and, based on pK_1 for 2-methylimidazole as 7.85 (H_2O), estimate pK_2 = 15.1 (H_2O) or 15.8 (D_2O).
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Adjacent Lone Pair (ALP) Effects in Heteroaromatic Systems. 2. Isotope Exchange of Ring Hydrogens in Nitro- and Fluoroimidazoles

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The ring protons of nitro- and fluoroimidazoles (and their *N*-methyl derivatives) undergo base-catalyzed exchange in D_2O by a combination of carbanion (C) and ylide (Y) pathways. In the C pathway, a proton is abstracted from the neutral imidazole species, and in the Y pathway, from the imidazolium ion. In 4-X-imidazoles, C exchange occurs more readily at C-5 than at C-2, log k_C correlating with σ_o^0 for the NH– and with σ_p^0 for the *N*-methyl series. For 1-methyl-4-nitroimidazole, $t_{1/2}$ = 2 min at C-5 (50 °C, 0.2 N NaOD). In 1-methyl-5-X-imidazoles, exchange at C-4 occurs only by the Y pathway, carbanion formation in the neutral species being retarded by the *adjacent lone pair* (ALP) effect at N-3. The same effect is seen in the lack of C exchange at C-4 in 1-methyl-2-X-imidazoles. The ALP effect is considerably weaker or nonexistent at C-2. Most exchanges *across* the ring show correlations of log *k* with σ_m^0. 4-Alkylimidazoles (but not 1,4-dialkylimidazoles) show enhanced C exchange at C-5, which may result from the existence of a trace concentration of the ketimine tautomer. Enhanced exchange at C-5 in 2-fluorohistidine is ascribed to a combination of the ketimine effect, C exchange involving catalysis by hydroxide ion *and* intramolecular general base catalysis by the side-chain primary amine function. The use of buffer catalysis for the tritium labeling of poorly reactive imidazoles is described.

In the first paper of this series,[2] we summarized present knowledge on pathways for isotopic exchange of ring hydrogens in imidazole, *N*-methylimidazole, and their *C*-methyl derivatives (Scheme I of preceding paper):[2] base-catalyzed exchange occurs by a carbanion (C) pathway, in which a proton is abstracted from the *neutral* imidazole species in the rate-limiting step, and/or an ylide (Y) pathway, involving base attack on the imidazolium *ion*. In addition, we established unequivocal assignments for the NMR signals of these hydrogens, presented new data on the rates of solvent–deuterium exchange, and demonstrated that considerable differences in proton acidity are observed at C-4 and C-5, positions which should be fairly equivalent in electron density. These differences were interpreted on the basis of the *adjacent lone pair* (ALP) effect: a ring-nitrogen atom bearing an sp^2 lone pair provides a sizable electrostatic obstacle to the generation of an sp^2 carbanion at an adjacent ring-carbon atom. While operation of the ALP effect is readily demonstrable at C-4 (adjacent to the lone pair at N-3), the magnitude of the effect at C-2 could not be evaluated because ylide exchange (Y) at the latter position may be 500–1000-fold faster than carbanion (C) exchange. Ylide exchange is not subject to the ALP effect because the lone pair at N-3 is utilized in formation of the imidazolium ion. We had hoped, therefore, that electronegative substituents at C-4 or C-5 might retard the Y pathway at C-2 and permit an evaluation of C exchange at the latter position. Further, it was conceivable that an electronegative group at C-5 might reduce or negate the ALP effect at C-4.

For various biological studies, we also needed practical routes to tritium-labeled fluoroimidazoles, as well as data on tritium loss from the labeled materials.[3] Initial studies had already indicated that the apparent acidities[4] of the ring hydrogens in these compounds are inconsistent with expectations based on nonfluorinated imidazoles. Thus, at pD 11 and 50 °C, $t_{1/2}$ = 7 h for exchange of H-2 in histidine,[5] while H-2 in 4(5)-fluorohistidine fails to exchange over a wide range in temperature or pD.[6] In contrast, H-5 in 2-fluorohistidine exchanges with $t_{1/2}$ = 20 h under the stated conditions, while H-5 in histidine is totally inert to exchange (except at very high temperatures). In our attempt to rationalize the behavior of the fluoroimidazoles, we were also led to examine imidazoles containing nitro[7] and several other substituents. Since alkylation of the imidazole NH eliminates complications due to ionization in basic media, 1-methyl-X-imidazoles (series **1–3**) were examined first. The principal compounds investigated are summarized in Chart I.

Chart I

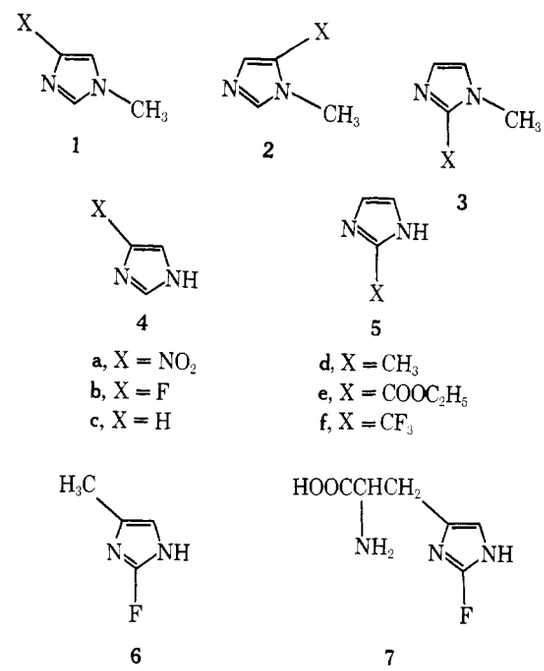


Table I. NMR Solvent Shifts ($\Delta\delta$) for *N*-Methylimidazoles[a]

Compd	Registry no.	position	δ, ppm			Δδ, ppm[b]	
			$CDCl_3$	Me_2SO-d_6	D_2O	Δ_1	Δ_2
1a	3034-41-1	H-2	7.44	7.82	7.74	−0.38	−0.03
		H-5	7.78	8.37	8.19	−0.59	−0.41
2a	3034-42-2	H-2	7.59	8.02	7.92	−0.43	−0.33
		H-4	8.05	8.02	8.11	+0.03	−0.06
3a	1671-82-5	H-4	7.17	7.19	7.20	−0.02	−0.03
		H-5	7.20	7.67	7.45	−0.47	−0.25
1b	66787-67-5	H-2	7.04	7.32	7.36	−0.28	−0.32
		H-5	6.43	6.85	6.81	−0.42	−0.38
2b	66787-68-6	H-2	7.42	7.58	7.50	−0.16	−0.08
		H-4	6.57	6.72	6.68	−0.15	−0.11
3b	66787-69-7	H-4	6.67	6.61	6.67	+0.06	0
		H-5	6.67	6.95	6.82	−0.28	−0.15
1e	41507-56-6	H-2	7.56	7.77	*c*	−0.21	
		H-5	7.66	8.02	*c*	−0.36	
2e	66787-70-0	H-2	7.63	7.97	*c*	−0.34	
		H-4	7.79	7.70	*c*	+0.09	
3e	30148-21-1	H-4	7.09	7.12	*c*	−0.03	
		H-5	7.17	7.50	*c*	−0.33	

[a] Parallel data for *N,C*-dimethylimidazoles are given in ref 2. [b] $\Delta_1 = \delta_{CDCl_3} - \delta_{Me_2SO\text{-}d_6}$; $\Delta_2 = \delta_{CDCl_3} - \delta_{D_2O}$. [c] Insufficiently soluble in D_2O to provide reliable δ values.

Results

General Methods. NMR Assignments. Identification of ring-proton NMR signals cannot be made unequivocally by application of electron density considerations,[8] and we relied on the techniques previously used[2] for the simpler *N*-methylimidazoles: (1) spin decoupling; (2) nuclear Overhauser enhancement; (3) solvent-dependent $\Delta\delta$ values; and (4) chemical transformation. The first two methods depend on the fact that four-bond coupling between the protons of the *N*-methyl group and any adjacent ring hydrogen is readily observed, while coupling to the distal hydrogen is not discernible. Thus, irradiation at the *N*-methyl frequency results in loss of fine structure and increase in peak height for adjacent protons, but is without effect on the signal for a distal proton. The third method is based on an empirical generalization: for protons adjacent to the *N*-methyl group, $\Delta\delta_1$ ($=\delta_{CDCl_3} - \delta_{Me_2SO\text{-}d_6}$) and $\Delta\delta_2$ [$=\delta_{CDCl_3} - \delta_{D_2O}$] have significant negative values (−0.10 to −0.60); for the remaining ring proton, these Δ values are usually less than ±0.10 (Table I).[2,9] To date, 1-alkyl-5-fluoroimidazoles (e.g., **2b**) are the only compounds which have given equivocal results in the solvent shift analysis. Identification of NMR signals in all fluoroimidazoles is confirmed, however, by spin decoupling and by examination of coupling constants: $J_{4(H)5(F} \simeq J_{4(F)5(H)} \simeq 7\text{–}8$ Hz; $J_{2(H)4(F)} \simeq J_{2(F)4(H)} \simeq 1\text{–}2$ Hz; $J_{2(H)5(F)} \simeq J_{2(F)5(H)} \simeq 0$ Hz.[10] While electronegativity considerations suggest that the imidazole proton closer to the nitro group should appear at lower field in **1a** and **2a**, such an argument is inapplicable to **3a**, making the $\Delta\delta$ criterion especially valuable in the latter case. For **1a**, additional verification was obtained by its transformation to **1b** following isotope exchange (see below).

Kinetic Analysis. Rates of exchange of imidazole-ring protons in D_2O (over a wide pD range) were obtained by integration of NMR peak areas at various time intervals and at reaction temperatures which provided conveniently measurable rates. For *N*-methylimidazole and its *C*-alkyl derivatives, exchange at C-2 occurs, overwhelmingly, via the imidazolium ion and the Y pathway [Y(2)].[2] At any pD more than 1.5 units above the p*K* of the compound, an increase in [OD^-] is directly offset by a decrease in [ImD^+], and further increase in the basicity of the exchange medium will have no effect on $k_{Y(obsd)}$ (ref 2, Figure 1B). By virtue of its inductive effect, an electronegative substituent at C-4 or C-5 should enhance the acidity of H-2; at the same time, however, $k_{Y(obsd)}$ may be reduced because of the reduction in p*K*. Thus, at a pD low enough to provide significant [ImD^+], [OD^-] may be vanishingly small. A priori, one cannot predict the net effect of these opposing factors on Y exchange. Values of k_{obsd} were obtained at pD 9.5–10, generally at 50 °C. In this pD range, $k_{Y(obsd)}$ has attained its maximum value and the contribution of $k_{C(obsd)}$ is negligible for most compounds. For the weakly basic fluoro- and nitroimidazoles, values of k_{obsd} at pD 5 or 7 showed little variation from those at the higer pD (as expected). For very reactive or poorly reactive compounds, extrapolation to 50 °C was calculated from data at other temperatures, using an average value of $E_a = 21$ kcal/mol. Temperature-dependence studies with three compounds provided E_a values in the range 20–22 kcal/mol. Specific rate constants (k_Y) were calculated from the equation

$$k_{Y(obsd)} = k_Y K_W/(K_1 + [D^+]) \quad (1)$$

in which K_W is the ion product of D_2O and K_1 is the dissociation constant for ImD^+, both constants estimated for the reaction temperature (see Experimental Section). Since $k_1 \gg [D^+]$ at pD 9.5–10, the contribution of [D^+] in eq 1 can usually be ignored. Exchange at C-4 or C-5 in *N*-methylimidazole also occurs by an ylide (Y) mechanism, but at a rate 10^4 to 10^5 slower than at C-2.[2] The same considerations regarding electronegative substituents should be applicable, although the inductive effect of the group should be felt more strongly at the adjacent ring position than at C-2. Values of $k_{Y(4)}$ and $k_{Y(5)}$ were obtained similarly to $k_{Y(2)}$ by use of eq 1 and $E_a = 21$ kcal/mol.

In *N*-methylimidazole, exchange at C-5 also occurs by a carbanion [C(5)] mechanism in strongly basic media; this pathway involves the neutral imidazole species, and k_{obsd} is directly proportional to [OD^-]. For this compound (in 1 N NaOD at 100 °C), C(5) exchange is ~15-fold faster than Y(5) exchange, ~40-fold faster than Y(4) exchange, but 800-fold slower than Y(2) exchange. Under these conditions, $t_{1/2} = 7$ h for C(5), while C(4) exchange could not be detected over 200 h. Values of total k_{obsd} were determined in alkaline media (0.05–1 N NaOD), both the temperature and pD range sometimes being limited by the stability of the compound to ring degradation or solvolysis of the substituent. Values of $k_{C(obsd)}$ were obtained by subtraction of $k_{Y(obsd)}$ (measured at pD 9.5–10) from total k_{obsd}. Plots of $k_{C(obsd)}$ vs. [OD^-] provided reasonably linear slopes with values = k_C. Even in

Table II. Values of k_{obsd}[a] and k[b] for Deuterium Isotope Exchange (50 °C) in Imidazoles

Series	Ring Site	Path	NO_2 10^5k_{obsd}	NO_2 k	F 10^5k_{obsd}	F k	H 10^6k_{obsd}	H k	CH_3 10^6k_{obsd}	CH_3 k	σ^0	ρ	log k_0	Figure
1	2	C[c]		4.33×10^{-2}		2.33×10^{-5}	d		d			e		
		Y	0.88	6.46×10^{9}	1.25	2.63×10^{7}	16500	1.62×10^{5}	9170	7.59×10^{4}	m	6.72	5.22	3A
	5	C		2.24		2.41×10^{-4}		2.08×10^{-5}		4.44×10^{-6}	p	6.00	-4.66	1A
		Y	10.7	7.76×10^{10}	1.46	3.02×10^{7}	1.41	13.8	0.19	1.55	o	7.10	1.17	2A
2	2	C		0.25		2.80×10^{-2}	d		d			e		
		Y	82.5	1.00×10^{9}	49.5	1.05×10^{7}	16500	1.62×10^{5}	14300	3.72×10^{4}	m	5.64	5.18	3B
	4	C	f		f		f		f					
		Y	0.37	4.47×10^{6}	0.17	3.63×10^{4}	0.52	5.13	0.46	1.18	o	4.28	0.72	2B
3	4	C	f		f		f		f					
		Y	g		g		0.52	5.13	0.52	0.68		e		
	5	C		1.16×10^{-2}		5.07×10^{-5}		2.09×10^{-5}		5.26×10^{-6}	p	3.42	-4.81	1B
		Y	g		g,h		1.41	13.8	0.52	0.68		e		
4[i]	2	C	g		g		d		d					
		Y[j]	0.20[k]	5.01×10^{8}	0.96	5.07×10^{6}	5630	7.59×10^{4}	4950	1.76×10^{4}	m	5.54	4.83	3C
	5[l]	C		1.40		3.96×10^{-2}		8.24×10^{-5}		2.90×10^{-4}	o	3.27	-4.08	2C
		Y	g,k		g		0.49	2.88[m]	0.44	1.38		e		
5[i]	5[n]	C		60.8		0.05		8.24×10^{-5}		1.38×10^{-5}	m	8.70	-4.17	3D
	4,5[o]	Y	g,k		g		0.49	2.88[m]	0.48	0.40[m]		e		
6[i]	5	C				3.08								
7[i,p]	5	C				2.83								
		C'[q]				1.37×10^{-3}								

[a] Min^{-1}. [b] $M^{-1}min^{-1}$. [c] For path C, k_{obsd} is a linear function of $[OD^-]$. [d] Masked by the much faster Y exchange. [e] Only two experimental points available. [f] No measurable exchange because of the ALP effect. [g] No measurable exchange in 30 d at 50° and/or 8 d at 100°. [h] Too unstable in D_2O to evaluate k_{obsd}. [i] Values of k_{obsd} and k include adjustment for f_{Im^-}. [j] For X = CF_3, $k_{obsd} = 3.89 \times 10^{-5}$ min^{-1} and $k = 3.31 \times 10^{7}$ $M^{-1}min^{-1}$. [k] Insoluble in D_2O alone; kinetics run in D_2O containing 20% DMSO-d_6, adjusted to pD 7 with CD_3COOD. [l] The 1,4-tautomer is considered the kinetically active species. [m] Since the kinetically active species is symmetrical, a statistical correction has been applied to k. [n] Although H-4 and H-5 are experimentally indistinguishable, the 1,4-tautomer is considered the active species. [o] H-4 and H-5 are experimentally indistinguishable. [p] Based on loss of tritium in H_2O. [q] Specific rate constant due to intramolecular general base catalysis by the side-chain primary amine function, in min^{-1}.

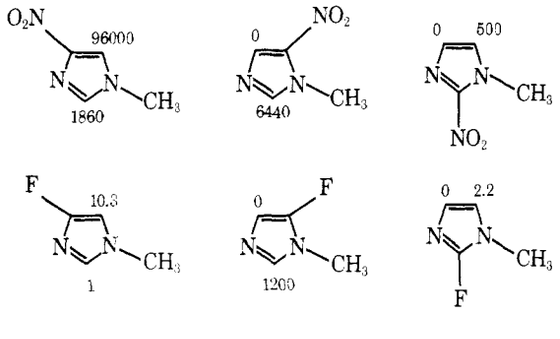


Figure 1. Relative rate constants (k_C) for carbanion exchange in nitro- and fluoro-1-methylimidazoles.

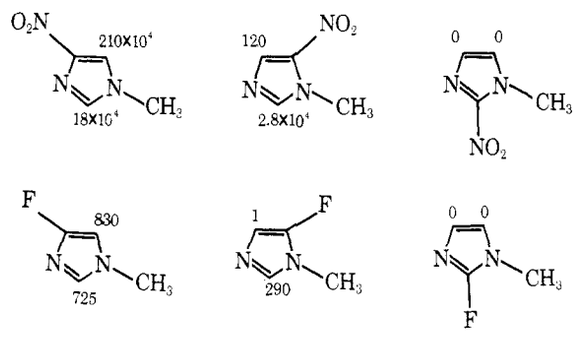


Figure 2. Relative rate constants (k_Y) for ylide exchange in nitro- and fluoro-1-methylimidazoles.

the most rapid exchanges, the contribution of the C pathway at pD 9.5–10 could be neglected. Values of k_C and k_Y are summarized in Table II, and relative rate constants for the two pathways are shown in Figures 1 and 2, respectively. The pK (H_2O, 25 °C) values used for Y pathway calculations are given in Table III, and methods for their conversion to pK (D_2O, 50 °C) are given in the Experimental Section. For the ylide pathways, values of k_{obsd} are also given in Table II to emphasize their lack of correlation with substituent parameters. Wherever "no detectable exchange" is indicated in Table II, runs were continued for 30–60 days at 50 °C and/or 8 days at 100 °C, stability permitting. The values of ρ in Table II are derived from the Hammett correlations of Figures 3–5, the latter being based on the set of σ^0 values proposed by Cohen and Takahashi (Table IV).[11]

1-Methyl-4-X-imidazoles (Series 1). Exchange at C-5 occurs by a combination of C and Y pathways, the former being far more significant in basic media. Thus, for X= NO_2, 0.02% of the total k_{obsd} is due to Y exchange in 0.2 N NaOD, while the fraction rises to 23% for X = F. In fact, H-5 in **1a** is remarkably acidic for a nonquaternized heterocycle with $t_{1/2} \simeq 2$ min at 50 °C in this medium. Introduction of a 4-nitro group into 1-methylimidazole increases total k_{obsd} at C-5

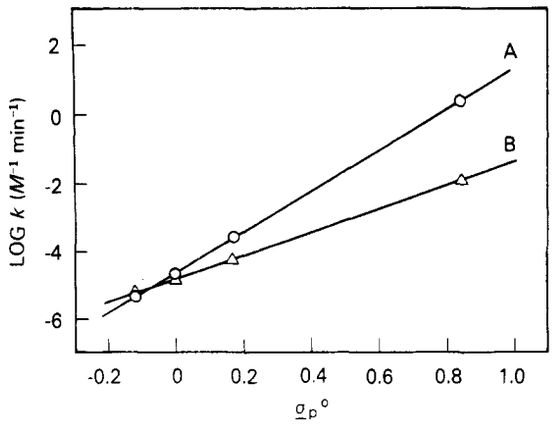


Figure 3. Hammett correlations of σ_p^0 for X vs. log k: A, series **1**, log $k_{C(5)}$; B, series **3**, log $k_{C(5)}$.

Table III. pK Values (25 °C) Used in Calculations

series	X = NO_2	F	H	CH_3	CF_3
1	−0.60[a]	1.90[b]	7.13[b]	7.20[b]	
2	2.13[c]	3.85[b]	7.13[b]	7.70[b]	
3	−0.44[a]	2.30[b]	7.13[b]	8.00[b]	
4 (pK_1)	−0.15[a]	2.44[d]	7.00[e]	7.56[b]	2.28[e]
4 (pK_2)	9.20[a]	11.92[b]	14.52[f]	15.10[e]	10.6[e]
5 (pK_1)	−0.20[g]	2.40[d]	7.00[e]	7.85[b]	
5 (pK_2)	7.15[a]	10.45[d]	14.52[f]	15.10[e]	
6 (pK_1)		3.06[d]			
6 (pK_2)		10.70[d]			
7 (pK_1)		1.22[d]			
7 (pK_2)		10.55[d]			

[a] Average of values given in ref 31. [b] Present investigation. [c] Reference 12. [d] H. J. C. Yeh, K. L. Kirk, L. A. Cohen, and J. S. Cohen, *J. Chem. Soc., Perkin Trans. 2*, 928 (1975). [e] L. A. Cohen and P. A. Cohen, manuscript in preparation. [f] D. J. Brown, *J. Chem. Soc.*, 1974 (1958). [g] E. Laviron, *Bull. Soc. Chem. Fr.*, 2840 (1963).

Table IV. σ^0 Values Used in Hammett Correlations[a]

σ^0	NO_2	F	CH_3	CF_3
σ_o^0	1.38[b]	0.88[b]	−0.16	0.91
σ_m^0	0.68	0.33	−0.07	0.48
σ_p^0	0.84[b]	0.17[b]	−0.12	0.54

[a] Reference 11. [b] Value for aqueous media.

86 000-fold in 0.2 N NaOD, but only 75-fold at pD 9.5; further, the nitro group is 7100-fold as effective as fluorine in promoting exchange at C-5 in 0.2 N NaOD, but only seven times as effective at pD 9.5. On the basis of the four substituents (including H) for which kinetic data has thus far been obtained, values of log $k_{C(5)}$ provide an acceptable Hammett correlation with aromatic σ_p^0 (Figure 3A); values of log $k_{Y(5)}$, on the other hand, correlate best with σ_o^0 (Figure 4A). In the latter scale, the contribution of σ^I is doubled[11] and, presumably, the change to the σ_o^0 scale is related to the presence of positive charge in the kinetically active species for ylide exchange. The correlation with full σ^0 ($\sigma^I + \sigma^R$) for both pathways shows that the kinetic acidity of the proton is determined by the *net* electron density at C-5. The magnitudes of the ρ values (Table II) show a high degree of sensitivity to electronic effects, paralleling those generally observed at an sp^2 carbon of the benzene ring.

In **1c** and **1d**, exchange at C-2 occurs overwhelmingly by the Y pathway; in fact, any contribution due to C exchange is indiscernible even in 1 N base. Introduction of electronegative

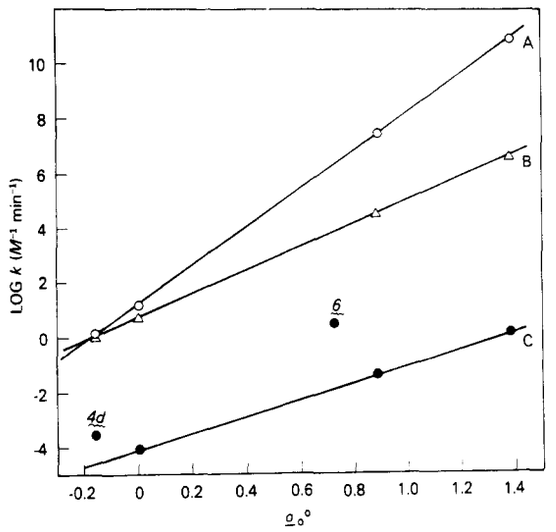


Figure 4. Hammett correlations of σ_o^0 for X vs. log k: A, series **1**, log $k_{Y(5)}$; B, series **2**, log $k_{Y(4)}$; C, series **4**, log $k_{C(5)}$.

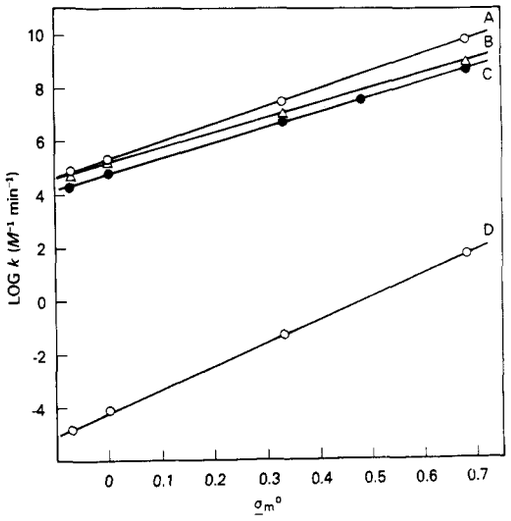


Figure 5. Hammett correlations of σ_m^0 for X vs. log k: A, series **1**, log $k_{Y(2)}$; B, series **2**, log $k_{Y(2)}$; C, series **4**, log $k_{Y(2)}$; D, series **5**, log $k_{C(5)}$.

substituents at C-4, however, markedly depresses $k_{Y(2)obsd}$; evidently, the reduction in pK_1 is more critical than inductive activation of H-2 by the group at C-4. Although $k_{Y(2)obsd}$ *decreases* with increasing electron withdrawal (Table II), $k_{Y(2)}$ (which takes account of the variations in K_1 and, thus, in [ImD$^+$]) shows an order consistent with electron withdrawal. Values of log $k_{Y(2)}$ correlate with σ_m^0 (Figure 5A). We were initially puzzled by the fact that values of $k_{Y(obsd)}$ for the two ring protons in series 1 show opposing trends; this phenomenon, however, is simply a consequence of the greater electron-withdrawing effect of 4-X at C-5 than at C-2. Electron withdrawal by the nitro and fluoro groups results in measurable C(2) exchange; log $k_{C(2)}$ may follow the σ_m^0 scale, as does log $k_{Y(2)}$, although only two experimental points are currently available. On the basis of these two points, $k_{C(2)obsd}$ for 1-methylimidazole (in 1 N NaOD at 50 °C) should be almost 10^6 slower than $k_{Y(2)obsd}$. For X = NO_2, H-5 is 52-fold as reactive as H-2 in the C pathway and 12-fold as reactive in the Y pathway. The lower reactivity at C-2 relative to C-5 is due to the greater distance between X and the proton undergoing exchange and, perhaps, to a partial ALP inhibition of carbanion formation at C-2.

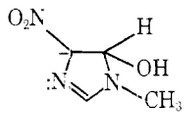
1-Methyl-5-X-imidazoles (Series 2). The magnitude of

the ALP effect at C-4 is strikingly evident in this series, since a C(4) pathway is not observed, *even* with a nitro group at C-5. Slow exchange via the Y(4) pathway is observed, however, and the substituent effect correlates with σ_o^0 (Figure 4B), as in series **1**. Interestingly, the ρ value is 2.8 units less than for series **1**, a factor which may result from the different sites of N-protonation relative to the substituent.

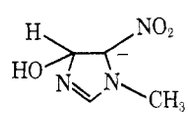
As in series **1**, the C(2) pathway can be observed only for X = NO_2 or F. The 5-nitro group is 3.5-fold as effective as 4-nitro in enhancing the acidity of H-2, possibly due to "para" resonance withdrawal in the former case; to our surprise, however, the 5-fluoro group is 1200-fold as effective as 4-fluoro. Hopefully, rate data for additional members of both series will help explain this unusual order of enhancements, which suggests that the magnitudes (or pathways) of electronic transmission from C-4 and C-5 to C-2 are significantly different; the nonequivalence in $J_{4(F)2(H)}$ and $J_{5(F)2(H)}$ has been noted earlier.[10] For series **2**, $k_{Y(2)}$ is consistently lower than for series **1**, while both series provide acceptable correlations of log $k_{Y(2)}$ with σ_m^0 (Figures 5B and 5A, respectively). The effect of higher pK_1 values in series **2** over series **1**[12] is seen in the values of $k_{Y(2)obsd}$, which are 94-fold greater for X = NO_2 and 40-fold for X = F.

1-Methyl-2-X-imidazoles (Series 3). C(5) exchange in **3a** is 13-fold slower than C(2) exchange in **2a** and 550-fold slower in **3b** than in **2b**. Presumably, the enhanced acidity at C-2 results from the extra inductive effect of N-3 and/or other factors (see Discussion); in addition, electronic transmission from X-5 to C-2 may be stronger than from X-2 to C-5, for reasons not yet obvious. In any case, it is clear that, if *any* ALP effect exists at C-2, it is considerably weaker than at C-4. Compound **3b** (X = F) is only 2.4-fold as reactive as **3c** (X = H) in C(5) exchange, and a Hammett correlation for this series can be achieved only with σ_p^0 (Figure 3B). It is noteworthy that σ_p^0 provides the best correlation for the two cases in which carbanion formation is required at C-5. This σ^0 scale does not hold for Y(5) exchange in series **1** or for C(5) exchange in the corresponding NH–imidazoles (see below); presumably, the *N*-methyl group serves to reduce electron density at C-5. Y(5) exchange cannot be detected in **3a** or **3b**, due to the combined effect of low pK and the distance of the substituent from the reaction site. For the same reasons, Y(4) exchange is not seen for either compound, while C(4) exchange is not detected for any member of the series because of the ALP effect. Based on the data for **3c** and **3d**, we estimate $t_{1/2} \simeq 1$ year (50 °C) for Y(5) exchange in **3a**, and even longer at C-4. Similar estimates suggest that Y(5) exchange should be reasonably observable for **3b**. Although the compound is sufficiently stable in 1 N NaOD (100 °C) to exhibit C(5) exchange, it decomposes too rapidly at pD 7–11 (50 °C) to provide rate data for Y(5) exchange. The instability of **3b** in the lower pD range arises from the fact that displacement of the 2-fluoro group occurs only when the ring is protonated.[13]

Instability of *N*-Alkylnitroimidazoles. Compounds **1a**, **2a**, and **3a** decompose in alkaline media, the rates of breakdown rising sharply with base concentration and with temperature. Under comparable conditions, **1a** and **3a** are 50–150-fold, respectively, more stable than **2a**. We consider the first step in breakdown of **1a** and **2a** to involve β addition of hydroxide ion to the 4,5-double bond, leading to the adducts **1a′** and **2a′**, respectively. The greater stability of **1a** may lie, therefore, in the fact that **1a′** cannot form as readily, being subject to an ALP effect not present in **2a′**. The onset of breakdown is readily detected by the appearance of new NMR signals; the multiplicity of the signals and their transience, however, prevented any speculation on the structures of intermediates. Ultimately, the *N*-methyl signal is lost completely, apparently by evaporation of methylamine. The breakdown of **3a** in base may involve an addition–elimination mechanism at C-2 but, in contrast with the behavior of **3b**, the nitro compound is stable at neutral pD. Apparently, the nitro group is sufficiently electron withdrawing to induce base attack on the neutral molecule, while ring protonation of the 2-fluoroimidazole is necessary to achieve adequate electron deficiency at C-2. A detailed study of these dual pathways is in progress.



1a′

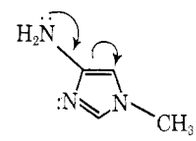


2a′

We have ignored consideration of isotope exchange via addition–elimination mechanisms, in which OD^- adds to the carbon atom carrying the electronegative group. Since nitro and fluoro are far better leaving groups than hydroxyl, it seems highly unlikely that the addition intermediates would revert to the starting compounds. Furthermore, such pathways cannot be considered for X = H or CH_3, and a duality of pathways is inconsistent with the linearity of the Hammett correlations.

Chemical Transformation. Although we had little reason to question the identity of the protons undergoing fast and slow exchange in **1a** and **2a**, chemical transformation provided a means for additional verification. Compound **1a** was converted to **1a**-d_2 by exhaustive exchange in 0.1 N NaOD (100 °C); the more labile deuterium atom was then back-exchanged in 0.1 N NaOH, and the resulting **1a**-*d* was converted into **1b**-*d* by zinc reduction, diazotization, and irradiation in fluoroboric acid. Since the product showed J_{HF} = 8.0 Hz, the hydrogen atom in **1b**-*d* must be adjacent to fluorine and, therefore, H-5 must be the more acidic proton.

Under the same exchange conditions, **2a** gave only a monodeuterated product, but the conversion of **2a** to **2b** has defied repeated efforts. Even when the intermediate 5-amino-1-methylimidazole (**9**) was generated from its stable *tert*-butoxycarbonyl derivative in fluoroboric acid, it failed to provide **2b** after diazotization and irradiation. Ultraviolet spectral analysis showed only traces of a diazonium chromophore after addition of nitrite, indicating **9** to be extremely unstable. The ALP effect may be operating to retard vinylamine resonance in **9**, but should have no effect in **8** and may even enhance resonance overlap in the latter case.[14,15]



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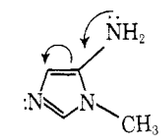


9

4-X-Imidazoles (Series 4). Kinetic analyses of isotopic exchanges in the NH–imidazoles must take account of ionization to their anions in alkaline media. Since the latter species appear to be resistant to exchange in the temperature range investigated, values of total k_{obsd} were adjusted for the fraction of NH species present in each medium, based on the pK_2 values given in Table III; specific rate constants were then calculated as for the *N*-methylimidazoles. It is assumed that the ALP effect is operative throughout the series and, therefore, that the 4-X tautomer is the only (or more) reactive species. Arguments have been advanced[16] that the 4-X tautomer is thermodynamically preferred for most substituents. Exchange at C-5 occurs predominantly by the C pathway, values of log $k_{C(5)}$ correlating with σ_o^0 (Figure 4C); this result stands in contrast with the σ_p^0 correlation required for the corresponding exchange in series **1**. Electronegative substitution has a stronger enhancement effect in this series than in series **1**, a factor which may again be due to the absence of

the N-methyl group. Figure 4C shows 4-methylimidazole to have an anomalously high rate of C(5) exchange, a phenomenon also observed with 2-fluoro-4-alkylimidazoles (see below). Y(5) exchange is apparently too slow to be measured for **4a** or **4b**; on the basis of the data obtained for **4c** and **4d** (Table II), we estimate the half-time for exchange of H-5 in **4a** (D_2O, 100 °C) at 5 years![17]

Carbanion exchange at C-2 could not be detected for any member of this series, while Y(2) exchange does occur and can be correlated with σ_m^0 (Figure 5C).[17] Values of $k_{Y(2)}$ are fairly similar to those for series **1** and the ρ values differ by 1.2 units.

2-X-Imidazoles (Series 5). Carbanion exchange at C-5 was observed for all members of the series, and log $k_{C(5)}$ values correlate with σ_m^0 (Figure 5D). Values of k_{obsd} for 2-X-imidazoles are lower than those for the 4-X series; after adjustment for NH ionization, however, values of $k_{C(5)}$ for the former series are impressive, that for **5a** being 43-fold that for **4a** and ~5000 times as great as for **3a**. This puzzling result is also observed with X = F, since **5b** is 1000-fold as reactive as **3b**. As in the case of series **4**, Y(5) exchange was not observed for **5a** or **5b**.

4-Alkylimidazoles. This series of studies had been undertaken originally in an attempt to account for the surprisingly facile tritium exchange at C-5 in 2-fluorohistidine (**7**); e.g., at pH 9 (50 °C) this compound exchanges 800-fold faster than does 2-fluoroimidazole. The complex pH dependence for exchange (Figure 6) is inconsistent with simple C or Y pathways, and suggests a role for an additional ionizing group. Indeed, the results are wholly in accord with C exchange involving a combination of hydroxide ion catalysis and intramolecular general base catalysis by the side-chain primary amine function.

$$k_{obsd} = \{k_C[OH^-] + k'_C[f_{RNH_2}]\}f_{Im} \quad (2)$$

In this rate expression, f_{RNH_2} = fraction of α-amino group in the unprotonated form (pK 8.85) and f_{Im} = fraction of neutral imidazole species (pK_2 10.55); k'_C is the specific rate constant for intramolecular general base catalysis of carbanion formation. An approximate value for k'_C was obtained by assuming the contribution of $k_C[OH^-]$ to k_{obsd} to be very small at the lower pH values. Curve-fitting was then performed by approximation, providing the values of $k'_C = 1.58 \times 10^{-4}$ min^{-1} (30 °C) and $k_C = 0.33$ M^{-1} min^{-1} (30 °C). For comparison with the data for other compounds, these values were adjusted to 50 °C (Table II), taking E_a = 21 kcal/mol. These comparisons have limited validity, since H/D and H/T isotope effects have not been evaluated. The rate of tritium exchange is enhanced in the presence of carbonate buffer; e.g., at pH 9.2 (0.1 M buffer), k_{obsd} is increased almost threefold.

After taking account of the contribution of an intramolecular pathway,[20] we find that k_C for H-5 in 2-fluorohistidine is still 50-fold greater than that for 2-fluoroimidazole. We were led, therefore, to examine the simpler analogue, 2-fluoro-4-methylimidazole (**6**); this compound also showed an unusually high value for $k_{C(5)}$, the latter being 60 times that for 2-fluoroimidazole and 250 times the predicted value (Figure 2C) based on $\Sigma\sigma^0$.

We have noted that k_{obsd} for C(5) exchange in 4-methylimidazole (**4d**) is also anomalously high, being *ca.* fourfold greater than the same exchange in imidazole and 21-fold greater than in 2-methylimidazole. For this compound, $k_{C(5)}$ is 10 times as great as the value predicted from Figure 4C. These three examples (**4d, 6,** and **7**) demonstrate that an alkyl group at C-4 provides a significant enhancement effect on C(5) exchange. There seems no obvious way for an alkyl group to stabilize an adjacent carbanion; therefore, we tentatively suggest an alternative pathway for exchange, via the still undetected tautomer, **10**.[19] It is noteworthy that rate enhancement is not seen with 1,4-dimethylimidazole, in which compound such tautomerism cannot occur.

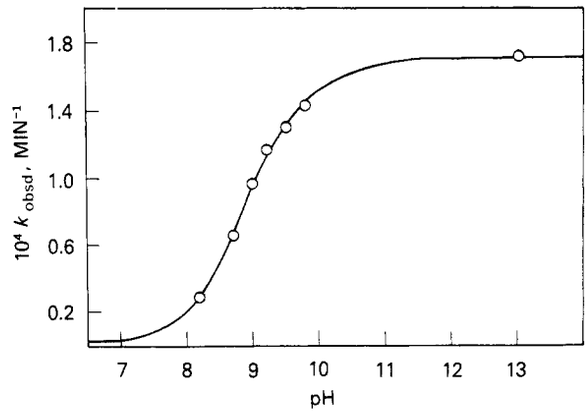
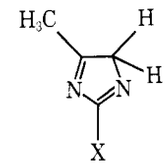


Figure 6. Dependence of total k_{obsd} on pH for loss of tritium from 2-fluorohistidine-5-^3H in H_2O at 30 °C: O, experimental values; —, curve calculated from eq 2 and specific rate constants cited in text.



10

Buffer Catalysis. Since the Y mechanism for exchange involves the attack of a base on the imidazolium ion, it is ideally suited for catalysis by buffer species. We have already reported that exchange of H-2 in N-methylimidazole is catalyzed by acetate buffer.[2] Tritium incorporation at H-2 of histidine is also promoted by phosphate and Tris buffers, these findings having been applied for preparative purposes.[20] Labeling at C-2 in 4-fluoroimidazole occurs at pD 3–10 by the Y pathway, with $t_{1/2}$ = 1200 h at 50 °C or 15 h at 100 °C; the exchange is even slower in more acidic or more alkaline media. Since pK_1 for **4b** is 2.44, chloroacetic acid (pK 2.88) was chosen for possible catalysis of Y exchange; in 1 M buffer (pD 2.44, 50 °C), a 32-fold enhancement was obtained. The same buffer system was then used to achieve tritium labeling at C-2 in 4-fluorohistidine under very mild and practical conditions.

In the chloroacetate buffer medium, exchange of H-5 in fluoroimidazole is also accelerated ($t_{1/2}$ = 13 h at 50 °C). In the absence of buffer, Y(5) exchange could not be observed at any pD; if the buffer species were catalyzing the Y pathway, extrapolation from the values of $k_{Y(5)}$ for **4c** and **4d** suggests a buffer enhancement factor for **4b** of 40 000! Since this factor seems unreasonably large, it may be the C(5) pathway which is being catalyzed by chloroacetate ion, providing a tenfold enhancement at pD 2.44 over k_{obsd} in 0.1 N NaOD; pending the acquisition of additional kinetic data, however, the role of the buffer catalyst at C-5 remains uncertain. Data were presented above for the intramolecular general base catalysis of C exchange in 2-fluorohistidine and, thus, it appears that both the C and Y pathways are sensitive to buffer catalysis.

Other Substituted Imidazoles. Studies with **4f** at pD 10 provided a value for Y(2) exchange (Table II and Figure 3C); however, the compound decomposes too rapidly in more alkaline media to provide data for C(2) exchange. The carbethoxyimidazoles (**1e, 2e,** and **3e**) failed to show Y exchange at 50 °C (pD 7–10); at 100 °C, ester hydrolysis occurred too rapidly to provide usable data.

Discussion

Certain of the k_Y values in Table II are close to the range for diffusion-controlled reactions.[21] Thus, $k_{Y(5)}$ for **1a** = 7.76

Table V. Half-Times for Exchange in 1-Methylimidazoles at 50 °C

	0.1 N NaOD			pD 9–10		
	H-2	H-4	H-5	H-2	H-4	H-5
none	42 min	2.5 yr	138 days	42 min	2.5 yr	1 yr
4-nitro	2.7 h		3 min	55 days		4.5 days
5-nitro	44 min	>2 yr		14 h	132 days	
2-nitro		>2 yr	10 h		>2 yr	>2 yr
4-fluoro	33 days		12 days	38 days		33 days
5-fluoro	3.5 h	>2 yr		23 h	285 days	
2-fluoro		>2 yr	97 days		>2 yr	>2 yr

$\times 10^{10}$ M^{-1} min^{-1} at 50 °C or 8.33×10^{7} M^{-1} s^{-1} at 25 °C. This rate constant for base-catalyzed formation of the vinyl carbanion is ca. $1/50$ of the k_{OH} value for proton loss from HCN.[22] Considering that C-5 in **1a** is subjected to the combined electron demands of the 4-nitro group, two ring nitrogen atoms, *and* a positive charge in the ring, a total electronegativity approaching that of the triply-bonded nitrogen in HCN is not unreasonable. Furthermore, in their review on base-catalyzed proton exchange in heterocycles,[23] Elvidge et al. have argued that, because vinyl carbanions are usually not resonance stabilized, their kinetic acidities should be compared with those of oxygen acids rather than those of the common carbon acids.

The kinetic results with nitro- and fluoroimidazoles (Table II, Figure 1) have clearly shown the existence of significant carbanion-mediated exchange at C-2. In view of the powerful ALP effect of N-3 in preventing carbanion formation at C-4, it is somewhat surprising that a C(2) pathway can be observed at all. We might argue that electron withdrawal by *two* ring nitrogen atoms can partially counteract the ALP effect at C-2; yet, it seems unreasonable that the magnitude of such withdrawal could so greatly exceed the combined electronegativities of N-3 and a 5-nitro group operating on C-4. Very strong bases (e.g., butyllithium in tetrahydrofuran) abstract H-2 from *N*-alkylimidazoles with essentially total specificity.[24] This fact appears to support the absence of a significant ALP effect at C-2; yet, we cannot rule out the possibility that proton abstraction is preceded by coordination of the lithium atom with the lone pair at N-3 and, thus, occurs by a Y rather than C pathway. It is also noteworthy that, in the presence of methoxide ion, C-2 in pyrimidine is the *least* acidic position in the ring;[25] this carbon atom is also flanked by two nitrogen atoms, but the corresponding carbanion would be subject to two ALP interactions.

It is also conceivable that the sp^2 carbanion at C-2 is electronically different from that at C-4 or that the imidazole ring becomes partially deformed from planarity when H-2 is lost, thus reducing lone-pair repulsion. Alternatively, we may invoke greater s character (hence, greater acidity) in the C(2)–H bond than in that at C-4;[25] this explanation is supported both by crystal structure data for imidazole[26] and by ^{13}C–^{1}H coupling constants.[27] At best, however, orbital interactions through bonds or space are not yet well understood,[28] and the imidazole case clearly demands further study.

These studies have demonstrated that both ylide and carbanion exchange in substituted imidazoles follow reasonably logical, but complex, patterns. Although we fully recognize that the Hammett correlations (based on four points) have only limited reliability, they have proved useful in predicting the conditions necessary to observe exchange with other substituted imidazoles. Further studies are in progress and, hopefully, the use of all three σ^0 scales will be supported with additional kinetic data. In addition to the large difference in ALP effect between C-2 and C-4, several phenomena have emerged which merit further exploration: (1) the enhancement effect of 4-alkyl substituents; (2) intramolecular general base catalysis in 2-fluorohistidine; and (3) buffer catalysis of both the C and Y pathways. Other surprising results have been obtained in studies of acid-catalyzed exchange; these results will be reported separately.

A wide variety of ring-substituted histamines and histidines have been prepared for biological studies (in progress). On the basis of the results herein reported, random or site-specific tritium labeling of the imidazole ring in these compounds has become attainable in practice. The very large spread in half-times for exchange (see examples in Table V) permits highly specific labeling in many cases. For poorly exchangeable protons, exchange is also attainable by the use of elevated temperatures or buffer catalysis; the optimum pH for such catalysis can be predicted from the pK value of the compound and the appropriate Hammett plot (Figures 3–5).

Experimental Section[29]

Materials. The following compounds were synthesized by known methods: **1a**,[30] **1d**,[2] **2a**,[30] **2d**,[2] **3a**,[31] **3e**,[32] **4b**,[33] **4d**,[2] **4e**,[34] **4f**,[35] **5a**,[36] **5b**,[33] and **7**.[13] Imidazole, 1-methylimidazole, 2-methylimidazole, 1,2-dimethylimidazole, and 4-nitroimidazole were obtained from commercial sources.

4-Fluoro-1-methylimidazole (1b). A solution of 5.08 g (0.04 mol) of **1a** in 120 mL of 48% aqueous fluoroboric acid was chilled to −10 to −15 °C with dry ice–acetone and 9.15 g (0.14 atom) of zinc powder was added over 30 min with stirring. At this point, the UV spectrum of the reaction mixture (measured on a small aliquot diluted with water) showed total loss of the nitro chromophore. The mixture was filtered through glass wool, and a solution of 3.2 g (0.048 mol) of sodium nitrite in 20 mL of water was added with stirring over 20 min at −10 °C. The solution was purged with nitrogen and was irradiated for 5 h by the procedure described previously.[33] The fluoroboric acid solution was then neutralized to pH 8 with concentrated sodium hydroxide (cold) and was subjected to continuous extraction with ethyl acetate for 48 h. The extract was evaporated to give a semisolid residue, which was chromatographed on 150 g of silica gel. Elution with ethyl acetate–ether (1:1) gave 1.0 g (25%) of **1b** as a pale yellow semisolid; NMR ($CDCl_3$) δ 3.66 (3 H, d, CH_3), 6.43 (1 H, q, H-5), 7.04 (1 H, m, H-2); $J_{4,5} = 8.0$, $J_{2,4} = 1.8$, and $J_{2,5} \simeq 1$ Hz.

The same compound was obtained by direct methylation of 4-fluoroimidazole with methyl iodide or dimethyl sulfate, using standard procedures.

4-Fluoro-1-methylimidazole-*d* (1b-*d*). 1-Methyl-4-nitroimidazole (0.5 g) was added to 50 mL of 0.1 N NaOD and the mixture was stirred at ambient temperature. When solution was complete (~15 min), NMR showed one proton to have exchanged completely. The solution was then heated at 100 °C for 1.5 h, at which point the remaining proton had exchanged completely. This product was isolated by extraction with ethyl acetate and the more labile deuterium atom washed out by exposure to 0.1 N NaOH for 15 min. The monodeuterio compound was converted to 4-fluoro-1-methylimidazole-*d* by the procedure described above. Since this product showed $J_{H,F} = 8.0$ Hz, the deuterium atom must be at C-2, and the very labile hydrogen atom in **1a** must be that at C-5.

5-Fluoro-1-methylimidazole (2b). Direct methylation of 4-fluoroimidazole with methyl iodide or dimethyl sulfate, under neutral or basic conditions, and in polar or nonpolar media, gave **1b** exclusively. Repeated efforts to prepare **2b** from **2a**, following the reduction–irradiation procedure used for the conversion of **1a** to **2a**, failed completely. Presumably, the intermediate 5-amino-1-methylimidazole is very short-lived, even at the low temperature of reduction. Alternatively, 5-amino-1-methylimidazole (**9**) was generated in fluoroboric acid solution from its *tert*-butoxycarbonyl derivative (see below), but again failed to produce **2b**. The only successful approach,

which follows, depends on a S_N1 rather than the common S_N2 pathway for nitrogen alkylation.

To a solution of 0.129 g (1.5 mmol) of 4-fluoroimidazole (**4b**) in 15 mL of dry acetonitrile was added a solution of 0.125 mL (2 mmol) of methyl iodide in 2 mL of acetonitrile, followed by portionwise addition of 0.414 g (2 mmol) of silver perchlorate. The mixture was stirred 1 h, another 0.125 mL of methyl iodide was added, and stirring was continued another hour at 40 °C. Two more portions of methyl iodide were added, with stirring for 1 h at 40 °C after each addition. The mixture was filtered and the filtrate was concentrated to a semisolid. This material was dissolved in 30 mL of ethyl acetate, the solution was washed with two 10-mL portions of saturated sodium bicarbonate, dried (Na_2SO_4), and evaporated to a colorless semisolid, 0.103 g (69%) of **2b**. Crystallization of the product from chloroform gave needles: mp 87–88 °C; NMR ($CDCl_3$) δ 3.62 (3 H, s, CH_3), 6.57 (1 H, d, H-4), and 7.42 (1 H, br, H-2); $J_{4,5}$ = 7.5, $J_{2,4}$ = 1.0, and $J_{2,5} \simeq 0$ Hz.

2-Fluoro-1-methylimidazole (3b). A. To a solution of 2-amino-1-methylimidazole (bisulfate)[37] (3.65 g, 0.025 mol) in 150 mL of 48% fluoroboric acid was added a solution of 1.90 g (0.0275 mol) of sodium nitrite in 5 mL of water, over 10 min with stirring and ice cooling. The mixture was irradiated for 3 h, at which point the diazonium chromophore at 306 nm had disappeared. The reaction mixture was neutralized with concentrated NaOH to pH [] (dry ice cooling); the solution was then extracted with five 60-mL portions of ether. The combined extracts were dried ($MgSO_4$) and evaporated to a semisolid residue. Chromatography on 150 g of silica gel and elution with chloroform (2% ethanol) gave **3b** as a pale yellow liquid: 0.87 g (35%); NMR ($CDCl_3$) δ 3.56 (3 H, s, CH_3), 6.67 (1 H, s, H-4), 6.67 (1 H, s, H-5); $J_{4,5}$ = 1.6, $J_{2,4}$ = 1.6, and $J_{2,5} \simeq 0$ Hz.

B. Direct methylation of 2-fluoroimidazole with dimethyl sulfate gave only the 1,3-dimethylimidazolium species, which underwent rapid loss of fluorine by solvolysis. The product was identified as 1,3-dimethyl-2-imidazolone.

N-Methylation of Ethyl Imidazole-4-carboxylate. To a solution of 4.20 g (0.03 mol) of **4e**[34] in 25 mL of methanol was added a solution of 8.52 g (0.06 mol) of methyl iodide in 10 mL of methanol, and the mixture was heated at reflux for 8 h. Evaporation of solvent gave a brown oil which was chromatographed on 120 g of silicic acid. Elution with chloroform (1.5% methanol) gave 1.82 g (40%) of **2e** as a pale yellow oil; NMR ($CDCl_3$) δ 1.38 (3 H, t, $CH_2\mathbf{CH_3}$), 3.96 (3 H, s, N-CH_3), 4.36 (2 H, q, $\mathbf{CH_2}CH_3$), 7.63 (1 H, m, H-2), 7.79 (1 H, d, H-4). Continued elution with the same solvent gave 0.22 g (5%) of **1e** as a pale yellow oil; NMR ($CDCl_3$) δ 1.37 (3 H, t, $CH_2\mathbf{CH_3}$), 3.81 (3 H, s, N-CH_3), 4.38 (2 H, q, $\mathbf{CH_2}CH_3$), 7.56 (1 H, m, H-2), 7.66 (1 H, d, H-5).

1-Methylimidazole-5-carbohydrazide. A solution of 2.31 g (0.015 mol) of **2e** in 5 mL of hydrazine hydrate was heated at 100 °C for 1 h. The solution was concentrated to ~2 mL under reduced pressure and chilled, giving 1.71 g (81%) of colorless prisms, mp 187–187.5 °C. Further concentration of the filtrate gave an additional 0.32 g (15%) of a less pure material.

***tert*-Butyl 1-Methylimidazole-5-carbamate.** To a solution of 1.40 g (0.01 mol) of 1-methylimidazole-5-carbohydrazide in 6 mL of water and 2 mL of concentrated hydrochloric acid was added dropwise over 10 min, with stirring at 0 °C, a solution of 1.04 g (0.015 mol) of sodium nitrite in 2 mL of water. The mixture was stirred 20 min at 0°, neutralized to pH 7 with 10% sodium hydroxide, and extracted with five 10-mL portions of ethyl acetate. The combined extracts were dried (Na_2SO_4) and evaporated to a pale brown semisolid, 1.41 g (93%). The acyl azide is unstable and was used immediately for the next step.

The total yield of crude azide was added to 20 mL of dry *tert*-butyl alcohol and the solution was heated at reflux for 2.5 h.[33] Evaporation of solvent gave a yellow solid which was crystallized twice from ethyl acetate and once from methanol to give 1.49 g (81%) of colorless leaflets, mp 173 °C.

Anal. Calcd for $C_9H_{15}N_3O_2$: C, 54.80; H, 7.67; N, 21.30. Found: C, 54.25; H, 7.28; N, 21.73.

This product was used to generate 5-amino-1-methylimidazole in fluoroboric acid solution. The aminoimidazole, however, failed to give **2b** when processed in a manner similar to that for the synthesis of **4b**.

2-Fluoro-4-methylimidazole (6). This compound was prepared from crude 2-amino-4-methylimidazole,[37] using the procedure and the scale described above for the preparation of **3b**. Total disappearance of the diazonium chromophore at 320 nm required irradiation for 1.5 h. The fluoroboric acid solution was neutralized to pH 7 (cold) and was extracted with five 100-mL portions of ethyl acetate. The combined extracts were dried (Na_2SO_4) and concentrated to a semisolid; chromatography on 59 g of silica gel and elution with ether gave a colorless powder, which was sublimed and recrystallized from ligroin–ether (4:1): mp 81–81.5 °C (10% yield based on aminoacetone hydrochloride hydrate, the precursor of 2-amino-4-methylimidazole); NMR ($CDCl_3$) δ 2.20 (3 H, t, CH_3), 6.40 (1 H, m, H-4 or H-5); $J_{2,4(5)}$ = 1.3 Hz.

Anal. Calcd for $C_4H_5N_2F$: C, 47.99; H, 5.03; N, 27.99; F, 18.98. Found: C, 47.87; H, 5.12; N, 28.77; F, 18.68.

2-Fluoro-L-histidine-5-^3H. To a solution of 75 mg of 2-fluoro-L-histidine (**7**) in 1 mL of tritiated water (5.0 Ci) was added 100 μL of triethylamine. The solution was stirred at ambient temperature for 4.5 days and was lyophilized. Normal water was added and the lyophilization repeated. The residue was treated with methanol and the solvent evaporated. Finally, the material was triturated with a small volume of cold methanol and filtered to give 32.5 mg of crystalline material with a specific activity of 40 mCi/mmol.

Tritium Loss From 2-Fluoro-L-histidine-5-^3H. A stock solution of 4.9 mg/mL of water of the labeled compound was prepared with specific acitvity of 3.9 μCi/μol. A 50-μL aliquot was added to 5.0 mL of 0.1 KCl. The pH was adjusted to the desired level with 0.05 N NaOH and was maintained at that level throughout the run by use of a Radiometer autoburette (Model ABU 12). The temperature was maintained at 30 °C by circulation of water from a Haake water bath through the jacketed reaction vessel. A slurry of one part Dowex 50 H^+x 8 (200–400 mesh) and three parts water was prepared; 1-mL aliquots of the slurry were added to Pasteur pipettes which had been loosely plugged with glass wool, and the columns were washed with water until the effluent was neutral. At various time intervals, 100-μL aliquots of the reaction mixture were transferred to the Dowex columns, the columns were washed with 5 × 0.5 mL of water, and the total effluent from each column was counted with a Perkin-Elmer liquid scintillation counter (Model 3375). Initial rates (up to ~10% exchange) were used to determine rate constants; initial and subsequent radioactivity counts were taken as measures of concentration of unreacted substrate.

pK Measurements. pK values were obtained for the new compounds and for others for which data were unavailable or literature values were in doubt. pK values were calculated from pH measurements in water at 25 °C (Corning pH meter, Model 101). Samples of 20–40 mg were used, and seven to ten aliquots of acid or base added. pK values were calculated for each addition and averaged to give the values in Table III; deviations were usually <0.10 unit. The effect of temperature on pK was determined (up to 70 °C) for several compounds by following the change in pH of a half-neutralized solution. The averaged results were considered applicable to all compounds in the study: for pK_1, $pK(50\ °C) = pK(25\ °C) - 0.50$ and $pK(100\ °C) = pK(25\ °C) - 0.30$.[38] Values of $pK(D_2O, 25\ °C)$ were calculated from the relationship $pK(D_2O) = 1.018\ pK(H_2O) + 0.43$ (Table III, footnote *d*). Temperature effects on $pK(D_2O)$ were assumed comparable to those in H_2O. For $pK_w(D_2O, 50\ °C)$, 14.18 was used;[39] for 100 °C, pK_w = 13.13 was estimated by extrapolation.

Kinetic Measurements. The techniques used to follow rates of exchange by NMR spectroscopy are described in the previous paper.[2] For series **4** and **5**, δ values are shifted in alkaline media, and may even become inverted in order. Upon completion of an exchange run, the solution was neutralized and the NMR spectrum compared with that of the original compound; since **4a** and **5a** are insoluble in water, the neutralized mixtures were saturated with NaCl and the compounds were extracted into Me_2SO-d_6 prior to spectral comparison. For C exchange, rate constants were obtained at three or four concentrations of NaOD, and k_C determined as the slope of a plot of $k_{C(obsd)}$ vs. [OD^-]. Ylide exchange was measured in D_2O solutions which were brought to pD 9.5–10 (25 °C) with 0.1 N NaOD. Specific rate constants for Y exchange were calculated according to eq 1.

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Registry No.—**1b** deuterium derivative, 23968-98-1; **1c**, 616-47-7; **1d**, 6338-45-0; **2d**, 10447-93-5; **3d**, 1739-84-0; **4b**, 30086-17-0; **4d**, 822-36-6; **4e**, 23785-21-9; **5d**, 693-98-1; **6**, 57212-35-8; **7**, 50444-78-5; **7** tritium derivative, 66787-71-1; 2-amino-1-methylimidazole (bisulfate), 66787-72-2; 1-methylimidazole-5-carbohydrazide, 23585-00-4; *tert*-butyl 1-methylimidazole-5-carbamate, 66787-73-3; 1-methylimidazole-5-methylazide, 66787-74-4; 5-amino-1-methylimidazole, 66787-75-5; 2-amino-4-methylimidazole, 6653-42-5.

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Spiro Meisenheimer Complexes from 7-(2-Hydroxyethoxy)-4-nitrobenzofurazan and 7-(2-Hydroxyethoxy)-4-nitrobenzofuroxan. A Kinetic Study in Aqueous Solution

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Cyclization of 7-(2-hydroxyethoxy)-4-nitrobenzofurazan (**3**) and 7-(2-hydroxyethoxy)-4-nitrobenzofurazan (**6**) occurs in aqueous solution containing base to give the spiro Meisenheimer-type complexes **5** and **8**, which have a high thermodynamic stability. A similar reaction occurs in Me_2SO where the structures of **5** and **8** could be fully characterized by ^1H NMR spectroscopy. The kinetics of formation and decomposition of **5** and **8** have been studied by the stopped-flow method between pH 1 and 12 in aqueous solution. It is found that **5** is only 2.5-fold more stable than **8** ($pK_a^5 = 6.86$; $pK_a^8 = 7.26$), but it forms and decomposes much faster than its furoxanic analogue. These differences in rates are attributed to the *N*-oxide group, which probably exerts a very unfavorable influence on the C–O bond-forming and bond-breaking processes associated with formation and decomposition of the furoxanic adduct **8**. The ring opening of **5** and **8** is subject to general acid catalysis in aqueous solution with a Brønsted coefficient α of 0.44. The results are discussed by comparison with those obtained for benzenic analogues.

The proposal[2–4] that the antileukemic activity of some benzofurazan and benzofuroxan derivatives may be due to their ability to easily form Meisenheimer-type complexes with essential cellular SH and/or amino groups has increased interest in the adducts obtained from covalent addition of nucleophiles to these compounds. There is now convincing structural evidence, mainly from NMR studies, that such adducts are formed in the reaction of a variety of mono- and dinitrobenzofurazans and -benzofuroxans with hydroxide and methoxide ions.[5–10] The thermodynamic and kinetic data for

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