Protonation Equilibria of Cardiotonic Polyaza Heterocycles

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The pK_a values of fifteen sulmazole analogues have been measured spectrophotometrically. The major protonation sites for most of these heterocycles were determined by ^{1}H and ^{13}C n.m.r. methods. Sulmazole (**1**), isomazole (**7**), 1*H*-imidazo[4,5-*d*]pyridazine (**14**), 1*H*-pyrrolo[2,3-*c*]pyridine (**17**), and 1*H*-pyrrolo[3,2-*c*]pyridine (**18**) underwent protonation at the pyridyl nitrogen. The purine (**11**) and 7*H*-imidazo[4,5-*e*]-1,2,4-triazine (**16**) were protonated mainly at N-1, and 1*H*-imidazo[4,5-*c*]pyridazine (**13**) at N-2. The benzimidazole (**4**) and 1*H*-imidazo[4,5-*b*]pyrazine (**15**) were protonated at the imidazole nitrogens. In some cases the various 1*H*- and 3*H*-tautomers were identified; their relative proportions were found to vary with the ring system.

The clinical evaluation of the cardiotonic drug sulmazole[1] (**1**) {2-(2-methoxy-4-methylsulphinylphenyl)-1*H*-imidazo-[4,5-*b*]pyridine} in patients with congestive heart failure has been terminated[2,3] because of undesirable toxicological effects and substantial metabolism. Potent cardiotonic agents which lack the drawbacks associated with sulmazole or digoxin are thus of much current interest.[4] Our initial approach to obtaining such an agent was to synthesise and determine the pharmacological profile of the sulmazole isomer (**7**). This 1*H*-imidazo[4,5-*c*]pyridine derivative† was found[5–7] to be a more potent cardiotonic agent than sulmazole itself. Stimulated by this finding we then undertook a wider-ranging investigation of the pharmacological and physicochemical properties of sulmazole analogues possessing a modified heterocyclic ring system. We now report the results of part of this study, which involved the synthesis of the heterocycles (**1**)—(**18**) and determination of their pK_a values and protonation sites. The work was undertaken with the ultimate aim of discovering whether the basicity properties of the heterocycles (**1**)—(**18**) were correlated with their cardiotonic activities. The pK_a values and protonation sites were therefore determined in aqueous solution whenever feasible so as to relate to the physiological situation as closely as possible.

Syntheses.—The preparation of heterocycles (**1**)—(**18**) will be described elsewhere.

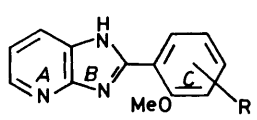
pK_a *Values and U.v. Spectra.*—The pK_a values were determined by the rapid spectrophotometric method[8] and are given in Table 1.

Where data were available for comparison, the pK_a values of the aryl-substituted heterocycles were found to be within at least 0.7 of the pK_a of the parent system. 2,4-Dimethoxyphenyl substitution consistently increased the pK_a by 0.2—0.7 whereas 2-methoxy-4-methylsulphinylphenyl substitution generally had little or no effect, *i.e.* $\Delta pK_a < 0.2$. One exception to the latter statement was observed however with the benzimidazole (**4**), which showed a pronounced decrease of 0.8 relative to the parent. This is in accord with previous observations[9] showing a ΔpK_a value of -0.3 for 2-phenyl substitution in benzimidazole. In contrast, 2-phenylation of imidazo[4,5-*c*]pyridine or purine shows little effect or a slight increase (ΔpK_a 0.3), respectively.

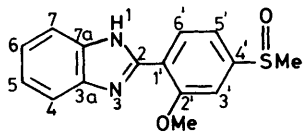
All heterocycles, with a single exception, underwent monoprotonation in aqueous solution as far as could be ascertained; no significant amounts of diprotonated species were detected by the u.v. or n.m.r. methods employed. The sterically hindered 2,6-dimethoxy analogue (**9**) was unusual in that it underwent diprotonation in aqueous solution (pK_a^2 2.0) near the limit of reliable measurement by the spectrophotometric method. N.m.r. studies with $(CD_3)_2SO$ as solvent, to which aliquots of D_2SO_4 were added, indicated that monoprotonation occurred predominantly at N-5 (pK_a^1 6.38), with subsequent protonation at the imidazole nitrogen (pK_a^2 2.0). It is probable that the other imidazo[4,5-*c*]pyridines in the series (**6**)—(**8**), (**10**) are also diprotonated in strongly acidic media, but have pK_a values below 2, precluding ready measurement in aqueous solution. Varying stoicheiometry has been observed[10] for the crystalline hydrochloride salts of a large number of 2-aryl-1*H*-imidazo-[4,5-*c*]pyridines, as deduced from microanalytical data, *i.e.* Base·*n*HCl where $1 < n < 3$. This may be due in some cases to full or partial formation of the dihydrochloride salt, while for other hydrochlorides some of the HCl appears to be loosely bound since heating under vacuum reduces *n*. In contrast the crystalline hydrochlorides of the corresponding 1*H*-imidazo-[4,5-*b*]pyridine analogues gave analysis results corresponding to Base·*n*HCl where $0 < n < 2$, but where *n* was predominantly 1. Loosely bound HCl was rarer for these salts. This suggests that diprotonation for 1*H*-imidazo[4,5-*b*]pyridines occurs only under more strongly acidic conditions than for the corresponding 1*H*-imidazo[4,5-*c*]pyridines. The absence of significant diprotonation in aqueous solution for 2-(2,6-dimethoxyphenyl)-1*H*-imidazo[4,5-*b*]pyridine (**3**) (as judged by u.v. measurements) supports this postulate. Although a pK_a^2 value of -0.5 for (**2**) has been reported,[11] no pK_a^2 value is yet available for (**8**), and so no firm conclusions can yet be drawn about pK_a^2 differences between the two series.

The 2,6-dimethoxy analogue (**9**) appeared to be significantly non-planar, as deduced from a comparison of its u.v. spectrum with that of the 2,4-dimethoxy analogue (**8**). The long-wavelength absorption bands of the analogue (**9**) show a hypsochromic shift and a decrease in absorption intensity relative to those of (**8**). The latter property suggests steric inhibition of conjugation of the chromophore leading to a non-planar conformation. Application of the Braude equation[12,13] $\varepsilon/\varepsilon_0 = \cos^2\theta$, where ε = molecular extinction coefficient of non-

† This compound was discovered independently by workers at Wellcome, E. Lilly, and E. Merck and is often referred to as BW746C or isomazole (LY-175326).



(1) R = 4' - S(O)Me
(2) R = 4' - OMe
(3) R = 6' - OMe



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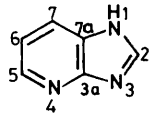

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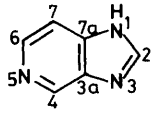

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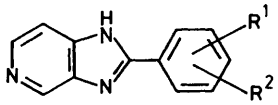
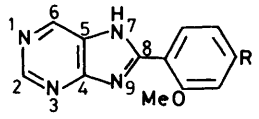
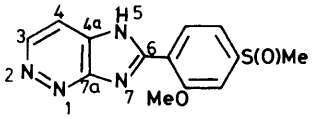




(7) R^1 = 2' -OMe, R^2 = 4' -S(O)Me
(8) R^1 = 2' -OMe, R^2 = 4' -OMe
(9) R^1 = 2' -OMe, R^2 = 6' -OMe
(10) R^1 = R^2 = H

(11) R = S(O)Me
(12) R = OMe



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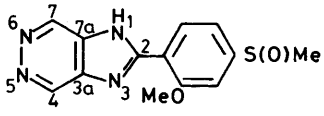

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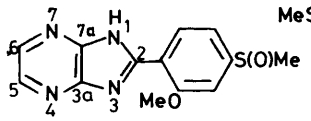

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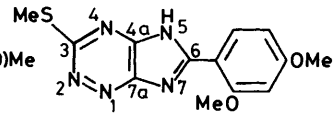
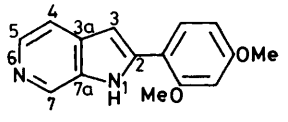


(16)



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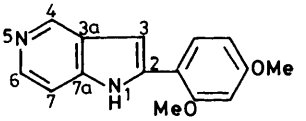


(18)

planar compound [*i.e.* (**9**)], ε_0 = molecular extinction coefficient of planar parent compound [*i.e.* (**8**)*], and θ = interplanar angle in the ground state, gives for (**9**) a value of θ of 42° (ε_0 = 20 155 at $\lambda_{max.}$ 313 nm; ε = 11 040 at $\lambda_{max.}$ 281 nm). This is to be compared with θ = 22° in the solid state as deduced from an *X*-ray crystallographic study[10] on the monoperchlorate salt of (**9**). This difference is not surprising in view of the approximate nature of the calculation and the possible effects of crystal packing.

N.m.r. Assignment Procedure.—^{1}H *N.m.r.* In all cases the ^{1}H spectra were assigned by inspection, on the basis of known substituent effects and values of spin coupling constants. In general the changes in proton chemical shift and coupling constant observed on protonation could not give an unambiguous assignment of the protonation site, though in some cases they provided confirmatory evidence. This interpretation is based upon the well known deshielding of protons attached to carbon adjacent to protonated nitrogen. In some cases diagnostic changes in coupling constants could also be characterised. The ^{1}H n.m.r. data are collected in Table 2.

^{13}C *N.m.r.* In many cases the ^{13}C spectra could again be assigned by inspection, on the basis of known substituent effects on ^{13}C chemical shifts. In cases where ambiguity remained some ^{13}C spectra were measured without broad-band ^{1}H decoupling, and diagnostic use of $^1J_{CH}$ and long-range J_{CH} values was employed. For example for (**2**) dissolved in $(CD_3)_2SO$ a plot was obtained of the residual J_{CH} value as a function of the proton frequency in a single-frequency decoupling experiment. Given the ^{1}H assignments by inspection, this allowed unambiguous assignment of the methine signals in the ^{13}C spectrum.

Also for (**2**), quaternary carbon signals were more difficult to assign and in this case chemical shift substituent effects and J_{CH} values were used. The signal at 110.4 p.p.m. in the spectrum of the base was easily assigned to C-1' because of the known shielding of an *ortho*-OMe group. The assignment was reinforced by the two long-range ^{13}C–^{1}H couplings observed to H-3' and H-5' of about 7 Hz. The signal at 129.4 p.p.m. was also assignable to C-7a because this nucleus is bonded to less deshielding substituents than the rest.

The assignments of C-2' and C-4' were based on the shifts in model compounds and on substituent effects. Thus, comparison of the benzene and anisole ^{13}C shifts[14] gives the OMe substituent effects as *ipso* +30.7; *ortho* −14.7; *meta* +0.4; *para* −8.4 p.p.m. Also with C-6' assigned (C–H) the effect of the heterocyclic system on an *ortho*-carbon can be determined as +1.8 p.p.m. From these values, the C-2' shift is predicted to be 161.4 p.p.m., close to the observed 162.4 p.p.m. With the *ortho* effect of the heterocyclic system small and positive, the *para* effect is likely to be essentially zero; thus the C-4' shift is predicted to be 159.6 p.p.m., close to the observed 158.4 p.p.m. These assignments were confirmed by elimination of those shifts due to C-3a and C-2 on the basis of long-range couplings.

The atom C-2 is remote from any hydrogen and thus is expected to show few long-range couplings. However C-3a is expected to be coupled significantly to at least H-5. This then enables assignment of the signal at 150.9 p.p.m. to C-2 and that at 152.5 p.p.m. to C-3a.

Similar arguments have been applied to all the molecules studied; the ^{13}C n.m.r. chemical shifts and coupling constants are collected in Table 3.

Determination of the Sites of Protonation.—The position of protonation has in general been determined by using the rule that protonation of heterocyclic nitrogen causes shielding of the α-carbon nuclei, usually of the order of 4—8 p.p.m.[15,16] Additional indications are the characteristic increases in coupling constants for *ortho* coupling between α- and β-protons,[17] and for the one-bond ^{13}C–^{1}H coupling at the α-position.[18]

The arylbenzimidazole (**4**), which can be protonated only at the imidazole nitrogen, was a useful reference. It shows the largest upfield shifts at C-2 and C-1' (−4.7 and −7.9 p.p.m., respectively) as expected, and also at C-3a and C-7a (−7.5 p.p.m.), confirming protonation at N-3. The hydrochloride salt of (**4**) shows a spectrum indicative of rapid rotational averaging such that the ^{1}H and ^{13}C shifts reflect the effective symmetry. Rapid imidazole tautomerisation in the base also gives an effective symmetry to the spin point group.

For the 1*H*-imidazo[4,5-*b*]pyridine (**2**) upfield shifts of −4.2

* An *X*-ray crystallographic study[7] of (**7**) has indicated that a hydrogen bond exists between the imidazole hydrogen and the methoxy oxygen, resulting in molecular planarity.

Table 1. pK_a Values for aryl heterocycles and some of the parent systems

Comp.	$pK_a(BH^+)$	I	$pK_a(B)$	I	Comment
(**1**)	3.91 ± 0.03	0.004	>11.5		
(**2**)	4.63 ± 0.02	0.002	>11.0		
(**3**)	4.85 ± 0.01	0.005	11.26 ± 0.006	0.004	
(**4**)	4.74 ± 0.08	0.002	>11.5		10% Me_2SO
(**7**)	6.17 ± 0.04	0.0005	>11.5		
(**8**)	6.52 ± 0.08	0.0004	>11.0		
(**9**)	6.38 ± 0.02	0.0003	10.84 ± 0.03	0.004	$pK_a^2(BH^+)$ = 2.0 ± 0.2 (I = 0.07)
(**10**)	6.00 ± 0.03	0.0001	10.3 ± 0.1	0.006	
(**11**)	2.69 ± 0.01	0.004	8.92 ± 0.03	0.000 08	
(**12**)	3.09 ± 0.05	0.003	9.92 ± 0.02	0.001	10% EtOH
(**13**)	3.65 ± 0.08	0.002	8.73 ± 0.09	0.000 06	
(**14**)	3.83 ± 0.03	0.0003	8.47 ± 0.03	0.000 05	
(**15**)	<1		9.00 ± 0.1	0.003	4% EtOH
(**16**)	<2		7.69 ± 0.07	0.009	
(**17**)	8.27 ± 0.04	0.0006	>12.0		
(**18**)	8.47 ± 0.08	0.0006	>12.0		
Purine	2.39		8.93		*a*
Benzimidazole	5.53		12.3		Ref. 24, *a*
(**5**)	3.95		11.08		*a*, *b*
(**6**)	6.00		10.30		*b*, *c*
5-Azaindole	8.26 ± 0.06				Ref. 24, *d*
6-Azaindole	7.95 ± 0.06				Ref. 24, *d*
2-Phenylbenzimidazole	5.23		11.91		H_2O, ref. 9
8-Phenylpurine	2.68		8.09		*a*

[a] S. F. Mason, *J. Chem. Soc.*, 1954, 2071. [b] 'Dissociation Constants of Organic Bases in Aqueous Solution,' ed. D. D. Perrin, Butterworths, London, 1965. [c] G. B. Barlin, *J. Chem. Soc. B*, 1966, 285. [d] A. Albert, in 'Physical Methods in Heterocyclic Chemistry,' ed. A. R. Katritzky, vol. III, Academic Press, New York, 1971, p. 64.

and −5.5 p.p.m. are observed at C-5 and C-3a, respectively, indicating protonation at N-4. The pattern of shifts is similar to that observed for pyridine[15] but smaller, and of similar magnitude to those observed in 1*H*-imidazo[4,5-*c*]pyridine (**6**).[19] Additional support for the protonation site being N-4 is provided by the 9.5 Hz increase in the one-bond ^{13}C–^1H coupling constant J[C(5)–H(5)] to 186.2 Hz and the 1 Hz increase in the *ortho* ^1H–^1H coupling J[H(5)—H(6)]. Sulmazole (**1**) shows shift and coupling constant changes similar to those of compound (**2**), indicating again N-4 protonation and showing that the change in 4′-substituent has no dramatic effect on protonation site.

Although (**2**) displays broadly similar upfield shifts at C-3a and C-5 (−5.5 and −4.2 p.p.m., respectively), the parent heterocycle (**5**) shows a much larger upfield shift at C-3a (−5.6 p.p.m.) than at C-5 (−2.8 p.p.m.). Also, while (**2**) has a downfield shift at C-2 of +4.2 p.p.m., in (**5**) this downfield shift at C-2 is reduced to +2 p.p.m. Benzimidazole (**4**), which is protonated fully at N-3, shows an upfield shift at C-2 of −4.7 p.p.m. These ^{13}C shifts indicate that for 1*H*-imidazo[4,5-*b*]pyridine protonation is not solely at N-4, and that there are contributing protonated imidazole forms. Thus while both (**1**) and (**2**) are protonated predominantly at N-4, as judged by ^{13}C n.m.r., the parent heterocycle (**5**) is protonated at both the pyridyl and imidazole nitrogens in the ratio *ca.* 2:1 as determined by ^{15}N n.m.r.[20] This suggests that 2-aryl substitution in 1*H*-imidazo[4,5-*b*]pyridines increases the degree of protonation at the pyridyl nitrogen. It is likely that ^{15}N n.m.r. would be able to substantiate this hypothesis.

The 1*H*-imidazo[4,5-*c*]pyridines (**7**) and (**8**) show ^{13}C chemical shift and coupling constant changes similar to those in the parent compound (**6**). Thus C-4 and C-6 signals are shifted approximately equally upfield [−5.1 and −5.2 p.p.m. for (**7**); −8.5 and −8.3 p.p.m. for (**8**); −4.9 and −6.6 p.p.m. for (**6**)] and comparable increases in J[H(6)–H(7)] are observed on protonation [0.8 Hz for (**6**) and 1.2 Hz for (**7**)]. From the similarities in the data it is concluded that these three compounds are all protonated at N-5. ^{15}N Studies[20] have shown unequivocally that (**6**) is protonated exclusively at N-5.

The 2,6-dimethoxy analogue (**9**), after an initial small addition of D_2SO_4 to its solution in $(CD_3)_2SO$, showed upfield ^{13}C chemical shifts at C-4 and C-6 and downfield shifts at C-2 and C-7a, consistent with protonation at N-5. After a second, larger addition of D_2SO_4, significant upfield shifts were observed at C-2, C-3a, and C-1′ (−2.0, −6.9, and −5.8 p.p.m., respectively) indicative of protonation at the imidazole nitrogens N-1/N-3.

The 8-arylpurine (**11**) shows the expected upfield ^{13}C shifts at C-2 and C-6 (−4.1 and −5.7 p.p.m., respectively) and a downfield shift at C-8 (+6.5 p.p.m.) indicative of protonation at N-1, the same as for purine itself.[16,18,21]

For the 1*H*-imidazo[4,5-*c*]pyridazine (**13**) an upfield ^{13}C chemical shift was only observed for C-3 (−4.2 p.p.m.), implying that the primary site of protonation was N-2. This was further supported by an increase of 0.9 Hz in the *ortho* ^1H–^1H coupling J[H(3)–H(4)] and an increase of 9.8 Hz in the one-bond ^{13}C–^1H coupling J[C(3)–H(3)]. Downfield shifts were observed for both C-6 (+8.1 or +3.8 p.p.m.) and C-7a (+0.9 or +5.2 p.p.m.); thus protonation at imidazole nitrogen or N-1, respectively, was not indicated.

Rapid tautomerisation and aryl rotation in the 1*H*-imidazo[4,5-*d*]pyridazine (**14**) gives an effective symmetry to the molecule on the n.m.r. time-scale for both the base and the salt. On acidification, deshielding effects were observed at C-2, C-3a, and C-7a (+1.3, 4.0, and 4.0 p.p.m., respectively) ruling out protonation at imidazole nitrogen. Only a small upfield effect (−0.6 p.p.m.) was observed for C-4 and C-7, and although protonation is occurring at N-5 or N-6 the shift change is much smaller than those observed for other heterocycles in the study. As it is well known[15] that protonation in pyridines causes a shielding of the α-carbon but a deshielding of the β-carbon atom, the small magnitude of the observed shift change

Table 2. ^{1}H N.m.r. data

	δ										J/Hz				
	H-2	H-3	H-4	H-5	H-6	H-7	H-3′	H-5′	H-6′	2′-OMe	4′-XMe	5, 6	5, 7	6, 7	3′, 5′	5′, 6′
(1) Base[a]				8.39	7.26	8.46	7.53	7.43	8.02	4.09	2.85	4.8		8.0		
Salt[a]				8.62	7.72	8.70	7.52	7.43	8.42	4.11	2.83	5.8	1.2	8.2		
(1) Base[b]				7.67	6.59	7.41	6.39	6.59	7.12	3.42	2.53			8.2		
Salt[b]				8.13	7.33	8.11	6.83	6.83	7.58	3.68	2.68	5.8	1.2	8.0		
(2) Base[a]				8.32	7.18	7.92	6.78	6.71	8.26	4.02	3.87	4.8	1.5	8.0	2.4	8.5
Salt[a]				8.52	7.52	8.34	6.85	6.84	8.30	4.10	3.93	5.8	1.3	8.3	2.3	8.2
(2) Base[b]				8.34	7.31	7.91	6.50	6.68	8.06	4.02	3.87	5.0		8.3		8.1
Salt[b]				8.51	7.69	8.30	6.39	6.58	7.88	4.13	3.92	5.9		8.2	2.2	8.8
(4) Base[a]			7.65	7.21	7.21	7.65	7.52	7.41	8.50	4.11	2.84					
Salt[a]			7.89	7.55	7.55	7.89	7.59	7.49	8.43	4.11	2.85					
(5) Base[b]	8.01			7.93	6.88	7.51						4.9	1.3	8.3		
Salt[b]	8.92			8.46	7.60	8.39						5.5	1.2	8.4		
(6) Base[b,d]	7.95		8.37		7.85	7.14								5.8		
Salt[b,e]	8.67		9.13		8.43	8.04								6.6		
(7) Base[a]			8.98		8.32	7.62	7.55	7.44	8.51	4.12	2.85			5.5		
Salt[a]			9.27		8.48*	8.16*	7.45	7.36	8.36	4.04	2.78			6.7		
(7) Base[b]			7.72		7.35	6.56	6.32	6.49	7.10	3.32	2.53			5.5		
Salt[b]			8.75		8.19	7.70	6.92	6.90	7.62	3.73	2.68			6.6		
(8) Base[b]			8.19		7.79	7.03	5.79	6.11	7.44	3.55*	3.39*					
Salt[b]			8.96		8.44	7.92	6.20	6.31	7.53	3.79*	3.55*					
(9) Base[a,f]			8.87		8.28	7.51	6.82	6.82		3.71				5.5		
Salt[a,c,g]			9.47		8.71	8.30	6.91	6.91		3.86				6.5		
(11) Base[b]	8.05				7.99		6.46	6.58	7.27	3.44	2.57					
Salt[b,h]	8.81				8.79		6.98	6.96	7.85	3.78	2.66					
(11) Base[a]	9.10				8.94		7.55	7.46	8.48	4.10	2.86					
Salt[a,i]	9.50				9.36		7.60	7.50	8.54	4.14	2.86					
(13) Base[b,j]		8.16	6.97				6.33	6.48	7.16	3.34	2.54					8.1
Salt[b,k]		9.13	8.21				7.00	6.93	7.79	3.76	2.66				1.3	8.2
(14) Base[b]			8.47			8.47	6.52	6.62	7.20	3.46	2.61					
Salt[b]			9.55			9.55	7.19	7.11	8.16	3.90	2.69					
(15) Base[b]				7.65	7.65		6.35	6.48	7.21	3.40	2.52					
Salt[b]				8.03	8.03		6.78	6.75	7.50	3.64	2.62					
(16) Base[a,i]							6.78	6.80	8.33	3.90*	4.02*					9.4
Salt[a,l]							6.71	6.75	8.26	3.84*	3.97*					8.9
(17) Base[a,m]		6.85	8.04	7.44		8.73	6.72	6.69	7.77	3.95*	3.83*					8.5
Salt[a,n]		7.20	8.11	7.91		8.97	6.70	6.67	7.91	3.95*	3.79*					
(18) Base[a]		6.96	8.68		8.00	7.62	6.45	6.49	7.50	3.79*	3.69*			6.0		8.5
Salt[a]		7.30	9.13		8.33	7.94	6.38	6.44	7.45	3.98*	3.86*			6.6		8.6

[a] $(CD_3)_2SO$. [b] D_2O. [c] Excess of D_2SO_4. [d] $J_{4,6}$ 1.1. [e] $J_{4,6}$ 0.9. [f] H-4′, δ 7.49. [g] H-4′, δ 7.63. [h] $J_{2,6}$ 1.1. [i] $J_{2,6}$ 1.0. [j] $J_{3,4}$ 5.4. [k] $J_{3,4}$ 6.3. [l] MeS, δ 2.64. [m] $J_{4,5}$ 5.3. [n] $J_{4,5}$ 6.5.

* Assignments in a horizontal row marked with an asterisk may be interchanged.

probably results from the near cancellation of two effects of opposite sign. This can be envisaged by considering either protonation at N-5 with rapid imidazole tautomerisation making C-4 and C-7 equivalent, or by simply considering protonation to be a rapid exchange process alternately at N-5 and N-6. Similar reasoning[15] has explained the much smaller shielding of the α-carbon atoms of pyridazine (−1.1 p.p.m.) relative to those of pyridine (−7.8 p.p.m.) on protonation.

The protonation behaviour of the 1*H*-imidazo[4,5-*b*]pyrazine (**15**) was similar to that of (**14**) in that only small ^{13}C shift changes were observed in D_2O. Downfield effects on protonation of (**15**) were observed for C-5 and C-6 (+1.4 p.p.m.), ruling out protonation in the six-membered ring. Small upfield effects were seen at C-2, C-3a, C-7a, and C-1′ (−1.1 or 0.2, −1.5, −1.5, and −1.3 p.p.m., respectively), indicating protonation in the imidazole ring. The small magnitude of the protonation shift changes could not be explained in terms of cancelling tautomeric effects as for (**14**). These observations are seen to be due to the low pK_a value because larger shifts were obtained in $(CD_3)_2SO$ with additional D_2SO_4, a medium in which complete protonation was achieved. The N-3 monoprotonation site for (**15**) is the same as that observed for 1-methylimidazo[4,5-*b*]pyrazine.[22]

The protonation of the weakly basic 7*H*-imidazo[4,5-*e*]-1,2,4-triazine (**16**) was studied with $(CD_3)_2SO$ containing an excess of D_2SO_4 as solvent. There proved to be no suitable coupling constants which were diagnostic[23] of the protonation site for this heterocycle. Protonation at N-1 was inferred, however, from the downfield shift (+6.1 p.p.m.) at C-6, the upfield shift (−4.0 p.p.m.) at C-7a, and the small upfield shift (−0.8 p.p.m.) at C-3. The assignments of C-4a and C-7a as shown in Table 3 gave a self-consistent set of shifts on protonation. In view of the importance of these assignments for interpreting the protonation site of (**16**), however, a definitive determination is desirable.

The 6-azaindole (**17**) was protonated exclusively at the pyridyl nitrogen as indicated by the characteristic upfield ^{13}C shifts at C-5 and C-7 (−8.1 and −9.3 p.p.m., respectively) and the increases in *J*[C(5)–H(5)] and *J*[H(4)–H(5)] of +12 and 1.2 Hz, respectively. Similarly the 5-azaindole (**18**) showed upfield shifts at C-4 and C-6 (−6.7 and −7.7 p.p.m.) and an increase in *J*[H(6)–H(7)] of 0.6 Hz, indicative of exclusive protonation at N-5. Thus the 2-arylazaindoles (**17**) and (**18**) are protonated at the same sites as the parent azaindoles.[24,25]

Observation of Individual Tautomers by ^{13}C *N.m.r.*—In some

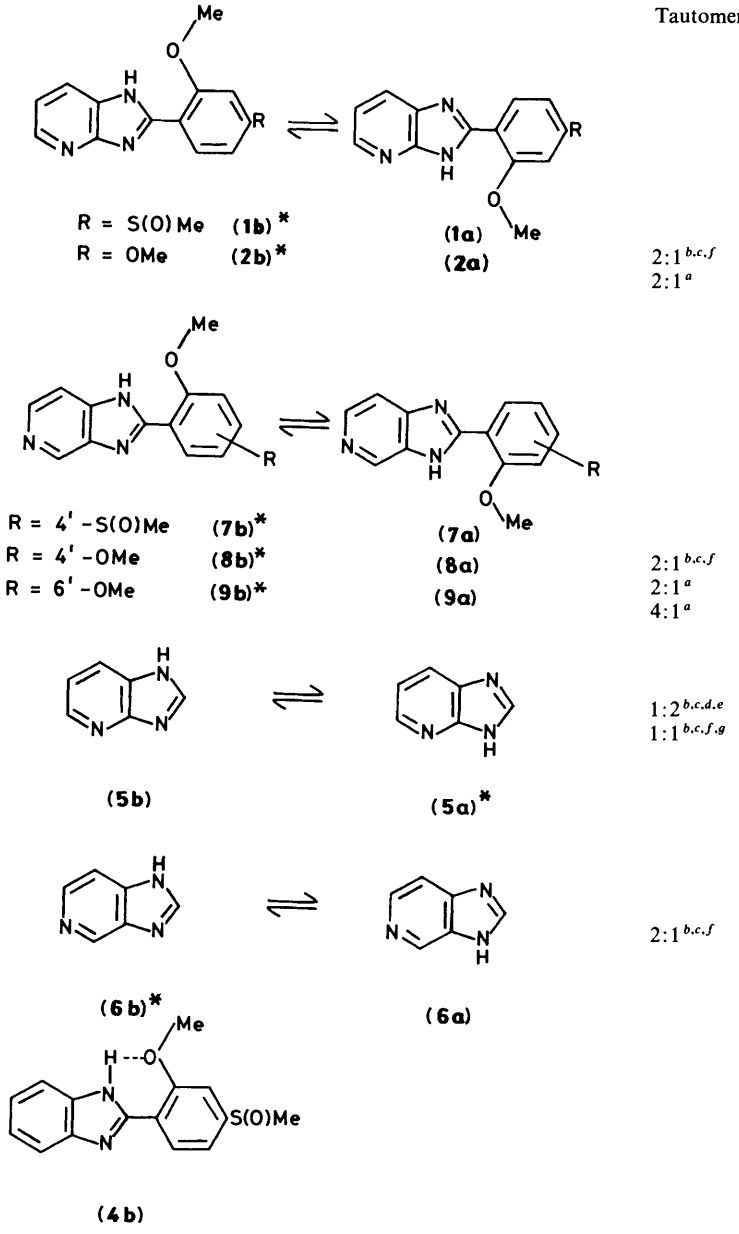
Table 3. ^{13}C N.m.r. data

	δ_C																J/Hz						
	C-2	C-3	C-4	C-5	C-6	C-7	C-3a	C-7a	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	2′-OCH_3	4′-XCH_3	C(3′)–H(3′)	C(5′)–H(5′)	C(6′)–H(6′)	C(4)–H(4)	C(5)–H(5)	C(6)–H(6)	C(7)–H(7)
(1) Base[b]	149.4			144.3	119.1	122.8	151.2	128.1	117.8	157.3	106.9	147.1	116.0	130.5	56.4	42.4							
Salt[b]	155.1			137.5	120.2	130.2	146.7	131.1	117.0	159.5	108.7	151.5	117.2	132.6	57.9	42.8							
(2) Base[a]	150.9			143.0	117.1	121.4	152.5	129.4	110.4	162.4	98.4	158.4	106.2	131.1	55.6*	55.3*	159.8	163.9	163.0		176.7	163.0	166.6
Salt[a]	155.1			138.8	120.1	127.6	147.0	129.9	106.2	166.3	99.3	161.2	108.7	132.9	57.4*	57.0*	160.9	164.8	163.0		186.2	167.8	169.7
(4) Base[a]	149.4*		115.4	122.1	122.1	115.4	138.7	138.7	120.3	157.2	107.2	148.0*	115.9	130.6	56.3	43.2							
Salt[a]	144.7		114.8	127.3	127.3	114.8	131.2*	131.2*	112.4	158.8	108.7	153.8	117.1	131.4*	58.1	43.7							
(5) Base[b,e]	144.8			144.6	119.4	124.5	151.2	129.2													180.1	165.4	166.9
Salt[b,f]	146.8			141.8	121.5	129.4	145.6	128.1													187.5	171.8	173.0
(6) Base[b]	146.4		139.0		141.0	110.7	137.3	142.4															
Salt[b]	151.0		134.1*		134.4*	112.9	137.9	146.0															
(7) Base[b]	150.7		137.5		140.5	110.1	136.2	142.0	117.7	157.5	107.2	147.9	116.2	130.8	56.4	42.4							
Salt[b]	156.6		132.4		135.3	113.0	135.6	146.3	116.6	159.7	108.9	151.9	117.4	132.7	57.9	42.8							
(8) Base[a]	151.2		138.8		140.9	108.4	137.9	141.6	110.2	162.7	98.6	158.5	106.5	131.3	55.4*	55.9*							
Salt[a]	157.0		130.3		132.6*	111.9	136.5	147.4	107.9	164.9	98.9	160.1	107.7	133.1	56.2*	56.6*							
(9) Base[a]	148.1		139.6		141.0	107.2	137.5	142.4	108.9	158.9	104.3	131.9	104.3	158.9	55.9		162.8	162.8		*		178*	166.7
Salt[a,c]	154.7		133.4*†		133.0*	110.8	138.2	145.5	106.6	158.9	104.6	133.0†	104.6	158.9	56.2								
Salt[a,d]	152.7		138.1*		133.0*	113.8	131.3	142.9	100.8	161.8	106.7	139.8	106.7	161.8	58.7		174	174		190†		186†	174
(11) Base[b,g]	152.1		156.4	126.8	142.8				116.7	158.0	107.3	149.3	116.3	131.4	56.9	42.4							
Salt[b,h]	148.0		159.8*	128.5	137.1				117.7	159.8*	108.7	151.8	117.2	133.1	57.6	42.7						196.6	
(13) Base[b,i]		145.0	112.0		154.1			157.0	116.2	158.1	107.3	149.6	116.2	130.7	56.8	42.3	162.9	167.1	165.5	175.0			
Salt[b,j]		140.8	116.1		162.2*			157.9*	116.9	160.1	108.6	152.6	117.2	133.4	57.7	42.7	165.6	169.7	167.2	182.9			
(14) Base[b]	157.9		141.3			141.3	136.1	136.1	116.7	157.9	107.7	149.6	116.7	131.3	57.1	42.7	163.7	168.9	166.2	186.6			186.6
Salt[b]	159.2		140.7			140.7	140.1	140.1	117.8	159.8	108.9	151.6	117.5	133.3	58.0	43.0	165.2	169.3	166.9	195.4			195.4
(15) Base[b]	152.2			139.6	139.6		143.3	143.3	117.0	157.9	107.2	148.9	116.2	131.0	58.3	42.4							
Salt[b]	151.1*			141.0	141.0		141.8	141.8	115.7	159.0	108.1	152.0*	116.8	131.6	57.4	42.6							
(15) Base[a]	152.5			138.9	138.9		144.9	144.9	119.2	157.8	107.1	151.4	115.7	131.3	56.2	43.1							
Salt[a,d]	150.2			141.3	141.3		139.2	139.2	113.4	158.5	107.6	154.3	115.8	131.9	56.8	42.6							
(16) Base[a,k]		165.7			158.4			150.6	108.6	164.8	98.5	160.2	107.2	132.9	56.0‡	55.7‡	161.0	164.9	163.6				
Salt[a,d,l]		164.9			164.5			146.6	105.8	167.3	97.9	161.9	108.6	134.1	56.4‡	56.1‡	162.4	166.0	163.6				
(17) Base[a]	132.4*	99.0	113.9	134.0		137.9	138.7*	133.4*	112.6	160.9	99.0	157.8	105.9	129.5	55.5†	55.8†	160.0	162.7	158.8		178.3		175.4
Salt[a]	131.8*	101.5	116.1	125.9		128.6	148.5*	137.7*	110.6	163.4	99.5	159.3	107.5	131.6	56.4†	56.8†	160.3	163.5	160.4	172.3	190.3		210
(18) Base[a]	125.7	99.5	141.2*		138.3*	107.3	140.3†	137.5†	112.7	161.2	99.3	158.0	106.3	129.6	55.6‡	56.1‡	159.0	164.7	159.2				
Salt[a,m]	125.3	101.6	134.5*		130.4*	109.1	142.8†	142.2†	111.2	162.4	99.4	158.6	106.7	130.6	56.1‡	56.5‡	164.2	164.5	159.0	188.5*			174.5

*†‡ Assignments in a horizontal row thus marked may be interchanged.

[a] $(CD_3)_2SO$. [b] D_2O. [c] <1 equiv. D_2SO_4. [d] Excess of D_2SO_4. [e] J[C(2)–H(2)] 210.6. [f] J[C(2)–H(2)] 217.9. [g] C-8, δ 152.8; J[C(2)–H(2)] 204.9; J[C(2)–H(6)] 10.4. [h] C-8, δ 159.3*; J[C(2)–H(2)] 217.6; J[C(2)–H(6)] 5.7. [i] C-4a, δ 130.6; J[C(3)–H(3)] 183.7. [j] C-4a, δ 137.8; J[C(3)–H(3)] 193.5. [k] C-4a, δ 148.8*; MeS, δ 13.7; J(C–H) 141.5. [l] C-4a, δ 152.1*; MeS, δ 13.4; J(C–H) 142.8. [m] J[C(3)–H(3)] 180.

Table 4. Individual tautomers



* Asterisks donate major tautomeric/rotameric form.

[a] $(CD_3)_2SO$. [b] D_2O. [c] Individual tautomers not observed because of rapid equilibration. Tautomeric ratio estimated by comparison of time-averaged shifts with those of *N*-methyl or 2-aryl derivatives. [d] Based on ^{15}N shift data of *ca.* 1M-solutions. [e] The 3*H*-tautomer has also been found to be the major species by dipole moment studies: Yu. M. Yutilov, N. R. Kal'nitskii, and R. M. Bystrova, *Khim. Geterotsikl. Soedin*, 1971, **10**, 1436. [f] Based on ^{13}C shift data of <0.1M-solutions. [g] The difference in tautomeric ratio values may reflect smaller errors in the ^{15}N method or be a concentration effect.

cases, it was possible to observe individual tautomers when low concentrations of free-base solute were used in $(CD_3)_2SO$ solution. Thus for (**2**), two species were observed where the imidazole tautomerism and aryl ring rotation could potentially lead to four species. This is in accord with the propensity for $(CD_3)_2SO$ to favour intramolecular hydrogen bonds and it is likely therefore that the two forms of (**2**) are (**2a**) and (**2b**) (see Table 4).

The ratio of the two forms is about 2:1. It is possible to assign many of the ^{13}C chemical shifts in the two species (**2a** and **b**), and thus to identify the major form. There is evidence[20] that substitution of NCH_3 for NH has little effect on ^{13}C chemical shifts in molecules of this type. Also examination of the ^{13}C shifts in 1*H*-imidazo[4,5-*c*]pyridine[19,20] shows that the signal of C-7a when adjacent to the imidazole N=C system will be about 8 p.p.m. to low field of its position when adjacent to NH. The same argument applies to C-3a. Thus for (**2a**), the C-7a signal will be about 8 p.p.m. to low field of that for (**2b**), and *vice versa* for C-3a. This is confirmed by observation and shows the major species to be (**2b**). The chemical shifts of the individual species of (**2**) are given in Table 5.

Similar results have been obtained with compounds (**8**) and

Table 5. ^{13}C Chemical shifts of tautomers of free bases

	(2)		(8)		(9)		(4)
	Major	Minor	Major	Minor	Major	Minor	Rotamer
C-2	151.4	150.6	150.8	152.0	148.2	148.1	149.4*
C-4			140.6	134.7	141.0*	134.1	118.5†
C-5	143.6	142.8					122.0
C-6	117.2	117.8	140.9	140.9	141.1*	140.5	122.0
C-7	119.3	125.3	107.0	112.8	106.6	113.6	112.0†
C-3a	155.6	148.9	139.2†	132.6	138.8†	132.2	142.8
C-7a	126.7	134.7	140.2†	147.3	141.0†	148.1	135.2
C-1′	110.2	110.7	110.2	110.2	108.9	108.9	120.3
C-2′	162.6	162.6	162.7	162.7	158.9	158.9	157.2
C-3′	98.6	98.4	98.6	98.6	104.3	104.3	107.2
C-4′	158.6	158.6	158.5	158.5	131.9	131.9	148.0*
C-5′	106.5	106.2	106.5	106.5	104.3	104.3	115.9
C-6′	131.5	131.0	131.3	131.3	158.9	158.9	130.6

*† Assignments thus marked may be interchanged.

(**9**) and identical arguments have been applied, enabling identification of the major forms as (**8b**) and (**9b**) (see Table 4).

Replacing the electron-donating 4′-OMe substituent by the electron-withdrawing 4′-S(O)Me group has the consequence of lowering the order of the C(2)–C(1′) bond and hence lowering the barrier to internal rotation. Thus individual rotamers were not generally observed, although in many cases diagnostic broadening of the resonances, especially for C-3a and C-7a, was used to aid the assignment process. An individual rotamer [probably (**4b**) (Table 4)] was observed, however, for the sulphoxide (**4**) in dilute Me_2SO solution at room temperature, as judged by ^{13}C n.m.r. (see Table 5). At higher concentrations and temperatures the coalescence of the C-3a/C-7a and C-4/C-7 signals indicated rapid tautomerisation and rotation about the C(2)–C(1′) bond. Comparison of the ^{13}C n.m.r. spectra of the heterocycles (**2**), (**8**), (**1**), and (**7**) in $(CD_3)_2SO$ showed that the tautomers were distinguished in (**2**) and (**8**) but not in (**1**) and (**7**). The tautomers of (**2**) and (**8**) could not be distinguished however from the ^{13}C spectra of dilute solutions in D_2O. The rate of tautomerisation thus varies with solvent as well as with 4′-substituent. Protonation of the heterocycles invariably led to faster equilibration of the cationic species.

Comparison of the aryl heterocycles with the parent compounds[20] shows that for the 1*H*-imidazo[4,5-*c*]pyridines (**6**), (**8**), and (**9**) the 1*H*-tautomers are the major species. In the 1*H*-imidazo[4,5-*b*]pyridines however the 1*H*-tautomer of the parent heterocycle (**5**) is not the predominant form but the 1*H* tautomer is the major species in (**2**).

Conclusion.—The pK_a values, major protonation sites, and in some cases tautomeric ratios of a comprehensive set of related heterocyclic bases have been determined. Where comparative data were available the pK_a values of the aryl heterocycles varied by less than 0.7 from those of the parent systems. Of the fifteen sulmazole analogues studied all but (**4**) and (**15**) were protonated mainly at a ring *A* nitrogen atom. The sterically crowded 1*H*-imidazo[4,5-*c*]pyridine (**9**) was significantly non-planar and underwent diprotonation in aqueous solution.

With these data to hand it became possible to investigate the relationship between the protonation equilibria of these sulmazole analogues and their cardiotonic activity. These results will be discussed in detail elsewhere.

Experimental

N.m.r. Spectroscopy.—^1H N.m.r. spectra were obtained at 200 and 360 MHz with Bruker AM-200 and WM-360 spectrometers. ^{13}C Spectra were measured with and without gated broad-band ^1H decoupling by use of the same instruments at 50 and 90.57 MHz. Solutions were made up in D_2O or $(CD_3)_2SO$ as specified in Tables 2 and 3, and salt or free-base forms were generated as appropriate by the addition of concentrated DCl or NaOD solution. In D_2O solutions, dioxane was used as internal reference (δ 3.53 for ^1H and 67.4 p.p.m. from Me_4Si for ^{13}C). In $(CD_3)_2SO$ solutions, CD_3SOCD_2H was used as internal reference for ^1H (at δ 2.50) and $(CD_3)_2SO$ as internal reference for ^{13}C (at 39.5 p.p.m. from Me_4Si). All spectra were obtained at ambient temperature: about 21 °C for ^1H and 35 °C for broad-band decoupled ^{13}C.

pK_a *Measurements.*—The pK_a values were determined spectrophotometrically[8] by use of a system developed at the Wellcome Research Laboratories. The pK_a measurement system is assembled around an Apple microcomputer, which has been interfaced to all other components in the system. Absorption spectra can be acquired semi-automatically by using a Beckman Acta CV instrument. This spectrometer is fitted with a flow cell which is connected to the titration vessel. Measurement of pH is carried out with a Beckman 4500 digital pH meter using a glass electrode with a calomel reference. The pH is controlled by adding acid or alkali to the titration vessel from Agla syringes mounted in computer-controlled syringe drives. Preliminary processing of the acquired spectra, each of which is labelled with the pH at which it was measured, is carried out on the Apple computer and the final pK_a is calculated by using a DEC PDP 11/84 computer. Solution concentrations for the pK_a measurements were typically 1 mg in 100 cm^3 of doubly distilled deionised water. Where necessary up to 10% of spectroscopic grade solvent was used to overcome solubility problems. Measurements were carried out at 25 °C under a flow of nitrogen. Concentrations of the hydrochloric acid and sodium hydroxide which were used as titrants varied from 0.2M to 10M depending on the required pH range of the titration.

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