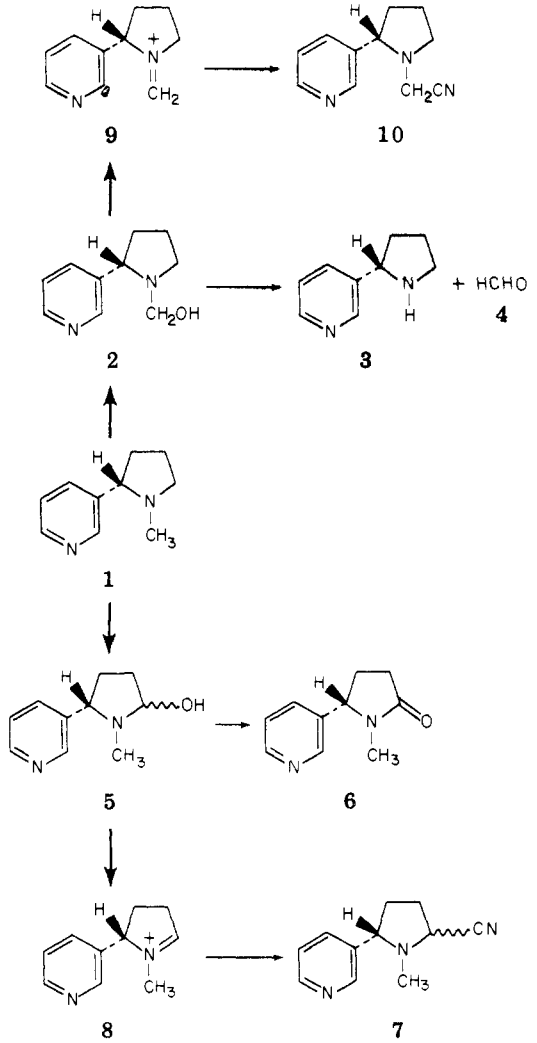
Metabolic N-Demethylation of Nicotine. Trapping of a Reactive Iminium Species with Cyanide Ion

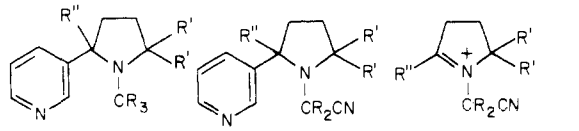
Sir:

Oxidative N-dealkylation is an important mixed function oxidase pathway for a variety of xenobiotics.[1] A number of chemical model[2] and metabolic[3] studies have attempted to reveal some of the mechanistic features of this conversion. Although the evidence is principally indirect, many investigators believe that the substrate, such as nicotine (1), undergoes carbon oxidation to form an intermediate carbinol 2 which subsequently breaks down to the dealkylated product 3 and an aldehyde 4.



Early studies on the in vitro metabolism of nicotine (1) in the presence of cyanide ion (an aldehyde oxidase inhibitor) led Hucker et al. to suggest that the carbinolamine 5 is an intermediate in the pathway to cotinine (6).[5] Employing similar incubation conditions, Murphy[4] isolated a compound for which the structure 5′-cyanonicotine (7) was proposed.[6] Murphy speculated that the cyano adduct would be formed by cyanide attack on the iminium species 8 which in turn would be generated in situ from the carbinolamine 5. We propose that if an analogous iminium species 9 is generated during the metabolic N-demethylation of nicotine, it might be trapped by cyanide ion as *N*-cyanomethylnornicotine (10). In this paper we report evidence consistent with this proposal.

Table I. GC-EI Mass Spectral Analysis of Metabolically Formed *N*-Cyanomethylnornicotines from Specifically Deuterium-Labeled Nicotines in the Presence of 0.01 M Sodium Cyanide



Substrate	Product	R	R′	R″	*m/e* for 11
1-d_0	10-d_0	H	H	H	109
1-5′,5′-d_2	10-5′,5′-d_2	H	D	H	111
1-2′,5′,5′-d_3	10-2′,5′,5′-d_3	H	D	D	112
1-*N*-methyl-d_3	10-cyanomethyl-d_2	D	H	H	111

We have examined the metabolism of nicotine (5×10^{-4} M) in a 10000g rabbit liver supernatant fraction (8 ml of homogenate, equivalent to 4 g of liver) containing NADPH (ca. 2×10^{-3} M), $MgCl_2$ (1.5×10^{-3} M), and NaCN (0.01 M). Analysis by GC–EI mass spectrometry of the bases isolated from the postincubate revealed the presence of cotinine (6) and two isomeric cyano derivatives of nicotine. The GC–EI mass spectrum of one of these compounds was the same as the spectrum reported by Murphy for his 5′-cyanonicotine (7). The GC–EI mass spectrum of the second compound displayed a parent ion at M^+ 187 (34%) and a single, prominent fragment ion at *m/e* 109 (100%). Based on the evidence presented below, this compound is assigned the structure *N*-cyanomethylnornicotine (10).

The following three specifically deuterium-labeled nicotine derivatives were available to us: nicotine-5′,5′-d_2 (1-5′,5′-d_2), nicotine-2′,5′,5′-d_3 (1-2′,5′,5′-d_3), and nicotine-*N*-methyl-d_3 (1-*N*-methyl-d_3). Each compound was incubated with the rabbit liver preparation and the bases isolated were analyzed by GC–EI mass spectrometry. By comparison with the well-documented mass fragmentation pattern of nicotine and related compounds,[7] the base peak at *m/e* 109 observed for the new cyano derivative must be due to a pyrrolinium ion which, in the case of compound 10-d_0, would be 11-d_0. Since fragment 11 retains all of the protons (or deuterons) of the pyrrolidinyl moiety of nicotine, except where replaced by the cyano group, it was possible to assign the location of the cyano group by determining the nominal masses of these ions. As summarized in Table I, the new cyano product retains the three deuterium atoms located at C-2′ and C-5′. The product isolated from the incubation of nicotine-*N*-methyl-d_3, however, retains only two deuterium atoms. Therefore, structure 10 is assigned to this product.

The synthesis of compound 10 was readily achieved in 80% yield by cyanomethylation of nornicotine. To a solution of nornicotine (740 mg, 5 mmol) and NaCN (490 mg, 10 mmol) in 166 ml of water was added dropwise 84 ml of 1.76% aqueous formaldehyde (1.5 g of H_2CO, 50 mmol) at room temperature with stirring and under nitrogen. After stirring an additional 10 min at room

temperature, the reaction mixture was extracted with five 120-ml portions of diethyl ether. The combined extracts were dried (Na_2SO_4), and the residue (750 mg) obtained after removing the solvent was distilled [bp 118–119 °C (0.3 mm)]: NMR ($CDCl_3$)[8] δ 1.6–2.5 (m, 4 H, C-3′ and C-4′), 2.5–3.1 (m, 1 H, C-5′), 3.1–3.5 (m, 1 H, C-5′), 3.6 (t, 1 H, J = 7.5 Hz, C-2′), 3.48 (center of AB q, 2 H, J = 17 Hz, $\Delta\nu_{AB}$ = 17 Hz, CH_2CN),[9] 7.0–9.0 ppm (3 m, 4 H, typical splitting for aromatic H of 3-substituted pyridine); ir (neat) ν 2240 cm^{-1} (weak C≡N stretch); uv (EtOH) λ_{max} 260 nm (ϵ 1880).[10] Anal. ($C_{11}H_{13}N_3$) C, H, N. The dipicrate (from 2-propanol) melted at 149.5–151.5 °C. Anal. ($C_{23}H_{19}N_9O_{14}$) C, H, N. The GC and EI mass spectral characteristics of synthetic **10** were identical with those observed for the compound isolated from the nicotine incubation mixture.

It was not clear from these experiments if the cyanomethyl compound was formed indirectly by a Mannich base type condensation of cyanide ion with metabolically formed nornicotine and formaldehyde or by some process not involving initial cleavage of the C–N bond. This question was investigated by incubating nicotine-*N-methyl-d*$_3$ (5×10^{-4} M) with the rabbit liver preparation in the presence of H_2CO (5×10^{-3} M). The H_2CO added would be expected to dilute any metabolically formed D_2CO at least 50-fold in which case **10**-*cyanomethyl-d*$_2$ should be almost negligible compared to **10**-*d*$_0$. The GC–EI mass spectrum of the isolated cyanomethyl compound was found to be a 1 to 1 mixture of **10**-*d*$_0$ and **10**-*cyanomethyl-d*$_2$ (*m/e* 109 vs. 111). Thus, to a significant extent, compound **10** appears to be formed without prior C–N bond cleavage suggesting that the reactive methyleniminium ion **9** is generated in the course of the in vitro metabolism of nicotine. The experimental details of these findings will be published separately.

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References and Notes

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(6) A recent paper by E. B. Sanders, J. F. DeBardeleben, and T. S. Osdene [*J. Org. Chem.*, **40**, 2818 (1975)] reports that the reaction sequence used by Murphy to synthesize 5′-cyanonicotine yields primarily 2′-cyanonicotine. Despite this discrepancy, Sanders et al. concluded that Murphy did in fact isolate authentic 5′-cyanonicotine under his reaction conditions and therefore the structure assignment for this compound appears to be correct.
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Book Reviews

Atomic Absorption Spectroscopy. Second Edition (Revised and Expanded). By James W. Robinson. Marcel Dekker, New York, N.Y. 1975. ix + 183 pp. $14.95.

The second edition of this work has been expanded to include chapters on nonflame atomizers and on atomic fluorescence. The remainder of the text has been revised slightly. The work is a clear and concise statement of the basic theory and problems with which anyone wishing to use atomic absorption should be conversant. No prior knowledge of the technique is assumed. The fundamentals of AA are explained from a practical vantage point and should be particularly helpful for a novice wishing to use the technique. Discussions include fundamental theory, design and use of flame atomizers, spectral and chemical interferences, techniques for improving analyses, and statistical considerations. The fourth chapter is an element by element tabulation of operating conditions used for flame procedures.

Part of the expansion deals with nonflame atomizers (carbon rods, tubes, furnaces, etc.) which are currently being used to greatly enhance the sensitivity of the technique. This chapter provides an excellent introduction to these methods.

The other added chapter covers the fundamentals of atomic fluorescence spectroscopy. The coverage is an adequate introduction although no discussion of saturated atomic fluorescence using high-intensity sources (currently the most useful AF technique) is included.

In summary, the text affords an excellent introduction to the technique. It is not, nor does it purport to be, a definitive text covering state of the art research interests. It is highly readable and provides surprisingly thorough coverage of practical AA considering its brevity.

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Structure–Activity Relationships for Some Conjugated Heteroenoid Compounds, Catechol Monoethers and Morphine Alkaloids. Volumes 1 and 2. By H. L. Holmes. Defence Research Establishment Suffield, Ralston, Alberta, Canada. 1975. 1498 pp. 21 × 28 cm. $30.00 (Canadian).

The two volumes achieve their principal objectives—to demonstrate whether the dominant factors, namely, the rate of