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Sites of Protonation in Cardiotonic Polyazaindolizines by NMR Spectroscopy

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The pK_a values of six sulmazole analogues were measured spectrophotometrically. The major protonation sites for most of these bridgehead nitrogen heterocycles were determined by ^{1}H and ^{13}C NMR methods. The aryl-substituted imidazo[1,2-*a*]pyrimidine (4), 8-methoxyimidazo[1,2-*a*]pyrazine (6), imidazo[1,2-*b*]pyridazine (9) and imidazo[1,2-*b*][1,2,4]triazine (11) undergo protonation at the imidazo nitrogen. The imidazo[1,2-*a*]pyrazine (5) protonates mainly at N-7. In some cases differences in basicity properties between these aryl analogues and the bridgehead heterocycles have been observed.

KEY WORDS Polyazaindolizines Protonation ^{13}C NMR ^{1}H NMR pK values

INTRODUCTION

The cardiotonic drugs sulmazole **1** and isomazole **2** are of much current interest,[1] and their physicochemical[2] and pharmacological[3,4] properties have been studied in some detail. We have recently established[2] by ^{1}H and ^{13}C NMR spectroscopy that sulmazole and isomazole undergo protonation at the pyridyl nitrogen, and the relationship between the basicity properties and inotropic activity of sulmazole and several of its analogues has also been discussed.[5] As part of a comprehensive study on the physicochemical properties of cardiotonic sulmazole analogues, we wished to investigate aryl-substituted heterocycles containing a bridgehead nitrogen atom. In particular, we wanted to determine the pK_a values and protonation sites of the sulmazole analogues **3–11**. All of these analogues, except **7** and **9**, displayed potent cardiotonic activities *in vitro*.[6–8] We were interested, therefore, to know what effects replacement of an imidazopyridine by a polyazaindolizine ring would have on the basicity properties of the resulting compounds, and whether these effects would be related to their inotropic activities.

RESULTS

Syntheses

Condensation of amine **12** with bromide **13** afforded **15**. Catalytic hydrogenation of **15** yielded the imidazo[1,2-*b*]pyridazine **9**. The sulphoxide **11** was prepared by oxidation of the sulphide **10**. This intermediate was obtained from the condensation of amine **16** with bromide **14**.[6–8] The structure was assigned as **11** rather than the isomeric **17** based on ^{1}H NOE NMR experiments. Preliminary accounts of the preparation of heterocycles **3–8** have been published.[6,7]

pK_a values

The pK_a values were determined by the rapid spectrophotometric method described previously[2] and are given in Table 1. Where data were available for comparison, the pK_a values for the aryl-substituted heterocycles were found to be lower than those of the parent systems. Thus, 2,4-dimethoxyphenyl substitution of imidazo[1,2-*a*]pyrimidine and imidazo[1,2-*b*]pyridazine gave rise to weaker bases (i.e. decreased their pK_a values by 0.5 and 0.4, respectively). For imidazo[1,2-*a*]pyrazine the effect of 2-methoxy-4-methylsulphinylphenyl substitution was also to reduce the basicity, but in this case the decrease in pK_a was 0.7. These findings are in contrast with those for 1*H*-imidazo[4,5-*b*]pyridine and 1*H*-imidazo[4,5-*c*]pyridines,[2] where 2-methoxy-4-methylsulphinylphenyl substitution had little or no effect and 2,4-dimethoxyphenyl substitution increased the basicity. Interestingly, the imidazo[1,2-*a*]pyrazine **6** is a weaker base than heterocycle **5**. However, in this case methoxy substitution α- to the heterocyclic nitrogen leads to a smaller change in pK_a (-0.42) than for pyridine ($\Delta pK_a = -1.95$) or pyrazine ($\Delta pK_a = +0.15$). These differing effects of methoxy substitution may reflect a change of protonation site and/or more complex electronic interactions occurring between the substituent and the heterocyclic ring for pyrazine and analogue **6**.

All the heterocycles examined, with a single exception, underwent monoprotonation in aqueous solution, as far as could be determined by the UV or NMR methods employed. Neither the pK_a value nor the protonation site of the imidazo[1,2-*c*]pyrimidine **8** could be determined. In acidic aqueous solution, and

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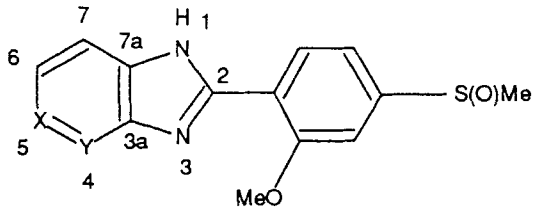
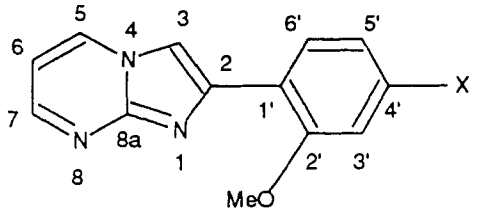
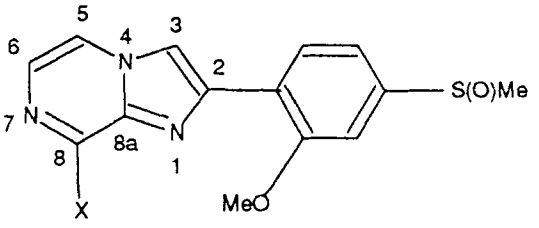


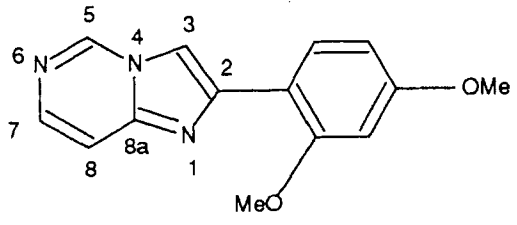
(1) X = CH, Y = N
(2) X = N, Y = CH



(3) X = O Me
(4) X = S(O)Me



(5) X = H
(6) X = O Me
(7) X = NH.Me



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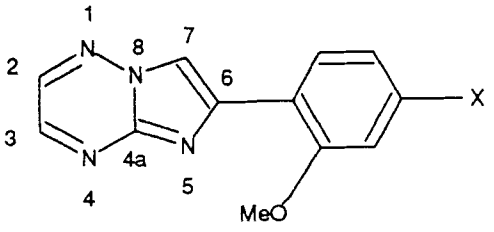
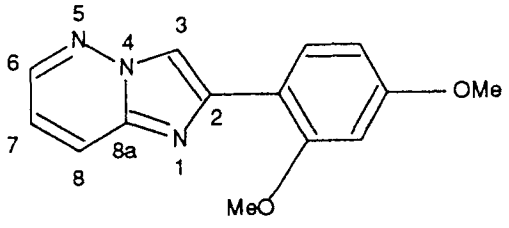


(10) X = SMe
(11) X = S(O)Me



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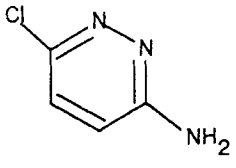
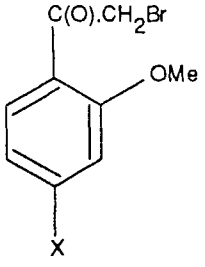
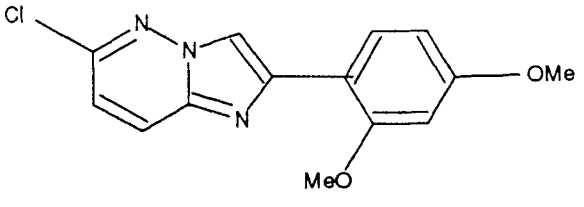


(12)



(13) X = OMe
(14) X = SMe



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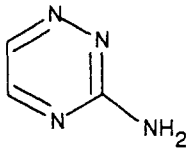

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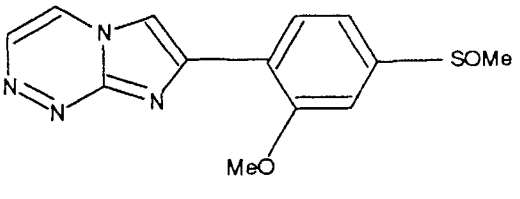

(17)

Table 1. pK_a values and protonation sites for aryl heterocycles and some of the parent systems

Compound	$pK_a(BH^+)$[a]	I[a]	Comment	Site of protonation
1	3.91 ± 0.03	0.004	—	N-4
2	6.17 ± 0.04	0.0005	—	N-5
3	4.34 ± 0.08	0.001	10% Me_2SO–H_2O	—
4	—	—	—	N-1
5	2.88 ± 0.02	0.002	—	N-7
6	2.46 ± 0.08	0.005	—	N-1
7	5.36 ● 0.03	0.006	—	—
8		—	Acid-catalysed decomposition occurs	—
9	4.17 ± 0.07	0.004	10% EtOH–H_2O	N-1
11	<2.5		10% EtOH–H_2O	N-5
Imidazo[1,2-*a*]pyrimidine	4.81 ± 0.02	0.01	[b]	N-1
Imadazo[1,2-*a*]pyrazine	3.59 ± 0.05	0.01	[b,c]	N-1
Imidazo[1,2-*b*]pyridazine	4.57 ± 0.03	0.01	[b]	N-1
2,3-Dimethyl-6-phenylimidazo[1,2-*b*][1,2,4]triazine	0.35	—	[d]	—
2-Methoxypyrazine	0.75		[e,f]	N-4
Pyrazine	0.60		[e]	N-1
2-Methoxypyridine	3.28		[e]	N-1
Pyridine	5.23		[e]	N-1

[a] Determined in aqueous solution at 25 °C unless stated otherwise. I = ionic strength.
[b] Ref. 9.
[c] Ref. 17.
[d] Ref. 18.
[e] Ref. 21.
[f] Ref. 22, 23.

more rapidly in acidic dimethyl sulphoxide, **8** underwent conversion to a mixture of two new products. The details of this chemical transformation will be reported elsewhere. This protonation behaviour is different from that of the parent heterocycle[9] ($pK_a = 4.41$, protonation at N-1) and may reflect increased ease of covalent hydration for **8** with subsequent hydrolysis and Dimroth rearrangement.

^{1}H NMR chemical shift assignments

These are given in Table 2. Generally assignments were made from the magnitude of proton–proton coupling constants and also from relative chemical shifts. For **4** and **5** assignments were in agreement with previous studies[10,11] on simpler systems. H-3 of **4** was found to exchange over several hours in the presence of DCl such that no proton resonance was observed and the C-3 resonance was greatly reduced in intensity. This observation is consistent with the acidic nature of protons α- to the bridgehead nitrogen atom in indolizine[9] and its aza derivatives.[12] Ambiguity initially remained over the assignments of H-5 and H-6 for analogues **5** and **6**. However, for **5** the signal at δ7.54 (base) was assigned to H-5 on the basis of its long-range coupling constant (1.4 Hz) to H-8 (observed by resolution enhancement). H-3 was also coupled to H-8 [$J(3,8) = 0.7$ Hz] and to H-5, but the latter coupling could not be accurately resolved. NOE experiments confirmed these assignments. Thus in acidified DMSO-d_6 (DCl was added to shift the H-3 signal away from that of H-5), low-power irradiation of the signal assigned to H-5 gave similar positive enhancements of the H-6 and H-3 signals. Irradiation at the H-6 signal gave a large positive emhancement at H-5 but a small diminution at H-3, the latter probably being due to a three-spin effect.

Similarly, when analogue **6** was dissolved in DMSO-d_6, low-power irradiation of the H-3 signal (δ8.50) gave a large positive NOE at the doublet resonance (δ8.21) which was therefore assigned to H-5. In D_2O, the chemical shift difference (0.02 ppm) between the H-3 and H-5 signals was too small for NOE experiments to be performed. It was therefore assumed that the relative assignments of H-5 and H-6 were as in DMSO-d_6.

In the spectrum of the imidazo[1,2-*b*]pyridazine **9** at 30 °C there was considerable overlap of the signals arising from the three pairs of protons, H-3/H-6, H-6′/H-8, and H-3′/H-5′. These resonances were distinguished, however, with the aid of decoupling and COSY experiments. At 70 °C the ^{1}H resonances were well resolved. For **11** the assignments proved straightforward with the exception of H-2 and H-3; the signals (δ8.60, 8.67) arising from these protons were not assigned unambiguously.

^{13}C NMR chemical shift assignments

The ^{13}C chemical shift values are given in Table 3. Assignment techniques were similar to those used previously.[2] For example, comparisons could be made with the established assignments for **1** and **2**. In addition, the ^{13}C shifts reported for imidazo[1,2-*a*]pyrimidine,[13] imidazo[1,2-*a*]pyrazine[14] and imidazo[1,2-*b*]pyridazine[15] provided useful reference data.

The ^{13}C signals in the spectrum of the imidazo[1,2-*a*]pyrimidine **4** were assigned by inspection and these assignments were confirmed by selective proton decoupling experiments. ^{13}C assignments for the imidazo[1,2-*a*]pyrazine **5** proved more difficult, however. Ternary carbon resonances were assigned from the corresponding proton resonances using a 2D ^{1}H–^{13}C correlation

Table 2. ^{1}H parameters for aryl heterocycles

Compound	Form	Chemical shifts (ppm)[a] H-3	H-4	H-5	H-6	H-7	H-8	H-3′	H-5′	H-6′	OMe	S(O)Me	Coupling constant (Hz) ^3J(HH)[b]
1, Sulmazole	base	—	—	7.67	6.59	7.41	—	6.39	6.59	7.12	3.42	2.53	$J(67) = 8.2$
	salt	—	—	8.13	7.33	8.11	6.83	—	6.83	7.58	3.68	2.68	$J(67) = 8.0$; $J(56) = 5.8$
2, Isomazole	base	—	7.72	—	7.35	6.56	—	6.32	6.49	7.10	3.32	2.53	$J(67) = 5.5$
	salt	—	8.75	—	8.19	7.70	—	6.92	6.90	7.62	3.73	2.68	$J(67) = 6.6$
4	base[c]	7.60	—	8.17	6.58	8.08	—	6.85	6.97	7.71	3.62	2.65	$J(56) = 6.7$; $J(67) = 4.3$
	salt[d]	8.35	—	8.91	7.43	8.79	—	7.25	7.20	7.74	3.87	2.71	$J(56) = 6.8$; $J(67) = 4.5$
5	base[e]	7.41	—	7.54	7.08	—	8.05	6.64	6.76	7.34	3.49	2.61	$J(56) = 4.6$
	salt	8.72	—	8.60[l]	8.00	—	9.18	7.26	7.20	7.96	3.87	2.71	$J(56) = 5.0$
6	base	6.89	—	6.87	6.36	—	—	6.42	6.61	7.16	3.44, 3.29	2.53	$J(56) = 4.7$
	salt	8.18	—	7.89	7.48	—	—	7.03	7.01	7.48	3.95, 3.76	2.67	$J(56) = 4.7$
6[f]	base	8.50	—	8.21	7.41[l]	—	—	7.38	7.35	8.42	4.04	2.81	$J(56) = 4.7$
	salt	8.70	—	8.36	7.64	—	—	7.39	7.34	8.22	4.07, 4.00	2.80	$J(56) = 4.6$
9[f,g]	base[h]	8.44	—	—	8.41	7.17	8.00	6.66[m]	6.66[m]	8.18	3.82[n], 3.97[o]	—	$J(78) = 9.2$
	salt[i]	8.66	—	—	8.85	7.74	8.44	6.66[m]	6.66[m]	8.40	3.76[n], 3.91[o]	—	$J(78) = 9.3$
11[f]	base[j]	8.60*	—	—	—	8.74	—	7.47	7.42	8.49	4.09	2.82	$J(23) = 2.1$
	salt[k]	8.92**	—	—	—	8.91	—	7.40	7.35	8.09	3.94	2.75	$J(23) = 2.0$[l]

[a] D_2O as solvent unless stated otherwise; compounds **1**, **2**, **4**, **5** at 360 MHz and **6**, **9** and **11** at 200 MHz. Asterisks indicate that assignments in a horizontal row may be interchanged.
[b] ^3J(HH) is the *ortho* coupling constant between the protons in the six-membered ring.
[c] $J(57)$ 2.0 Hz.
[d] $J(57)$ 1.7 Hz.
[e] $J(58)$ 1.4; $J(38)$ 0.7 Hz.
[f] DMSO-d_6.
[g] 343 *K*.
[h] $J(67)$ 4.5; $J(68)$ 1.5; $J(38)$ 0.7 Hz.
[i] $J(67)$ 4.5; $J(68)$ 1.5.
[j] H-2 8.67*.
[k] H-2 8.99**.
[l] Approximate value.
[m] Overlapped signals.
[n] OMe at C-2′.
[o] OMe at C-4′.

experiment. This technique permitted the assignment of C-5 (120.2 ppm) to low frequency of C-6 (128.4 ppm), consistent with the assignments of these carbons in the parent compound. Phenyl quaternary carbon resonances were assigned by comparison with other analogues.[2] The signal at 144.2 ppm was assigned to C-4′, this being close to the chemical shift observed for this nucleus in **4** (144.5 ppm) and **6** (143.1 ppm). The broadness of the 144.2 ppm resonance was a further diagnostic feature, this being expected for C-4′ because of long-range coupling to the methyl protons. The two remaining carbon resonances (at 140.5 and 138.5 ppm) were therefore assigned to C-2 and C-8a. In the spectrum of the parent compound the signal at 140.4 ppm has been assigned[14] to C-8a, i.e. to high frequency of the signal at 135.2 ppm, which is assigned to C-2. Since in the spectrum of **5** the chemical shift difference between these two resonances is much smaller than for the parent compound, the assignments could not be made unequivocally for **5**. Both the carbon atoms giving rise to the above resonances show long-range coupling to several protons. Perhaps not surprisingly, selective proton decoupling experiments failed to provide an unambiguous assignment.

Similar arguments to the above were applied to aid assignments for the 8-methoxy derivative **6** in DMSO-d_6. Thus, assignment of the phenyl carbons was again made by comparison with other analogues and one-bond C–H coupling constants were also used to confirm the assignments of ternary carbon atoms. The greater magnitude of J(CH) for the ternary carbon atoms adjacent to a nitrogen atom [e.g. J(C-3, H-3) = 200.7 Hz] readily allowed these carbons to be distinguished from those of the phenyl ring [e.g. J(C-3′, H-3′) = 162.0 Hz]. The signals due to C-5 and C-6 were distinguished by selective irradiation of the proton doublet H-5 (δ8.2), which gave a decoupled resonance at 115.7 ppm but not at 125.5 ppm. The former was therefore assigned to C-5 and the latter to C-6. The signal arising from C-3 was assigned by comparison with the corresponding shifts in the spectrum of the parent compound[14] and also from its one-bond coupling constant, this being larger than at C-5 (190.2 Hz) or C-6 (186.0 or 192.2 Hz), as in related compounds.[14]

Of the six quaternary carbon atoms, C-8 and C-2′ were distinguished by irradiation of the methoxy protons, which led to decoupling of the two highest frequency quaternary resonances at 153.8 and 156.8 ppm. C-2′ was expected at higher frequency than C-8, by analogy with **5**, and was therefore assigned to the resonance at 156.8 ppm. Selective irradiation of the overlapping resonances arising from H-6′ and H-3 led to decoupling of the resonances at 139.3 and 131.9 ppm, which were then assigned to C-2 and C-8a because of

Table 3. ^{13}C NMR data for aryl heterocycles

		^{13}C chemical shifts (ppm)[a]														
Compound	Form	C-2	C-3	C-5	C-6	C-7	C-8	C-8a	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	OMe	S(O)Me
1, Sulmazole	base[b]	149.4	—	144.3	119.1	122.8			117.8	157.3	106.9	147.1	116.0	130.5	56.4	42.4
	salt[c]	155.1	—	137.5	120.2	130.2			117.0	159.5	108.7	151.5	117.2	132.6	57.9	42.6
2, Isomazole	base[d]	150.7	—	—	140.5	110.1			117.7	157.5	107.2	147.9	116.2	130.8	56.4	42.4
	salt[e]	156.6	—	—	135.3	113.0			116.6	159.7	108.9	151.9	117.4	132.7	57.9	42.8
4	base	140.2	113.6	136.3	110.2	152.7		147.8	124.8	158.1	107.6	144.5	117.0	129.9	56.6	42.5
	salt	132.6	113.2	138.4	115.3	159.0		144.1	118.1	158.6	108.8	148.7	117.6	130.6	57.5	42.6
5	base[f]	138.5*	115.3	120.2	128.4		141.2	140.5*	122.6	157.1	106.5	144.2	116.0	128.6	55.9	42.3
	salt[g]	142.8*	118.0	122.5	126.5		138.3	137.2*	120.9	158.0	107.5	146.8	116.7	129.8	56.7	42.5
6[h]	base[i]	139.3	116.0	115.7*	125.5		153.8	131.9	123.7	156.8	106.5	147.0	115.6*	128.6	56.0, 53.6	43.3
	salt	135.3	116.8*	116.0*	128.7**		152.2	129.6	119.7	157.3	107.1	148.9	116.8*	129.2**	56.6, 54.8	43.4
6	base[j]	138.6	116.4	115.8*	125.1		153.4	131.2	123.1	156.9	106.1	143.1	115.4*	128.4	55.7, 54.7	42.3
	salt[k]	133.3[q]	117.2	116.2	131.4[q]		151.8	128.1[q]	117.6	158.0	107.8	147.6[q]	116.8	129.7[m]	57.1, 56.4	42.6
9[h,l]	base[m]	140.7	114.6		143.0	117.6	124.5	137.5	114.5	160.8	98.6	157.9	105.8	128.8	55.5[r]/55.7*	
	salt[n]	135.8	115.6		148.6	121.5	125.4	133.3	111.4	163.7	99.7	159.1	107.6	130.3	56.6[r]/57.0*	
11	base[o]	143.3*	139.2*		140.8**	115.4			123.1	157.3	106.6	148.1	115.8	128.8	56.1	43.2
	salt[p]	144.8*	151.1*		133.6	117.3			118.0	158.8	108.8	150.4	117.6	131.1	58.1	44.0

[a] D_2O as solvent unless stated otherwise; compounds **1**, **2**, **4**, **5** at 90 MHz and **6**, **9**, **11** at 50 MHz. Asterisks indicate that assignments in a horizontal row may be interchanged.
[b] C-3a 151.2, C-7a 128.1.
[c] C-3a 146.7, C-7a 131.1.
[d] C-4 137.5, C-3a 136.2, C-7a 142.0.
[e] C-4 132.4, C-3a 135.6, C-7a 146.3.
[f] J(C-3, H-3) 202.6, J(C-5, H-5) 189.8, J(C-6, H-6) 188.0, J(C-6, H-5) 4.9, J(C-6, H-8) 12.2, J(C-8, H-8) 188.0, J(C-3′, H-3′) 162.4, J(C-3′, H-5′) 6.1, J(C-5′, H-5′) 166.0, J(C-5′, H-3′) 6.1, J(C-6′, H-6′) 163.6 Hz.
[g] J(C-3, H-3) 206.3, J(C-5, H-5) 195.3, J(C-6, H-6) 194.1, J(C-6, H-5) 6.1, J(C-6, H-8) 9.8, J(C-8, H-8) 195.0, J(C-3′, H-3′) 163.6, J(C-3′, H-5′) 6.1, J(C-5′, H-5′) 168.5, J(C-5′, H-3′) 6.1, J(C-6′, H-6′) 164.8 Hz.
[h] DMSO-d_6.
[i] J(C-3, H-3) 200.7, J(C-5, H-5) 190.2, J(C-5, H-6) 12.7, J(C-6, H-6), 186.0 or 192.2, J(C-6, H-5) 6.2, J(C-8, H-6) 10.7, J(C-2′, H-6′) 7.6, J(C-3′, H-3′) 162.0, J(C-3′, H-5′) 5.8, J(C-5′, H-5′) 161.9 or 169.3, J(C-5′, H-3′) 7, J(C-6′, H-6′) 163.6 Hz.
[j] J(C-3, H-3) 202.6–203.3, J(C-5, H-5) 190.7–191.2, J(C-6, H-6) 186 Hz.
[k] J(C-3, H-3) 211, J(C-5, H-5) 198.9, J(C-6, H-6) > 190 Hz.
[l] 70 °C.
[m] J(C-3, H-3) 200, J(C-6, H-6) 183, J(C-7, H-7) 171, J(C-8, H-8) 171, J(C-3′, H-3′) 159, J(C-5′, H-5′) 163, J(C-6′, H-6′) 161, J(C-$2'_{OMe}$, H-$2'_{OMe}$) 144, J(C-$4'_{OMe}$, H-$4'_{OMe}$) 145 Hz.
[n] J(C-3, H-3) 208, J(C-6, H-6) 191, J(C-7, H-7) 180, J(C-8, H-8) 177, J(C-3′, H-3′) 161, J(C-5′, H-5′) 164, J(C-6′, H-6′) 160, J(C-$2'_{OMe}$, H-$2'_{OMe}$) 146, J(C-$4'_{OMe}$, H-$4'_{OMe}$) 146 Hz.
[o] δ(C-4a) 141.2**, J(C-7, H-7) 203.7, J(C-2, H-2) 200, J(C-3, H-3) 200, J(C-3′, H-3′) 162.5, J(C-5′, H-5′) 166.8, J(C-6′, H-6′) 163.9 Hz.
[p] δ(C-4a) 140.6, J(C-7, H-7) 212, J(C-2, H-2) 198.1***, J(C-3, H-3) 196.7***, J(C-3′, H-3′) 164.8, J(C-5′, H-5′) 166.6, J(C-6′, H-6′) 163.9 Hz.
[q] Approximate values.
[r] OMe on C-2′.
[s] OMe on C-4′.

their long-range couplings over three bonds to H-6′ and H-3, respectively. (Long-range couplings between ^{13}C and ^{1}H are usually greater over three bonds than over two or four bonds). C-8a was expected to absorb to low frequency of C-2 because of the large shift effect to lower frequency of the OMe substituent. C-4′ was excluded from these two assignments since it would remain broadened from coupling to methyl protons; it was therefore assigned to the signal at 147.0 ppm. Similarly, the signal due to C-1′ is characterized by long-range coupling to H-3′ and H-5′ and was assigned to the resonance at lower frequency (123.7 ppm).

Correlation experiments aided assignments for the imidazo[1,2-*b*]pyridazine **9**. Thus the signal at 133.3 ppm (salt) was assigned to C-8a because of the three-bond coupling of this carbon to H-7 (δ7.74). Similarly, C-2 (135.8 ppm, salt) was found to be coupled to H-6′ (δ8.40). It was noted that the difference (7.2 ppm, base) in chemical shift between C-2 of **9** and the parent heterocycle was larger than that for the other carbons. The two methoxy groups were also distinguished by correlation experiments involving both one- and three-bond couplings of the C′–O–C–H system. C-2′ (160.8 ppm, base), for example, was found to couple to the methyl protons at δ3.82. These protons were also coupled [^{1}J(CH) = 144 Hz] to the adjacent methyl carbon (55.5 ppm).

For the imidazo[1,2-*b*][1,2,4]triazine **11**, similar reasoning to that used above permitted unambiguous assignment of the signals due to the phenyl ring carbon atoms. The signal arising from C-5′ (115.8 ppm) was distinguished from that due to C-7 (115.4 ppm) by the magnitude of ^{1}J(CH), that for C-7 being 37 Hz larger. The remaining resonances arising from non-quaternary carbon atoms (139.2 and 143.3 ppm) are then due to C-2 and C-3. These two signals were not assigned unambiguously, although C-2, which is closer to N-1 and N-8, might be expected to correspond to the higher frequency resonance. The signals due to the quaternary carbon atoms C-6 and C-4a were not conclusively dis-

tinguished in the spectrum of the base because of the closeness of their chemical shifts (140.8 and 141.2 ppm). The lower intensity of the signal at 141.2 ppm in the proton-decoupled spectrum did suggest that it was due to C-4a, since this carbon would not be expected to gain in intensity on decoupling as much as C-6. In the spectrum of the salt form, however, a distinction was possible by means of a selective decoupling experiment, irradiating at the H-6′ signal. This showed the C-6 resonance to be that at 133.6 ppm (a singlet) and the resonance now assigned to C-4a at 140.6 ppm was a doublet of low intensity.

Determination of the sites of protonation

The sites of protonation were determined from the degree of shielding of the carbon atoms adjacent to the protonated nitrogen atom. Increases in the *ortho* proton–proton spin-coupling constant for the α- and β-protons and for the one-bond ^{13}C–^{1}H coupling at the α-carbon were also characteristic in some cases.

Using these criteria, protonation was found to occur at the imidazo nitrogen, N-1 for the imidazo[1,2-*a*]pyrimidine **4**. The most diagnostic changes on protonation were the low-frequency shifts at C-2, C-1′ and C-8a (−7.6, −6.7 and −3.7 ppm, respectively). No marked increase in ^{3}J(H-6, H-7) was observed for **4**, ruling out substantial protonation at N-8. Studies[16] of the parent compound indicated that protonation occurred at N-1, or at N-1 and N-8 with rapid tautomerism.

Addition of DCl to the D_2O solution of the imidazo[1,2-*a*]pyrazine **5** caused low-frequency shifts of −1.9 and −2.9 ppm at C-6 and C-8, respectively, and an increase of 0.4 Hz in ^{3}J(H-5, H-6). These changes are indicative of protonation at the pyrazinyl nitrogen, N-7, and further support for this conclusion is provided by the observation that the largest increase in ^{1}J(CH) (+7.0 Hz) was observed at C-8. The expected absence of protonation at N-4 was inferred from the high-frequency shifts (+2.7 and +2.3 ppm) observed at C-3 and C-5, respectively. Protonation at the imidazo nitrogen N-1 would be expected to give large low-frequency shifts at C-2, C-1′ and C-8a (as for **6**). Since these are not observed, it is deduced that protonation at N-1 does not occur to any appreciable extent. Studies of imidazo[1,2-*a*]pyrazine by ^{13}C and ^{15}N NMR methods[17] indicate that protonation occurs predominantly at N-1, with the level of protonation at N-7 being less than 10% of that at N-1. 2-Aryl substitution thus appears to favour protonation at N-7 rather than at N-1.

The imidazo[1,2-*a*]pyrazine **6**, in contrast to **5**, displayed low-frequency shifts for C-2, C-1′ and C-8a (−5.3, −5.5 and −2.9 ppm, respectively) and the fact that ^{3}J(H-5,H-6) did not change indicated that no substantial protonation had occurred at N-7. The 8-methoxy substituent thus has the effect of changing the protonation site in these imidazo[1,2-*a*]pyrazines from N-7 to N-1.

For the imidazo[1,2-*b*]pyridazine **9**, protonation chiefly at the imidazo nitrogen N-1 was inferred from the low-frequency shifts at C-2, C-8a and C-1′ (−4.9, −4.2 and −3.1 ppm, respectively). The high-frequency shift (+5.6 and +1.0 ppm) observed at C-6 and C-3, together with the lack of significant change in ^{3}J(H-6, H-7), indicated the absence of substantial protonation at N-4 or N-5. Low-frequency shifts were observed at C-1′, C-6 and C-4a (−5.1, −7.2, −1.4 ppm) for the imidazo[1,2-*b*][1,2,4]triazine **11**, suggesting protonation at N-5. High-frequency shifts at C-2 and C-3 and no increase in ^{3}J(H-2, H-3) ruled out significant protonation at N-1 or N-4. The changes in ^{1}J(CH) for **11**, even when compared with those of **5** and **9**, are not particularly diagnostic. Thus an increase of 8 Hz in ^{1}J(C-7, H-7) and decreases of 2.3 Hz in ^{1}J(C-2, H-1) and ^{1}J(C-3, H-3) are observed for **11**. For **5**, however, ^{1}J(C-3, H-3) increases by 3.7 Hz, ^{1}J(C-8, H-8) by 7 Hz and ^{1}J(C-6,H-6) by 6.1 Hz. For **9**, ^{1}J(C-3, H-3) increases by 3 Hz, ^{1}J(C-7, H-7) increases by 1 Hz and ^{1}J(C-6, H-6) increases by 3 Hz.

CONCLUSIONS

The pK_a values and major protonation sites of a set of diazaindolizines and a triazaindolizine have been determined. Where comparative data were available the basicities of the aryl heterocycles were lower than those of the parent systems (pK_a values lower by $\leqslant 0.7$). In two cases the protonation characteristics of the aryl heterocycles were markedly different from those of the unsubstituted compounds. Thus, the imidazo[1,2-*a*]pyrazine **5** underwent protonation at N-7, in contrast to the parent compound, which protonated predominantly at the imidazo nitrogen (N-1). In addition, the imidazo[1,2-*c*]pyrimidine **8** underwent acid-catalysed decomposition, whereas the unsubstituted heterocycle protonated at N-1. All the other analogues investigated **4**, **6**, **9** and **11** were protonated at an imidazo nitrogen atom. The imidazo[1,2-*a*]pyrazine **6**, unlike the unsubstituted compound **5**, protonated at N-1. This may not be surprising when one considers that the effect of —OMe substitution *ortho* to a nitrogen in a pyridine ring is to lower substantially the basicity of that nitrogen ($\Delta pK_a = -1.95$). If a similar substituent effect occurs in going from analogue **5** to **6**, the pyrazinyl nitrogen may become less basic, with the result that the imidazo nitrogen becomes the more basic nitrogen in **6**. A similar reduction in basicity and change in protonation site from N-4 to N-1 ($\Delta pK_a = -0.3$)[18], was observed on 5-methoxy substitution of sulmazole.[19] The relationship between the protonation equilibria and the inotropic activities of these sulmazole analogues will be discussed elsewhere.

EXPERIMENTAL

^{1}H NMR spectra were obtained at 200 and 360 MHz using Bruker AM-200 and AM-360 spectrometers, respectively. ^{13}C NMR spectra were measured with and without gated broad-band ^{1}H decoupling using the same instruments at 50.4 and 90.6 MHz, respectively. Solutions were made up in D_2O or $(CD_3)_2SO$ and the

salt or free-base forms generated as appropriate by the addition of concentrated DCl or NaOD solution. In D_2O solutions, dioxane was used as the internal reference (δ3.53 ppm for ^1H and δ67.4 ppm from TMS for ^{13}C). In $(CD_3)_2SO$ solutions, CD_3SOCD_2H was used as an internal reference for ^1H (at δ2.50) and $(CD_3)_2SO$ as internal reference for ^{13}C (at 39.5 ppm from Me_4Si). Most ^1H NMR spectra were obtained at about 21 °C; spectra for **9** were obtained at 70 °C. Broad-band decoupled ^{13}C NMR spectra were obtained at 35 °C.

6-Chloro-2-(2-4-dimethoxyphenyl)imidazo[1,2-*b*] pyridazine 15

3-Amino-6-chloropyridazine **12** (0.75 g, 5.8 mmol), α-bromo-2',4'-dimethoxyacetophenone **13** (1.50 g, 5.8 mmol and dry ethanol (30 ml) were stirred and heated at reflux for 6 h. After cooling to 0 °C, the solid which deposited was collected by filtration and washed with cold ethanol to give 1.49 g (70%) of the hydrobromide of **15**, m.p. 238–240 °C. Found, C 45.7, H 3.68, N 11.2, Cl 10.2; $C_{14}H_{12}ClN_3O_2$·HBr requires C 45.4, H 3.54, N 11.3, Cl 9.57%. This salt underwent some decomposition on standing and was therefore converted to the stable free base by washing a chloroform solution of this material with saturated aqueous sodium hydrogencarbonate (2 × 40 ml) and then with water. Evaporation of the dried extract ($MgSO_4$) gave 0.92 g (55%) of **15**, m.p. 193–194 °C. Found, C 57.8, H 4.34, N 14.4, Cl 11.9, $C_{14}H_{12}ClN_3O_2$ requires C 58.0, H 4.17, N 14.5, Cl 12.2%.

2-(2,4-Dimethoxyphenyl)imidazo[1,2-*b*]pyridazine 9 hydrochloride

Chloroheterocycle **15** (0.91 g, 3.1 mmol), ethanol (40 ml) and 10% palladium–charcoal catalyst were stirred under hydrogen at 1 atm and room temperature until uptake of hydrogen had ceased (*ca.* 3 h). The catalyst was removed by filtration and the filtrate evaporated. The residue was dissolved in chloroform (100 ml) and the resulting solution washed with saturated aqueous sodium hydrogencarbonate (2 × 25 ml) and then with water (25 ml). After drying over $MgSO_4$, the organic layer was evaporated and the residue recrystallized from ethanol to give 0.46 g (57%) of **9**, m.p. 140–142 °C. Found, C 65.7, H 5.24, N 16.2; $C_{14}H_{13}N_3O_2$ requires C 65.9, H 5.13, N 16.5%. A solution of **9** in acetone was treated with ethereal hydrogen chloride to give **9** hydrochloride, m.p. 251–252 °C (decomp.). Found, C 57.4, H 4.67, N 14.1; $C_{14}H_{13}N_3O_2$·HCl requires C 57.6, H 4.84, N 14.4%.

6-(2-Methoxy-4-methylsulphinylphenyl)imidazo[1,2-*b*] [1,2,4]triazine 11

3-Amino-1,2,4-triazine **16** (1.54 g, 16 mmol), α-bromo-2'-methoxy-4'-methylthioacetophenone **14**[6–8] (4.06 g, 15 mmol) and dry ethanol (50 ml) were heated at reflux for 4 h. More **16** was then added (0.5 g, 5.2 mmol) to the reaction mixture and heating continued for a further 2 h. After cooling, the mixture was evaporated and the residue partitioned between chloroform and saturated aqueous sodium hydrogencarbonate. The chloroform layer was separated, the aqueous phase extracted twice with chloroform–ethanol and the organic extracts were combined. These extracts were then washed with brine, dried over Na_2SO_4 and evaporated. The dark residue was purified by chromatography on silica, eluting with chloroform (AnalaR grade) to give 0.66 g (16%) of 6-(2-methoxy-4-methylthiophenyl)imidazo[1,2-*b*]-[1,2,4]triazine **10**. A solution of *m*-chloroperbenzoic acid (0.44 g, 85%, 2.2 mmol) in chloroform (15 ml) was added dropwise over 30 min to a stirred solution of **10** (0.60 g, 2.2 mmol) in chloroform (20 ml) at 0 °C. The reaction mixture was stirred for a further 2 h at 0 °C and then poured into saturated aqueous sodium hydrogencarbonate. The organic layer was separated, dried over Na_2SO_4 and evaporated. The residual solid was purified by chromatography on silica, eluting with chloroform–methanol (50:1) to give 0.40 g (63%) of **11**, m.p. 213–217 °C (chloroform–ethanol). Found, C 54.3, H 4.16, N 19.4, S 10.9; $C_{13}H_{12}N_4O_2S$ requires C 54.2, H 4.20, N 19.4, S 11.1%.

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