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The carbon-13 and proton nmr spectra of nicotine in aqueous solution have been ivnestigated. The changes resulting from alteration of pD level are detailed. In the case of the carbon-13 spectrum, these changes are essentially independent of the acid present. Changes in previously published assignments for carbons (2) and (6) and protons (2) and (6) are presented.

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Introduction.

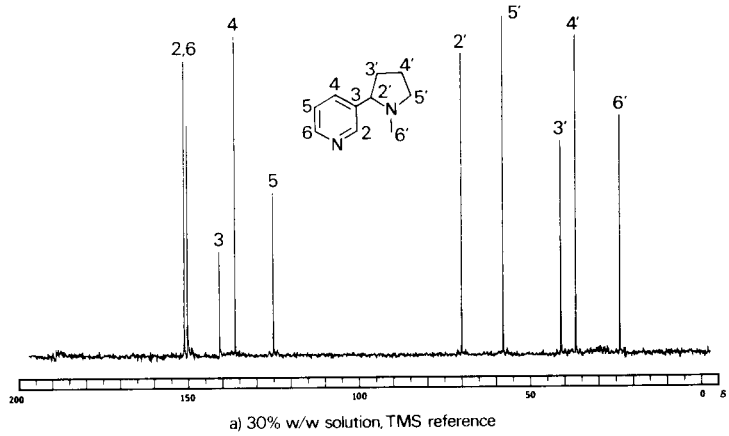
The physical, chemical and biochemical properties of nicotine are of great importance to researchers in various fields of study concerned with tobacco. In recent years, increased use has been made of carbon-13 and proton nmr spectroscopy to study biological precursors of nicotine [1-5], its degradation products and metabolites [6,7] and its solution structure [8-12]. Implicit in these works is the assumption that an unambiguous assignment of resonances in the relevant spectra can be made. In the case of the carbon-13 spectrum of nicotine, disagreement [1,13] over the assignments of carbons 2 and 6 was apparently resolved in 1978 [14]. However, additional work presented here shows that the positions of the resonances are highly pD dependent and under alkaline conditions are at variance with previous assignments [14]. Additionally, it has been found that the proton spectrum exhibits a similar dependence and previously published [9,14-16] assignments must be reconsidered in terms of solvent and concentration dependence.

Results and Discussion.

Carbon-13 Spectra.

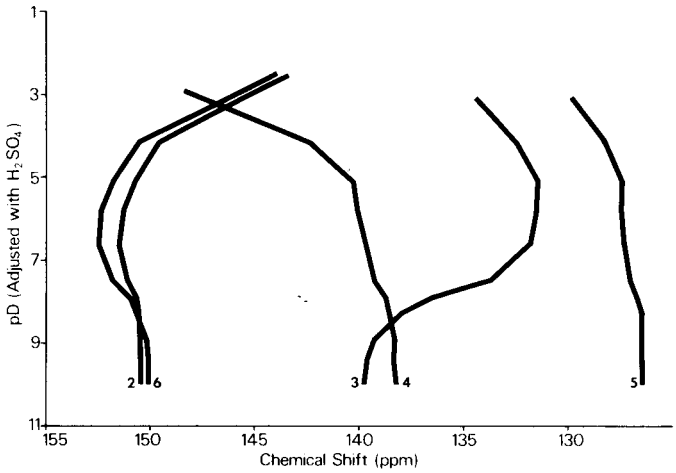
The carbon-13 nmr spectrum of nicotine as obtained in acetone-d_6 is shown in Figure 1. Resonance assignments have previously been made based on multiplicities, intensities, double resonance techniques and by analogy to 3-picoline [1,13]. The closely spaced C(2) and C(6) resonances could not be differentiated by these methods and Pitner, Seeman and Whidby [14] resorted to the use of selective population transfer and selective decoupling techniques to make assignments in a variety of media. Table 1 shows their assignments of the pyridyl carbons at three pD levels. These assignments indicate that in all three media, C(2) resonates at a higher field than C(6). In organic solvents (acetone-d_6, DMSO-d_6, pyridine-d_6), Pitner, *et al.* found this relationship reversed. To further understand the influence of protonation on the nicotine spectrum, a more detailed analysis at several more pD levels has been carried out. Figure 2 indicates the shifts of the pyridyl carbons as the pD is incrementally varied from 10.4 to 2.2. The position of the C(4) resonance is most profoundly affected, shifting 11.2 ppm downfield. In the process of doing so, it moves downfield of carbons (2), (6) and (3). Also of interest is the large upfield shift of C(3) in the region from pD 8 to 6 indicating that protonation of pyrrolidine ring results in increased electron density at this position. In addition, Figure 2 indicates a crossover of C(2) and C(6) under slightly alkaline conditions. This crossover can be found to occur at exactly pD = 8.28, where, as shown in Figure 3, the two resonances overlap. This effect was not

Figure 1. Carbon-13 NMR of Nicotine in d_6-Acetone[a]



a) 30% w/w solution, TMS reference

Figure 2. pD Dependence of Carbon-13 NMR Pyridyl Carbons[a]



a) initial 30% w/w solution, conc. H_2SO_4 added incrementally, DSS reference

Table 1

Carbon-13 Chemical Shifts and One Bond Coupling Constants for Nicotine in Deuterium Oxide - Pyridyl Carbons

	pD 10.7		pD 5.4		pD > 1	
	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
C(2)	148.9	179.5	150.0	179.4	142.8	189.7
C(3)	138.7	—	129.9	—	133.8	—
C(4)	137.1	162.1	138.4	163.8	148.2	170.0
C(5)	127.3	164.2	125.9	168.3	129.3	178.6
C(6)	149.2	179.5	151.2	182.6	143.7	193.6

From T. P. Pitner, J. I. Seeman and J. F. Whidby, *J. Heterocyclic Chem.*, **15**, 585 (1978).

specific for sulfuric acid but was also noted for several others including acetic, malic, tartaric, gluconic and hydrochloric. In fact, shift changes induced by pD adjustments with different acids are remarkably similar throughout the spectrum. This point is illustrated in Table 2. Interestingly, the largest effect in the pyrrolidine ring is seen at C(3). This may reflect a change in ring conformation or the orientation of the pyridine. Since chemical shifts are indicative of electronic and steric effects within the molecule it can be seen that the nature of the anion has little influence on the structure of the monoprotonated nicotine moiety in aqueous solution. This is the reverse of the situation in the solid state where nicotine salts form in several stoichiometries which are postulated to be intimately connected with different solid state structures [17].

Figure 3. Carbon-13 NMR Spectrum of Nicotine at Several pD's - Pyridyl Carbons

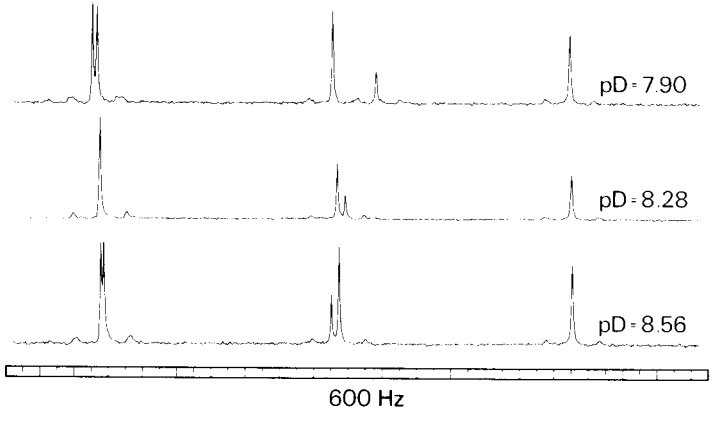


Table 2

Acid Induced CArbon-13 Shifts [a] of Nicotine

	HCl	ACetic	Tartaric	H_2SO_4
C(2)	0.97	1.05	.73	1.31
C(3)	−8.37	−8.20	−7.92	5.98
C(4)	1.56	1.28	0.20	0.90
C(5)	0.95	0.78	0.33	0.51
C(6)	2.58	2.56	2.06	1.71
C(2')	1.37	1.15	0.75	0.67
C(3')	−3.18	−3.19	−3.21	−2.31
C(4')	−0.03	−0.03	−0.03	−0.25
C(5')	0.06	−0.15	−0.42	−0.15
C(6')	−0.81	−0.95	−1.15	−0.78

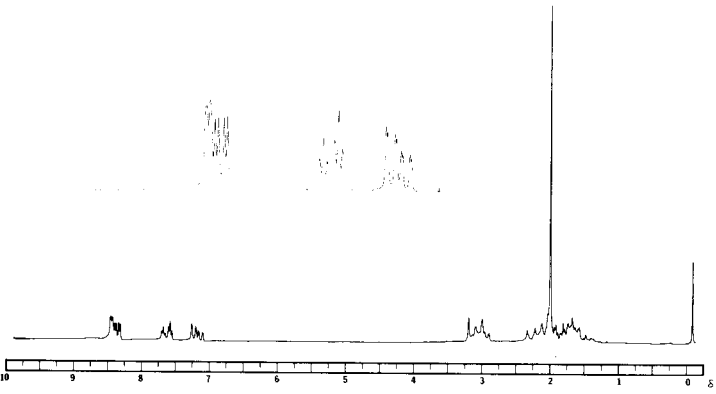
[a] Thirty three % w/w nicotine/deuterium oxide, plus 1 equivalent acid, a negative value denotes an upfield shift.

Proton Spectrum.

The published assignments [14] of the nicotine carbons (2) and (6) depended on techniques which involved irradiation of specific nicotine protons. Thus, a reexamination of the proton spectrum was undertaken to remove any remaining ambiguity. Figure 4 shows the spectrum as it appears in acetone-d_6. Assignments of the pyridyl protons in this and similar media can be made on the basis of chemical shifts, multiplicities and coupling constants in a straightforward manner. H(2) and H(6) are quite close in chemical shifts, analogous to the situation for their respective carbons.

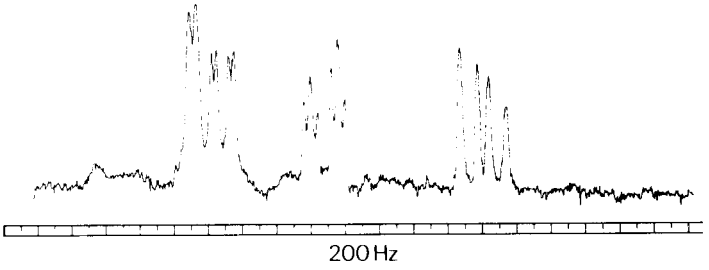
Figures 5, 6, and 7 show the pyridyl protons in deuterium oxide at several pD levels. At low pD, the appearance of the nicotine salt is very similar to that of neutral nicotine in organic solvents. However, neutral nicotine in aqueous solution (pD = 10.2, 10.7) has a quite different set of multiplicities arising from H(2) and H(6). Computer simulations (Figure 6b, 7b) indicate that at pD = 10.2, H(2) and H(6) resonate at exactly the same frequency while at 10.7 H(2) is approximately 4 Hz upfield of H(6). The fact that exact coupling constants can be obtained from the other pyridyl protons allows this unambiguous computer simulation. The crossover of H(2) and H(6) could give rise to erroneous assignments of C(2) and C(6) when a transfer of spin saturation *via* irradiation of the carbon-13 coupled proton satellite resonances is employed [14] in this particular case.

Figure 4. Proton NMR Spectrum of Nicotine in d_6 - Acetone (Insert: Pyridyl Protons, 200 Hz)[a]



a) 30% w/w solution, TMS reference

Figure 5. Proton NMR Spectrum of Nicotine in Deuterium Oxide (pD = 4.6), Pyridyl Resonances Only[a]



a) 30% w/w nicotine solution, H_2SO_4 added, DSS reference

Figure 6. (a) Pyridyl Protons at pD = 10.2. (b) Computer Simulated Spectrum.

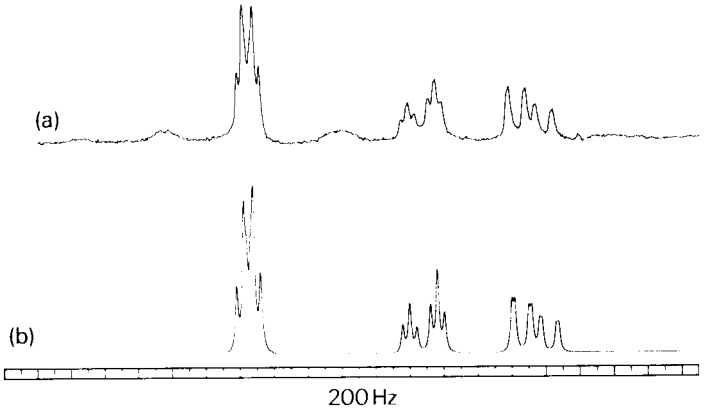
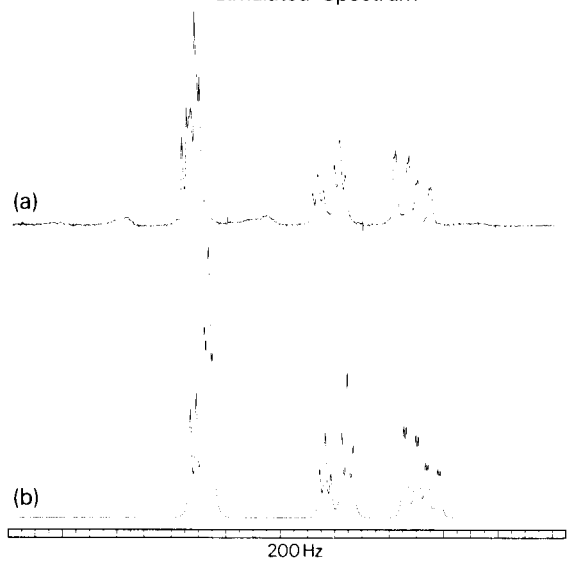


Figure 7. (a) Pyridyl Protons at pD = 10.7 (b) Computer Simulated Spectrum



Conclusions.

These observations may have important implications for understanding the conformation of nicotine and nicotine salts in aqueous solution. A large amount of nmr work [8-12] has been carried out to determine the most favored conformation of nicotine in solution. However, these experiments have been carried out on alkaloidal nicotine in deuteriochloroform. The changes in the carbon-13 and proton spectra detailed here may be of importance in further studies in aqueous solution, especially at physiological pH levels. Past work which is affected include the assignments of isotope distributions in a catalytic tritiation study [16].

EXPERIMENTAL

Apparatus.

Proton and carbon-13 nmr spectra were obtained on a Varian CFT-20 spectrometer equipped with a proton/carbon-13 dual probe and 24K memory. The pD measurements were made at 22°C using an Ingold 6030-04 electrode and a Fisher Accumet 142 meter.

Chemicals.

Commercial nicotine (Eastman) was purified by treatment with sodium hydroxide, filtration and vacuum distillation. Acids were reagent grade or better and used without further purification.

Spectroscopy.

All solutions were degassed with nitrogen prior to data acquisition. The carbon-13 spectra were obtained at 20.2 MHz utilizing 8K data points over a 5 KHz spectral window. The acquisition time was 2.047 seconds with no pulse delay and a tip angle of 41 (10 μ second pulse). Proton spectra were produced at 80 MHz with 4K data points, a 1 KHz window, 0.819 second acquisition time and a 90° tip angle (47 μ second).

Simulation spectra were generated with a SIMEQ program provided for the CFT-20 by Varian Associates.

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