Mass Spectra of Tobacco Alkaloid-Derived Nitrosamines, their Metabolites, and Related Compounds*

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INTRODUCTION

Nitrosamines derived from the tobacco alkaloids are present in tobacco and tobacco smoke. The principal alkaloid-derived nitrosamines which have been considered in studies to date are *N'*-nitrosanornicotine [NNN], 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK], 4-(methylnitrosamino)-4-(3-pyridyl)butanal [NNA], *N'*-nitrosoanabasine [NAB], and *N'*-nitrosoanatabine [NAtB] (1).

NNN, NNK and NNA are formed in the nitrosation of nicotine. NAB and NAtB are derived from anabasine and anatabine. NNN, NNK and NAtB have been detected in tobacco and tobacco smoke (2).

The mass spectral data presented in this report will be useful in further studies on the occurrence and metabolism of the tobacco alkaloid-derived nitrosamines as well as in studies on related derivatives of the tobacco alkaloids.

MATERIALS AND METHODS

Apparatus

Mass spectrometry (MS) and gas-liquid chromatography in combination with mass spectrometry (GLC-MS) were run with a Hewlett-Packard Model 5982A dual source

instrument with a membrane separator. For GLC-MS, a 6 ft. × 0.25 inch column filled with 10% UCW-98 on Gas-Chrom Q was used, with helium as carrier gas (flow rate: 50 ml/min). High resolution mass spectra were determined on an AEI MS-30 instrument (Associated Electrical Industries) by Shrader Analytical and Consulting Laboratories, Inc., Detroit, Michigan.

Chemicals

Numbers in italics and square brackets refer to structures in Schemes 1 and 2. All compounds were synthesized: NNN (3a); NNK, NNA, 4-hydroxy-1-(3-pyridyl)-1-butanone [10] (4); NAB (5); NAtB (2, 3); 3'-hydroxy-NNN [3], 4'-hydroxy-NNN [4], NNN-1-*N*-oxide [1], 3'-hydroxymyosmine, 4'-hydroxymyosmine, 3'-hydroxynornicotine, 4'-hydroxynornicotine (6); 5-(3-pyridyl)-2-hydroxytetrahydrofuran [12], 4-hydroxy-1-(3-pyridyl)-1-butanone-2,4-dinitrophenylhydrazone, 4-hydroxy-4-(3-pyridyl)-1-butanal-2,4-dinitrophenylhydrazone (7); 4-oxo-4-(3-pyridyl)butanal [22], 4-oxo-4-(3-pyridyl)butanal-bis-2,4-dinitrophenylhydrazone [24], 4-hydroxy-1-(3-pyridyl)butane-1-ol [14], 4-(methylnitrosamino)-1-(3-pyridyl)butane-1-ol [18], 4-(methylnitrosamino)-1-(3-pyridyl)-*N*-oxide-1-butanone (NNK-1-*N*-oxide [17]) (8); 2'-acetoxy-*N'*-nitrosanornicotine, 4-(*N*-carbethoxy-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone [21], 5'-acetoxy-*N'*-nitrosanornicotine (9); *N'*-nitrosanornicotine, 1-(3-pyridyl)-2-butene-1-one (10); cyclopropyl(3-pyridyl)ketone (11); norcotinine [6] (12); 4-oxo-4-(3-pyridyl)butyric acid [13], 4-hydroxy-4-(3-pyridyl)butyric acid [15] (13).

Silylation was performed with bis(trimethylsilyl)trifluoroacetamide plus 1% trimethylchlorosilane (Regisil RC-2), obtained from Regis Chemical Company, Morton Grove, Ill. The purity of all compounds was assured by analysis by GLC, thin-layer chromatography (TLC), or high-pressure liquid chromatography (HPLC) prior to determination of mass spectra.

Mass Spectrometry

Mass spectra were determined by direct introduction (ion source temperature, 180 °C; ionizing voltage, 70 eV)

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Scheme 1. Metabolism of *N'*-nitrosornicotine [NNN] in the F-344 rat.

Figure 1. Mass spectrum of *N'*-nitrosornicotine [NNN].

Figure 2. Mass spectrum of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK].

Figure 3. Mass spectrum of 4-(methylnitrosamino)-4-(3-pyridyl)butanal [NNA].

or, for the silylated compounds, by combined GLC-MS (150 °C for 8 min, then 4°/min to 240 °C). High resolution mass spectra were obtained by direct introduction of selected compounds (ion source temperature, 200 °C; ionizing voltage, 70 eV; accelerating voltage, 4000 V). All elemental compositions of fragment ions discussed in the text have been confirmed by high resolution mass spectrometry.

Figure 4. Mass spectrum of *N'*-nitrosoanabasine [NAB].

Figure 5. Mass spectrum of *N'*-nitrosoanatabine [NAtB].

RESULTS AND DISCUSSION

The mass spectra of NNN, NNK, NNA, NAB and NAtB are shown in Figures 1–5. Mammalian metabolites of NNN and NNK are summarized in Schemes 1 and 2. Among the NNN metabolites, 3'-hydroxy-NNN [3], 4'-hydroxy-NNN [4], NNN-1-*N*-oxide [1], norcotine [6], keto acid [13], diol [14], hydroxy acid [15], and lactone [16] are urinary metabolites of NNN in the F-344 rat whereas myosmine [7], keto alcohol [10], lactol [12], 3'-hydroxy-NNN [3], 4'-hydroxy-NNN [4], and NNN-1-*N*-oxide [1] were observed when NNN was incubated with rat liver microsomes; [10] and [12] were also produced by human liver microsomes (6, 7, 14). The NNK metabolites NNK-1-*N*-oxide [17] and 4-(methylnitrosamino)-1-(3-pyridyl)butane-1-ol [18] as well as keto acid [13], diol [14] and hydroxy acid [15] were detected in the urine of NNK-treated F-344 rats, whereas [17], [18], myosmine [7], keto alcohol [10], and keto aldehyde [22] were formed when NNK was incubated with rat liver microsomes (8). The mass spectra of myosmine [7], norcotine [6], keto acid [13], lactone [16] and hydroxy acid [15] were presented in a previous report on mammalian metabolites of nicotine (15). The mass spectra of the other metabolites are shown in Figures 6–14.

Scheme 2. Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK] in the F-344 rat.

Figure 6. Mass spectrum of *N*'-nitrososornicotine-1-*N*-oxide [NNN-1-*N*-oxide].

Figure 7. Mass spectrum of 3'-hydroxy-*N*'-nitrososornicotine.

Figure 8. Mass spectrum of 4'-hydroxy-*N*'-nitrososornicotine.

Derivatization has been useful in the identification of metabolites of NNN and NNK. The keto alcohol [10], lactol [12], and keto aldehyde [22] are most readily isolated and quantified as the corresponding 2,4-dinitrophenylhydrazones, the mass spectra of which are presented in Figures 15–17. Trimethylsilylation has been effective for the identification of metabolites by GLC–MS. The mass spectra of silylated metabolites are shown in Figures 18–25.

Figure 9. Mass spectrum of 4-hydroxy-1-(3-pyridyl)-1-butanone.

Figure 10. Mass spectrum of 5-(3-pyridyl)-2-hydroxytetrahydrofuran.

Figure 11. Mass spectrum of 4-hydroxy-1-(3-pyridyl)butane-1-ol.

Figures 26–35 present the mass spectra of a number of compounds related to the tobacco alkaloid-derived nitrosamines and their metabolites. 2'-Acetoxy-NNN (Fig. 26) and 4-(*N*-carbethoxy-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (Fig. 27) are model compounds for the unstable metabolites [2], [8], and [25] resulting from 2'-hydroxylation of NNN or methyl hydroxylation of NNK. 5'-Acetoxy-NNN (Fig. 28) is a model compound for 5'-hydroxy-NNN [5]. 3'-Hydroxymyosmine, 3'-hy-

Figure 12. Mass spectrum of 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanone [NNK-1-*N*-oxide].

Figure 13. Mass spectrum of 4-(methylnitrosamino)-1-(3-pyridyl)butane-1-ol.

Figure 14. Mass spectrum of 4-oxo-4-(3-pyridyl)butanal.

Figure 15. Mass spectrum of 4-hydroxy-1-(3-pyridyl)-1-butanone-2,4-dinitrophenylhydrazone.

Figure 16. Mass spectrum of 4-hydroxy-4-(3-pyridyl)-1-butanal-2,4-dinitrophenylhydrazone.

droxynornicotine, 4'-hydroxymyosmine and 4'-hydroxynornicotine (Figures 29–32) are intermediates in the syntheses of 3'-hydroxy-NNN and 4'-hydroxy-NNN and are potential metabolites of myosmine, nornicotine or NNN. Nitrosonornicotine (Fig. 33) is a potential nitrosation product of cotinine and nornicotine. It is unstable in water giving rise to lactone [16]. 1-(3-Pyridyl)-2-butene-1-one and cyclopropyl(3-pyridyl)ketone (Figures 34–35) have been detected as minor products in the decomposition of the model compounds for α -hydroxylation of NNN and NNK but have not been identified with certainty as metabolites (9).

The mass spectra of the compounds with a carbonyl

group adjacent to the pyridine ring are characterized by intense ions at m/e 78 ($C_5H_4N^+$) and m/e 106 ($pyr-C=O^+$). In addition, McLafferty rearrangement ions at m/e 121 are frequently observed. In [14] and [18] (Figures 11 and 13), ions are observed at m/e 108, corresponding to $(pyr-CHOH)^+$. The lactol [12] (Fig. 10) also gives an intense m/e 108 peak, which may arise after ring opening to the corresponding tautomer, 4-(3-pyridyl)-4-hydroxybutyraldehyde [11].

The mass spectra of the cyclic nitrosamines (Figures 1, 4–8, 26, 28) are generally characterized by loss of NO (M^+-30) followed by fragmentation of the pyrrolidine or piperidine ring. In NNN, major fragments are ob-

[illegible]

Chemical structure of compound 11: CC1(C)C(C(C1)O)C2=CC=CC=C2N2C(=O)N2

Mass spectrum of compound 11 (m/e vs. Rel. Intensity):

m/e	Rel. Intensity (%)
73	~30
92	~40
119	100
235	~20

Chemical structure of compound 119: C[Si](C)(C)O[N+]1CCCN1c2ccncc2

Mass spectrum data (approximate relative intensities):

m/e	Rel. intensity
73	25
92	30
101	15
119	100
130	15
145	15
235	15
265	10

Chemical structure of compound 10: C[Si](C)(C)C1COC(C1)c2ccncc2

Mass spectrum of compound 10. The x-axis is m/e (0 to 320) and the y-axis is Rel. intensity (0 to 100). The base peak is at m/e 75. Other significant peaks are labeled at m/e 118, 145, 219, and 234.

Chemical structure of compound 10: CCCC(=O)c1ccncc1OSi(C)(C)C

Mass spectrum of compound 10 (m/e vs. Rel. intensity):

m/e	Rel. intensity (%)
51	~10
75	100
78	~60
101	~60
106	~60
116	~60
121	~40
148	~50
194	~20
222	~70
237	~10

Chemical structure of compound 10: C[Si](C)(C)O[C@H]1O[C@H](c2ccncc2)[C@H](O1)

Mass spectrum of compound 10 (m/e vs. Rel. Intensity):

m/e	Rel. Intensity (%)
75	~75
119	100
130	~35
148	~25
222	~25
237	~15

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Figure 23. Mass spectrum of 4-hydroxy-1-(3-pyridyl)butane-1-ol-bis(trimethylsilyl) ether.

Figure 24. Mass spectrum of 4-oxo-4-(3-pyridyl)butyric acid trimethylsilyl ester.

Figure 25. Mass spectrum of 4-hydroxy-4-(3-pyridyl)butyric acid bis(trimethylsilyl)-ester ether.

The spectra of 3'-hydroxy-NNN [3] and 4'-hydroxy-NNN [4] contrast markedly. A prominent ion in the spectrum of 4'-hydroxy-NNN is m/e 145 ($C_9H_9N_2$), which may result from sequential loss of NO and H_2O .

Figure 26. Mass spectrum of 2'-acetoxy-N'-nitrosonor-nicotine.

Figure 27. Mass spectrum of 4-(N-carbethoxy-N-nitroso-amino)-1-(3-pyridyl)-1-butanone.

Figure 28. Mass spectrum of 5'-acetoxy-N'-nitrosonor-nicotine.

The m/e 145 ion is not observed as a major fragment in the spectrum of 3'-hydroxy-NNN. Analogous fragmentations occur in the spectra of 3'-hydroxymyosmine and 4'-hydroxymyosmine. These differences may be a consequence of facile cleavage of the pyrrolidine ring of [3] or of 3'-hydroxymyosmine, at the 2'-3' positions, a process which is apparently favored by the neighboring 2'-(3-pyridyl) and 3'-hydroxy groups. A second important distinction between the spectra of 3'-hydroxy-

Figure 29. Mass spectrum of 3'-hydroxymyosmine.

Figure 30. Mass spectrum of 3'-hydroxynornicotine.

Figure 31. Mass spectrum of 4'-hydroxymyosmine.

Figure 32. Mass spectrum of 4'-hydroxynornicotine.

Figure 33. Mass spectrum of N'-nitrosomycosmine.

Figure 34. Mass spectrum of 1-(3-pyridyl)-2-butene-1-one.

Figure 35. Mass spectrum of cyclopropyl(3-pyridyl)ketone.

NNN and 4'-hydroxy-NNN is the loss of NO, which is inhibited in the latter. In this respect, 4'-hydroxy-NNN resembles NNN-1-N-oxide; their intense molecular ions are unusual for nitrosamines. The mechanistic basis for these differences requires further investigation.

SUMMARY

The mass spectra of the tobacco alkaloid - derived nitrosamines NNN, NNK, NNA, NAB and NATB and of known mammalian metabolites of NNN and NNK are presented. Mass spectra of synthetic derivatives related to these nitrosamines and their metabolites are also included. These spectra will be useful in studies on the occurrence and metabolism of the tobacco alkaloid-derived nitrosamines and in investigations of other compounds related to the tobacco alkaloids.

ZUSAMMENFASSUNG

Für die von Tabakalkaloiden abgeleiteten Nitrosamine NNN, NNK, NNA, NAB und NATB sowie für die bekannten Säugetiermetaboliten von NNN und NNK werden die Massenspektren dargestellt. Auch werden die Massenspektren synthetischer Derivate dieser Nitrosamine und ihrer Metaboliten vorgestellt. Bei der Untersuchung des Vorkommens und des Stoffwechsels von Nitrosaminen, die sich von Tabakalkaloiden ableiten, und bei der Erforschung anderer mit den Tabakalkaloiden verwandter Verbindungen können die massenspektrometrischen Daten von Nutzen sein.

RÉSUMÉ

Les spectres de masse des nitrosamines NNN, NNK, NNA, NAB et NATB, dérivées des alcaloïdes du tabac, ainsi que des métabolites connus de mammifères de NNN et NNK sont présentés dans l'article. Les spectres de masse des dérivés synthétiques apparentés à ces nitrosamines et à leurs métabolites sont également traités. Ces spectres seront utiles dans le cadre des études sur les nitrosamines dérivées des alcaloïdes du tabac et leur métabolisme, et dans les recherches relatives à d'autres composés apparentés aux alcaloïdes du tabac.

REFERENCES

1. Hecht, S. S., C. B. Chen and D. Hoffmann: Tobacco specific nitrosamines: Occurrence, formation, carcinogenicity, and metabolism; *Acc. Chem. Res.* 12 (1979) 92-98.
2. Hoffmann, D., J. D. Adams, K. D. Brunnemann and S. S. Hecht: Chemical studies on tobacco smoke, LXII. Assessment of tobacco specific *N*-nitrosamines in tobacco products; *Cancer Res.* 39 (1979) 2505-2509.
3. a) Hu, M. W., W. E. Bondinell and D. Hoffmann: Synthesis of carbon-14-labelled myosmine, nornicotine and *N'*-nitrosonornicotine; *J. Labelled Compd.* 10 (1974) 79-88.
b) Quan, P. M., T. K. B. Karns and L. D. Quin: The synthesis of anatabine and related compounds; *J. Org. Chem.* 30 (1965) 2769-2772.
4. a) Hecht, S. S., C. B. Chen, M. Dong, R. M. Orna, D. Hoffmann and T. C. Tso: Studies on non-volatile nitrosamines in tobacco; *Beitr. Tabakforsch.* 9 (1977) 1-6.
b) Späth, E., and H. Bretschneider: Eine neue Synthese des Nikotins und einige Bemerkungen zu den Arbeiten Nagais über Ephedrine; *Ber. Dtsch. Chem. Ges.* 61 (1928) 327-334.
c) Haines, P. G., and A. Eisner: Identification of pseudo-oxynicotine and its conversion to *N*-methylmyosmine; *J. Am. Chem. Soc.* 72 (1950) 1719-1721.
5. Hecht, S. S., R. M. Orna and D. Hoffmann: Chemical studies on tobacco smoke, XXXIII. *N'*-Nitrosonornicotine in tobacco - Analysis of possible contributing factors and biological implications; *J. Natl. Cancer Inst.* 54 (1975) 1237-1244.
6. Hecht, S. S., C. B. Chen and D. Hoffmann: A study of chemical carcinogenesis, 29. Metabolic β -hydroxylation and *N*-oxidation of *N'*-nitrosonornicotine; *J. Med. Chem.* 23 (1980) 1175-1178.
7. Chen, C. B., S. S. Hecht and D. Hoffmann: A study of chemical carcinogenesis, 11. Metabolic α -hydroxylation of the tobacco specific carcinogen *N'*-nitrosonornicotine; *Cancer Res.* 38 (1978) 3639-3645.
8. Hecht, S. S., R. Young and C. B. Chen: A study of chemical carcinogenesis, 31. Metabolism in the F-344 rat of 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco specific carcinogen; *Cancer Res.* 40 (1980) 4144-4150.
9. Hecht, S. S., and C. B. Chen: A study of chemical carcinogenesis, 14. Hydrolysis of model compounds for α -hydroxylation of the carcinogens, *N*-nitrosopyrrolidine and *N'*-nitrosonornicotine; *J. Org. Chem.* 44 (1979) 1563-1566.
10. LaVoie, E. J., R. Young, S. S. Hecht and D. Hoffmann: unpublished.
11. Edwards, W. B.: Synthesis of pyridyl- and picolyl-cyclopropyl ketones; *J. Heterocycl. Chem.* 12 (1975) 413-416.
12. McKennis, H., Jr., L. B. Turnbull, E. R. Bowman and E. Wada: Demethylation of cotinine *in vivo*; *J. Am. Chem. Soc.* 81 (1959) 3951-3954.
13. McKennis, H., Jr., S. L. Schwartz, L. B. Turnbull, E. Tamaki and E. R. Bowman: The metabolic formation of γ -(3-pyridyl)- γ -hydroxybutyric acid and its possible intermediary role in the mammalian metabolism of nicotine; *J. Biol. Chem.* 239 (1964) 3981-3989.
14. Hecht, S. S., C. B. Chen, G. D. McCoy, D. Hoffmann and L. Domellof: A study of chemical carcinogenesis, 18. α -Hydroxylation of *N*-nitrosopyrrolidine and *N'*-nitrosonornicotine by human liver microsomes; *Cancer Lett.* 8 (1979) 35-41.
15. Pilotti, A., C. Enzell, H. McKennis, Jr., E. R. Bowman, E. Dufva and B. Homstedt: Studies on the identification of tobacco alkaloids, their mammalian metabolites and related compounds by gas chromatography-mass spectrometry; *Beitr. Tabakforsch.* 8 (1976) 339-349.
16. Glenn, D. F., and W. B. Edwards: Synthesis and mass spectrometry of some structurally related nicotinoids; *J. Org. Chem.* 43 (1978) 2860-2870.

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