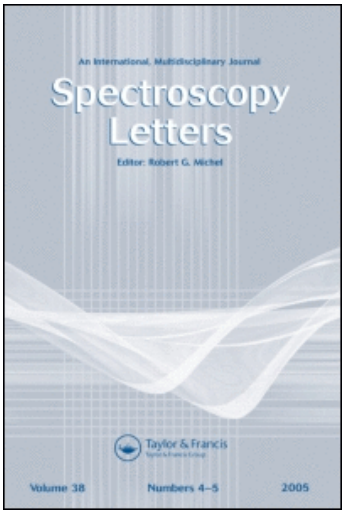

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An Investigation of Protonation in 2-Methoxy Pyrazine Using ^{13}C AND ^{15}N NMR Spectroscopy

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AN INVESTIGATION OF PROTONATION IN 2-METHOXY PYRAZINE USING ^{13}C AND ^{15}N NMR SPECTROSCOPY

Key Words: protonation, ^{15}N, NMR, pyrazines

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Abstract

Through the synthesis of a model compund and the use of a ^{15}N NMR spectroscopic titration together with ^{13}C and ^{1}H NMR the site of protonation of 2-methoxypyrazine has been determined to be N4.

INTRODUCTION

We have studied a wide range of cardiotonic polyaza heterocyclic bases in order to determine their preferred site of protonation in solution. The principal interest in these substances lies in their positive inotropic actions and the relationship between their physicochemical properties, especially their protonation behaviour, and their biological effects[1-5]. During a recent study[6] we noticed that methoxyl substitution ortho to a pyrazinyl nitrogen of an imidazo [1,2-*a*] pyrazine derivative caused the protonation to shift from that site to the imidazo nitrogen in the molecule such that the reduction in pK_a was not very marked. This finding contrasts with the situation in pyridine and 2-methoxypyridine[7] where the methoxy substituent causes a lowering of the pK_a by 1.95. Hence, it appears that, in the former case, such substitution would lower the pK_a of an adjacent nitrogen so much that it is possible for another nitrogen in the molecule to become the more basic. We have tested this

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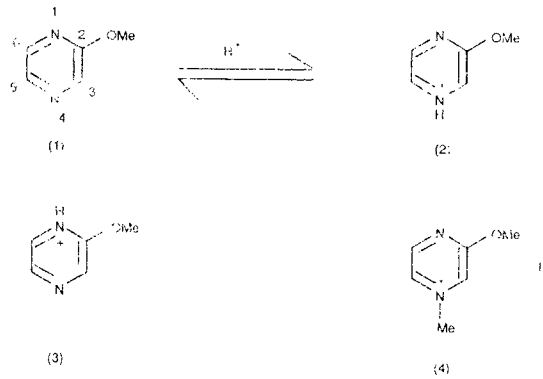
hypothesis by measuring the site of protonation of a suitable reference compound 2-methoxy pyrazine (1). Although the pK_a has been determined[7,8], the nature of the protonated species, either (2) or (3) was not known. We have elucidated the protonation site of (1) by measuring ^{15}N and ^{13}C spectra of (1) as a function of added acid and of 4-methyl-2-methoxy pyrazinium iodide (4).



(1) (2) (3) (4)

EXPERIMENTAL

2-methoxypyrazine (1) was obtained from Aldrich and used without further purification. 4-N-methyl-2-methoxypyrazinium iodide (4), m.p. 132-134°C was synthesised by the reaction of (1) with methyl iodide according to the procedure of Barlin and Benbow[9]. Its structure was determined unequivocally by ^1H NOE experiments since it was previously described only as 2-methoxypyrazine methiodide. Thus irradiation of the N-methyl signal in the ^1H NMR spectrum gave positive NOE enhancements at two of the ring proton signals, namely those assigned to H3 and H5 on the basis of coupling constant values. This structure was also confirmed by analysis of the long range ^1H-^{15}N couplings observed in the coupled ^{15}N NMR spectrum. The NMR spectra were measured on a Bruker AM-360 instrument at 298K. No ^1H decoupling was used for the ^{15}N NMR spectra in order to avoid signal suppression due to partial negative NOEs. The chemical shifts in the ^1H spectra were referenced to dmso-d_5 at δ2.50 from TMS, in the ^{13}C spectra to dmso-d_6 at 39.5ppm from TMS and in the ^{15}N spectra to external CH_3NO_2 at 0ppm. Positive shifts are to high frequency. The titration of (1) was achieved using 98% H_2SO_4 in H_2O and the protonation monitored using ^{15}N NMR on a 2M solution in dmso-d_6. "pH" values were obtained from an EIL7050 pH meter with a micro electrode inserted directly into the 10mm NMR tube. ^1H and ^{15}N spectra were measured after each addition of H_2SO_4 and ^{13}C spectra were measured only on the base and fully protonated forms.

RESULTS

The ^{15}N NMR spectrum of (1) consists of two peaks which are easily assigned to N1 (-100.7ppm) and N4 (-37.6ppm) on the basis of the well known shielding effect of ortho methoxyl substitution. This assignment has been confirmed by the analysis of long range ^{15}N-^1H coupling constants. On addition of aliquots of H_2SO_4 the N4 resonance becomes increasingly shielded, eventually moving to -128.7ppm a chemical shift change of 91.1ppm indicating

TABLE 1

^{15}N Chemical shifts of (1) as a function of added H_2SO_4

"pH"	N1	N4	No of $[H^+]$ Equivalents Added
10.8	-100.7	-37.6	
1.64	-100.5	-39.3	0.20
1.23	-99.9	-42.6	0.61
1.02	-99.4	-46.4	1.01
0.95	-98.6	-50.6	1.40
0.86	-97.7	-55.9	1.79
0.65	-95.0	-71.9	3.36
0.35	-91.9	-89.5	3.85
0.22	-89.3	-104.8	4.36
0.02	-86.9	-118.3	4.77
<0	-85.1	-128.5	5.08
<0	-81.1	-128.7	7.83

TABLE 2

^{13}C and ^{15}N NMR parameters for (1) and related compounds

	Pyrazine[10]	(1)*	(2)	(4)
N1	-46.9	-100.7	-85.1	-85.1
N4	-46.9	-37.6	-128.7	-128.4
C2	145.0	160.0	165.8	162.3
C3	145.0	135.2	130.6	130.6
C5	145.0	136.5	129.4	129.0
C6	145.0	140.6	150.6	146.5
OMe	-	53.1	59.1	56.0
Me	-	-	-	48.4

* Literature values are N1: -101.6, N4: -38.6ppm[11], C2: 160.7, C3: 136.1, C5: 136.5, C6: 140.6ppm[10]

unambiguously protonation at N4. The N1 signal also changes its chemical shift being progressively deshielded by 15.6ppm. The two resonances exhibit the same mid points showing that the N1 shift is monitoring the same protonation effect as N4 although indirectly. The titrated chemical shifts and the numbers of equivalents of added acid is shown in Table 1. Confirmation of the site of protonation is gained by a comparison of the ^{15}N chemical shifts of the salt of (1) and (4) which has unambiguously shown to be 4-methyl-2-methoxy pyrazinium iodide. We have already shown that N-protonation and N-methylation produce similar chemical shifts at the directly bonded nitrogen and at adjacent carbons in the same ring[1-5]. An examination of the ^{13}C chemical shifts in Table 2 therefore also confirms the site of protonation.

The pK_a of the protonation equilibrium can be estimated both from the mid-point of the chemical shift/pH titration curve which yields a value of 0.5 or by calculating the equilibrium constant of the protonation reaction knowing the

intial concentration of the base (2M) and calculating the number of equivalents of H^+ from the integral of the exchange proton peak in the ^1H NMR spectrum. This latter method yields a value of 0.9. This figure measured in dmso-d_6 is remarkably close to the true pK measured in water at low concentration (0.75)[8].

Methoxy substitution will alter the ring charges in two ways, firstly from a direct electromagnetic effect leading to δ^- at N1 and δ^+ at N4, and secondly from p–π conjugation giving δ^+ at N1 and δ^- at N4. Hence it appears to be this latter electronic effect which is dominant in determining the protonation site as N4, ignoring any steric contribution.

Comparing the ^{13}C chemical shifts in the salt and base, it can be seen that the effect of protonation on C3/C5 and C2/C6 is not symmetrical in that C5 is shielded by 7.1ppm but C3 is only shielded by 4.6ppm. Similarly C6 is deshielded by 10.0ppm but C2 is only deshielded by 5.9ppm. This implies a different substituent effect of OMe in the salt and base forms such that the opposing effects of the electronegativity and p-π conjugation are altered differently at positions ortho and para to the methoxy group. The assymetric effect of the methyl substituent in (4) is seen in the unusual observation of spin-spin coupling to the ^{14}N nucleus of N4 which gives rise to 1:1:1 triplets in the broadband decoupled ^{13}C spectrum and in the proton spectrum. ^1J(C3-N4) = 11.1Hz and ^1J(C5-N4) = 8.3Hz with the coupling to the methyl resonance being about 4Hz. Also in the ^1H spectrum, resolution enhancement allowed the measurement of ^2J(H3-N4) = 0.9Hz, ^2J(H5-N4) ~0.8Hz with the ^{14}N coupling to the methyl protons being even smaller.

REFERENCES

1. Lindon J.C., Williams J.M. & Barraclough P. ^1H, ^{13}C and ^{15}N observations on the protonation of 1- and 3-deazapurine Magn. Reson.Chem., 1986; 24: 55-58.

2. Barraclough P., Firmin D., Iyer R., King W.R., Lindon J.C., Nobbs M.S., Smith S., Wharton C.J. & Williams J.M. Protonation equilibria of cardiotonic polyaza heterocycles. J.Chem.Soc. Perkin II, 1988; 1839-1846.

3. Barraclough P., Lindon J.C., Nobbs M.S. & Williams J.M. ^{13}C NMR studies of tautomerism in imidazo[4,5-*c*]pyridines. Magn.Reson.Chem., 1989; 27: 992-994.

4. Lindon J.C., Williams J.M., Barraclough P., King W.R. & Nobbs M.S. ^1H and ^{13}C NMR studies of the protonation of isomeric methoxysulmazole analogues. Magn.Reson.Chem., 1990; 28: 573-575.

5. Sanderson P.N., Farrant R.D., Lindon J.C. & Barraclough P. A multinuclear magnetic resonance study of protonation in imidazo [1,2-*a*]pyrazine. Magn.Reson.Chem., 1990; 28: 874-877.

6. Barraclough P., Firmin D., Lindon J.C., Nobbs M.S., Sanderson P.N., Smith S. & Gillam J.M. Sites of protonation in cardiotonic polyazaindolizines by NMR spectroscopy. Magn.Reson.Chem., in the press.

7. Albert A. & Phillips J.N. Ionization constants of heterocyclic substances II. Hydroxy-derivatives of nitrogenous six-membered ring compounds. J.Chem.Soc. 1956; 1294-1304.

8. Ul'yanova T.N., Dvoryantseva G.G., Sheinker Yu.N., Elina A.S. & Musatova I.S. Structure of cations of N-oxides of monosubstituted pyrazines and quinoxalines studied by PMR. Khim.Geterotsikl, Soedein. 1973; 8: 1115-1123.

9. Barlin G.B. & Benbow J.A. Kinetics of reactions in heterocycles. Part XIV. Reactions of 2- and 4-amino-, -methylamino-, and dimethylamino-pyridine methiodides and 2-methylthiopyrimidine methiodide with hydroxide ions in water. J.Chem.Soc. Perkin II. 1975; 1385-1388.

10. Tsujimoto T., Nomura T., Iifuru M. & Sasaki Y. Studies on ^{13}C magnetic resonance spectroscopy. XIII. ^{13}C and ^{1}H NMR of 4-substituted pyrazine and 2-substituted pyrazine derivatives. Chem.Pharm.Bull. 1979; 27: 1169-1175.

11. Tobins S., Schmitt P. & Günther H. Substituenteffekte in monosubstituierten pyrazinen. Chem.Ber. 1982; 115: 2015-2021.

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