal raw materials of the tobacco industry (Akehurst, 1981). Hypothetically, this well-known and extremely well studied (Seeman, 1987; Seeman, 1984; Seeman, 1983) substance can exist in one or more (Schmeltz and Hoffmann, 1977; Stedman, 1968) of three forms in cigarette blend components: nonprotonated nicotine (**1**), monoprotonated nicotine (**2**), and diprotonated nicotine (**3**) (Brunnemann and Hoffmann, 1974). (In all structures herein, "Z" refers to a general anion, not necessarily identical to other Z's within the scheme.)

The efficiency of transfer of nicotine from tobacco to smoke has been the subject of considerable discussion and attention in the past few years (Kessler et al., 1996; Kessler et al., 1997; Freedman, 1995; U.S. Food and Drug Administration, 1996). In this paper, we present



evidence which indicates (1) that nicotine is present primarily in one or both protonated forms in tobacco and is likely to be diprotonated when heated to temperatures required to release nicotine from tobacco, (2) that nonprotonated nicotine (**1**) and the nicotine carboxylic acid salts such as those found in tobacco (**2** and **3**) transfer nicotine to the gas phase at temperatures below about 220 °C, and (3) that nicotine salts in tobacco and nonprotonated nicotine, if it exists in tobacco at all, are likely to transfer nicotine to the gas phase during the smoking process with comparable yields and efficiencies. Evidence will also be presented that implies a facile proton-transfer between **1**, **2**, and **3** under thermal or smoking conditions.

## MATERIALS AND METHODS

**Thermogravimetric/Differential Thermal Analysis/Mass Spectrometry (TG/DTA/MS).** A VG Instruments quadrupole mass spectrometer (EGA300SL) was interfaced to a Seiko simultaneous TG/DTA unit (TG/DTA 300) via a flexible fused silica-lined stainless steel capillary (0.3 mm i.d. × 6 ft

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long). No modification to the Seiko balance was necessary, because coupling was made using a Teflon ball joint directly attached to the end of a quartz furnace tube. An open sample pan configuration with horizontal purge gas flow across the sample facilitated sample/purge gas interaction and rapid escape of gaseous reaction decomposition products. One end of the capillary was positioned in the furnace tube downstream of the sample pan, and the other end of the capillary was connected to the mass spectrometer through a molecular leak (silicon carbide frit) backed by a rotary pump.

This system provides minimal transit time of evolved gas from the TG/DTA to the mass spectrometer. To prevent condensation, the transfer line was heated to about 170 °C by applying an ac voltage across the length of the stainless steel capillary sheath. Samples, approximately 3–10 mg for nicotine and the nicotine acid salts and 20 mg for tobacco samples, were heated in platinum pans at rates of 5 °C min<sup>-1</sup> under about a 100 mL min<sup>-1</sup> flow of either helium or a mixture of 21% oxygen in helium (hereafter sometimes referred to as “21% oxygen”), in case oxidative loss of nicotine was to be significant. MS data were collected using a multiple ion monitoring (MIM) mode in which specific ions were monitored as discussed below (section 3b). Peak nicotine appearance temperatures were determined by recording the maxima of the MS ion fragment of mass-to-charge ratio (*m/z*) 84, the most intense peak of the nicotine mass spectrum. In some cases, particularly when interference at *m/z* 84 might be anticipated, the second most abundant nicotine mass spectral fragmentation peak, at *m/z* 133, was also followed. In the figures herein, only selected ion fragments are shown. MS and TG/DTA data were converted to ASCII files, and data reduction/overlap was performed in Microsoft EXCEL.

Kinetic data were obtained using the Seiko TG/DTA unit. Nicotine and (*S*)-nicotine bis[(2*R*,3*R*)-hydrogen tartrate] dihydrate were each subjected to TGA scans at heating rates of 5, 10, 15, and 20 °C min<sup>-1</sup> under flowing compressed air at rates of 100 and 500 mL min<sup>-1</sup>. The TG curves were normalized to a given sample weight prior to calculating activation energies and degradation/volatilization times (lifetimes) by use of a kinetics package, provided by Seiko, which utilizes the nonisothermal method of Ozawa (Ozawa, 1965).

**Evolved Gas Analysis/Mass Spectrometry (EGA/MS).** EGA/MS experiments were conducted by heating samples at 20 °C min<sup>-1</sup> under flowing He (~200 mL min<sup>-1</sup>). Samples were contained in either a quartz boat (if solid) or on quartz wool (if liquid) inside an 8 mm i.d. quartz tube which was heated by a gold reflectance tube furnace. For tobacco samples, a starting mass of about 500 mg was used. For the nicotine carboxylic acid salts, a starting mass of about 25 mg was used. A portion of the exiting gases was directed into a Balzer QMG511 mass spectrometer utilizing the Balzer capillary inlet system. The mass spectrometer was programmed to scan over a mass range of 0–255 amu with an operating ionization energy of 18 eV. Data, at 2 °C or 4 °C intervals, were collected, stored, compiled, and plotted by a PDP-1103 computer system. Again, nicotine evolution was followed by monitoring the ion fragment at *m/z* 84.

**Preparation and Characterization of Compounds.**  
**Warning:** Nicotine is severely toxic in the quantities described below. Due care must be exercised while handling pure nicotine and nicotine salts.

(*S*)-Nicotine Bis[(2*R*,3*R*)-hydrogen tartrate] Dihydrate (**2c–3c**). (Sometimes **2c–3c** is referred to hereafter as “nicotine bitartrate” or “the bitartrate salt” or “nicotine bis-bitartrate”.) A solution of commercially available (*S*)-(-)-nicotine (**1**) (114.98 g, 0.7087 mol) in 95% ethanol (490 mL) was stirred magnetically at room temperature and treated with a boiling-hot nearly saturated solution of (2*R*,3*R*)-(+)-tartaric acid (213 g, 1.42 mol) in water (115 mL), added over about a minute as rapidly as exothermicity would permit. The acid solution was rinsed in with ethanol (10 mL, 95%). The mixture was seeded; then it was eventually stirred manually to promote crystallization of the product in an easily filtered form. The mixture was chilled in an ice bath to complete the crystallization; then the solids were filtered off (glass Büchner funnel) and rinsed

with ice-cold 95% ethanol, until the rinsings came through colorless, and no more color was present in the filtercake. Impurity minor alkaloids remain in solution and were removed by the filtration process: the dark color of unpurified nicotine served as an internal indicator for completion of the washing process. The solids were dried in air to yield 325 g of **2c–3c** (92%), mp: 89.5–92.5 °C (dec). Lit. mp: 88–89 °C (Pictet and Rotschy, 1904). <sup>13</sup>C NMR (5% w/w in D<sub>2</sub>O, internal standard sodium 3-trimethylsilylpropionate, TSP) δ 179.46\*, 149.46, 148.41, 145.32, 133.92, 129.61, 75.84\*, 72.17, 59.26, 41.36, 33.24, 24.45 (asterisk (\*) indicates tartrate carbons). This crystalline nicotine bis-bitartrate contains both monoprotonated (**2c**) and diprotonated (**3c**) nicotine in the crystal unit cell and is thus referred to as **2c–3c** in this report.

This material was used as described below to prepare pure nicotine. Samples of **2c–3c** have remained stable in our laboratory for more than a decade; the salt is, therefore, an effective means of storing nicotine. A sample (10.31 g) was dissolved in water (16.6 mL, plus 3.4 mL for rinsings) and gravity filtered through fluted Whatman No. 1 paper. The aqueous solution was placed in a Petri dish in a desiccator, accompanied by two Petri dishes containing ethanol (125 mL total, 95%). Over a period of weeks, coarse crystals formed. These were harvested as needed and dried on filter paper. The recrystallized material was used for the TG/DTA/MS studies and for an X-ray structural determination.

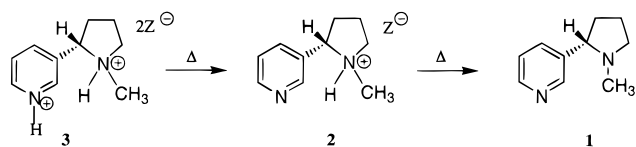
**Purified (*S*)-(-)-Nicotine (**1**).** Nicotine bis-bitartrate dihydrate (**2c–3c**) (115.04 g, 0.2308 mol) and sodium hydroxide pellets (40.13 g, ca. 1 mol) were treated with water (100 mL). Oily aqueous nicotine separated from the hot mixture. Hexane (100 mL) was added, and the mixture was chilled on ice. The mixture was swirled periodically, until the three liquid phases had become two. The pale organic phase was isolated, decanted from any traces of aqueous phase, and then concentrated on a rotary evaporator. A second hexane extract (100 mL) was treated likewise. The combined product was distilled [Kugelrohr, bp: 101–142 °C (oven)/0.9 Torr]. Yield: 36.05 g (96%). Optical rotation: [α]<sub>589</sub><sup>20</sup> -170.61 (lit.: -169.4) (Tate and Warren, 1937); [α]<sub>578</sub><sup>20</sup> -179.53, [α]<sub>546</sub><sup>20</sup> -205.51 (lit.: -204.2) (Tate and Warren, 1937) (neat, 10.000 cm cell). <sup>13</sup>C NMR (H<sub>2</sub>O, external TMS, TSP internal standard) δ 148.24, 147.74, 137.33, 136.53, 124.33, 68.18, 56.15, 38.88, 33.09, 21.64; TSP at δ -2.86. <sup>13</sup>C NMR (neat liquid): δ 152.53, 151.56, 141.82, 137.26, 126.21, 71.51, 59.65, 43.04, 38.55, 25.57. (*S*)-(-)-Nicotine prepared in this manner was found to be free of detectable minor alkaloids, as evidenced by GC/MS analysis as well as by <sup>13</sup>C NMR. The colorless product was stored under nitrogen, protected from light.

**Association of (*S*)-(-)-Nicotine with Acetic Acid (**2a**).** (*S*)-(-)-Nicotine (0.815 g, 0.0050 mol) and glacial acetic acid (0.905 g, 0.0151 mol) were weighed together and swirled until homogeneous. The molar ratio of acetic acid to nicotine was 3.00. The resultant oily **2a** was used directly in the TG/DTA/MS experiments. For literature characterizations, see Perfetti (Perfetti, 1983) and Dezelic and Nicolin (Dezelic and Nicolin, 1967).

**Nicotine Salts with (*S*)-(-)-Malic Acid.** (a) 1:0.56 Nicotine/malic acid salt, as prepared by titration of nicotine with malic acid to a pH of 5.60 (**2b**). (*S*)-(-)-Nicotine (3.255 g, 0.0201 mol) was dissolved in water (10.00 g) in a 100 mL graduated cylinder, fitted with a magnetic stirring bar. A pH electrode was inserted, reading 9.68 initially. A solution of (*S*)-(-)-malic acid (9.65 g) in water to give a total of 20.80 g of solution was added dropwise to the stirred nicotine solution until the pH permanently fell to 5.60. A total of 3.28 g of malic acid solution was required (0.0113 mol). The molar ratio of nicotine to malic acid in the resulting solution was 1.77. The resulting solution was allowed to evaporate in air to afford a yellowish syrup. This noncrystalline 1:0.56 nicotine:malic acid salt may be heterogeneous at room temperature, but because an aqueous solution of this malate has a pH of 5.6, it is referred to as **2b** herein.

(b) 1:1.0 Nicotine:malic acid salt (**2b–3b**). (*S*)-(-)-Malic acid (2.83 g, 0.0211 mol) was dissolved in 100% ethanol (5 mL)

## Scheme 1



(boiling water bath). (*S*)-(-)-Nicotine (3.255 g, 0.0201 mol, purified as above) was added to the solution, followed by ethyl acetate (ca. 5.5 mL), added until opalescence nearly persisted. The solution was seeded (the seeds having been obtained by slow evaporation of a 1:1 stoichiometric solution of nicotine and malic acid in water) and allowed to crystallize. The solids were filtered off and rinsed with ethyl acetate/100% ethanol (1:1, v/v, 2 × 20 mL) and then ethyl acetate (2 × 10 mL). The solids were dried under suction. Yield: 5.07 g (85%). Mp 112.5–121.4 °C (dec). Anal. Calcd for C<sub>14</sub>H<sub>20</sub>N<sub>2</sub>O<sub>5</sub>: C, 56.75; H, 6.80; N, 9.45. Found: C, 56.01, 56.08; H, 6.92, 6.96; N, 9.25, 9.03. <sup>13</sup>C NMR (D<sub>2</sub>O, TSP internal standard): δ 182.35, 179.68, 152.38, 151.15, 141.48, 132.29, 128.29, 72.58, 71.76, 59.01, 43.34, 41.11, 33.07, 24.41. <sup>13</sup>C NMR (solid state): δ 180.21, 175.33, 152.16\*, 135.20, 131.14, 123.96, 69.92\*, 55.43, 41.19\*, 33.28, 22.63 (11 peaks for 14 carbons: based on peak shape and chemical logic, the peaks indicated with an asterisk are believed to be doubly degenerate). The NMR chemical shifts of the crystalline solid are consistent with monoprotonation of nicotine within the crystal unit cell; however, the melt of this salt is believed to contain both mono- and diprotonated nicotine, and it is therefore referred to as **2b–3b**.

1:2.0 Nicotine:malic acid salt (**3b**). Nicotine and (*S*)-(-)-malic acid do not appear to form a homogeneous 1:2 salt. A solution of nicotine (3.26 g, 0.02 mol) and (*S*)-(-)-malic acid (5.40 g, 0.04 mol) in water and ethanol was permitted to evaporate at room temperature. The residue formed a suspension of solids in syrup when seeded with the 1:1 malate salt. Because of the molar excess of malic acid in this salt and in the absence of any data to the contrary, it is assigned to be primarily the diprotonated nicotine salt **3b**, when in solution or in a homogeneous melt. A representative sample of this mixture was examined by TG/DTA/MS.

## RESULTS AND DISCUSSION

**1. Goals of This Study.** Scheme 1 depicts the fate of the nicotine molecule, as the NH protons are formally successively lost from the diprotonated salt **3** due to dissociation of the salt or decomposition of the counterions. This scheme summarizes the two major questions addressed herein. (1) What are the forms of nicotine present in tobacco (**1**, and/or **2**, and/or **3**)? (2) If more than one form of nicotine is present in tobacco, do they thermally transfer to nicotine in the gas phase with comparable yield and efficiency?

As will be seen below, the answers to these two questions can be obtained using much of the same experimental data.

**2. "pH of Tobacco" and Implications for the Degree of Protonation of Nicotine in Tobacco.** The determination of the form(s) (**1** versus **2** versus **3**) of nicotine in tobacco is complicated as tobacco is not a homogeneous entity and any isolation method would de facto disrupt the endogenous state of the system (Tso, 1990). Tobacco cell walls and structural components can create microenvironments which could, in principle, inhibit or prevent the interaction among various tobacco constituents. Thus, the tobacco matrix is such that all tobacco components may not necessarily be in equilibrium with each other. However, extensive chemical changes occur during the curing of the tobacco (Tso, 1990; Peele et al., 1995; Wiernik et al., 1995). In addition, chemical and physical changes occur to tobacco

**Table 1. Representative Literature Values of the "pH of Tobacco"**

tobacco leaf type	pH <sup>a,b</sup>
aromatic (Oriental)	4.9
flue cured (Bright)	5.5
Burley	5.8

<sup>a</sup> The procedure to obtain the "pH of tobacco" is typically as follows: an aqueous extract of the tobacco is obtained and filtered; the "pH of tobacco" is the pH of the resultant aqueous solution. <sup>b</sup> See: Wynder and Hoffmann, 1967; Akehurst, 1981; Browne, 1990.

during the cigarette manufacturing process, which include repeated heating, moistening, drying, and mixing steps. Modifications which occur both during the curing of tobacco and during the manufacture of cigarettes likely increase the mobility of nicotine and other compounds within the tobacco matrix and may help to establish an equilibrium between the tobacco alkaloids and the natural tobacco acids.

Can pH measurements of tobacco extracts be useful models or indicators of the form of nicotine in tobacco? One means of approaching an understanding of the relative concentrations of acids and bases in tobacco and an understanding of the extent of N-protonation of nicotine as contained in tobacco is to measure the pH of aqueous extracts of tobacco. While the literature is replete with references to the "pH of tobacco" (Browne, 1990; Williamson and Chaplin, 1981; Pankow et al., 1997; Liang and Pankow, 1996; Akehurst, 1981; Wynder and Hoffmann, 1967), the concept of "tobacco pH" does not have precise meaning. The "pH of tobacco" is typically determined by extracting a tobacco sample with water, perhaps filtering the resultant mixture and measuring the pH of the resultant dilute aqueous solution. Thus, "pH of tobacco" is not an intrinsic property of matter but rather an arbitrary experimental construct that varies with the experimental conditions chosen.

While tobacco can have a high equilibrium moisture content (10% or more) (Akehurst, 1981), tobacco is, by no means, a dilute aqueous solution. Any use of the "pH of tobacco" must be done with the understanding that it is only a rough approximation of the hydrogen ion activity within tobacco. An observed pH value of a tobacco extract is significantly influenced by a number of factors, including the volume of water employed to extract the quantity of tobacco taken as well as the relative water extractability and solubility of the various acids and bases in the tobacco. Nonetheless, the "pH of tobacco" may be a useful, though certainly limited, conceptual device. It is crucial that any use of the "pH of tobacco" takes into consideration its mode of experimental determination and all assumptions underlying both its experimental determination and any application.

Table 1 lists representative determinations of the "pH of various tobaccos" (Browne, 1990; Akehurst, 1981). Table 2 summarizes the relative proportions of nicotine (**1**) and its protonated forms (**2**, **3**) in dilute aqueous solution at selected values of pH. Given the observation that aqueous extracts of cigarette tobaccos are acidic (Table 1) (Akehurst, 1981; Browne, 1990; Wynder and Hoffmann, 1967), it can be deduced reliably that the underlying tobaccos are acidic as well and that the nicotine contained therein will at least be formally monoprotonated if not diprotonated.

One aspect of this work is the evaluation of the effect of the degree of protonation of nicotine (e.g., from

**Table 2. Relative Amounts of Nonprotonated and Protonated Nicotine as a Function of pH in a Dilute Aqueous Solution<sup>a</sup>**

pH	% nonprotonated nicotine (1)	% monoprotated nicotine (2)
5.8	0	100 <sup>b</sup>
6.0	1	99
6.4	2	98
6.6	4	96
6.8	6	94
7.0	9	91
7.2	13	87

<sup>a</sup> Based on  $pK_a$ 's for nicotine of 8.0 and 3.1. <sup>b</sup> At pH 5.3, 0.6% of the total nicotine alkaloid is in the diprotonated form **3**; as pH drops below 5.3, the percentage of **3** rises rapidly. At pH 3.1, 50% of the alkaloid is **3** and 50% is **2**.

substrates **1–3** or from the tobacco matrix) on the ease of thermal transfer to nicotine in the gas phase. The pH of an aqueous tobacco extract does not incorporate the changes to the tobacco caused by heat: evaporation of volatile substances, including water; increased fluidity of materials within the tobacco; pyrolytic changes. A discussion of acidity functions (Nigretto and Jozefowicz, 1973; Cox and Yates, 1983; Rochester, 1970) and the meaning of acidity under nonaqueous conditions is outside the scope of this study. Consequently, the form(s) of nicotine just prior to the transfer to the smoke (or gas phase) is as complex a question as the characterization of the heated material itself.

Additional evidence of nicotine's protonated state in tobacco is the observation that nicotine neither substantially evaporates nor significantly autoxidizes while in storage in cured tobacco. By contrast, nonprotonated nicotine exposed to air is vulnerable to both evaporation and/or autoxidation.

**3. TG/DTA/MS Analysis.** (a) *Choice of Substrates.* To explore Scheme 1 chemistry, several salts or associations of nicotine with carboxylic acids were prepared and subjected to thermal analysis in tandem with mass-spectral monitoring. In this work, the primary tactic was to examine the thermal properties of monoprotated and diprotonated nicotine carboxylic acid salts in which the acid was either identical to or else very similar to the carboxylic acids found in tobacco. The salts selected were designed to shed light as to the temperature ranges such salts release nicotine (**1**) to the gas phase, given considerations of variable acid strength, volatility, and stability.

While the preparation of various nicotine acid salts has been known for many years, many of these are salts of inorganic acids (Johnstone and Plimmer, 1959; Stedman, 1968). Salts of nicotine with truly strong inorganic acids (hydrochloric, nitric, sulfuric, phosphoric, and derivatized phosphoric) are not found in tobacco because these acids are more than adequately neutralized in tobacco by an excess content of strong inorganic bases. The combined ion charge of potassium, calcium, and magnesium ions in tobacco significantly exceeds the ion charge of the combined strong inorganic acids present (Wynder and Hoffmann, 1967; Bokelman and Ryan, 1985). Consequently, the thermal chemistry of salts such as nicotinium sulfate, nicotinium chloride, or nicotinium nitrate was not examined in this study.

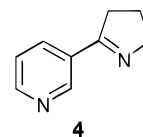
In tobacco, the natural acids associated with nicotine are of the carboxylic acid type (Akehurst, 1981; Wynder and Hoffmann, 1967). Nicotine salts of organic acids are frequently not crystalline and can have a variety of stoichiometries (Perfetti, 1983). As a representative of

a volatile, thermally stable, relatively weak organic carboxylic acid, the nicotine "salt" with acetic acid was examined. The literature suggests that this salt contains nicotine and acetic acid in a 1:3 molar ratio (Perfetti, 1983). Mixing nicotine and acetic acid in this ratio results in mild exothermicity and the formation of a viscous oil. Acetic acid serves as the prototype for the behavior of the class of alkanic acids higher than formic acid such as those that occur in tobacco and tobacco smoke (Stedman, 1968).

By abundance and acid strength, the polycarboxylic acids such as malic and citric acids are the dominant acids in tobacco. Polycarboxylic acids as a class are generally stronger than the monocarboxylic acids. Importantly, polycarboxylic acids have greatly reduced volatility relative to monocarboxylic acids. Whereas nicotine citrates and malates are highly water soluble, nicotine salts of some of the other acids found in tobacco can have very low water solubility (Perfetti, 1983; Crooks and Lynn, 1992).

(*S*)-Nicotine bis[(2*R*,3*R*)-hydrogen tartrate] dihydrate (**2c–3c**) was selected to provide an example of a bis-protonated nicotine cation. Tartaric acid was chosen as an example of a polyfunctional carboxylic acid because this salt with nicotine is crystalline and well characterized. Note, however, that tartaric acid is not a significant component of tobacco. Thus, in addition, three different salts with an important tobacco component, (*S*)-(–)-malic acid, **2b**, **2b–3b**, and **3b**, were prepared for examination. The malate salts had different nicotine: malic acid ratios, in an attempt to examine both monoprotated and diprotonated substances. Only one of these three malate salts was crystalline, this having a 1:1 stoichiometry between nicotine and malic acid.

(b) *Observed Behavior of the Salts and Burley Tobacco under TG/DTA/MS Conditions.* In the TG/DTA experiments, the weight loss as well as the temperature differential between the sample and a reference (alumina) are recorded as a function of temperature during the dissociation/volatilization/decomposition of the starting sample. Interfacing the MS to the TG/DTA unit provides a means of identifying (qualitatively) these gas-phase dissociation/volatilization/decomposition products. Especially important are the maxima of the MS data curves which typically correspond to the weight loss rate maxima (differential thermogravimetry, DTG). Due to chromatographic effects of the silica transfer line and residues in the TG/DTA furnace tube, some peak-tailing is observed in the MS data curves. Table 3 shows selected masses with reported abundances (range of 1–999) for nicotine and possible pyrolysis products of the nicotine carboxylic acid salts. The most abundant mass-spectral ion fragment of nicotine appears at a mass-to-charge ratio,  $m/z$ , of 84 (Duffield et al., 1965; Glenn and Edwards, 1978). Myosmine is a major decomposition product of nicotine at temperatures greater than 300 °C in air (Kobashi et al., 1963) and above 600 °C in an inert atmosphere (Woodward et al., 1944; Jarboe and Rosene, 1961). It should be noted that under the conditions used, recordable levels of myosmine (**4**) were not observed ( $m/z$  146).



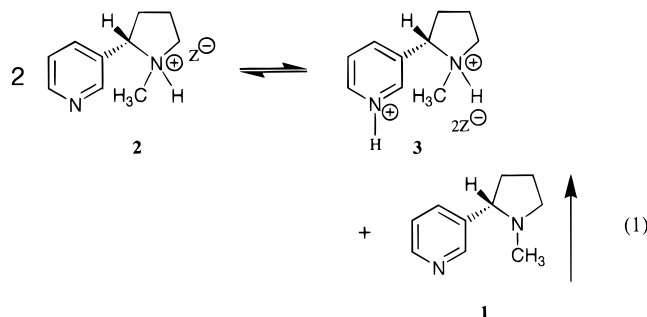
**Table 3. Mass Spectral Features of Nicotine and Possible Pyrolysis Products in the Thermal Treatment of 1, 2a, 2b, 2b-3b, 3b, 2c-3c, and Burley Tobacco**

compound	masses <sup>a</sup>												
	17	26	29	43	44	45	54	60	72	76	84	133	146
nicotine		8		28	34		21				999	308	
acetaldehyde		57	999	328	814	17							
acetic acid	28	12	132	999	47	874		570					
maleic acid		810	180	60	100	780	300		999				
maleic anhydride		999	23	1	83	6	471						
malic acid		390	23		54	8	999	3					
tartaric acid		3	160	115	62	155		80	2	999			
acrylic acid		543	99	118	125	350	17	26	759				
carbon dioxide					999	11							
water	212												
myosmine													858

<sup>a</sup> From NIST/EPA/MSDC Mass Spectral Database Version 2.0, November, 1988.

Since anionic decomposition has a major bearing on the nicotine thermal evolution profile from nicotine carboxylic acid salts, the mass spectral monitoring was adjusted to cater to the specific decomposition chemistry of the various counterions. Thus, the system with acetic acid monitored the ion fragments at  $m/z$  43 and 60, principal ions of acetic acid's mass spectrum. For tartaric acid, ion fragments specific to acetaldehyde at  $m/z$  29 and 44 (also the dominant ion for carbon dioxide) were followed, since tartaric acid appears to decarboxylatively dehydrate to form formylacetic acid, which is further decarboxylated to give acetaldehyde. Malate systems followed the ion fragments to be expected for maleic anhydride ( $m/z$  54) and acrylic acid ( $m/z$  72), both anticipated decomposition products of the malate anion. In all runs, ion fragments specific to water at  $m/z$  17 and carbon dioxide at  $m/z$  44 were also monitored.

In the temperature range in which nonprotonated nicotine evaporates, two general classes of complex chemical reactions can be expected to occur with nicotine/carboxylic acid salts. The first involves dissociation and/or dehydration and decomposition of the carboxylic counterion (Scheme 1). The second class of chemical reaction involves proton transfer between the various nicotine species. In the case of monoprotonated salts, a disproportionation reaction can convert them to a mixture of the diprotonated salt and free nicotine (eq 1).



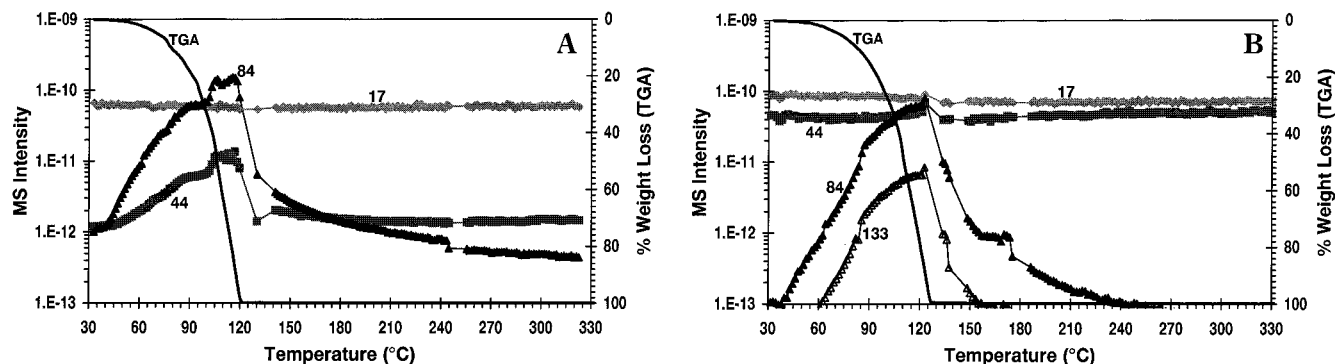
The disproportionation equilibrium is displaced by the evaporation of nonprotonated nicotine (1). To the extent that the nicotine dication (3) is not volatile, the temperature range of the ultimate release of nicotine from 3 is dictated by the stability of the counterion. Equation 1 will be equally valid whether proton transfer occurs directly between the participants as shown or whether such proton transfer occurs via other species contained within the medium or tobacco matrix. One of the

mechanistic pathways embedded in eq 1 is the association-dissociation reaction between nicotine and acid exemplified in Scheme 1. Dissociation becomes more important for salts or associations of nicotine with weaker acids.

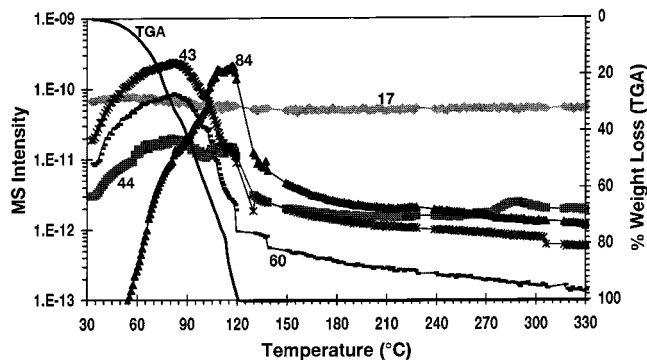
Figures 1A and 1B show overlays of TG and MS curves for nicotine (1) run under helium and 21% oxygen, respectively. TG/MS results indicate that under the conditions used, the peak transfer of nicotine to the gas phase occurs between about 110 and 120 °C, well below nicotine's boiling point of 247 °C.

The TG and MS curves for nicotine acetate (2a) run under helium are shown in Figure 2. Initial weight loss appears to be due to the dissociation of the amine salt and subsequent volatilization of acetic acid ( $m/z$  43 and 60) at a peak temperature of about 80 °C. A peak temperature of about 115 °C is observed for the volatilization of nicotine; interestingly, this temperature is essentially that observed for the simple evaporation of free nicotine (1) (Figure 1A). Therefore, the acetate salt of nicotine does not appear to be stable at temperatures above ca. 90 °C, even starting with as many as 3 mol equiv of acetic acid relative to nicotine. Similar results were obtained for nicotine acetate (2a) run in 21% oxygen.

(S)-Nicotine bis[(2*R*,3*R*)-hydrogen tartrate] dihydrate (2c-3c) is a crystalline salt, the X-ray structure of which indicates that in the solid state not all of the nitrogen atoms of nicotine are protonated, despite the stoichiometry. The unit cell was found to contain four nicotine molecules, equivalent in pairs, varying in conformation and degree of protonation on nitrogen. Two of the nicotine molecules were monoprotonated (on the pyrrolidine ring, e.g., 2c); the other two were diprotonated (3c). In a melt, nearly all of the nicotine is likely to be diprotonated 3c. Thus, the X-ray structure has little relevance to the ensuing pyrolysis chemistry. Figures 3A and 3B show TG and MS curves for this salt run under helium and 21% oxygen, respectively. MS results indicate that the initial weight loss at about 80 °C corresponds to initial dehydration ( $m/z$  17, about 5% of the total weight loss) followed by decomposition/dissociation of the nicotine bitartrate salt into water ( $m/z$  17), carbon dioxide ( $m/z$  44), acetaldehyde ( $m/z$  29), and nicotine ( $m/z$  84). Importantly, nicotine evolution peaks at about 200 °C. Thermal loss of water from the hydrated salts (and from the tobacco matrix, see experiments below) occurs prior to transfer of nicotine to the gas phase. It appears that greater than 95% of the total material is transferred to the gas/aerosol phase by ca.



**Figure 1.** (A) TG/MS of nicotine in helium as a function of temperature. (B) TG/MS of nicotine in 21% oxygen as a function of temperature.



**Figure 2.** TG/MS of a nicotine acetate salt in helium as a function of temperature.

230 °C, i.e., a carbonaceous residue of less than 5% of the original weight remained to be decomposed at higher temperatures in oxygen.

Figure 4 shows the TG/DTA for (*S*)-nicotine bis-[(2*R*,3*R*)-hydrogen tartrate] dihydrate (**2c–3c**) run under 21% oxygen. The overall DTA curve for the decomposition of the nicotine bitartrate salt is generally endothermic, due to the volatilization of water and nicotine; however, the discontinuity in the curve is indicative of the exothermic nature expected for the tartrate moiety decomposition. The similar nature of the data curves run in helium and in 21% oxygen are suggestive of the thermal stability of the nicotine bitartrate salt with respect to oxidation.

Figures 5–7 show the TG and MS curves for the three different nicotine malate salts heated in helium, these having malic acid:nicotine molar ratios of 0.56, 1, and 2, respectively. The decomposition/dissociation of the nicotine malate salts proceeds via a more elaborate route than the nicotine acetate and bitartrate salts. Figure 5 shows the TG/MS curves for the 1:0.56 nicotine:malic acid salt mixture. The MS data indicate that the initial 40% weight loss, in the temperature range of about 50–150 °C, is due to the evaporation of water (*m/z* 17) followed by the volatilization of nicotine (*m/z* 84, peaking at about 110 °C). At temperatures in excess of 150 °C, the salt begins to decompose/dissociate into water, carbon dioxide, maleic anhydride (*m/z* 54), acrylic acid (*m/z* 72), and nicotine. Two additional peaks for nicotine evolution are observed at about 165 and 200 °C. The presence of maleic anhydride and acrylic acid were confirmed by separate pyrolysis GC/MS work.

The 1:1 nicotine:malic acid crystalline salt (**2b–3b**) heated under helium is shown in Figure 6. The initial weight loss at about 120 °C is attributed to the volatilization of nicotine (*m/z* 84). At temperatures in excess

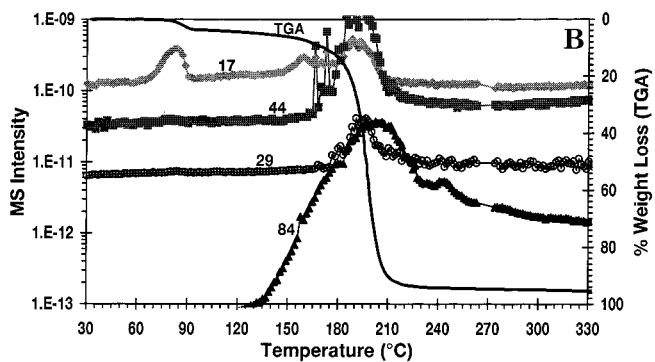
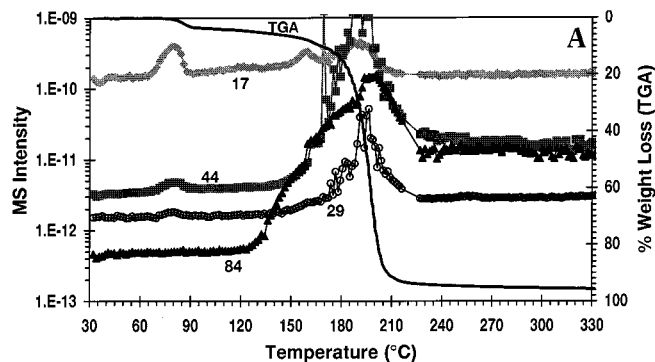
of 150 °C, the data again indicate the decomposition/dissociation of the salt to water, carbon dioxide, maleic anhydride (*m/z* 54), acrylic acid (*m/z* 72), and nicotine. The two additional peaks for nicotine evolution at about 165 and 200 °C observed during the decomposition/dissociation of the 1:0.56 nicotine:malic acid salt mixture are again observed for the 1:1 crystalline salt.

The TG/MS data for the 1:2 nicotine:malic acid salt, shown in Figure 7, is observed to be similar to those of the 1:1 crystalline nicotine malate salt, with the exception that the low-temperature (~120 °C) nicotine peak is now absent. The TG shows major weight loss in the 150–220 °C temperature range due to the decomposition/dissociation of the salt. Only two nicotine (*m/z* 84) evolution peaks are observed at about 165 and 205 °C.

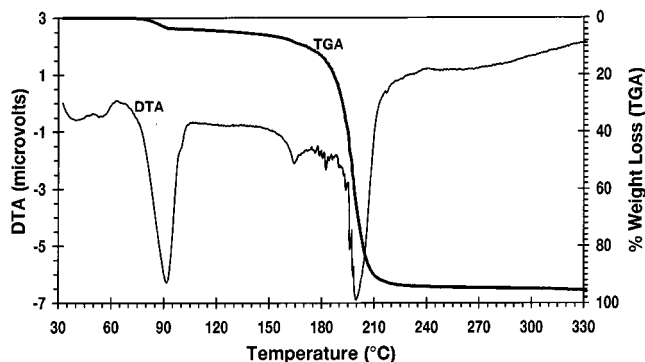
Carbonaceous residues with weights of between 4% and 9% of the original weight were observed at temperatures of 330 °C for each of the nicotine malate salts. These residues, primarily polymerization products of the dicarboxylic acids, combusted (*m/z* 44) in oxygen at temperatures around 500 °C (data not shown).

The low-temperature (115 ± 5 °C) transfer of nicotine from both the 1:0.56 and 1:1 nicotine:malic acid salt mixtures is essentially the temperature range observed for the volatilization of nonprotonated nicotine in helium as well as in 21% oxygen. The source of nonprotonated nicotine from the nicotine malate salts in this low-temperature region may well be volatilization arising from the disproportionation of monoprotonated salt (eq 1). Enough malic acid was present to monoprotonate nicotine but not enough to totally diprotonate it. A disproportionation reaction yields, for two molecules of monoprotonated salt, one molecule each of the diprotonated salt and free nicotine. A disproportionation reaction also explains the presence of a peak appearance temperature of nicotine in the 200 °C region for the 1:0.56 salt, a compound which prior to thermal treatment is likely not to have any diprotonated salts present.

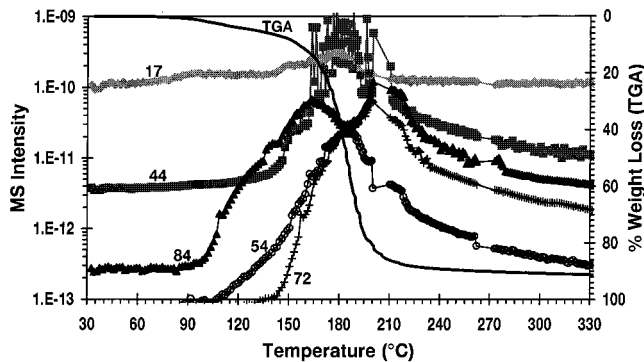
Figure 8 shows an overlay of the nicotine (*m/z* 84) evolution curves for the three nicotine malate salts. For the salts which contain significant concentrations of monoprotonated salt **2b**, these data are consistent with the proposed disproportionation of monoprotonated nicotine (**2**) to diprotonated nicotine (**3**) and nonprotonated nicotine (**1**) (which volatilizes in the same low-temperature region as that observed for nonprotonated nicotine), followed by further release of nicotine via decomposition/dissociation of the resultant diprotonated salt(s). The two peaks, at about 165 and 200 °C, observed in the nicotine evolution curves of Figures 5–8 can be attributed to the decomposition/dissociation of



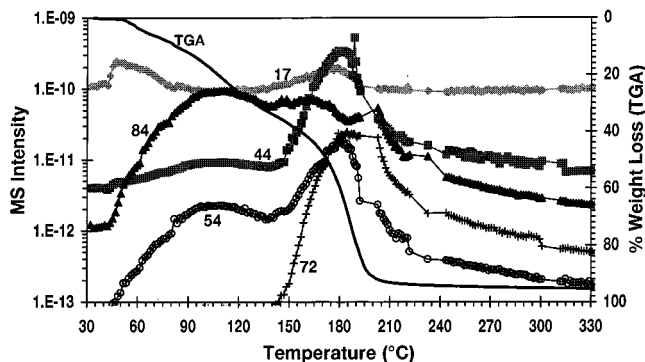
**Figure 3.** (A) TG/MS of nicotine bitartrate in helium as a function of temperature. (B) TG/MS of nicotine bitartrate in 21% oxygen as a function of temperature.



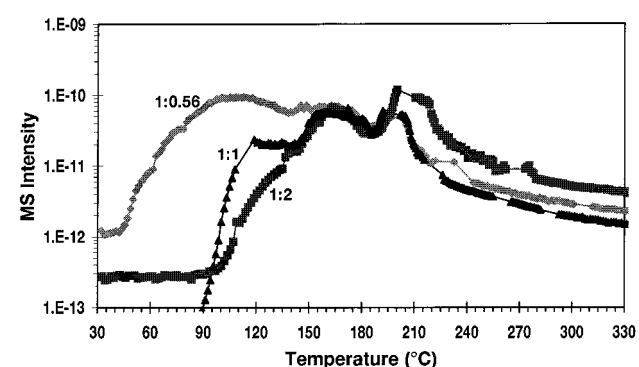
**Figure 4.** TG/DTA of nicotine bitartrate in 21% oxygen as a function of temperature.



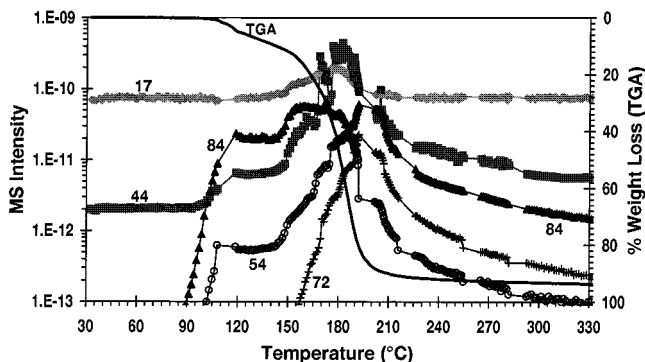
**Figure 7.** TG/MS of a nicotine malate salt (1:2) in helium as a function of temperature.



**Figure 5.** TG/MS of a nicotine malate salt (1:0.56) in helium as a function of temperature.



**Figure 8.** Nicotine evolution,  $m/z$  84, of the three nicotine malate salts in helium as a function of temperature.



**Figure 6.** TG/MS of a nicotine malate salt (1:1) in helium as a function of temperature.

diprotinated nicotine malate resulting in the formation and further decomposition of nicotine maleate and/or nicotine fumarate. It is of interest to note that the nicotine malate salt prepared with excess malic acid (1:

2) does not show a nicotine peak in the low-temperature range of about 115 °C, suggesting the nicotine in this sample is totally diprotinated (consistent with the TG/MS data of the diprotinated nicotine bitartrate).

The disproportionation reaction of eq 1 ensures that any volatile carboxylic acid too weak to diprotinate nicotine effectively cannot prevent the volatility of all of the nicotine at temperatures comparable to those required to evaporate nicotine. Any dication that does form as a result of disproportionation may dissociate back to the monocation, allowing the circle leading to complete dissociation to continue. Competing with any disproportionation of a monoprotonated nicotine salt (2) with a weak acid is the direct dissociation reaction into free nicotine (1) and free carboxylic acid HZ, portrayed in Scheme 1. Such dissociation can be driven to completion by removal of the acid from the system by simple evaporation should the acid be significantly more volatile than nicotine. Acetic acid was found to be such an acid, significantly preceding nicotine into the gas phase.

**Table 4. Peak Appearance Temperatures of Nicotine from 1, 2a, 2b, 2b–3b, 3b, 2c–3c, and Burley Tobacco**

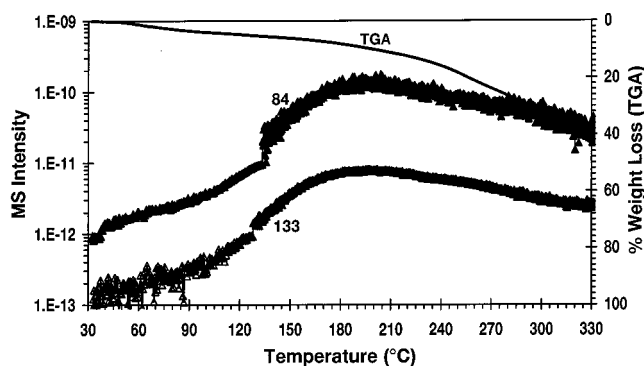
substrate	atmosphere <sup>a</sup>	peak appearance temp (°C) of nicotine <sup>b</sup>	figure
nicotine ( <b>1</b> )	helium	110 ± 5	1A
nicotine ( <b>1</b> )	21% oxygen/He	115 ± 5	1B
nicotine acetate ( <b>2a</b> )	helium	115 ± 5	2
nicotine acetate ( <b>2a</b> )	21% oxygen/He	120 ± 5	
nicotine bis[(2 <i>R</i> ,3 <i>R</i> )-hydrogen tartrate] dihydrate ( <b>2c–3c</b> )	helium	200 ± 5	3A
nicotine bis[(2 <i>R</i> ,3 <i>R</i> )-hydrogen tartrate] dihydrate ( <b>2c–3c</b> )	21% oxygen/He	200 ± 5	3B, 4
nicotine ( <i>S</i> )-malate ( <b>2b</b> ) 1/0.56 nicotine/malic acid	helium	110 ± 5, 165 ± 5, 200 ± 5	5, 8
nicotine ( <i>S</i> )-malate ( <b>2b</b> ) 1/0.56 nicotine/malic acid	21% oxygen/He	120 ± 5, 165 ± 5, 190 ± 5	
nicotine ( <i>S</i> )-malate ( <b>2b–3b</b> ) 1/1.0 nicotine/malic acid	helium	120 ± 5, 165 ± 5, 200 ± 5	6, 8
nicotine ( <i>S</i> )-malate ( <b>2b–3b</b> ) 1/1.0 nicotine/malic acid	21% oxygen/He	120 ± 5, 170 ± 5, 200 ± 5	
nicotine ( <i>S</i> )-malate ( <b>3b</b> ) 1/2.0 nicotine/malic acid	helium	165 ± 5, 205 ± 5	7, 8
nicotine ( <i>S</i> )-malate ( <b>3b</b> ) 1/2.0 nicotine/malic acid	21% oxygen/He	170 ± 5, 205 ± 5	
Burley tobacco <sup>c</sup>	helium	195 ± 15	9

<sup>a</sup> Flow ~100 mL min<sup>-1</sup>. <sup>b</sup> Samples heated at ~5 °C min<sup>-1</sup>. <sup>c</sup> Large sample size used.

On the basis of the results reported herein, the highest temperature of free nicotine release, which will be from its diprotonated form (**3**), will depend on the conjugate acidic strength, the volatility, and the thermal stability of the acid counterion. If the intrinsic temperature of decomposition of a given organic counterion is below the intrinsic dissociation temperature of diprotonated nicotine (**3**), it will be the counterion decomposition temperature that dictates the volatilization upper temperature ranges of free nicotine. The amount of heat needed to volatilize these polycarboxylic acids intact is greatly in excess of that needed to effect their decomposition by dehydration and/or decarboxylation and/or cyclic anhydride formation, which leads to the formation of weaker monocarboxylic acids (e.g., acrylic acid) or nonacids (e.g., acetaldehyde and maleic anhydride).

The peak appearance temperatures for nicotine from **1**, **2a**, **2b**, **2b–3b**, **3b**, and **2c–3c** determined in this study are summarized in Table 4. These results are consistent with reports by Lawson, Bullings, and Perfetti in the patent literature (Lawson et al., 1989a; Lawson et al., 1989b) which report, without either specific data or experimental details, that organic acid salts of nicotine decompose below 200 °C. Essentially three temperature ranges for the appearance of nicotine were observed for the various salts studied: 115 ± 5, 165 ± 5, and 200 ± 5 °C. It would have been attractive to suppose that these three ranges corresponded to the release of nicotine from nonprotonated nicotine, monoprotonated nicotine, and diprotonated nicotine, respectively. However, more likely, based on the observed temperature profile of mass spectral ion evolution of the substrates examined, it is that they encompass two true ranges. The lower range (115 ± 5 °C) would correspond to evaporation/volatilization of nonprotonated nicotine (**1**) available from the system either by dissociation from a weak acid (as is the case with acetic acid) or from disproportionation of the monocation (**2**). The higher range (160–210 °C) would reflect nicotine release from mostly dicationic forms **3** as a result of variable temperatures of anion decomposition, which is dependent on the thermal stability of the counterion or of counterions derived from those originally present.

Figure 9 shows the TG/MS curves for Burley tobacco leaf run under helium. To obtain a recordable MS intensity for nicotine (*m/z* 84 and 133), a deeper TG sample pan was filled with the Burley tobacco sample and the voltage to the secondary electron multiplier of the MS was increased. This accounts for the observed higher baseline and greater tailing effects in the MS

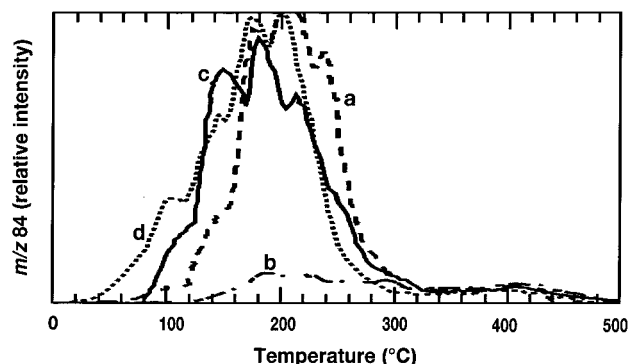


**Figure 9.** TG/MS of Burley tobacco leaf in helium as a function of temperature.

curves. The peak appearance temperature of nicotine occurs between about 180 and 210 °C. These temperatures fall well within the range observed for the nicotine dicarboxylate salts studied here. The lack of a significant nicotine appearance at ca. 115 °C indicates that there is no nonprotonated nicotine available in the tobacco matrix at that stage and given that a disproportionation reaction (eq 1) would form nonprotonated nicotine, peaks at ca. 115 °C would have been observed. We therefore infer that nicotine, at these elevated temperatures in the tobacco, is diprotonated. A more detailed discussion of this point is presented in section 5.

**4. Evolved Gas Analysis.** Transfer of nicotine to the gas phase from various tobacco types was evaluated using evolved gas analysis/mass spectrometry (EGA/MS). The EGA/MS system had a much larger sample capacity compared to that of the TG/MS system, therefore, making it more suitable for studying tobacco samples which cannot be analyzed conveniently using TG/MS techniques. Figure 10 (curves a–d) depict the transfer of nicotine to the gas phase from normal Burley tobacco, a low-alkaloid Burley, low-alkaloid Burley with added nicotine citrate (1:2), and low-alkaloid Burley with added nonprotonated nicotine, respectively. Normal Burley tobacco, when heated, resulted in a broad “triplet” peak with maxima at about 180 and 210 °C and a shoulder at 240 °C (Figure 10, curve a). A low-alkaloid Burley, when heated, shows (Figure 10, curve b) a weak “doublet” with maxima at about 190 and 220 °C. This experiment serves as a control for the two “nicotine addition” experiments, shown in Figure 10 (curves c and d). When nicotine citrate was added to the low-alkaloid





**Figure 10.** Nicotine evolution,  $m/z$  84, of normal Burley (a), a low-alkaloid Burley (b), low-alkaloid Burley with added nicotine citrate (c), and low-alkaloid Burley with added nonprotonated nicotine (d) in helium as a function of temperature.

**Table 5. Peak Appearance Temperatures of Nicotine from Various Tobacco Samples, As Determined by Evolved Gas Analysis (EGA)**

source of nicotine	peak appearance temp (°C) of nicotine <sup>a,b</sup>	figure
Burley Tobacco Samples		
Burley	180(m), 210(s), 240(m)	10a
low-alkaloid Burley	190(w), 220(w)	10b
low-alkaloid Burley + nicotine citrate	150(m), 180(s), 215(m)	10c
low-alkaloid Burley + nonprotonated nicotine	105(w), 145(m), 175(s), 200(s)	10d
Bright Tobacco Samples		
Bright	160(s), 180(s), 210(m), 240(m)	
low-alkaloid Bright	180(w), 210(w)	
low-alkaloid Bright + nicotine citrate	160(m), 195(s), 235(m)	
Oriental Tobacco Samples		
low-alkaloid Oriental	180(w), 220(w)	
low-alkaloid Oriental + nicotine citrate	160(s), 185(s), 220(w)	

<sup>a</sup> Performed under an atmosphere of helium and at a thermal temperature heating rate of 20 °C min<sup>-1</sup>. <sup>b</sup> w = peak of weak or low intensity; m = peak of medium or moderate intensity; s = peak of strong or high intensity.

Burley sample and heated, a "triplet" (150, 180, and 215 °C) was seen (Figure 10, curve c) similar to that observed in normal Burley (Figure 10, curve a), consistent with nicotine transfer temperatures observed for protonated nictines. When nonprotonated nicotine was added to the low-alkaloid Burley, a "triplet" was observed (Figure 10, curve d) with maxima at about 145, 175, and 200 °C. The weak shoulder observed at about 105 °C in Figure 10 (curve d) can be attributed to the presence of residual nonprotonated nicotine and/or the disproportionation of monoprotonated nicotine. These results suggest that sufficient acidity is present in the low-alkaloid Burley to convert exogenous nicotine (1) to its monoprotonated (2) and/or diprotonated (3) form(s). Similar studies were performed with both Bright and Oriental tobaccos (the data are summarized in Table 5).

The EGA data are consistent with the TG/MS results which show the peak nicotine evolution temperatures from Burley tobacco leaf in the range of 180–210 °C. The EGA results are also consistent with work reported in 1960 by Kobashi and co-workers (Kobashi et al., 1960; Kobashi and Sakaguchi, 1960) who found that addition of nonprotonated nicotine or, separately, nicotine tartrate to a low-alkaloid filler led to cigarette models

which had the same nicotine transfer efficiency to mainstream smoke. (Mainstream smoke is the smoke which travels down the cigarette rod and through the cigarette filter.) Note that evidence has been presented (Jenkins and Comes, 1976) that the transfer of nicotine to mainstream smoke is the same from endogenous nicotine and exogenously added nicotine.

**5. Stability of Nicotine under Pyrolytic Conditions. Transfer of Nicotine to the Gas Phase.** It is crucial to demonstrate that nicotine itself is stable under the thermal conditions required to volatilize it from nonprotonated nicotine itself or the thermal conditions required to form it and volatilize it from the various protonated nictines 2 and 3. Recently, it was found that heating samples of nicotine, (*S*)-nicotine bis[(2*R*,3*R*)-hydrogen tartrate] dihydrate, and the 1:0.56 and 1:1 nicotine malates individually from ambient temperature, at approximately 400 °C min<sup>-1</sup>, under a 500 mL min<sup>-1</sup> flow of either air or argon led to 92–100% transfer efficiency (or yield) (Fournier et al., 1998). A number of studies have shown that nicotine in the absence of oxygen will not begin to decompose thermally until temperatures in excess of 600 °C are reached (von Euler, 1965; Balasubrahmanyam and Quin, 1962; Jarboe and Rosene, 1961). Nicotine is stable in air until temperatures exceed 300–350 °C with a 2-s contact time on a glass wool surface (von Euler, 1965; Kobashi et al., 1963). As nicotine volatilizes well below its boiling point of 247 °C (see Tables 4 and 5), pyrolysis studies at higher temperatures must be performed by injecting nicotine into a preheated tube in one of a number of fashions (Balasubrahmanyam and Quin, 1962; Jarboe and Rosene, 1961; Schmeltz et al., 1979; von Euler, 1965). Additionally, in the TG/MS studies reported herein, no mass spectral fragmentations were observed for the ions which are markers for myosmine ( $m/z$  146) (Duffield et al., 1965), the major thermal decomposition product of nicotine (von Euler, 1965; Balasubrahmanyam and Quin, 1962; Jarboe and Rosene, 1961).

The term "transfer of nicotine to the gas phase" does not mean that all of the nicotine remains in the gas phase during, or after, a pyrolysis experiment (or in the burning/puffing of a cigarette). Rather, it is an estimate that the initial thermal process puts the nicotine into the gas phase near the heat source (or the coal). Subsequently, the travel of smoke along the pyrolysis tube and cooling (or down the cigarette rod) causes condensation (sometimes referred to as "raining out" or precipitation) of most of the nicotine out of the gas phase and into an aerosol (Jenkins and Comes, 1976; Houseman, 1973; Crooks and Lynn, 1992).

Because thermally induced water evolution occurs at low temperatures, the water content of the tobacco drops significantly and the effective acidity of the residual tobacco may increase during the heating process. As discussed in sections 3 and 4, evolution peaks at ca. 110 °C (for nonprotonated nicotine) are not seen in the thermal treatments of the tobacco samples except when excess nonprotonated nicotine is added to the tobacco sample. We therefore conclude that endogenous nicotine in tobacco in the region about to release nicotine due to the approaching heat is likely to be substantially diprotonated.

Under smoking conditions, the heat of the approaching coal would be expected to expel much of the water content of tobacco, before nicotine release became significant. The results above emphasize that nicotine

in tobacco closely resembles the polycarboxylate salts of nicotine in the thermal requirements for nicotine release to the gas phase and is significantly different from the behavior to be expected for monocarboxylic acid salts of nicotine, as exemplified by the acetic acid–nicotine system. It is the stronger (and significantly abundant) polycarboxylic acids (Wynder and Hoffmann, 1967) whose anions will resist protonation more than those of the weaker monocarboxylic acids. This work has demonstrated that weak low molecular weight aliphatic acids (such as acetic acid) precede nicotine into the gas phase.

Importantly, whatever carboxylic acids that are formed during the smoking process from acidic precursors are likely to be weaker than their precursors. To the extent that acids condense in the cooler regions of the cigarette, new nicotine carboxylic acid salts may be formed. The re-evaporation of nicotine from such salts will likely be more facile than for the original release process, as our results with nicotine acetate vs the nicotine polycarboxylates have demonstrated above.

The appearance temperatures of nicotine (Tables 4 and 5) are significantly lower than the temperatures observed near the coal of a puffing cigarette, ca. 600–950 °C (Baker, 1975, 1981, 1987; Kobashi et al., 1960; McRae et al., 1987). These factors (i.e., the stability of the nicotine ring system and the high temperature ranges of a burning cigarette) combined with the mass of the tobacco allow the dissociation and/or decomposition and/or disproportionation of nicotine carboxylic acid volatilization to nicotine in the gas state. In conclusion, the TG/DTA/MS results, combined with the thermal stability of the nicotine ring structure in both its nonprotonated and carboxylic acid salt forms, clearly indicate that nicotine carboxylic acid salts will transfer nicotine to the gas phase in thermolysis experiments and in the smoking process with efficiency comparable to that from nonprotonated nicotine itself.

**6. Transfer of Nicotine to the Gas Phase from the Lower Temperature Regions of a Puffing Cigarette.** A more subtle question is now addressed: Is it possible that within portions of the puffing cigarette, intermediate temperatures will be experienced (e.g., in the range of ca. 125–250 °C) such that an enhanced amount of nicotine will volatilize from nonprotonated nicotine (**1**) but not from monoprotonated (**2**) and/or diprotonated (**3**) nicotine(s)?

*i. Transfer Kinetics.* The time available during the puffing of a cigarette compared with the rates of volatilization can be evaluated. The Federal Trade Commission (FTC) method defines a standard model for experimental smoking comparisons as follows: a 35 mL puff taken over a 2-s interval, followed by a 58 s static burn (Federal Register, 1967; Pillsbury et al., 1969). That is, the time during which a puff is taking place and mainstream smoke is being generated is modeled as 2 s.

It is useful to determine the “reaction fraction dissociation/decomposition/volatilization times” for **1–3**, that is, the time it would take to convert and/or transfer **1–3** to nicotine in the gas phase at various temperatures. To evaluate this, nicotine (**1**) and (*S*)-nicotine bis-[(2*R*,3*R*)-hydrogen tartrate] dihydrate (**2c–3c**) were each subjected to TG scans at four heating rates (5, 10, 15, and 20 °C min<sup>-1</sup>) under flowing compressed air at rates of 100 and 500 mL min<sup>-1</sup>. A kinetic software package (utilizing the Ozawa method) was used to

**Table 6. Calculated Reaction Fraction Decomposition Time (s) and Energy of Activation ( $E_a$ ) To Transfer Nicotine to the Gas Phase from Nicotine (**1**) and (*S*)-Nicotine Bis[(2*R*,3*R*)-hydrogen tartrate] Dihydrate (**2c–3c**) under a Compressed Air Flow of 100 mL min<sup>-1</sup>**

temp (°C)	% transfer to nicotine in the gas phase					
	from nicotine ( <b>1</b> )			from nicotine bitartrate ( <b>2c–3c</b> )		
	10%	20%	50%	10%	20%	50%
	time (s) to reach specified percentage of nicotine in the gas phase					
150	30	55	130	1200	1900	3300
200	5.8	11	24	43	61	104
250	1.6	2.8	6.4	3.0	3.8	6.4
300	0.5	0.9	2.1	0.3	0.4	0.6
$E_a$	55 kJ/mol			115 kJ/mol		

**Table 7. Calculated Reaction Fraction Decomposition Time (s) and Energy of Activation ( $E_a$ ) To Transfer Nicotine to the Gas Phase from Nicotine (**1**) and (*S*)-Nicotine Bis[(2*R*,3*R*)-hydrogen tartrate] Dihydrate (**2c–3c**) under a Compressed Air Flow of 500 mL min<sup>-1</sup>**

temp (°C)	% transfer to nicotine in the gas phase					
	from nicotine ( <b>1</b> )			from nicotine bitartrate ( <b>2c–3c</b> )		
	10%	20%	50%	10%	20%	50%
	time (s) to reach specified percentage of nicotine in the gas phase					
150	16	34	85	940	1600	2900
200	2.5	5.6	14	31	48	82
250	0.6	1.3	3.3	2.0	2.8	4.6
300	0.2	0.4	1.0	0.2	0.3	0.4
$E_a$	60 kJ/mol			116 kJ/mol		

calculate activation energies and degradation/volatilization times (lifetimes). The data calculated at the two different flow rates are presented in Tables 6 and 7. For appearance of nicotine, a value of ca. 55–60 kJ/mol was determined, consistent with the literature value for the heat of vaporization for nicotine of 51.6 kJ/mol (CRC Handbook of Chemistry and Physics, 1973). The activation energy determined for the melting of the nicotine bitartrate (**2c–3c**) and subsequent decomposition and transfer of nicotine to the gas phase was about 115 kJ/mol.

Degradation/volatilization times for nicotine and nicotine bitartrate were calculated for temperatures of 150, 200, 250, and 300 °C at reaction fractions of 10%, 20%, and 50%. Since the weight loss observed for nicotine is via evaporation, rather than decomposition followed by volatilization, as observed for the bitartrate salt, it follows (as observed in Tables 6 and 7) that flow rate should have a greater influence on the degradation/volatilization times of free nicotine than that of the salt.

The data in Tables 6 and 7 can be used to extrapolate degradation/volatilization times for nicotine and the natural tobacco carboxylic acid salts of nicotine in a cigarette during puffing (1050 mL min<sup>-1</sup> at FTC conditions of a 2 s 35 mL puff). It should be noted that the diprotonated bitartrate salt is thermally more stable than the protonated salts of the other carboxylic acids investigated; therefore, it would be expected that the degradation/volatilization times of other protonated salts (e.g., malates, acetates, citrates) would be less than that of the bitartrate salt.

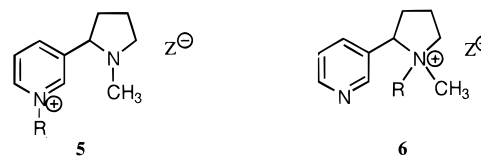
In the temperature range of 250–300 °C, the data in Tables 6 and 7 suggest that the transfer rates of nicotine to the gas phase from nonprotonated nicotine and the carboxylic acid salts of nicotine are comparable. It is only within the temperature range from about 125 to

250 °C that differences in the transfer rate of nicotine to smoke from protonated nicotine and nonprotonated nicotine (if any nonprotonated nicotine is, in fact, present) could be expected. However, within the 125–250 °C region of a cigarette rod during puffing, the rate of volatilization of nonprotonated nicotine is expected to be small. For example, extrapolating the data in Tables 6 and 7, one can estimate that in the 200 °C region of a puffing cigarette, it would take about 4–5 s to volatilize 20% of any nonprotonated nicotine present. Therefore, during the relatively short time of a puff, significantly less than 20% of any nonprotonated nicotine in the 125–250 °C region could volatilize. Moreover, this particular temperature zone of the cigarette rod is not only narrow (Baker, 1975, 1981, 1987), but is also in motion (Crooks and Lynn, 1992), as the coal region chases it down the rod in response to puffing action. The cigarette's dynamic burn further decreases the time any particular zone of tobacco is exposed to this particular temperature range to significantly less time than the duration of a puff. The combined effect of (1) the small volume of tobacco within the 125–250 °C region of a puffing cigarette, (2) the brief time a particular zone of tobacco is exposed to this temperature range, (3) the relatively low temperature (and available energy) of the region, and (4) the small content of nonprotonated nicotine multiplied together indicate a minute possibility of significant transfer of nicotine to smoke from this temperature region. Furthermore, there is likely to be net condensation of nicotine within the lower temperature part of this region as the aerosol begins to form (see below).

*ii. Condensation of Smoke Nicotine onto the Tobacco.* As nicotine travels down the rod during puffing, it is known that some of it condenses onto, or is otherwise entrained by, the tobacco as the temperature of cigarette smoke decreases (Jenkins and Comes, 1976; Houseman, 1973; Crooks and Lynn, 1992). Analysis of puff-by-puff nicotine delivery also confirmed the condensation phenomena (Crooks and Lynn, 1992). Thus, there is significant flux of nicotine *onto* the tobacco in lower temperature regions of a cigarette during a puff rather than volatilization of nicotine off of the tobacco, i.e., a net deposition of nicotine in these lower temperature regions is what occurs. Greater than 95% of the nicotine in mainstream smoke at the exit point of a cigarette is in the particulate phase, as would be expected from the low vapor pressure of nicotine at 30 °C (Ogden et al., 1993).

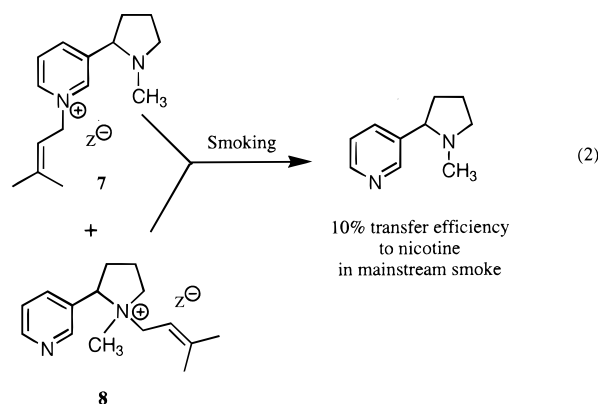
**7. Formation of and Transfer of Nicotine to the Gas Phase from Covalently Bound Nicotine Precursors.** The claim has been made that protonated nicotine in tobacco is "bound" and does not transfer to the smoker during the smoking process (Kessler et al., 1996, 1997; Kessler, 1994). One might ask to what extent is protonated nicotine "bound" or unable to be volatilized thermally? The results described above provide strong evidence that protonated nicotine is fully capable upon mild (ca. 200 °C) thermal treatment to transfer nicotine to the gas phase.

Another way of examining this question would be to ask, if one wanted to construct a "bound" nicotine, what might it be? One experimental answer is to examine the smoke chemistry of a nicotine derivative such as **5** and/or **6**, where R is a covalently bound substituent other than a proton (in these cases, forming a N–C bond).

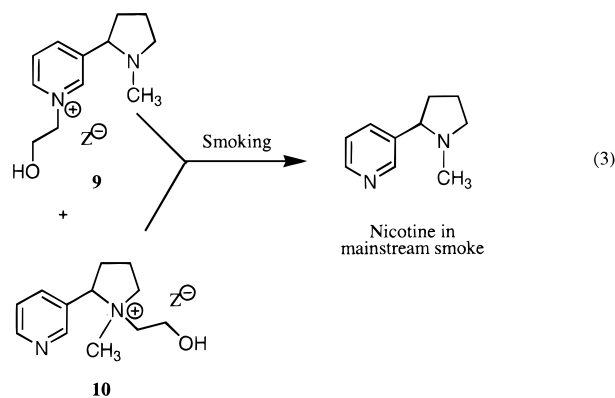


To evaluate smoke chemistry, researchers from government, academia, and industry have used the standard FTC smoking paradigm to quantify nicotine and "tar" deliveries. Under these conditions, it is known that the transfer efficiency of nicotine in commercial cigarettes is between 1% and 10% (Hoffmann et al., 1995). Transfer efficiency means the amount of nicotine delivered to mainstream smoke divided by the amount of alkaloid present in the tobacco in the cigarette before smoking. Transfer efficiency is analogous to the yield of a chemical reaction.

An aqueous solution of *N*- and *N*-(3-methyl-2-but-1-enyl)nicotinium citrate (**7** and **8**, eq 2) was added to a low-alkaloid Burley–Bright tobacco mixture (1:1).



The resultant tobacco was equilibrated overnight and handmade into nonfiltered cigarettes. These cigarettes were found to yield an additional amount of nicotine to the mainstream smoke, equal to a 10% transfer efficiency, based on the amount of **7** and **8** that was added to each cigarette filler (Seeman, 1982). As the "net" transfer efficiency to nicotine in the mainstream smoke for **7** and **8** is within the experimental range of transfer efficiencies for nicotine itself (Perfetti et al., 1998), **7** and **8** cannot be considered "bound", if by "bound" one implies an inability for nicotine to be transferred to the smoke. Similar results have been reported for the transfer to nicotine in smoke from the reaction products of nicotine with ethylene oxide (**9** and **10**, eq 3) in the tobacco matrix (Obi et al., 1968).



## SUMMARY, CONCLUSIONS, AND IMPLICATIONS OF THIS WORK

The nicotine ring system is thermally quite stable. Nicotine carboxylic acid salts transfer nicotine to the gas phase by three types of mechanisms: deprotonation, decomposition of the carboxylic acid anion, and disproportionation. Under the TG/DTA/MS conditions used in these studies, the peak transfer temperatures from nicotine itself and from the various nicotine carboxylic acid salts to nicotine in the gas phase are nicotine and nicotine acetate, both ca. 110–125 °C; nicotine malates, ca. 110–210 °C for nicotine to malic acid ratios of 1:0.56 and 1:1 and ca. 160–210 °C for a nicotine to malic acid ratio of 1:2; and (*S*)-nicotine bis[(2*R*,3*R*)-hydrogen tartrate] dihydrate, ca. 195–210 °C. Decomposition products of nicotine were not observed, even in 21% oxygen.

The TG/DTA/MS and EGA/MS experiments show peak nicotine evolution temperatures from tobacco samples in the range of ca. 160–220 °C, the same range as the nicotine polycarboxylic acid salts. EGA/MS results also show that addition of modest amounts of nonprotonated nicotine to low-alkaloid tobaccos results in nicotine evolution peaks similar to those observed for normal tobaccos. Under smoking conditions, nicotine release occurs after much of what water was originally present has first evaporated. Nicotine in cigarette tobacco is present largely (>95%, perhaps even >99%) in a protonated state at room temperature. Thermally induced water evolution occurs at temperatures lower than those required for nicotine release, possibly increasing the effective acidity of the medium. At the temperature range where thermal release of nicotine from tobacco occurs, evidence reported herein suggests that nicotine is diprotonated.

Nonprotonated nicotine and nicotine carboxylic acid salts, such as those found in tobacco, are likely to transfer nicotine to the gas phase during the smoking process with comparable yield and efficiency. Mild heat (300–400 °C) should be sufficient to transfer nicotine to the gas phase. It follows that the acidity of the tobacco, as examined herein, is of little consequence for delivery of nicotine into the gas and, subsequently, aerosol (or particulate) phase.

Given that the principal temperature range of nicotine evolution from both tobacco and the diprotonated nicotine polycarboxylic acid salts is essentially the same, it is likely the tobacco polycarboxylic acids (e.g., malic acid and citric acid) determine the nicotine evaporation temperature ranges from the tobacco matrix (less than about 220 °C, considerably less than temperatures observed around the coal of a burning cigarette). The polycarboxylic acids are thermally labile and decarboxylatively dehydrate or eliminate to form less acidic products, e.g., monocarboxylic acids.

The kinetic results from the TGA study suggest that given the contact time available under smoking conditions, whatever nonprotonated nicotine that might exist in the tobacco would only be transferred to smoke in significant amounts at temperatures in excess of about 300 °C. At these temperatures there is little difference in the rates of volatilization of nicotine from nicotine carboxylic acid salts and evaporation of nonprotonated nicotine. The zone of a puffing cigarette that encompasses the temperature range 125–250 °C is physically very small, and the rate of transfer of nicotine to the gas phase is low; therefore, any nonprotonated nicotine contained within the lower temperature regions (should

some exist) would likely have insufficient time to volatilize. Volatilization of nicotine from lower temperature regions will be further minimized because the smoke passing through these regions is already saturated with nicotine originating from the higher temperature regions nearer the coal. Indeed, because of condensation of nicotine from the hot smoke coming from the higher temperature regions to the lower temperature regions of the cigarette rod during puffing, a net flux of nicotine *onto* the tobacco in the lower temperature regions is likely, rather than the reverse.

Nicotine covalently bound as *N*- and *N*-alkylnicotinium quaternary salts were found from previous work to transfer nicotine to the smoke from a puffing cigarette in yields similar to that found for the transfer of endogenous nicotine to nicotine in smoke. Protonated nicotine cannot be considered "bound", if by the term "bound" it is meant a form of nicotine which will not readily transfer nicotine to smoke.

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