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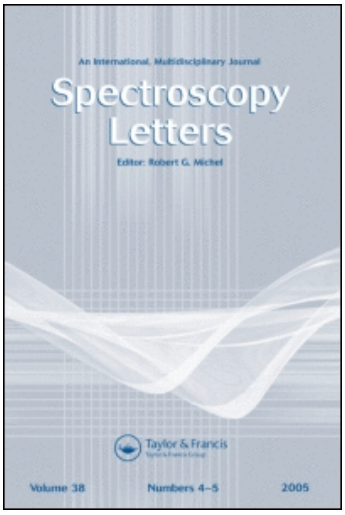

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The Use of Protonation Induced Changes in Carbon-13 NMR Chemical Shifts to Investigate the Solution Microscopic Structure of Partially Protonated Polybasic Molecules

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THE USE OF PROTONATION INDUCED CHANGES IN CARBON-13 NMR CHEMICAL SHIFTS TO INVESTIGATE THE SOLUTION MICROSCOPIC STRUCTURE OF PARTIALLY PROTONATED POLYBASIC MOLECULES

Keywords: Protonation, DTPA, 4-Pyridone, Carbon-13, NMR

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ABSTRACT

Changes in the NMR chemical shift of carbon-13 nuclei upon protonation of a nearby basic center are shown to be a useful method of probing the microscopic site of protonation of molecules containing multiple basic centers, such as diethylenetriaminepentaacetic acid (DTPA). This approach is also shown to support the predominance of the pyridine amine protonated tautomer in the 4-pyridone ↔ 4-hydroxypyridine equilibrium.

INTRODUCTION

Acid-base properties in solution are generally formulated in terms of ionization constants (pK_a's) which describe the affinity of a base for a hydrogen ion. For polybasic molecules these macroscopic constants are often not fully descriptive since they do not define which basic center (or centers) are being protonated at a given pK_a. The microscopic ionization constants which describe the proton affinity of the individual basic centers at the molecular level are chemically more informative. To define the microscopic protonation behavior of polybasic molecules one must be able to determine fractional protonation of each basic center at a given

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macroscopic pK_a. A number of experimental procedures have been used to acquire this fundamental information and these have been reviewed elsewhere.[1,2]

In this paper we describe the use of carbon-13 NMR spectra to examine the microscopic protonation of the conjugate base of diethylenetraaminepentaacetic acid (DTPA) and the tautomeric equilibrium in 4-hydroxypyridine ↔ 4-pyridone. NMR is a useful probe of micro species in solution since chemical shifts of magnetic nuclei near basic sites are often markedly influenced by the ionization state of the basic center. Proton NMR was shown to be very useful for such work some years ago[3,4] and only recently has carbon-13 spectrometry[5] begun to see application in this area. Carbon-13 NMR possesses certain advantages over proton NMR and ^{13}C spectra should see considerably more use in these applications. First ^{13}C has an inherently larger shift range than ^{1}H, 200 ppm vs 10 ppm, making ^{13}C potentially more sensitive to protonation. A comparison of data for ^{13}C and ^{1}H nuclei on amine protonation reveals 0.5 ppm and 0.3 ppm downfield shifts for α and β protons on primary amine protonation,[3] while ^{13}C nuclei α and β show 2 and 5 ppm upfield protonation shifts.[6] Secondly carbon NMR spectra run normally with proton noise decoupling give carbon resonances which are sharp singlets; often proton spectra are complex due to significant spin-spin coupling interactions and overlapping of multiplets. A third advantage of carbon NMR exists for molecules bearing few (or no) protons near the basic centers to be monitored, e.g. in purine bases and nucleotides.

EXPERIMENTAL

Details regarding solution preparation and NMR spectral measurements were identical to those cited previously.[6] Reagents were obtained commercially. The ^{13}C spectra were measured at 25.2 MHz.

pH measurements were made with an Orion model 601 digital pH meter using a combination electrode. pH data presented in this paper are direct instrument readings and have not been corrected for the presence of D_2O.

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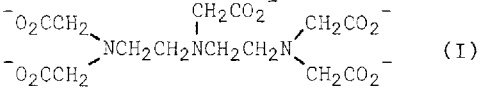
The analysis of the NMR-pH titration data shown in Figure 2 was performed with a non-linear least squares program written in these laboratories. This program fits the chemical shift vs pH data to obtain the chemical shifts for each line of each species and all pK_a's. Initial estimates of the chemical shifts and pK_a's are input. The program then modifies these estimates, attempting to minimize the root-mean-square deviation between the calculated and observed chemical shifts. Convergence is rapid, usually within 4 or 5 iterations, even for systems with 5 basic sites. Important points to note are that the program weights all points equally, and that all lines are handled simultaneously. We feel that this yields macroscopic pK_a's which are truly representative of all the data, and are not biased by one line. Agreement of pK_a's derived from this analysis with earlier literature data (10.56, 8.69, 4.37, 2.87, 1.94)[7] is quite good.

RESULTS AND DISCUSSION

The conjugate base (I) of diethylenetriaminetetraacetic acid is a species which can accept an equivalent



DTPA^{5-} (I)

of acid to form a number of microscopically distinct species as is depicted schematically in Figure 1. Earlier characterization of the ^{13}C protonation shifts of aliphatic amines[6] and carboxylic acids[8] suggests that a study of the variation of the ^{13}C shifts with pH can aid in evaluating the microscopic basicity of the various basic centers of DTPA. Assignment of the ^{13}C spectrum of DTPA based on relative intensity of signals (terminal acetate signals being four times as intense as signals from central acetate arm) and comparison of chemical shifts with model compounds; this is given on Figure 2 which also depicts the effect of pH on the individual resonances.

The initial addition of acid to DTPA^{5-} produces pronounced upfield shifts of the terminal and central carboxylate carbon

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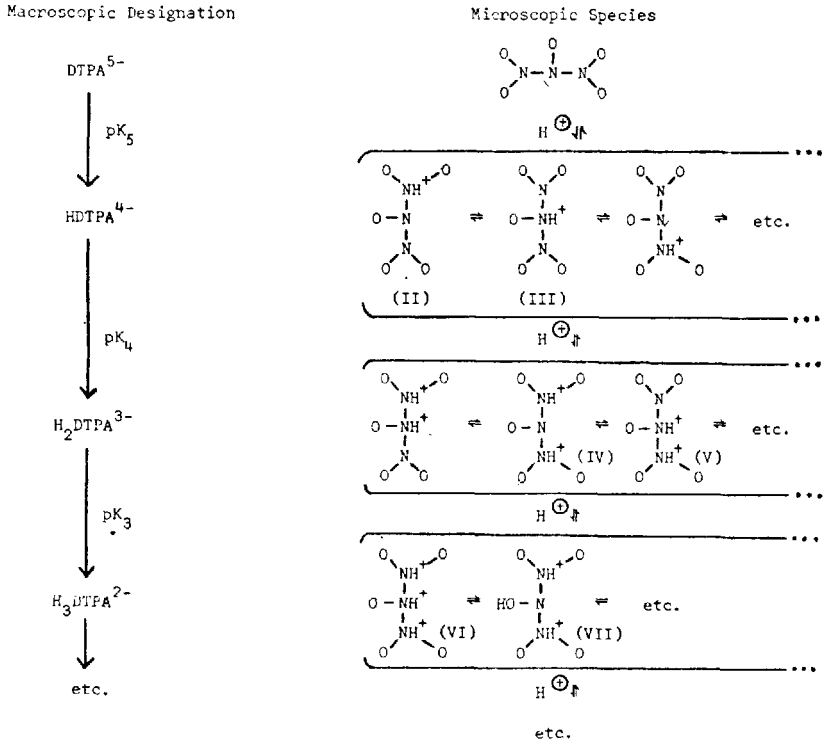

FIG. 1 Microscopic Protonated Forms of DTPA

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resonances. This result is consistent with the ^{13}C protonation shifts observed for amine protonation in α-amino acids, an example of which is N,N-dimethylglycine (N,N-Me_2gly):

TABLE 1

25.2 MHz Carbon-13 Protonation Shifts
(Hz; positive shift = downfield) for N,N-dimethylglycine

Group Protonated	$(CH_3)_2$ - N - CH_2 - $COO^{\ominus}$		
$NR_3 \xrightarrow{H^{\oplus}} NR_3H^{\oplus}$	+17	+73	+201
$COO^- \xrightarrow{H^{\oplus}} COOH$	-7	+66	+57

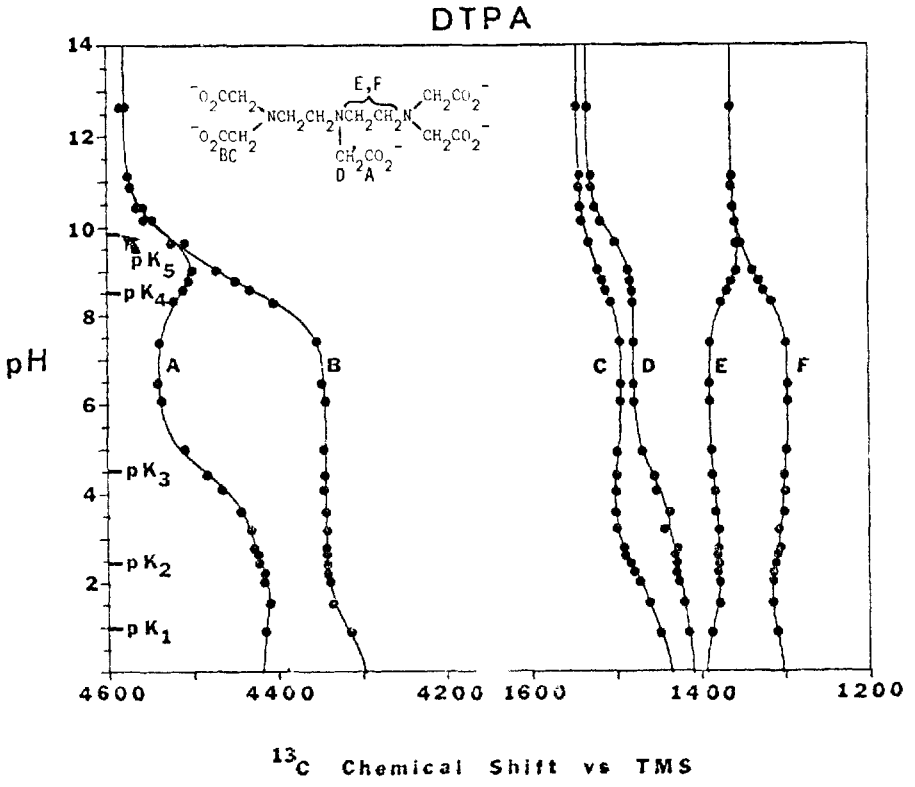

FIG. 2 Effect of pH on the Carbon-13 Resonances of DTPA

In the region of pK_5 the two kinds of acetate methylene carbons also exhibit smaller upfield shifts. These data shown in Figure 2 indicate protonation of both the central and terminal amino groups (II and III) at pK_5. The similar microscopic basicity of these two types of tertiary amines is not unexpected.

Addition of a second equivalent of acid causes a reversal in the protonation shift of the central carboxylate carbon resonance which the first equivalent of acid had produced. Simultaneously the terminal C=O resonance continues its upfield shift indicative of protonation of the terminal amine nitrogens. These observations

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suggest that diprotonation of $DTPA^{5-}$ occurs preferentially on the terminal nitrogens (IV). Thus some of the $HDTPA^{4-}$ originally protonated at the central amino group (III) at pK_5 rearranges upon further addition of acid to give the di-terminally protonated ion (IV); however, it appears that some vicinally diprotonated ion (V) might also be present because the shift of the central C=O at pH = 6.5 (2 equivalents acid added) is still 40 Hz upfield from the chemical shift of the $DTPA^{5-}$ species (pH = 12.7) where the central amino group is completely unprotonated. One must be mindful that conformational differences between $DTPA^{5-}$ and H_2DTPA^{3-} (even if totally in form IV) could also account in part for this 40 Hz difference in shifts observed for the central C=O resonance. Nevertheless the data indicate that the terminal amino nitrogens are significantly more basic than the central amine at pK_4. This finding is in agreement with earlier postulates based on proton NMR data.[3] This behavior may arise as a means of gaining maximum separation of the positively charged ammonium centers in H_2DTPA^{3-} or, perhaps, to allow the two protons to be stabilized as chelated protons by the iminodiacetate-type terminal groupings.

With the addition of a third equivalent of acid the central C=O resonance once again experiences a large upfield protonation shift; its acetate methylene carbon also shows an upfield movement. Significantly the chemical shifts of terminal acetates' carbon-13 resonances show little influence of the added acid in the region of pK_3. These findings are compatible with protonation at the central amine nitrogen to give species (VI). However, some protonation of the central carboxylate group (VII) would also be expected to produce similar results (see data for N,N-Me_2gly). The two microscopic forms cannot be quantitated from the present data but the magnitude of protonation shift for the C=O resonance with addition of the third equivalent of acid, ≈100 Hz, appears to favor amine protonation even though this species (VI) would justapose three positive charges on the alkyl chain.

Further equivalents of acid produce much smaller protonation shifts in the C=O region and involve predominantly protonation of the carboxylate groups. Interpretation of protonation shifts in

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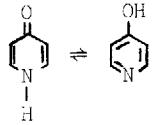
this region appear to be complicated by conformational influences and will not be discussed further here.

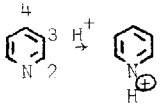
Another situation where ^{13}C protonation shifts could contribute to defining microscopic molecular properties in solution is in the tautomeric equilibrium, 4-pyridone ⇌ 4-hydroxypyridine (VIII). A



(VIII)

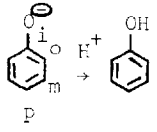
number of spectroscopic and theoretical studies have investigated the tautomeric equilibria in this compound (as reviewed in Ref. 9) and other similar aza-aromatic ring systems, e.g. the purine bases. More recently proton,[10,11] carbon-13[11,12] and nitrogen-14[13] NMR studies have focused on the pyridone ↔ hydroxypyridine equilibria. By comparing chemical shifts of various magnetic nuclei in the parent molecule and N- and O- methylated derivatives the 4-pyridone formulation seems microscopically appropriate in acetone-methanol[13] solutions and in dimethylsulphoxide.[11]

Some recent studies we have begun on the carbon-13 protonation shifts experienced by aromatic ring carbons in pyridine molecules and phenolic systems[14] suggest that protonation shifts may be a useful approach to examine the pyridone ↔ hydroxypyridine tautomeric equilibrium. Protonation of a pyridine type nitrogen in aqueous solution engenders characteristic shifts in the ring carbon shifts; the ^{13}C protonation shifts for pyridine serve as a representative example:



Protonation Shifts (+ = downfield)	
C-4	+262 Hz
C-3	+87 Hz
C-2	-185 Hz

Similarly phenolate ions when protonated produce characteristic ring carbon resonance shifts, as typified by the unsubstituted phenolate ion:



Protonation Shifts	
C_i	-277 Hz
C_o	-91 Hz
C_m	0 Hz
C_p	+155 Hz

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Although the absolute magnitude of the protonation shifts in phenols and pyridines vary somewhat with the introduction of ring substituents[14] the observed trends are generally followed.

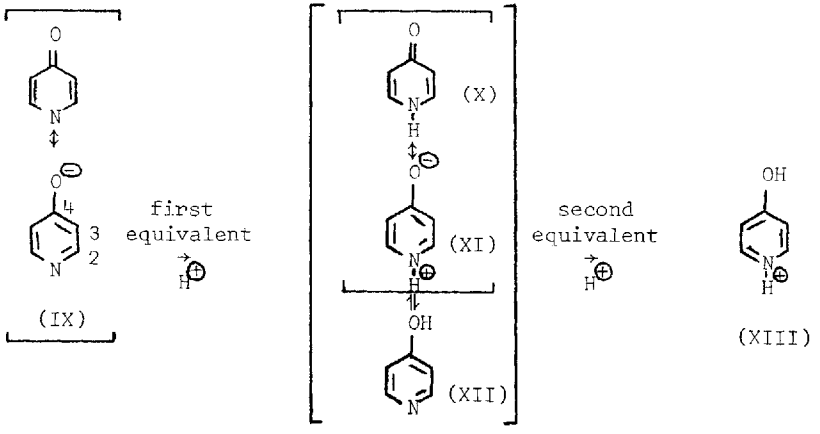
We have studied the 4-pyridone ↔ 4-hydroxypyridine question by taking the deprotonated anion of this compound in D_2O (pH 13.5) and have added successively one equivalent of acid, then a second, to this species. The carbon-13 protonation shifts associated with each protonation step were monitored. The experimental findings are summarized below:

TABLE 2

^{13}C Protonation Shifts (Hz) for  (positive = downfield)

	Protonation Shifts		
	C-2	C-3	C-4
1st equivalent of acid	-255	+14	+142
2nd equivalent of acid	-78	-66	-227

These data can be interpreted on the scheme



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The first equivalent of acid produces ^{13}C protonation shifts indicative of predominant pyridine nitrogen protonation. The C-2 and C-4 protonation shifts resemble quite closely the data for the model system, pyridine; the C-4 resonance should be most sensitive to oxygen protonation shows the opposite direction protonation shift vs phenol C_i. Thus the protonation shifts favor a nitrogen protonated species, the 4-pyridone (X) or its resonance hybrid (XI), in aqueous solution. The 4-pyridone formulation has been reported to predominate in other non-aqueous systems.[11,13]

Addition of a second equivalent of acid gives protonation shifts indicative of oxygen protonation, quite like phenolate ion itself. It is not possible from this data to conclude which microscopic protonation step best describes our findings, (XI) → (XIII) or (X) → (XIII), although the protonation shifts of C-4 and C-3 resemble strongly those of C_i and C_o in the phenolate ion protonation.

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