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The Tautomeric Structures of 5-Hydroxycotinine, a Secondary Mammalian Metabolite of Nicotine

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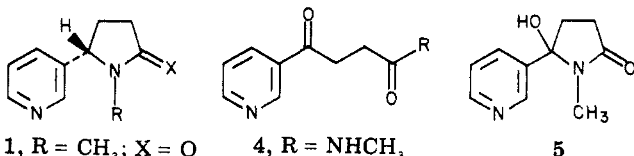
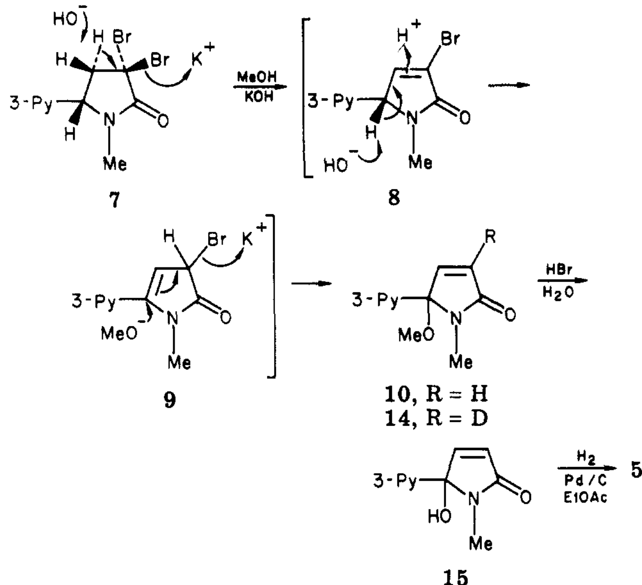
A novel synthesis of 5-hydroxycotinine has provided a homogenous product which has been examined spectroscopically in order to assess the possible equilibrium of the hydroxy lactam with the tautomeric keto amide structure originally proposed for this urinary metabolite of the tobacco alkaloid nicotine. Treatment of the readily available (*S*)-3,3-dibromocotinine with methanolic KOH led to (*R,S*)-1-methyl-5-(3'-pyridyl)-5-methoxypyrrolin-2-one which upon cleavage of the enol ether and catalytic hydrogenation of the double bond provided the desired product as the pure crystalline hydroxy lactam. The equilibrium of the hydroxy lactam with the ring-open keto amide tautomer was examined by NMR and was shown to proceed slowly in deuteriochloroform and rapidly in water. The equilibrium mixtures in both solvents consist of more than 80% of the cyclic tautomer. The product isolated from the urine of a Rhesus monkey treated with (*S*)-cotinine displayed spectral properties identical with those of the synthetic material.

Many metabolic oxidative conversions of xenobiotics can be regarded formally as carbon or heteroatom hydroxylations.[1-3] The oxidation of cyclic amines to lactams is likely to involve initial α-carbon hydroxylation followed by a second enzymatic step which converts the α-hydroxylated intermediate to the corresponding lactam.[4] Further metabolism of N-substituted lactams by hydroxylation at the N-substituent is believed to lead to intermediate carbinols which subsequently undergo N–C cleavage.[5-8] When the ring carbon atom α to the lactam nitrogen atom is attacked, the resulting hydroxy lactam may ring open to the corresponding amino aldehyde or amino ketone. The equilibria between the closed and the open ring forms of these systems[9-12] and related cyclic phosphoramides[13] have been the subject of several studies.

The lactam (*S*)-cotinine (**1**), the major mammalian metabolite of the tobacco alkaloid (*S*)-nicotine (**2**),[14] is known to undergo oxidative N-demethylation to form demethylcotinine (**3**).[15-18] The product of C_5 ring hydroxylation of (*S*)-cotinine was reported to be γ-(3-pyridyl)-γ-oxo-*N*-methylbutyramide (**4**).[19] Without supporting experimental evidence it was suggested that **4** exists in equilibrium with the closed ring form, 5-hydroxycotinine (**5**).[20] The open-ring structure was preferred because the metabolite exhibited properties characteristic of ketones and had physicochemical properties matching those of a synthetic sample of the keto amide obtained by methylaminolysis of the corresponding keto ester **6**.



Scheme I. Reaction Pathway to 5-Hydroxycotinine (5)



During the course of our studies on the in vivo metabolism of (*S*)-cotinine (**1**) in the Rhesus monkey[21] we found

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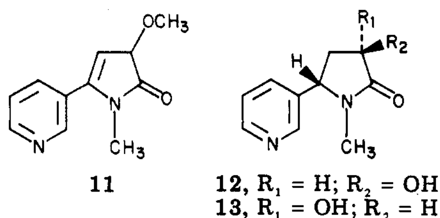
that material with the same TLC R_f value as that reported for keto amide 4 did not give consistent direct-insertion EI mass spectra. In general two types of spectra were observed. In some experiments the EIMS of the isolate displayed a weak molecular ion at mass 192 (5%) and fragment ions at *m/e* 174 (25%), 163 (51%), 114 (24%), 106 (100%), 86 (27%), 79 (38%), and 78 (62%) (type A spectrum), while on other occasions the EI mass spectrum displayed a relatively prominent molecular ion at mass 192 (15%) and fragment ions at *m/e* 163 (27%), 114 (100%), 106 (70%), 86 (15%), 79 (86%), and 78 (45%) (type B spectrum). The possibility that the differences in the mass spectra were due to the presence of varying amounts of the open-chain (4) and cyclic (5) tautomers prompted the present investigation.

The synthesis of what McKennis originally proposed[19] to be the keto amide 4 was repeated and provided a product in poor yield which gave a complex ^1H NMR spectrum. The salient features of the spectrum in $CDCl_3$ included an intense singlet at 2.65 ppm and a weakly intense doublet (J = 6 Hz) which slowly collapsed to a singlet (2.80 ppm) after treatment with D_2O. These data suggested the presence of two types of *N*-methyl groups—a tertiary *N*-methyl group (singlet at 2.65 ppm) and a secondary *N*-methyl group (doublet centered at 2.80 ppm) which is spin coupled to an exchangeable proton. In addition the presence of a weakly intense triplet centered at 3.3 ppm was taken as evidence for the presence of a second component.

In order to further characterize the properties of this metabolite an independent synthesis designed to yield 5-hydroxycotinine (5) was undertaken (Scheme I). Treatment of (*S*)-3,3-dibromocotinine (7) with methanolic KOH gave a light yellow oil displaying a parent ion under EI conditions at M^+ 204. The absence of doublet or cluster ions established that both bromine atoms of 7 had been lost. The ^1H NMR spectrum of this oil displayed, in addition to the characteristic aromatic pattern for a 3-substituted pyridine moiety, two 3-proton singlets at 2.62 (NCH_3) and 3.25 ppm (OCH_3) and an AB quartet centered at 6.67 ppm (J_{AB} = 6 Hz, $\Delta\nu$ = 33 Hz). These data are consistent with the 5-methoxy-3-pyrrolin-2-one 10 and the isomeric 3-methoxy compound 11. Compound 11 was ruled out since upon ether cleavage and double-bond reduction the product obtained was different from the known *trans*- and *cis*-3-hydroxycotinine (12 and 13, respectively).



The mechanism for the conversion of 7 to 10 is likely to involve base-promoted dehydrohalogenation of 7 to 8 followed by double-bond migration to form the allylic bromo derivative 9. S_N2' attack by methoxide ion would lead to the final product 10. As expected, when the reaction was repeated with KOD in CH_3OD, the resulting product, 14, contained one deuterium atom (M^+ 205) and the ^1H NMR spectrum displayed only one coupled olefinic proton at 6.85 ppm. The pyrrolinone 10 did not rotate the plane of polarized light at the sodium D line, consistent with the loss of chirality in the formation of intermediate 9.

Cleavage of the allylic methyl ether with aqeuous HBr proceeded smoothly to give 1-methyl-5-hydroxy-5-(3'-pyridyl)-3-pyrrolin-2-one (15). The cyclic structure was indicated by the ^1H NMR spectrum in $CDCl_3$ which displayed the *N*-methyl proton resonance as a sharp singlet at 2.72 ppm and by ^{13}C NMR spectra which showed the presence of only one carbonyl carbon atom signal at 170.0 ppm in $CDCl_3$ and at 172.6 ppm in D_2O. These results are consistent with the report that compounds of this type exist exclusively in the cyclic form.[11]

Reduction of 15 with Pd/C gave a product which was obtained as a white crystalline solid. The IR spectrum of the freshly prepared crystalline synthetic material in $CHCl_3$ showed only one intense absorption band in the carbonyl-stretching region (1685 cm^{-1}). The absence of an N–H bending vibration in the region 1550–1510 cm^{-1} seemed to rule out the presence of the open-chain tautomer 4. This conclusion was further supported by the presence of a sharp single band of medium intensity at 1430 cm^{-1} which was assigned to the in-plane OH bending vibration of a tertiary alcohol.[22,23]

Attempts to characterize this product by mass spectrometry were only partially successful. Spectra obtained under EI conditions were not reproducible and varied between the type A and type B spectra already observed with the metabolite. We were unable to relate these changes to source temperature, to the nature of the solvent used in applying the sample to the probe, or to the history of the sample. CI spectra displayed only a pseudomolecular ion at *m/e* 193 (MH^+) and provided no additional structural information. The EI spectra of the Me_3Si derivative of the metabolite and this synthetic material displayed a prominent fragment ion at *m/e* 175 corresponding to the loss of a (trimethylsilyl)oxy group from the molecular ion. This fragmentation is consistent with the hydroxy lactam structure 5.

The proton NMR spectrum of the crystalline product in $CDCl_3$ displayed a sharp *N*-methyl signal at 2.65 ppm but showed neither the triplet centered at 3.3 ppm nor the doublet centered at 2.80, both of which were present in the $CDCl_3$ spectrum of the product synthesized by the route described by McKennis. However, after 4 days in $CDCl_3$ at room temperature or when the crystalline material was dissolved in water and then extracted into $CDCl_3$, these signals were present. The resulting ^1H NMR spectra were essentially identical with those obtained with the McKennis product. The ^1H NMR spectrum of the *N,N*-dimethyl keto amide 16, which is structurally similar to 4 but which can exist only in a ring-open form, also displays a triplet centered at 3.3 ppm. We have assigned this signal in all of these spectra to the methylene protons α to the keto carbonyl carbon atoms of 16 and 4. These data suggest that hydroxy lactam 5 equilibrates slowly with 4 in $CDCl_3$. This equilibrium is established rapidly in water since the signal centered at 3.3 ppm is present even in freshly prepared D_2O solutions of 5. By integration of this signal it was estimated that at equilibrium the ring-open form accounts for rougly 15% of the material in both solvents.

Further evidence of the presence of the ring-open form in aqueous media was obtained in an exchange experiment. Treatment of a solution of 5 in D_2O with 1 drop of 4% NaOD in D_2O caused the signals between 2 and 3.5 ppm of the ^1H NMR spectrum to disappear over a period of 5 min. Additionally, a broad signal centered at 2.62 ppm

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originally integrating for 5 protons integrated for 3 protons following NaOD treatment. The EIMS of this material showed that the molecular ion had shifted to three mass units higher. We interpret these results in terms of a base-catalyzed incorporation of two deuterium atoms at the methylene carbon atoms α to the keto group of **4**. The exchange of protons at C-3 of compound **5** was ruled out since the analogous exchange of cotinine with D_2O in the presence of K_2CO_3 is very slow, requiring several days at 100 °C.[21] When cotinine was subjected to the same exchange conditions as employed with **5**, no change was observed in the ^1H NMR spectrum, even after 68 h.

Finally the results from the proton NMR spectra were confirmed by ^{13}C NMR. A freshly prepared solution of crystalline **5** in $CDCl_3$ showed the following 10 well-defined signals, one for each carbon atom in **5**: 175.5 (N—C=O), 148.9, 147.4, 139.0, 134.0, 123.7 (5 pyridine carbons atoms), 91.6, (C—OH), 37.4, 29.5, 25.2 ppm (3 aliphatic carbon atoms). However, after equilibration in $CDCl_3$ or when the spectrum was taken in D_2O the following additional signals were observed (in $CDCl_3$): 198.0 (pyr C=O), 172.7 (N—C=O), 153.4, 149.5, 135.7 (5 pyridine carbon atoms), 33.7, 29.7, 26.5 ppm (3 aliphatic carbon atoms).

The results of these studies provide experimental support for the statement by McKennis that metabolic C-5 hydroxylation of (*S*)-cotinine leads to a product which in water consists of a tautomeric mixture of the hydroxy lactam **5** and keto amide **4**. The hydroxy lactam tautomer is energetically favored and can be obtained in pure crystalline form by the synthetic route described in this paper which, because of the exclusive use of aprotic solvents, avoids the rapid tautomerism facilitated by aqueous solvents. Since equilibration of **5** in $CDCl_3$ is a slow process it is likely that the cyclic form will be the predominant if not the exclusive form of this metabolite in lipophilic environments.

Experimental Section

All reactions were carried out under a nitrogen atmosphere unless otherwise stated. Melting points and boiling points are uncorrected. All chemicals used were reagent grade unless otherwise specified. Infrared (IR) spectra were taken on a Perkin-Elmer 337 grating spectrophotometer. Intensities of IR absorption bands are given as (sh) sharp, (s) strong, (m) medium, (w) weak, and (br) broad. A Varian 60A instrument was used to obtain proton magnetic resonance (^1H NMR) spectra. Chemical shifts are given in parts per million (ppm) downfield from internal tetramethylsilane in all solvents except D_2O where sodium 2,2-dimethyl-2-silapentane-5-sulfonate (SDSS) was used. Spin multiplicity is given as (s) singlet, (d) doublet, (t) triplet, (q) quartet, (p) pentet or (m) multiplet. A Varian FT-80A instrument was used to obtain decoupled ^{13}C magentic resonance (^{13}C NMR) spectra. Chemical shifts are given in parts per million (ppm) downfield from internal Me_4Si in $CDCl_3$ and are referenced to dioxane at 67.39 ppm in D_2O. Electron-impact mass spectra (EI mass spectra) were obtained by direct insertion on an AEI MS-12 mass spectrometer which is interfaced to a PDP 8/I computer using the DS-30 software. Unless otherwise stated, mass spectra were taken with an 8-kV accelerating voltage, a trap current of 500 μA, an electron beam energy of 70 eV, a source temperature of 200 °C, and a resolving power of 1200. Chemical-ionization mass spectra (CI mass spectra) were obtained by direct insertion on an AEI MS 902 instrument modified for CI mass spectra and using isobutane as reactant gas.

1-Methyl-5-methoxy-5-(3′-pyridyl)-3-pyrrolin-2-one (10). KOH pellets (4.2 g, 75 mmol) were added to a well-stirred solution of (*S*)-3,3-dibromocotinine (**6**, 10 g, 30 mmol) in 125 mL of methanol at −10 °C. The mixture was cooled in a dry ice–2-propanol bath as necessary to keep the temperature below −5 °C. When all of the KOH pellets had dissolved, the mixture was allowed to warm to room temperature. After 2 h the KBr precipitate (2.6 g) was removed by filtration and the methanol was removed by rotary evaporation at reduced pressure. To the residue was added chloroform (100 mL) and the insoluble KBr (2.5 g) was separated by filtration. The chloroform solution was washed once with water, decolorized with charcoal, dried, and evaporated to give **10** as a light yellow oil (5.5 g, 90%): EI mass spectrum, *m/e* (relative intensity) 204 (M^+, 39%), 173 (100%), 126 (96%); ^1H NMR ($CDCl_3$, Me_4Si) δ 2.62 (s, 3 H, NCH_3), 3.25 (s, 3 H, OCH_3), 6.67 (AB q, J_{AB} = 6 Hz, $\Delta\nu$ = 33 Hz, 2 H, C_3 and C_4), 7.0–9.0 (4 H, typical 3-substituted pyridine pattern); ^{13}C NMR ($CDCl_3$) δ 23.7 (NCH_3), 50.4 (OCH_3), 95.0 ($COCH_3$), 123.4, 128.7, 132.2, 133.8, 147.8, 148.1, 150.0 (vinylic and pyridine carbon atoms), 169.6 (N—C=O); IR ($CHCl_3$) ν 1700 cm^{-1} (s, C=O).

Anal. Calcd for $C_{17}H_{15}N_5O_9$ (picrate, mp 142 °C): C, 47.12; H, 3.49; N, 16.16. Found: C, 46.75; H, 3.45; N, 16.08.

1-Methyl-5-hydroxy-5-(3′-pyridyl)-3-pyrrolin-2-one (15). Compound **10** (5.9 g, 28.9 mmol) in 15% aqueous HBr (45 mL) was heated at 95 °C for 2 h. The reaction mixture was then cooled, neutralized with $NaHCO_3$, and extracted with CH_2Cl_2. The combined extracts were decolorized on charcoal. After solvent removal, **15** was obtained as a semicrystalline solid. A crystalline material was obtained from benzene (4.5 g, 82%) which after several recrystallizations gave a mp of 145–146 °C: EI mass spectrum, *m/e* (relative intensity) 190 (M^+, 72.8%), 112 (93.8%), 106 (39.5%), 79 (100%), 78 (73.6%); IR ($CHCl_3$) ν 1700 cm^{-1} (s, C=O); ^1H NMR ($CDCl_3$) δ 2.72 (s, 3 H, NCH_3), 6.60 (AB q, J_{AB} = 6 Hz, 2 H, $\Delta\nu_{AB}$ = 33 Hz, 2 H, C_3 and C_4), 7.0–9.0 (4 H, typical 3-substituted pyridine pattern); ^{13}C NMR ($CDCl_3$) δ 23.6 (NCH_3), 91.1 (COH) 123.8, 125.8, 133.4, 134.5, 147.5, 149.1, 150.8 (vinylic and pyridyl carbon atoms), 169.9 (N—C=O); ^{13}C NMR (D_2O) 24.2 (NCH_3), 92.3 (COH), 125.3, 126.1, 133.4, 135.9, 135.9, 147.3, 150.0, 151.9 (vinylic and pyridyl carbon atoms), 172.6 (N—C=O).

Anal. Calcd for $C_{10}H_{10}N_2O_2$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.34; H, 5.34; N, 14.84.

1-Methyl-5-hydroxy-5-(3′-pyridyl)-2-pyrrolidinone (5-Hydroxycotinine, 5). Compound **15** (1.45 g, 7.6 mmol) in 100 mL of EtOAc was reduced with H_2 at atmospheric pressure, using 10% Pd/C (375 mg) as catalyst. At the end of 20 h the mixture was filtered. Upon removal of the EtOAc a white crystalline material (1.35 g, 92%) was obtained which gave a melting point of 126–127 °C after several recrystallizations from benzene/hexane: EI mass spectrum, see text; IR ($CHCl_3$) 1685 (s, C=O), 1430 cm^{-1} (m, OH); ^1H NMR ($CDCl_3$) δ 2.3–3.0 (a complex m, 4 H, C_3 and C_4), 2.65 (intense s, 3 H, NCH_3) 7.0 (br s, 1 H, OH), 7.3–9.0 (4 H, typical 3-substituted pyridine pattern); ^{13}C NMR ($CDCl_3$), see text; ^{13}C NMR (D_2O, dioxane) δ 25.7, 29.7, 37.2 (3 aliphatic carbon atoms), 92.8 (COH), 125.1, 135.5, 139.0, 146.9, 149.5 (5 pyridine carbon atoms), 178.3 (N—C=O); in addition to the above signals, peaks at lower intensities were seen at 26.7, 30.0, 34.7, 137.4, 149.3, 153.6, 175.4, and 201.1 ppm.

Anal. Calcd for $C_{10}H_{12}N_2O_2$: C, 62.49; H, 6.29; N, 14.57. Found: C, 62.54; H, 6.32; N, 14.72.

***N,N*-Dimethyl-4-(3-pyridyl)-4-oxobutanamide (16).** An ether solution of diazomethane was added dropwise to 4-(3-pyridyl)-4-oxobutanoic acid[24] (235 mg, 131 mmol) until the color persisted. Evaporation gave a crystalline solid (not isolated) to which was added 143 mg of an aqueous solution containing 59 mg (1.31 mmol) of dimethylamine. After 4 days at room temperature this was extracted with dichloromethane. Evaporation of the extracts gave the product as a yellow oil (27 mg, 10%): EI mass spectrum, *m/e* (relative intensity) 206 (M^+, 17%), 162 (67%), 161 (77%), 134 (34%), 106 (100%), 100 (37%), 78 (71%), 72 (54%); ^1H NMR ($CDCl_3$) δ 2.80 (t, 2 H, CH_2CON), 2.95 (s, 3 H, NCH_3), 3.09 (s, 3 H, NCH_3), 3.33 (t, 2 H, PyCOCH2), and 7.3–9.0 (4 H, typical 3-substituted pyridine pattern); high-resolution mass spectrum, calcd for $C_{11}H_{14}N_2O_2$ *m/e* 206.1055, obsd *m/e* 206.1049.

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Registry No. 5, 75919-05-0; 7, 74093-56-4; 10, 75919-07-2; 10 picrate, 75919-08-3; 15, 75919-09-4; 16, 75919-10-7; 4-(3-pyridyl)-4-oxobutanoic acid, 4192-31-8.

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