Studies on the Pyrrolinone Metabolites Derived from the Tobacco Alkaloid 1-Methyl-2-(3-pyridinyl)pyrrole (*β*-Nicotyrine)

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Previous studies have established that the tobacco alkaloid 1-methyl-2-(3-pyridyl)pyrrole (*β*-nicotyrine) is biotransformed by rabbit lung and liver microsomal preparations to an equilibrium mixture of the corresponding 3- and 4-pyrrolin-2-ones. Autoxidation of these pyrrolin-2-ones generates the chemically stable 5-hydroxy-5-(3-pyridinyl)-3-pyrrolin-2-one. This paper summarizes efforts to document more completely the pathway leading to this hydroxypyrrolinone. Chemical and spectroscopic evidence implicates the 2-hydroxy-1-methyl-5-(3-pyridinyl)pyrrole (2-hydroxy-*β*-nicotyrine) as the key intermediate in this reaction pathway. Of potential toxicological interest is the detection of radical species derived from the autoxidation of this compound.

Introduction

The pharmacological (*1*) and toxicological (*1*−*4*) properties of tobacco products have prompted extensive studies on the metabolic fate of (*S*)-nicotine (**1**) (*5*−*9*), the principal pharmacologically active alkaloid present in tobacco products. A more limited effort, which includes our studies on 1-methyl-4-(3-pyridinyl)pyrrole [*β*-nicotyrine (**2**)] (*10*), has focused on the metabolism of some of the minor tobacco alkaloids (*11*). Interest in *β*-nicotyrine is based in part on reports that structurally related five-membered heteroarenes may be biotransformed to reactive metabolites that contribute to their toxic properties (*12*−*16*). It has been reported that *β*-nicotyrine forms from the autoxidation of nicotine under sunlight (*17*) and also is a urinary metabolite of (*S*)-nicotine in dogs and rats (*18*). Results of preliminary studies in which cytochrome P450 rich Clara cells isolated from rabbit lung were used suggest that *β*-nicotyrine is bioactivated in an NADPH-dependent process to pneumotoxic metabolites.[1]

Previous studies in which NADPH-supplemented rabbit liver and lung microsomal preparations were used and a GC/EIMS assay have led to the characterization of isomeric pyrrolinones **3** and **4** as metabolites of *β*-nicotyrine (Scheme 1) (*19*). Following their isolation from the incubation mixtures, these pyrrolinones were found to undergo autoxidation to yield 5-hydroxy-1-methyl-5-(3-pyridinyl)-3-pyrrolin-2-one (**9**). We postulated a free radical pathway for the conversion of **3** and **4** to **9** involving a single electron transfer to dioxygen from the oxyanion **6** of the 2-hydroxypyrrole derivative **5**, the enol tautomer of the pyrrolinones, which yields the resonance-stabilized radical **7** and superoxide radical anion $O_2^{\bullet-}$. Subsequent radical recombination of **7b** with HOO$^\bullet$ (the conjugate acid of $O_2^{\bullet-}$) and hydrolysis of the resulting hydroperoxide **8** would yield **9** (Scheme 1). Mass spectral evidence also suggested the presence of an isomeric hydroxypyrrolinone (possibly **11**) that could be derived from the isomeric radical recombination product **10** (*19*). This paper describes the results of studies designed to evaluate the pathway leading from the pyrrolinones **3** and **4** to the 5-hydroxypyrrolinone **9**.

Materials and Methods

Chemistry. Syntheses were carried out under a nitrogen atmosphere. All starting materials and the spin trapping reagents *N*-*tert*-butyl-α-phenylnitrone [PBN[2] (**20**)] and 5,5-dimethyl-1-pyrroline *N*-oxide [DMPO (**21**)] were purchased from Aldrich Chemical Co. (Milwaukee, WI), while TRIS, HEPES, potassium chloride, EGTA, acetonitrile, triethylamine, and acetic acid were obtained from Fisher Chemical Co. (Pittsburgh, PA). $NADP^+$, magnesium chloride, D-glucose 6-phosphate, glucose-6-phosphate dehydrogenase, and mono-, di-, and tribasic phosphates were purchased from Sigma Chemical Co. (St. Louis, MO). The tartrate salt of *β*-nicotyrine (**2**) (*10*), 3,3-dibromocotinine (*20*), 5-hydroxycotinine (**16**) (*21*) (improved synthesis described later in the text), and 5-methoxy-1-methyl-5-(3-pyridyl)-3-pyrrolin-2-one (**13**) (*21*) were synthesized as described previously.

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[1] W. K. Nichols, M. O. Covington, G. S. Yost, X. Liu, and N. Castagnoli, Jr. (1990) Bioactiviation of the tobacco alkaloid *β*-nicotyrine in isolated Clara cells (unpublished data).

[2] Abbreviations: a_N, α nitrogen hyperfine splitting constant; a_H^β, *β* hydrogen hyperfine splitting constant; DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; DMSO, dimethyl sulfoxide; ESR, electron spin resonance; GC/MS, gas chromatography/mass spectrometry; $NADP^+$, nicotinamide adenine dinucleotide phosphate; NADPH, 1,4-dihydronicotinamide adenine dinucleotide phosphate; PBN, N-*tert*-butyl-α-phenylnitrone.

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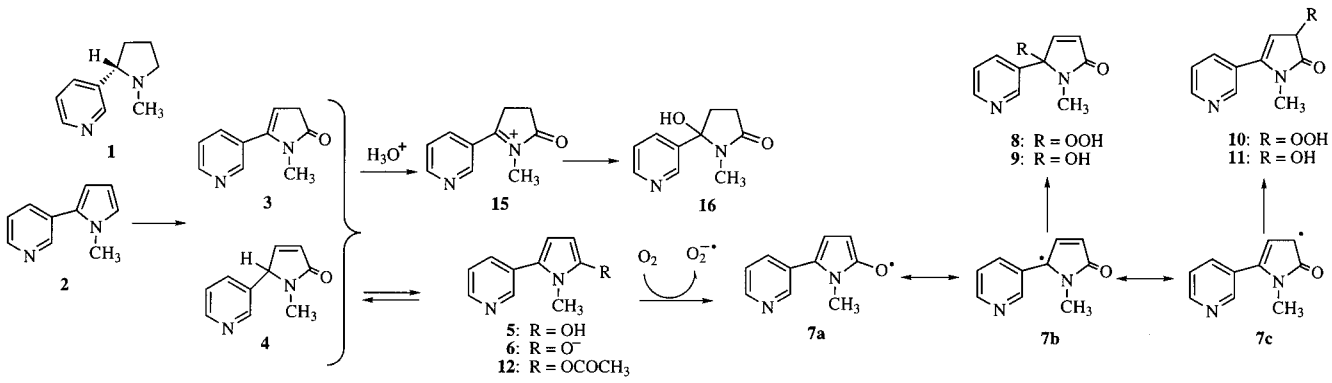
Scheme 1. In Vitro Metabolic Fate of β-Nicotyrine



Proton NMR spectra were recorded on a Bruker WP 270 MHz spectrometer linked to an Aspect 2000 computer. Chemical shifts are reported in parts per million relative to Me_4Si in $CDCl_3$ or to 3-(trimethylsilyl)propionic-2,2,3,3-acid-d_4, sodium salt (TSP), in D_2O and CD_3OD. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or b (broad). ESR spectra were performed on a Bruker ER 200 D instrument. UV spectra were recorded on a Beckman DU 50 spectrophotometer. GC/EI mass spectral analyses were performed on an HP 5970 mass selective detector interfaced with an HP 5890 gas chromatograph equipped with an HP-1 100% dimethylpolysiloxane capillary column (12.5 m × 0.2 mm × 0.33 μm). The analyses were performed using a temperature program starting at 100 °C and, following a 1 min hold, increasing the temperature to 275 °C at a rate of 25 °C/min. HPLC/diode array analyses were performed with a dual (Beckman 114M) pump system at a flow rate of 1 mL/min, a Beckman 421A controller, a C8 Alltech Econosil (25 cm × 4.6 mm × 10 μm) column, and an HP 1040A diode array detector. Elution proceeded first with 100% mobile phase A [90:10:0.5:0.1 (v/v) water/acetonitrile/acetic acid/triethylamine] for 10 min employing a 10 min ramp and ended with 40% mobile phase B (100% acetonitrile). After elution for 10 min with 40% B, the mobile phase was ramped back to 100% A over a 5 min period, after which the elution was continued for another 10 min, allowing the column to equilibrate. The total eluting time was 45 min. The effluent was monitored simultaneously at 260 and 282 nm. Typical retention times were as follows: β-nicotyrine, 23.5 min; pyrrolinone **3**, 21.5 min; pyrrolinone **4**, 20.5 min; 5-hydroxypyrrolinone **9**, 13.5 min; and 5-hydroxycotinine (**16**), 11 min. Nicotinamide (8.5 min) was observed in both control and experimental incubations.

1-Methyl-5-methoxy-5-(3-pyridyl)-2-pyrrolidinone [5-Methoxycotinine (14)]. A solution of 1-methyl-5-methoxy-5-(3-pyridyl)-3-pyrrolin-2-one (**13**, 10.2 g, 100 mmol) in 300 mL of methanol containing 2 g of 10% Pd/C was stirred vigorously under hydrogen at atmospheric pressure and room temperature for 24 h. After filtration of the catalyst and removal of the solvent by evaporation under vacuum, 10.3 g (50 mmol, 100%) of a pale yellow oil was obtained. The product solidified upon storage in the refrigerator. Recrystallization from ethyl acetate yielded cubic colorless crystals that were suitable for analyses: mp 75−76 °C; GC/EIMS (t_R = 3.7 min; relative intensity) *m*/*z* 206 (5%), 175 (100%), 149 (10%), 128 (10%), 100 (18%), 78 (30%); ^{1}H NMR ($CDCl_3$) δ 8.67 (1H, d, C2′-H), 8.59 (1H, d of d, C6′-H), 7.70 (1H, t of d, C4′-H), 7.32 (1H, m, C5′-H), 3.27 (3H, s, O-CH_3), 2.69−2.60 (2H, m, C3-H), 2.58 (3H, s, N-CH_3), 2.52−2.41 (1H, m, C4-H), 2.32−2.15 (1H, m, C4-H); UV (in CH_3OH) λ_{max} 216 (ε = 7300), 260 nm (ε = 4500). Anal. Calcd for $C_{11}H_{14}N_2O_2$: C, 64.06; H, 6.84; N, 13.58. Found: C, 64.16; H, 6.86; N, 13.64.

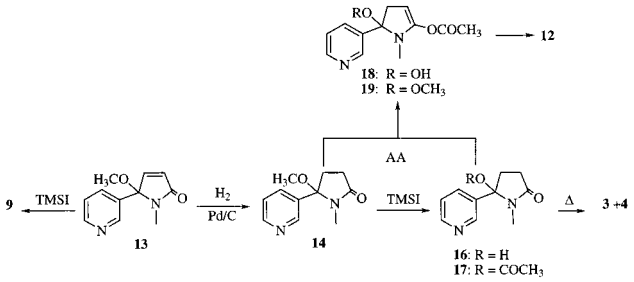
5-Hydroxy-1-methyl-5-(3-pyridyl)-2-pyrrolidinone [5-Hydroxycotinine (16)]. A solution of 5-methoxycotinine (**14**, 1.03 g, 5 mmol) and trimethylsilyl iodide (0.925 mL or 1.3 g, 6.5 mmol) in 2 mL of chloroform was stirred at room temperature for 24 h (*22*). After the addition of 0.82 mL of methanol (0.64 g, 20 mmol), the solvent was removed under vacuum at 50 °C. The residue was treated with saturated sodium bicarbonate and the neutralized solution extracted with chloroform. The extract was washed with aqueous solutions of sodium sulfite and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum to leave a light brown residue which, after crystallization from diethyl ether, provided pure 5-hydroxycotinine (0.87 g, 4.5 mmol, 90%) which was identical in all respects to the previously reported product which was obtained by catalytic reduction of the corresponding hydroxypyrrolinone **9** (*21*).

5-Hydroxy-1-methyl-5-(3-pyridyl)-3-pyrrolin-2-one (9). Following the procedure for the O-demethylation of 5-methoxycotinine (**14**), 5-hydroxy-1-methyl-5-(3-pyridyl)-3-pyrrolin-2-one (**9**) was obtained from the corresponding methyl ether **13** as yellow crystals in 80% yield. This product was identical in every respect to that obtained by treatment of **13** with HBr.

Mixture of 1-Methyl-5-(3-pyridyl)-4-pyrrolin-2-one (3) and 1-Methyl-5-(3-pyridyl)-3-pyrrolin-2-one (4). 5-Hydroxycotinine (**16**, 87.2 mg, 0.454 mmol) was placed in a flask connected to a 50 cm long pyrolysis tube wrapped with electrothermal wire that was connected to a cold trap cooled in a dry ice/acetone bath. The whole system was purged with nitrogen. The flask containing the 5-hydroxycotinine was heated slowly in an oil bath to a final temperature of 135 °C, while the pyrolysis tube was kept at 280 °C. The process was carried out under vacuum (0.12−0.14 mmHg) for 1.5 h and resulted in a dark purple-colored condensate containing a mixture of the two pyrrolinones **3** and **4** in a ratio of 3:1 as determined by GC/MS (9 mg, 11.4%): ^{1}H NMR ($CDCl_3$) of 1-methyl-5-(3-pyridyl)-4-pyrrolin-2-one (**2**) δ 8.67 (m, 2H, C2′- and C6′-H), 7.69 (d, 1H, C4′-H), 7.35 (dt, 1H, C5′-H), 5.38 (t, 1H, C4-H), 3.22 (d, 2H, C3-H), 3.02 (s, 3H, N-Me); ^{1}H NMR ($CDCl_3$) of 1-methyl-5-(3-pyridyl)-3-pyrrolin-2-one (**4**) δ 8.50 (m, 2H, C2′- and C6′-H), 7.65 (dt, 1H, C4′-H), 7.30 (m, 1H, C5′-H), 7.03 (dd, 1H, C3-H), 6.30 (dd, 1H, C4-H), 5.01 (t, 1H, C5-H), 2.83 (s, 3H, N-Me). Direct insertion probe high-resolution EI-MS ($M^{\bullet+}$) calcd for $C_{10}H_{10}N_2O$ 174.0793131, found 174.078354.

2-Acetoxy-1-methyl-5-(3-pyridyl)pyrrole (12). A solution of 5-methoxycotinine (**14**, 1.03 g, 5 mmol) in 5 mL (100 mmol) of acetic anhydride was heated to 80 °C in an oil bath for 6 h and then allowed to remain at room temperature overnight. The reaction was monitored by GC/EIMS. When the starting material (retention time of 3.7 min) had been fully consumed, the GC/EIMS showed only one peak at *m*/*z* 216 (retention time of 4.4 min). After removal of the remaining acetic anhydride, the brown oily residue was purified first by passage through a neutral alumina column with $CHCl_3$ followed by chromatography on a silica gel column (1:20 w/w), again eluting with $CHCl_3$. The first fraction gave 0.83 g (76.5%) of 2-acetoxy-1-methyl-5-(3-pyridyl)pyrrole (**12**) as a pale yellow oil: GC/EIMS (t_R = 4.5 min; relative intensity) *m*/*z* 216 (10%), 174 (100%), 145 (10%), 131 (10%), 104 (10%), 78 (10%); ^{1}H NMR ($CDCl_3$) δ 8.66 (1H, d, C2′-H), 8.52 (1H, q, C6′-H), 7.68 (1H, q, C4′-H), 7.32 (1H, q, C5′-H), 6.21 (1H, d, C3-H), 5.95 (1H, d, C4-H), 3.45 (3H, s, NCH_3), 2.35 (3H, s, $COCH_3$); UV (in the HPLC mobile phase of 50:50:0.1:0.5 v/v water/acetonitrile/triethylamine/acetic acid at pH 3.8)

Scheme 2. Synthetic Routes to Compounds Discussed in the Text



λ_{max} 230 (ϵ = 8600), 290 nm (ϵ = 15 000). Anal. Calcd for $C_{12}H_{12}N_2O_2 \cdot 0.15H_2O$: C, 65.83; H, 5.59; N, 12.79. Found: C, 65.62; H, 5.53; N, 12.81.

Hydrolysis of 2-Acetoxy-1-methyl-5-(3-pyridyl)pyrrole (12). Solutions of **12** (10 mg in 0.5 mL) were prepared in 10% DCl in D_2O and 0.1 M phosphate buffer in D_2O (pH 7.4). The reaction mixtures were monitored by ^{1}H NMR at room temperature and the products analyzed by GC/MS.

Metabolism. Rabbit liver microsomes were prepared from male New Zealand white rabbits as described previously (*20*). Incubation mixtures contained rabbit lung (4 mg of protein/mL) or liver microsomes (5 mg of protein/mL), β-nicotyrine tartrate (154 mg/mL, 0.5 mM), and an NADPH-regenerating system (0.5 mM $NADP^+$, 8 mM glucose 6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase, and 4 mM $MgCl_2$) in a total of 4 mL of a 0.1 M Tris-HCl buffer (pH 7.4) containing 0.1 mM EGTA. Incubations were carried out at 37 °C for 75 min in a metabolic shaker at 60 rpm. At the end of the specified incubation period, the mixtures were added to an equal volume of ice-cold acetonitrile. The quenched mixtures were centrifuged at 16000*g* for 5 min, and the resulting supernatants were centrifuged at 16000*g* for an additional 5 min and filtered to remove the precipitated protein fraction. The final supernatants were analyzed by the HPLC method described above.

ESR Studies. All studies were carried out at room temperature. Solutions of the acetoxypyrrole **12** (6 mM in 0.1 mM phosphate buffer at pH 7.0) contained 0.3 mM purified DMPO in water or saturated PBN in water (both at 1:1 v/v) were used in these studies. Control studies were carried out in which the trapping reagents or **12** was omitted. Additional controls included incubations in which **12** was replaced with 13 mM β-nicotyrine (**2**). Some incubations also contained 2 mM ethanol, a hydroxyl radical scavenger. Each sample was siphoned into 2 in. long capillary tubes (inside diameter of 1 mm), and the tubes were sealed. Unless stated otherwise, the ESR parameters were set as follows: X-band, 9.3 GHz, 100 kHz modulation frequency; microwave power of 20 mW; modulation amplitude of 2.0 G; time constant of 1.25 s; scan time of 500 s; receiver gain of 8.0×10^5; central field of 3483 G; and scan width of 100 G. The line width of the ESR was measured with an E-500 gauss meter.

Results and Discussion

Chemistry. Synthetic standards of compounds **3**, **4**, **9**, and **16** were required for HPLC and ^{1}H NMR studies. The synthesis of the 5-hydroxypyrrolinone **9** was achieved in good yield by treatment of the readily available methoxypyrrolinone **13** (*21*) with trimethylsilyl iodide to cleave the methyl ether (Scheme 2). Catalytic hydrogenation of **13** followed by ether cleavage of the resulting 5-methoxycotinine **14** with trimethylsilyl iodide, rather than with HBr, provided an improved route to 5-hydroxycotinine (**16**).

We have earlier reported the synthesis of the 4-pyrrolinone **3** via 3-phenylselenylation of cotinine followed by elimination of the corresponding phenylselenyl oxide and rearrangement of the initially formed 3-pyrrolinone **4** (*19*, *23*). The overall yield, however, was very low, and our attempts to prepare useful quantities of both **3** and **4** by this approach were unsuccessful. A mixture of **3** and **4** could be obtained by vacuum tube pyrolysis of 5-hydroxycotinine (**16**). GC/EIMS and ^{1}H NMR analyses of the distillate gave spectra which were in agreement with this earlier report. Solutions of this mixture in anhydrous, argon-purged $CHCl_3$ slowly turned a deep purple color which disappeared within hours upon storage due to decomposition. The chemical instability of these pyrrolinones has been taken as evidence of their equilibration with the highly electron rich and, presumably, unstable 5-hydroxypyrrole derivative **5** as proposed in Scheme 1.

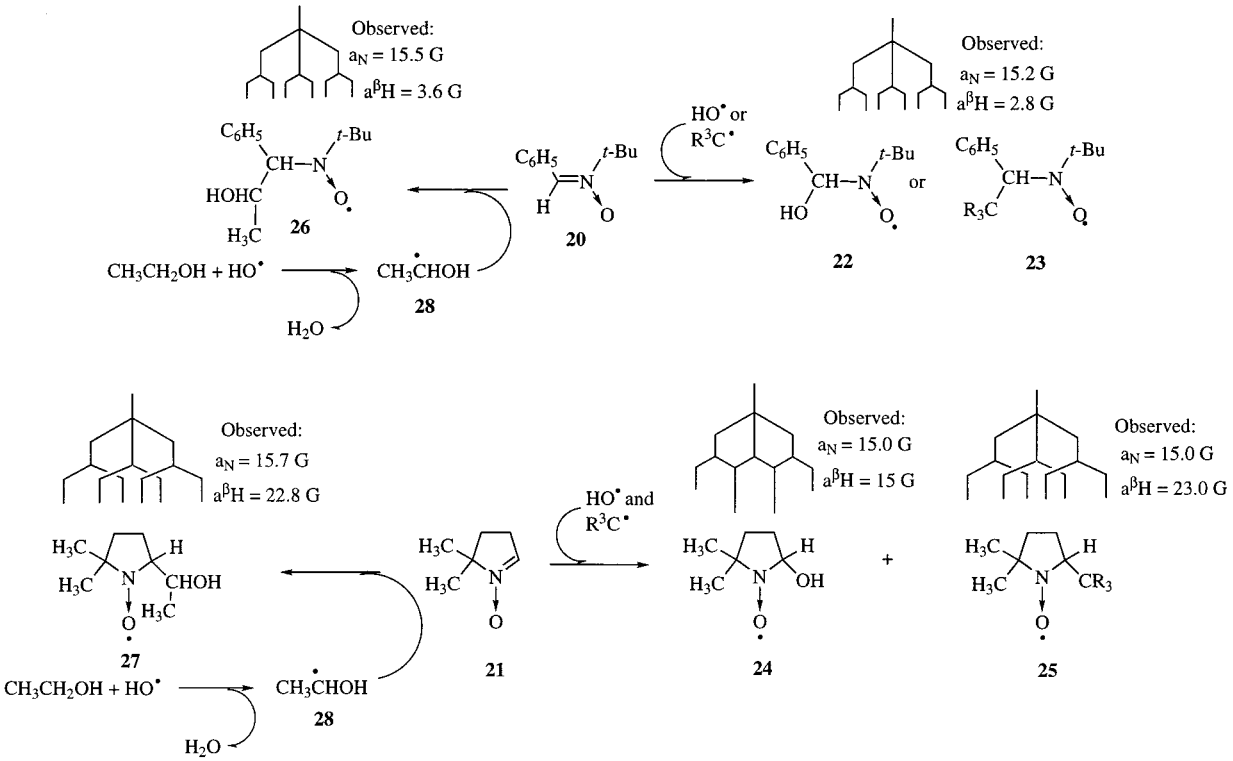
An alternative approach to the preparation of **3** that was examined involved reaction of 5-hydroxycotinine (**16**) with acetic anhydride with the hope that the expected 5-acetoxy species **21**, containing a better leaving group, would undergo elimination to form **3**. GC/EI mass spectral analysis of the principal product isolated from this reaction, however, displayed a parent ion at *m*/*z* 216 rather than at *m*/*z* 174 as expected for **3**. Proton NMR and elemental analyses (see Materials and Methods) led to the characterization of this product as 2-acetoxy-1-methyl-5-(3-pyridinyl)pyrrole (**12**). Since 5-methoxycotinine (**14**) was an even better substrate for this reaction, it seems likely that the pathways to **12** proceed through pyrrolinyl acetate **18** or **19** (Scheme 2) followed by loss of water (from **18**) or methanol (from **19**).

Metabolism. Consistent with earlier results, β-nicotyrine was metabolized efficiently in NADPH-supplemented rabbit liver microsomal preparations with essentially 100% of the substrate being consumed within 75 min. HPLC/diode array analysis of the incubation mixture and synthetic standards established the two pyrrolinones **3** and **4** as major metabolites. Two additional minor peaks were shown to be due to the hydroxypyrrolinone **9** and 5-hydroxycotinine (**16**). As will become evident from the discussion below, compounds **9** and **16** are likely to be derived from the pyrrolinones **3** and **4**. No metabolite was formed in the absence of microsomes or NADPH.

The availability of **12** provided an opportunity to examine the chemical behavior of the putative hydroxypyrrole metabolite **5** (Scheme 1) which should be formed following hydrolysis of the ester group. We monitored the fate of **12** in deuterated phosphate buffer (pH 7.4) by ^{1}H NMR spectroscopy. The unique chemical shift values for the signals for the *N*-methyl groups for the compounds of interest [δ 3.52 (**12**), 3.06 (**3**), 2.88 (**4**), 2.74 (**9**), and 2.65 ppm (**16**)] provided an opportunity to make rough estimates of the concentrations of reactant and products over the time course of the incubations. Under neutral conditions, the hydrolysis of **12** was very slow, perhaps due to its limited water solubility. Signals corresponding to the hydroxypyrrole **5** were not observed at any time. Instead, the principal products formed early in the reaction were the two pyrrolinones **3** and **4** which were present in approximately equal amounts during the course of the 8 day incubation. The ^{1}H NMR signals for pyrrolinones were slowly replaced with signals corresponding to the hydroxypyrrolinone **9** (20% yield) and 5-hydroxycotinine [**16** (65% yield)].

The rate of hydrolysis of **12** was dramatically enhanced at pH 1 (5% HCl/H_2O), conditions under which **12** was soluble. ^{1}H NMR signals for the starting material could

Scheme 3. Proposed Structures of the Trapped Radicals Derived from 5-Hydroxy-β-nicotyrine



not be detected after 10 min. At this time, 3-pyrrolinone **4** (2%), 4-pyrrolinone **3** (37%), and 5-hydroxycotinine [**16** (59%)] were present; no signals reflecting the formation of the hydroxypyrrolinone **9** were observed. By 70 min, 5-hydroxycotinine accounted for all of the starting material. The conversion of the pyrrolinones to 5-hydroxycotinine presumably proceeds via the protonation of the amidoiminium intermediate **15** (Scheme 1).

The results from these studies suggest that the putative 5-hydroxypyrrole metabolite **5** (derived chemically from the corresponding acetoxy compound **12** and metabolically from β-nicotyrine) exists predominantly as the equilibrium mixture of pyrrolinones **3** and **4** in aqueous solution. These pyrrolinones undergo acid-catalyzed hydration, to yield 5-hydroxycotinine **16**, and autoxidation, to yield the 5-hydroxypyrrolinone **9**.

The autoxidative pathway proposed to account for the conversion of the pyrrolinones **3** and **4** to the hydroxypyrrolinone **9** (Scheme 1) leads to $O_2^{\bullet -}$ and the free radical species **7**. Evidence to support this proposal was sought with the aid of the acetoxypyrrole **12** which was incubated at pH 7.0 and room temperature in the presence of the spin trapping reagent PBN (**20**) or DMPO (**21**). No ESR signals were observed in several control incubations performed in the absence of the spin trapping reagents or **12**. Furthermore, even after a 5 day incubation period, no ESR signals were observed when β-nicotyrine replaced **12**. Incubation mixtures containing PBN and **12** slowly developed a six-line ESR pattern after 24 h. This pattern is that expected for a nitroxyl radical in which the unpaired electron is split into a triplet by the α-nitrogen atom (hyperfine splitting constant $a_N = 15.2$ G) with each line of the triplet being split further by the β-hydrogen ($a^\beta_H = 2.8$ G). Although these spectral data are not definitive, they are consistent with the hydroxyl radical adduct **22** ($a_N = 15.2$ G, and $a^\beta_H = 2.75$ G) (*24*). An alternative interpretation is an adduct **23** (Scheme 3), resulting from trapping of a carbon-centered radical (reported $a_N = 14-16.2$ G, and $a^\beta_H = 2.64-4.35$ G) (*24*). Favoring the carbon radical adduct is the chemical instability of **22** (*25*). The absence of evidence of further coupling with the OH proton ($a^g_H = 0.21$ G) (*26*) present in **22** also is support for the carbon adduct. A similar experiment carried out with DMPO (**21**) gave a weak 10-line spectrum after 2 h. The spectrum may be interpreted as a mixture of a four-line pattern ($a_N = 15$ G, and $a^\beta_H = 15$ G), expected for the hydroxyl radical adduct **24**, with a six-line pattern ($a_N = 15.9$ G, and $a^\beta_H = 23.0$ G) (*24*), expected for a carbon radical adduct (**25**). The absence of adducts resulting from trapping of the hydroperoxyl radical (HOO•) is expected because of the short half-life and ease with which HOO• is reduced to HO• (*27*, *28*). The absence of evidence for the formation of an adduct between the form of **7** in which the radical is centered on oxygen (**7a**) also would be expected because the greater electronegativity of oxygen versus that of carbon makes alkoxyl radicals less stable than carbon radicals.

In an effort to provide further information on the putative hydroxyl radical pathway, ethanol, a specific hydroxyl radical scavenger (*29*), was added to the reaction mixtures containing **12** and either spin trap. The DMPO-containing system gave a six-line ESR signal ($a_N = 15.7$, and $a^\beta_H = 22.8$), while the PBN-containing system gave another six-line ESR signal ($a_N = 15.5$, and $a^\beta_H = 3.6$). Comparison with literature values (*29*) led us to conclude that these ESR signals correspond to adducts **26** and **27** formed between the hydroxyl radical-generated methyl hydroxyl carbinyl radical (**28**) and the spin traps DMPO and PBN, respectively. Again, no ESR signals were observed in the control samples which did not contain **12**.

Summary. The results of the studies reported here confirm previous observations documenting the efficient

NADPH-dependent oxidation of β-nicotyrine by rabbit liver and lung microsomal preparations in forming a mixture of the chemically labile pyrrolinones **3** and **4**. These pyrrolinones may undergo hydration, in an acid-dependent reaction, to form the known (*S*)-nicotine metabolite 5-hydroxycotinine (**16**), which has been reported to be a urinary metabolite of (*S*)-nicotine, or autoxidation to form the hydroxypyrrolinone **9**. Evidence obtained with synthetic 5-acetoxy-1-methyl-2-(3-pyridinyl)pyrrole (**12**) suggests that the pyrrolinones **3** and **4** are derived from the corresponding 5-hydroxypyrrole derivative **5**. The autoxidation leading to the hydroxypyrrolinone **9** is likely to proceed via the conjugate base **6** derived from **5** since assignable ESR signals were detected when **12** underwent hydrolysis in the presence of radical spin trapping reagents. Studies on the in vivo metabolic fate of β-nicotyrine are underway.

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