Use of the ^3J(C, H) Coupling Constant for Quantitative Determination of Protonation Sites in Nitrogen Heterocycles

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It has been shown unambiguously that the quantitative evaluation of the population of protonated forms in pyrimidines by the use of the ^3J(C, H) coupling constant must take into account the difference between the methylated and protonated systems.

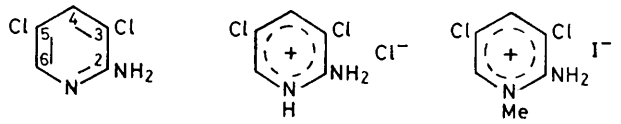
In the biochemistry of nitrogen heterocycles, the basic character of the heteroatom plays a predominant role. Considerable attention has been devoted to the determination of the protonation site in these systems by various techniques.[1]

The usefulness of ^{13}C n.m.r. parameters such as chemical shifts or (^{13}C, ^1H) coupling constants for the detection of protonation sites has been recognized.[2–4] Carbon-13 chemical shifts, which are very sensitive to the effects of protonation, can be used to evaluate the relative percentage of monoprotonated forms, *e.g.*, in pyrimidines, the (N-1–H) and (N-3–H) species.[1] Another method, based on the decrease of the vicinal (^{13}C, ^1H) coupling-constant values across the proton-

Table 1. J(C, H) Coupling constants[a] for neutral, protonated, and N-methylated 2-amino-3,5-dichloropyridine (Hz).

Compound	^3J(C-2, H-4)	^3J(C-2, H-6)	^3J(C-3, H-4)	^4J(C-3, H-6)	^1J(C-4, H-4)	^3J(C-4, H-6)	^3J(C-5, H-4)	^2J(C-5, H-6)	^1J(C-6, H-6)	^2J(C-6, H-4)
(1)	5.9	12.3	4.5	2.0	170.1	5.2	4.0	4.0	184.2	5.2
(2)	6.6	8.0	5.1	1.7	175.8	5.4	4.5	≤0.5	194.1	4.6
(3)	6.6	6.6	4.7	1.2	176.8	5.4	5.0	1.4	194.9	4.2

[a] Coupling constants are accurate to ±0.2 Hz. The long-range coupling constants with the NMe group in (3) are: ^3J(C-2, NMe) 3.3 and ^3J(C-6, NMe) 4.2 Hz.



(1) (2) (3)

ated nitrogen,[2–7] has recently been investigated for 2,4-diaminopyrimidines.[8] Like the chemical shift approach,[1] this method makes use of model compounds for the determination of protonation effects on ^3J(C, H) since the (N-1–H) and (N-3–H) species undergo rapid exchange in the n.m.r. time scale. The models investigated were the N-1- and N-3-methylpyrimidinium iodides, and it was assumed the effects of protonation and methylation were similar. However, the fact that methylation and protonation effects on ^3J(C, H) were found to be identical for a doubly charged species does not prove that they are similar for a singly charged species.[9] Moreover, for neutral heterocycles, significant differences between the NH and NMe effects are observed for some proton and carbon chemical shifts and also for some J(H, H) and J(C, H) coupling constants.[10] Furthermore a comparison of the methylation and protonation effects on the chemical shift of the α-carbon shows a difference that can be as much as 3.95 p.p.m. for pyridine and 2.47 p.p.m. for pyrimidine.[11] Finally the coupling constant ^2J(N, H-2) in N-methylpyridinium iodide is 4 Hz larger than the value in protonated pyridine.[12]

We therefore decided to determine the protonation and methylation effects on a singly charged species. We chose as the parent compound 2-amino-3,5-dichloropyridine for two reasons: (i) only one nitrogen atom is involved in the processes under examination; (ii) the spectra for compound (1), its hydrochloride (2), and its N-methyl iodide (3), obtained at 20 MHz (spectrometer Varian CFT 20), can be analysed on a first-order basis (this was carefully checked by simulation of spectra).

Compound (1) was commercially available and derivatives (2) and (3) were obtained by passing a current of dry hydrogen chloride gas [for (2)] or by adding methyl iodide [for (3)] to a stirred diethyl ether solution of (1).

In order to avoid solvent effects, compounds (1), (2), and (3) were studied in the same solvent, $(CD_3)_2SO$. As deprotonation of the hydrochloride (2) occurs in this solvent, complete protonation was achieved by an addition of hydrochloric acid to the solution, monitored by changes in the carbon chemical shifts. These changes indicate a partial deprotonation of the hydrochloride in $(CD_3)_2SO$ that amounts to *ca.* 25%. Therefore the pK_a of the pyridine (1) in $(CD_3)_2SO$ should be less than 1.

The assignment of chemical shifts and coupling constants was made by analogy with data for neutral and protonated pyridine.[2,6,13] The coupling constant values for compounds (1), (2), and (3) are summarized in Table 1. Analysis of these values shows that ^3J(C-2, H-6) is greater in protonated pyridine (8.0 Hz) than in N-methylated pyridine (6.6 Hz) whereas ^3J(C-2, H-4) is 6.6 Hz in both cases. Therefore, the NMe group causes a decrease of *ca.* 1.4 Hz for ^3J(C-2, H-6) with respect to the NH group. A similar decrease is also observed for the analogous ^3J(C, H) of N-methyl derivatives of 2-hydroxypyridine, 2-mercaptopyridine, and 4-oxopyrimidine (−1.8, −2.3, and −1.3 Hz, respectively).[10] Moreover, for imidazole the N-methyl group reduces the ^3J(C, H) coupling through the nitrogen by 0.6 and 0.8 Hz compared to the NH group for the neutral and monoprotonated molecules, respectively.[7] These data show that the substitution effect on going from an NH to an NMe group is always negative (−0.6 to −2.3 Hz). Therefore it is necessary to take into account a correction from the model compound (N–Me) considered when ^3J(C, H) coupling constants are used for accurate quantitative evaluation of the population of the monoprotonated forms, as carried out recently.[7] Owing to the similarity of the results obtained for six-membered nitrogen heterocycles (pyridine and pyrimidine) the correction of −1.4 Hz found for compound (1) was used for 2-aminopyrimidines. The calculation of the populations in 2,4-diaminopyrimidine was made with a correction of −1.4 Hz applied to the ^3J(C-2, H-6) value of N-1-methyl-2,4-diaminopyrimidinium iodide, used as the model compound.[8] The result from this calculation was 100% of the (N-1–H) form. A similar calculation on 2-amino-4-methylpyrimidine gives 66% of the (N-1–H) form. Owing to the negative sign of the correction, these populations are greater than those reported previously[8] and are in better agreement with the values obtained from carbon chemical shifts.[1]

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