Assignment and Solvent Dependence of the Carbon-13 Nuclear Magnetic Resonance Spectrum of Nicotine

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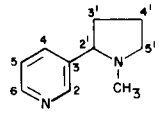
Received January 16, 1978

The ^{13}C nmr spectrum of nicotine is assigned in Acetone-d_6, DMSO-d_6, Pyridine-d_5, and deuterium oxide (pD 10.7, 5.4, < 1). Attention is focused on assignment of the closely-spaced C(2) and C(6) resonances, using selective decoupling, population transfer, and long-range coupling constant measurements. C(2) resonates at lower field in the organic solvents but at higher field in deuterium oxide at all pD values investigated.

J. Heterocyclic Chem., **15**, 585 (1978)

Accurate assignment of ^{13}C nmr peaks is prerequisite to structural, conformational, and biosynthetic studies by nmr. Closely spaced peaks cannot be assigned by relying on chemical shift model compounds of similar structure, since slight variations in structure or substituents can reverse the chemical shifts of carbons in nearly equivalent magnetic environments. It was for this reason that Crain, *et al.*, (1) were not able to assign unambigously the C(2) and C(6) resonances of nicotine (**1**) in deuteriochloroform



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by comparing the spectrum of nicotine with that of 3-picoline (2). Hutchinson, *et al.*, (3) have recently assigned the C(2) and C(6) resonances of monoprotonated nicotine in deuterium oxide employing a double resonance technique (4). Although it appeared that the assignments were reversed by the two groups of investigators, it was not clear whether the assignments by Crain, *et al.*, (1) were in error or whether the change in solvent from deuteriochloroform to deuterium oxide had resulted in chemical shift reversal for C(2) and C(6). It is the purpose of this paper to clarify the ^{13}C assignments of nicotine in these solvents and to extend the assignments to acetone-d_6 (Figure 1), DMSO-d_6, pyridine-d_5 and deuterium oxide (at various degrees of nicotine protonation) (5).

The C(3), C(4), and C(5) resonances of nicotine can be assigned readily by comparison with 3-picoline (2) (Tables I and II) as accomplished previously (1). In addition, the C(3) resonance can be identified since it is significantly lower in intensity than the other aromatic peaks; the absence of a directly bonded hydrogen increases the spin-lattice relaxation time of this carbon. Other methods are required to assign the closely spaced C(2) and C(6) peaks. Hutchinson, *et al.*, employed the method described by Birdsall, *et al.*, (4) in which the residual one bond ^{13}C-^{1}H coupling is monitored as a function of the frequency of monochromatic proton decoupling.

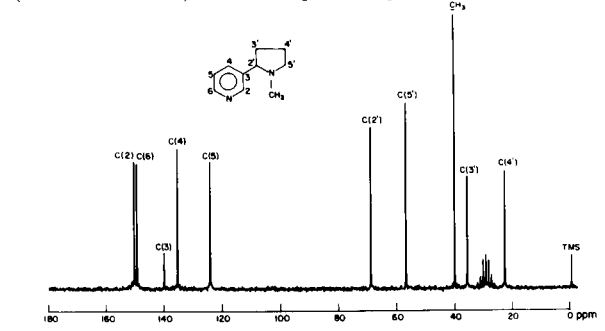

Figure 1. Proton decoupled 20.1 MHz ^{13}C FT nmr spectrum of nicotine in acetone-d_6 (33% V/V). Experimental conditions: Bruker WP-80; 100 pulses; 10 mm sample tube.

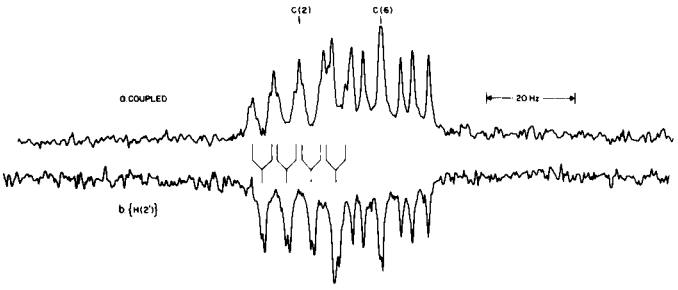

Figure 2. a). Coupled $^{13}C(2)$ and $^{13}C(6)$ resonances of nicotine in acetone-d_6 (20.1 MHz); b) effect of decoupling $^{1}H(2')$.

C(2) and C(6) may also be distinguished by single-frequency decoupling of H(2') (Figure 2). By analogy with 3-picoline (6) the exocyclic H(2') couples significant-

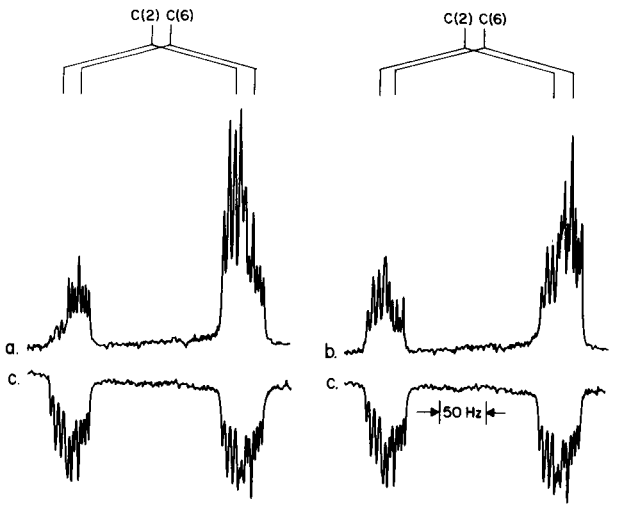

Figure 3. $^{13}C(2)$ and $^{13}C(6)$ resonances of nicotine (20.1 MHz): a) low field satellite of $^{1}H(2)$ saturated; b) low field satellite of $^{1}H(6)$ saturated; c) unperturbed spectrum.

ly with C(2) but not C(6). We observe major perturbations only in the lower field resonance upon irradiation of H(2′), allowing assignment of this resonance to C(2).

C(2) and C(6) may also be delineated by selective population transfer (7-12), if the ^{13}C satellites of H(2) and H(6) are reasonably well separated. This experiment involves low-power saturation of one of the ^{13}C satellites of either H(2) or H(6), which in turn causes intensity perturbations in the corresponding ^{13}C resonances. Saturation of the low field ^{13}C satellite of H(2) caused magnetization transfer from the low field multiplet to the high field multiplet of one of the carbons, allowing assignment of this carbon as C(2) (Figure 3). A similar approach is taken to confirm the assignment of C(6).

Table I

^{13}C Chemical Shifts and Coupling Constants of the Pyridine Carbons of 3-Picoline in Acetone-d_6

δ (ppm) (a)	J (Hz)				
	H(2)	H(4)	H(5)	H(6)	H(exocyclic)
C(2) 151.1	175.0	5.6	0.9	11.1	5.6
C(3) 133.7					
C(4) 136.7	5.1	160.5		6.4	5.1
C(5) 123.7	1.6		162.4	9.1	
C(6) 147.7	11.1	6.5	3.6	177.4	0.9

(a) TMS internal reference.

Table II

^{13}C Chemical Shifts and Coupling Constants of the Pyridine Carbons of Nicotine in Acetone-d_6

δ (ppm) (a)	J (Hz)				
	H(2)	H(4)	H(5)	H(6)	H(exocyclic) (b)
C(2) 150.2	176.0	5.6	0.9	11.2	4.6
C(3) 139.9					
C(4) 135.3	5.3	161.8		6.4	4.4
C(5) 124.1	1.6		162.6	9.1	
C(6) 149.2	11.2	6.6	3.8	177.2	0.3

(a) TMS internal reference. (b) H(2′)

Takeushi (6) has presented coupling constant measurements for a wide variety of substituted pyridines. We have repeated his measurements for 3-picoline in acetone-d_6 at higher resolution (Table I). By examining the ^{13}C-^{1}H splitting patterns (see example Figure 4), the coupling constants for nicotine in acetone-d_6 were determined (Table II). Selective decoupling of H(2′) aids in confirming these long-range coupling constant assignments. Although the C(4) couplings can be extracted from the splitting patterns, the resonances of this carbon are not as symmetrical in appearance as those of the other carbons; this results from higher order effects due to overlap of the lower field ^{13}C satellite of H(4) with the H(2) and H(6) resonances.

Examination of the coupling constants in Table II allows differentiation between some of the pyridyl carbons. The one bond ^{13}C-^{1}H coupling constants offer a clear distinction between carbons α to the pyridyl nitrogen and those β or γ, since the former are 10-15 Hz larger. If a case arose in which C(4) and C(5) could not be distinquished by a more straightforward method, it is clear that a comparison of long-range coupling constants would offer such a delineation. It is not possible, however, to differentiate between C(2) and C(6) of nicotine by a comparison of the coupling constants with those of 3-picoline, unless specific splittings have been assigned to specific protons. However, with all the nonaqueous solvents investigated in this study, the C(2) resonances of nicotine have the same appearance (three triplets flanked by two doublets, Figure 4); the C(6) resonances also have the same appearance from solvent to solvent (Figure 4). This

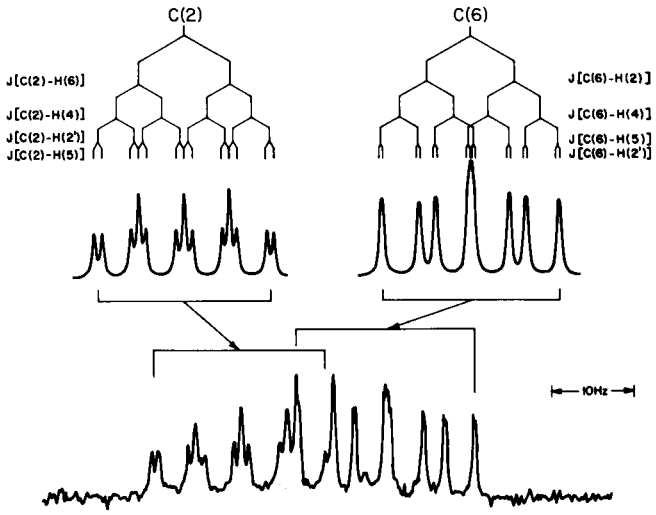

Figure 4. Coupled $^{13}C(2)$ and $^{13}C(6)$ resonances of nicotine in acetone-d_6 (20.1 MHz). The low field portions of these resonances are presented together with computer simulated spectra. Experimental conditions: decoupler gated off during acquisition; 5000 pulses.

suggests the possibility that the C(2) and C(6) resonances can be assigned by a simple "fingerprint" technique in certain solvents.

The ^{13}C resonance assignments of nicotine in acetone-d_6, deuteriochloroform, pyridine-d_5, DMSO-d_6 and deuterium oxide (pD 10.7, 5.4 < 1: meter reading + 0.4) (Table III) were made on the basis of H(2′) decoupling and saturation transfer. The pyrrolidine carbon assignments are those of Crain, *et al.*, (1) and have been confirmed in this laboratory by synthesis and ^{13}C nmr analysis of nicotine-4′,4′,5′,5′-d_4. In the four organic solvents C(2) resonates at lower field than C(6), whereas in deuterium oxide at all degrees of protonation, C(2) resonates at higher field.

Table III

^{13}C Chemical Shifts (ppm) and One Bond Coupling Constants (Hz) for Nicotine in Several Solvents (a)

	Acetone-d_6		Deuterio-chloroform		Pyridine-d_5		DMSO-d_6		Deuterium oxide (pD 10.7)		Deuterium oxide (pD 5.4)		Deuterium oxide (pD < 1)	
	δ (b)	J	δ (b)	J	δ (b)	J	δ (b)	J	δ (c)	J	δ (c)	J	δ (c)	J
C(2)	150.2	176.0	149.7	176.1	150.1	175.5	149.3	176.0	148.9	179.5 (d)	150.0	179.4	142.8	189.7
C(3)	139.9	----	139.1	----	139.6	----	139.0	----	138.7	----	129.9	----	133.8	----
C(4)	135.3	161.8	134.8	161.7	134.9	161.4	134.8	161.8	137.1	162.1	138.4	163.8	148.2	170.0
C(5)	124.1	162.6	123.5	162.7	123.8	162.8	123.7	163.1	125.3	164.2	125.9	168.3	129.3	178.6
C(6)	149.2	177.2	148.7	177.6	149.1	177.3	148.6	177.5	149.2	179.5 (d)	151.2	182.6	143.7	193.6
CH_3	40.5	----	40.3	----	40.3	----	40.1	----	40.1	----	39.0	----	39.7	----
C(2′)	69.3	----	68.8	----	68.8	----	68.3	----	69.3	----	70.4	----	69.4	----
C(3′)	36.1	----	35.4	----	35.8	----	35.3	----	34.6	----	31.1	----	31.6	----
C(4′)	23.2	----	22.7	----	23.0	----	22.5	----	22.6	----	22.3	----	22.5	----
C(5′)	57.4	----	57.0	----	57.0	----	56.6	----	57.2	----	56.9	----	57.5	----

(a) Concentrations are 33% by volume, except for deuterium oxide pD 5.4 (30%) and deuterium oxide pD < 1 (27%). (b) TMS-internal reference (0.0 ppm). (c) Dioxane internal reference (67.4 ppm). (d) Measured between centers of nearly over lapping C(2) and C(6) multiplets.

We have noticed a concentration dependence of the pyridine carbon chemical shifts in deuterium oxide, so that care must be exercised to assign these peaks at the concentration of interest. Shown also in Table III are the one bond ^{13}C-^{1}H pyridine coupling constants. These are virtually identical in the four organic solvents shown, but exhibit a dependence upon protonation of the pyrrolidine ring (pD 5.4) and pyridine ring (pD < 1). The effect of protonation on pyridyl couplings has been noted previously (13).

In our hands, the techniques used in this paper have proven very fruitful in confirming ambigous ^{13}C assignments of nicotine as well as other tobacco alkalloids (14).

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(14) Following completion of this investigation, we were informed by Dr. Edward Leete that he and his co-workers had confirmed the tentative assignments of Crain, *et al.*, (1) for C(2) and C(6) in chloroform. Peaks were assigned using enriched nicotine obtained biosynthetically from the specifically labeled precursor [5,6-$^{13}C_2$]nicotinic acid. We thank Dr. Leete for making available to us a preprint of a manuscript which has since appeared: *Bioorg. Chem.*, **6**, 273 (1977).