^{19}F and ^{1}H Nuclear Magnetic Resonance Studies of Ring-fluorinated Imidazoles and Histidines

By **Herman J. C. Yeh, Kenneth L. Kirk,** and **Louis A. Cohen,*** Laboratory of Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014, U.S.A.

Jack S. Cohen, Reproductive Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20014, U.S.A.

^{1}H Titration curves (in D_2O) and ^{19}F titration curves (in H_2O) have been obtained for 2- and 4-fluoro-imidazole and -histidine. By use of computer-assisted curve fitting, pK values were derived for the several dissociation steps in these compounds. Fluoroimidazoles are weaker bases and stronger acids than bromoimidazoles, indicating that the inductive effect of the halogen greatly overshadows its resonance effect. Introduction of an alkyl group at C-5 displaces the F-4 signal upfield (as in *o*-fluorotoluene) but that of F-2 downfield, suggesting further that -4 and -5 are coupled both by induction and resonance while C-2 and -5 are coupled only by induction. Introduction of fluorine displaces the H-2 and -4 signals upfield, in parallel with the behaviour of fluorobenzene. The shielding effect of the magnetic anisotropy of ^{19}F evidently counteracts the deshielding effect of its electronegativity. Protonation of the imidazole ring causes the ^{19}F signal to shift downfield at F-2 but upfield at F-4; conversely, the formation of the imidazole anion produces an upfield shift at F-4 but downfield at F-2. In the fluorohistidines, there is some evidence for field perturbation of the ^{19}F signal by the charge on the amino-acid side chain; however, inconsistencies in the direction of signal displacement due to σ, π, and charge variation within the imidazole ring render difficult the analysis of possible field effects. These data are intended as a basis for extensive ^{19}F n.m.r. studies of fluorohistidines in polypeptides and proteins.

THE combination of ^{1}H n.m.r. and pH variation has been used extensively in studies of substances of biological interest.[1-3] In such studies, the chemical shifts of one or more *reporter* protons are used to monitor structural and electronic changes which may occur within a molecule as a consequence of the formation or neutralization of charge. One significant value of the method lies in the fact that while the reporter proton may be many bond lengths from the group being titrated, it may still be subject to Coulombic field perturbation. The imidazole ring is a group of wide occurrence and considerable importance in biological structure and function and is well suited to act as a built-in probe. In particular, the resonances of the C-2 and -4 ring protons differ significantly from those of most other protons in proteins, as well as from each other, and have been studied extensively by n.m.r. titration.[1c,2]

Changes in the net charge of titratable groups can perturb both the ionization equilibria of the imidazole ring and the chemical shifts of its protons. In an n.m.r. titration curve (*e.g.*, Figure 1), such change may produce a purely Coulombic effect, which would be reflected in a horizontal displacement of the curve, *i.e.*, a change in the pK value. Alternatively, changes in charge can result in electronic shielding or deshielding of the reporter proton, such perturbation being reflected in a vertical displacement of the n.m.r. titration curve. In general, the effect on the chemical shift of a proton perturbed by a change in nearby charge is quite small. For example, changes in the state of ionization of the primary amino- or carboxy-groups in histidine produce a change of $<0{\cdot}2$ p.p.m. in the chemical shift of either ring *C*-proton.[2] Nevertheless, the histidine ring protons have proved to be rather useful reporters of charge perturbation phenomena in proteins.[3]

It is possible to use other magnetic nuclei, such as ^{13}C and ^{19}F, as potentially more responsive reporters, since these nuclei are known to be more sensitive than the proton to environmental perturbation or electron density changes.[4] The carbon isotope, of course, should invoke almost no alteration in native structure of a protein while fluorine, by virtue of its small size, may sometimes perform almost as well. To this end, incorporation of suitably labelled amino-acids into new protein has been achieved biosynthetically;[5] in the average case, however, the multiplicity of the common amino-acids in a polypeptide sequence can lead to n.m.r. spectra with signals approaching those of the proton in number and complexity. The alternative technique of attaching a labelled reagent to a unique site of the protein by chemical modification invites the danger of significant conformational change.[6] Unfortunately, the total synthesis of a protein or protein fragment containing an n.m.r.-sensitive nucleus in a single and specific position is not readily accomplished with present methods,

[1] See, *e.g.* (*a*) S. S. Danyluk and F. E. Hruska, *Biochemistry*, 1968, **7**, 1038; (*b*) O. A. Gansow and R. H. Holm, *Tetrahedron*, 1968, **24**, 4477; (*c*) G. C. K. Roberts and O. Jardetzky, *Adv. Protein Chem.*, 1970, **24**, 447.

[2] (*a*) D. H. Sachs, A. N. Schechter, and J. S. Cohen, *J. Biol. Chem.*, 1971, **246**, 6576; (*b*) R. I. Shrager, J. S. Cohen, S. R. Heller, D. H. Sachs, and A. N. Schechter, *Biochemistry*, 1972, **11**, 541.

[3] (*a*) J. H. Griffin, J. S. Cohen, and A. N. Schechter, *Biochemistry*, 1973, **12**, 2096; (*b*) J. S. Cohen, W. R. Fisher, and A. N. Schechter, *J. Biol. Chem.*, 1974, **249**, 1113.

[4] (*a*) A. R. Quirt, J. R. Lyerla, jun., I. R. Peat, J. S. Cohen, W. F. Reynolds, and M. H. Freedman, *J. Amer. Chem. Soc.*, 1974, **96**, 570; (*b*) M. H. Freedman, J. R. Lyerla, jun., I. M. Chaiken, and J. S. Cohen, *Eur. J. Biochem.*, 1973, **32**, 215; (*c*) F. Millett and M. A. Raftery, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 625.

[5] (*a*) D. T. Browne, G. L. Kenyon, E. L. Packer, H. Sternlicht, and D. M. Wilson, *J. Amer. Chem. Soc.*, 1973, **95**, 1316; (*b*) M. W. Hunkapiller, S. H. Smallcombe, D. R. Whitaker, and J. H. Richards, *Biochemistry*, 1973, **12**, 4732; (*c*) B. D. Sykes, H. I. Weingarten, and M. J. Schlesinger, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 469.

[6] (*a*) L. A. Cohen, in ' The Enzymes,' ed. P. D. Boyer, Academic Press, New York, 1970, 3rd edn., ch. 3; (*b*) A. F. S. A. Habeeb, in ' Chemistry of the Cell Interface,' ed. H. D. Brown, Academic Press, New York, 1971, Part B, ch. 8.

although several specifically labelled oligopeptides have already been prepared by synthesis.[7]

Recently, a variety of ring-fluorinated imidazoles, including histamines and histidines, have become available by a photochemical synthesis.[8] Such compounds were expected to have significant application in biochemical and pharmacological studies, and early results have already justified these expectations.[7a,9] Of obvious interest is the incorporation of the fluorohistidines into polypeptides and proteins. Synthetic studies with 4-fluorohistidine in the ribonuclease series suggest that the introduction of fluorine may, in some cases, have little disruptive effect on the tertiary structure of the protein.[7a] Furthermore, preliminary results indicate that fluorohistidine is incorporated into new protein, both in animal and bacterial systems.

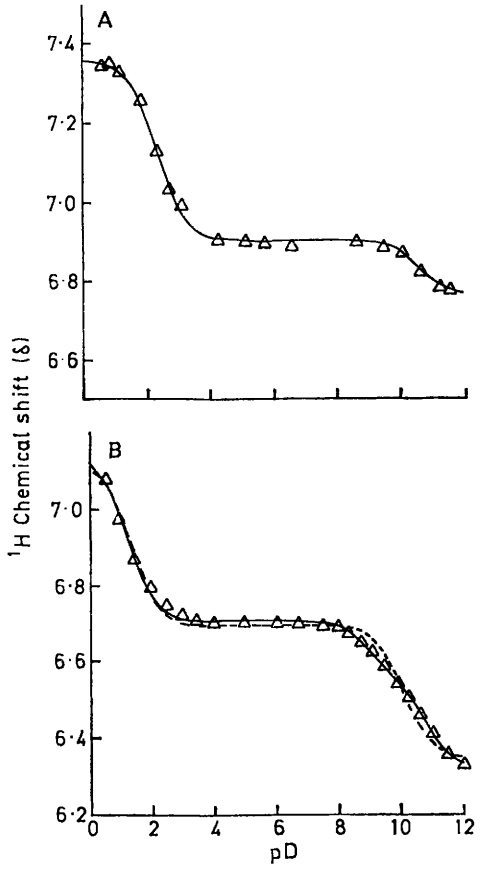
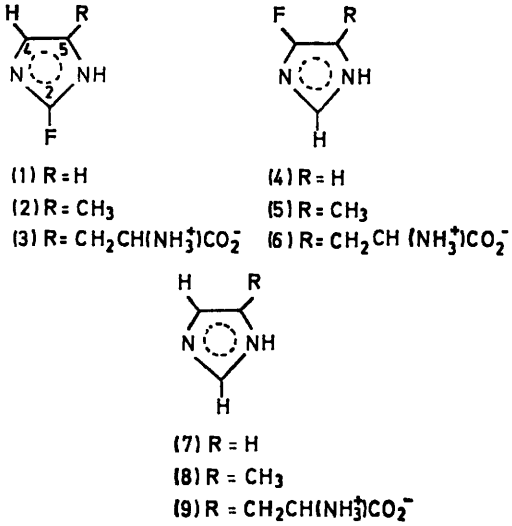


FIGURE 1 Variation of ^{1}H chemical shift (H-4) as a function of pD (uncorrected) for A, 2-fluoroimidazole and B, 2-fluorohistidine: △, experimental values of δ; lines, theoretical titration curves calculated by computer-assisted curve-fitting analysis,[2b] solid, three transitions; dashed, two transitions

In preparation for studies of polypeptides and proteins, ^{19}F and ^{1}H n.m.r. data have been obtained for 2-fluoroimidazole (1),[8b] 2-fluoro-5-methylimidazole (2),[10] 2-fluorohistidine (3),[8d] 4-fluoroimidazole (4),[8b] 4-fluoro-5-methylimidazole (5),[8c] and 4-fluorohistidine (6).[8b] The



effects of creation and neutralization of nearby formal charge on the ^{19}F and ^{1}H titration curves are presented and discussed. From such titration curves, pK values were calculated by computer simulation[2b] and are compared with several values obtained at the glass electrode. Patterns in the variation of chemical shift with electronic and Coulombic changes are summarized and discussed.

EXPERIMENTAL

N.m.r. Measurements.—^{1}H and ^{19}F n.m.r. spectra were obtained using a Varian HA-100 spectrometer operating at 100·00 and 94·077 MHz, respectively. A capillary containing 5% tetramethylsilane in carbon tetrachloride was centred in the sample tube and was used as an external ^{1}H lock and reference. For the ^{19}F n.m.r. measurements, either 10% C_6F_6 in bromoform or 10% $(CF_2CO_2H)_2$ in water was used as an external lock signal, depending on the frequency range being examined. The ^{19}F signal for $(CF_2CO_2H)_2$ is 50·4 p.p.m. downfield from C_6F_6; this value was introduced as necessary so that all ^{19}F chemical shifts are reported downfield from C_6F_6. No corrections were made for magnetic susceptibility. Room temperature was maintained at 25° while probe temperature was 30°.

Samples were 0·1M in D_2O (for ^{1}H spectra) or in H_2O (for ^{19}F spectra). Adjustments in pD were made with 1M-NaOD or DCl. pH and pD readings were made directly on a model TTT-1c Radiometer pH meter, equipped with a scale expander.

*p*K *Determinations.*—The pK values calculated from ^{1}H n.m.r. titration curves in D_2O [2b] (Figures 1 and 3) are based on measurement of apparent pD. Normally, such measurements require a correction of +0·40 units (at 25°) to obtain

[7] (*a*) B. M. Dunn, C. DiBello, K. L. Kirk, L. A. Cohen, and I. M. Chaiken, *J. Biol. Chem.*, 1974, **249**, 6295; (*b*) I. M. Chaiken, M. H. Freedman, J. R. Lyerla, jun., and J. S. Cohen, *ibid.*, 1973, **248**, 884; (*c*) I. M. Chaiken, J. S. Cohen, and E. A. Sokolovski, *J. Amer. Chem. Soc.*, 1974, **96**, 4703; (*d*) W. H. Vine, D. A. Bruckner, P. Needleman, and G. R. Marshall, *Biochemistry*, 1973, **12**, 1630.

[8] K. L. Kirk and L. A. Cohen (*a*) *J. Amer. Chem. Soc.*, 1971, **93**, 3060; (*b*) *ibid.*, 1973, **95**, 4619; (*c*) *J. Org. Chem.*, 1973, **38**, 3647; (*d*) K. L. Kirk, W. Nagai, and L. A. Cohen, *J. Amer. Chem. Soc.*, 1973, **95**, 8389.

[9] (*a*) B. M. Dunn, C. DiBello, and I. M. Chaiken, *Fed. Proc. Amer. Soc. Experimental Biol. Medicin.*, 1973, **32**, 541; (*b*) C. B. Klee, K. L. Kirk, L. A. Cohen, and P. MePhie, *J. Biol. Chem.*, in the press.

[10] Y. Takeuchi, K. L. Kirk, and L. A. Cohen, in preparation.

TABLE 1

^{1}H N.m.r. data for fluoroimidazoles (in D_2O)

Compound	Nucleus observed	Figure	δ [a]	J/Hz	Δ_1 [b]	Δ_3	Δ_4
(1)	4,5-H_2	1A	6·89 (d)	HF 0·5	0·46		0·14
(2)	4-H		6·47 (d)	HF 1·5	0·49		0·33
(3) [c]	4-H	1B	6·70 (d)	HF 1·5	0·45	0·15	0·23
(4)	2-H	3A	7·22 (t)	HH 1·5 HF 1·5	1·16		0·69
	4-H	3C	6·56 (dd)	HH 1·5 HF 7·8	0·57		0·68
(5)	2-H		7·27 (d)	HF 1·5	1·14		0·46
(6) [d]	2-H	3B	7·32 (d)	HF 1·5	1·16	0·07	0·60
(7)	2-H		7·91 (t)	HH 1	0·92		0·45
	4,5-H_2		7·28 (d)		0·38		0·33
(8)	2-H		7·56 (d)	HH ~0·5	0·96		0·42
	4-H		6·74 (d)		0·41		0·35
(9)	2-H		7·78 (d)	HH ~0·5	1·04		0·42
	4-H		7·03 (d)		0·41		0·31

[a] Chemical shift for the uncharged imidazole ring. [b] For definitions of Δ, see text; positive Δ values indicate an upfield displacement. [c] Side-chain α-H, δ 3·90; β-H_2, 3·00. [d] Side-chain α-H, δ 4·00; β-H_2, 3·16.

true pD values.[11] At the same time, ionization equilibria in D_2O differ from those in H_2O by an amount dependent on the acidity of the ionizable group. For many compounds, pK^D and pK^H may be related by equation (1).[12] Fortuitously, this correction is of opposite sign and is approximately equal to the electrode correction at 25°. When pK values are derived from glass electrode measurements in D_2O, neglect of both corrections will provide a figure within 0·1—0·2 units of the true pK^H, for pK values below 9. In the past, various imidazole pK values have been obtained by ^{1}H n.m.r. titration [2] and have been reported without correction. Comparison of pK values obtained from n.m.r. and glass electrode data shows this practice to be quite acceptable. Since we wished to express all our values as pK^H, and since some of the pK values exceed 9, we have performed both corrections routinely. Thus, the values given in Table 3 are pK^H values; the figures in parentheses indicate the amount by which apparent pK values (in D_2O) were reduced in order to obtain pK^H values. The pD values in Figures 1 and 3 are those actually measured in D_2O solution at a glass electrode, and do not include any electrode correction for the solvent.

$$pK^D = 1{\cdot}018\,pK^H + 0{\cdot}43 \qquad (1)$$

RESULTS AND DISCUSSION

The ^{1}H and ^{19}F n.m.r. data are summarized in Tables 1 and 2, respectively The δ values are those for the uncharged imidazole ring, as measured from the plateaux in the titration curves (Figures 1—4) at *ca.* pH or pD 6. The Δ values in these Tables indicate the total displacement in chemical shift resulting from each ionic transformation, and were calculated in the direction of increasing pH or pD: thus, $\Delta_1 = \delta_{ImH^+} - \delta_{Im}$ and $\Delta_4 = \delta_{Im} - \delta_{Im^-}$; for the amino-acids, $\Delta_3 = \delta_{Im(NH_3^+)} - \delta_{Im(NH_2)}$. On the basis of these conventions, positive Δ values indicate an upfield displacement in chemical shift (and

TABLE 2

^{19}F N.m.r. data for fluoroimidazoles (in H_2O)

Compound	Figure	δ [a]	J/Hz	Δ_1 [b]	Δ_3	Δ_4
(1)	2A	56·60 (t)	HF 0·5	3·91		−2·66
(2)		58·59 (d)	HF 1·5	3·55		−3·10
(3)	2B	59·80 (d)	HF 1·5	4·49	0·57	−2·15
(4)	4A	23·43 (dd)	H_2F 1·5 H_5F 7·8	−5·56		5·74
(5)		16·91 (d)	HF 1·5	−5·12		4·12
(6)	4B	20·25 (d)	HF 1·5	−3·74	1·74	5·11

[a] Values for the uncharged imidazole ring, downfield from the external standard (10% C_6F_6 in bromoform.) [b] Positive Δ values indicate an upfield displacement; for definitions of Δ, see text.

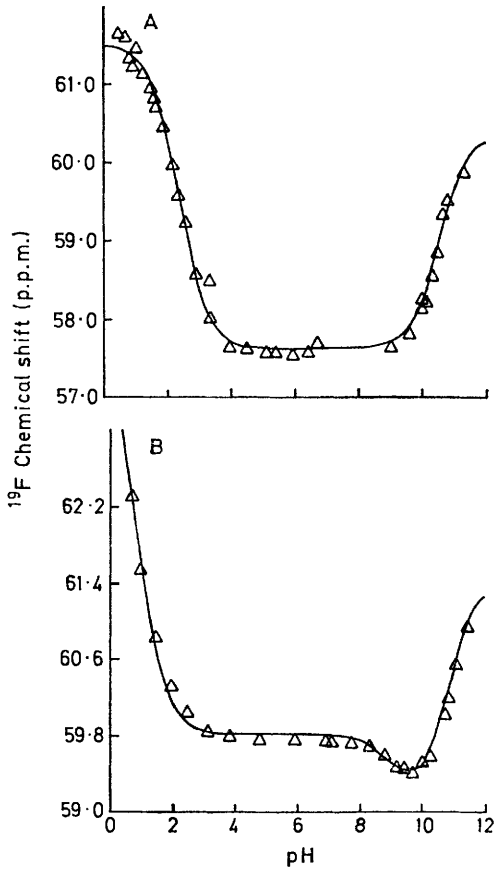


FIGURE 2 Variation of ^{19}F chemical shift as a function of pH for A, 2-fluoroimidazole and B, 2-fluorohistidine: △, experimental values of δ; solid line, theoretical titration curve

[11] T. H. Fife and T. C. Bruice, *J. Phys. Chem.*, 1961, **65**, 1079.

[12] Equation (1) was obtained from a least squares analysis of literature pK^H and pK^D data for *ca.* 20 compounds: see, *e.g.*, R. P. Bell and A. T. Kuhn, *Trans. Faraday Soc.*, 1963, **59**, 1789; C. A. Bunton and V. J. Shiner, *J. Amer. Chem. Soc.*, 1961, **83**, 42.

vice versa). Values of $\Delta_2(CO_2H \longrightarrow CO_2^-)$ are not given for the amino-acids since this transition is almost coincident with and is overshadowed by Δ_1; the latter values, therefore, should be viewed as composites of Δ_1 and Δ_2.

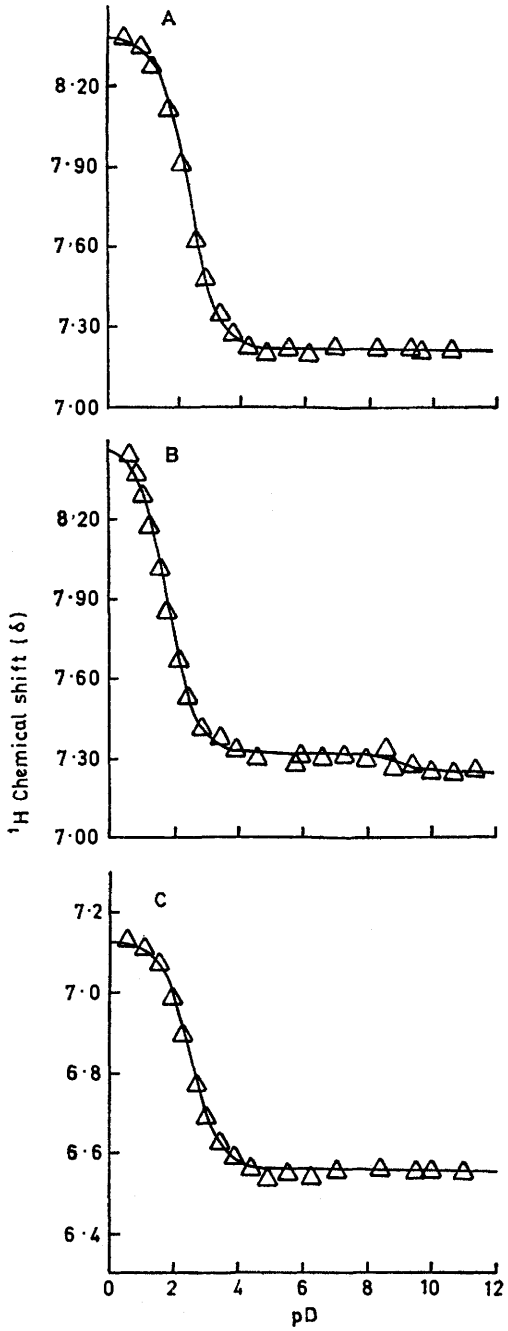


FIGURE 3 Variation of ^{1}H chemical shift as a function of pD (uncorrected) for A, 4-fluoroimidazole (H-2), B, 4-fluorohistidine, and C, 4-fluoromidazole (H-5): △, experimental values of δ: solid line, theoretical titration curve

The pK values, adjusted for the requisite corrections (see Experimental section), are given in Table 3. The pK subscripts correspond to the equilibria defined in equations (2)—(5); for the reasons given above, pK_2 values could not be derived from n.m.r. data, nor were they evaluated by other means. For 2-fluorohistidine (3), the ^{1}H n.m.r. transition above pD 8 (Figure 1B) does not correspond to the theoretical curve for a single transition;

$$ImH^+ \overset{K_1}{\rightleftharpoons} Im + H^+ \quad (2)$$

$$Im \overset{K_4}{\rightleftharpoons} Im^- + H^+ \quad (3)$$

$$ImCH_2\underset{NH_3^+}{CHCO_2H} \overset{K_2}{\rightleftharpoons} ImCH_2\underset{NH_3^+}{CHCO_2^-} + H^+ \quad (4)$$

$$ImCH_2\underset{NH_3^+}{CHCO_2^-} \overset{K_3}{\rightleftharpoons} ImCH_2\underset{NH_2}{CHCO_2^-} + H^+ \quad (5)$$

curve fitting by computer simulation provided the pK values 8·85 and 10·55, which are assigned to K_3 and K_4

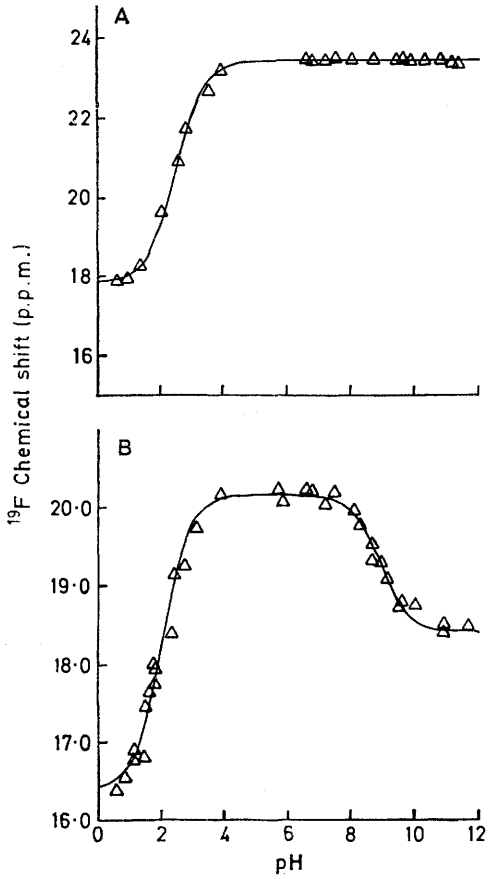


FIGURE 4 Variation of ^{19}F chemical shift as a function of pH for A, 4-fluoroimidazole and B, 4-fluorohistidine: △, experimental values of δ; solid line, theoretical titration curve

respectively. As may be seen in Figure 2B, these transitions are totally separate in the ^{19}F titration curve for the amino-acid. For the series of 4-fluoroimidazoles, pK_4 values lie beyond 11·5; because of the errors in pH or pD measurement in highly alkaline media, no attempt was made to obtain pK_4 values from titration curves for these compounds. Approximate values of pK_4 for (4) and (5) were obtained at the glass electrode by half-neutralization. The agreement in pK values obtained

by the two n.m.r. methods is quite satisfactory, and rather impressive in several cases. For the sake of comparison, pK values for (1) and (4) were also obtained at the glass electrode by titration in H_2O; the results are consistent with those calculated from n.m.r. data.

TABLE 3

pK Values for fluoroimidazoles [a,b]

Compound	Nucleus observed	pK_1	pK_3	pK_4
(1)	H-4,5	2·38 ± 0·02 (0·08)		10·36 ± 0·01 (0·20)
	F-2	2·42 ± 0·04		10·54 ± 0·08
	Titr.	2·51		10·41
(2)	Titr.	3·06		10·70
(3)	H-4	1·22 ± 0·06 (0·06)	8·85 ± 0·14 (0·20)	10·55 ± 0·11 (0·23)
	F-2	0·92 ± 0·05	8·90 ± 0·22	10·94 ± 0·07
(4)	H-2	2·42 ± 0·01 (0·08)		[c]
	H-5	2·44 ± 0·01 (0·08)		[c]
	F-4	2·46 ± 0·02		[c]
	Titr.	2·48		11·92
(5)	Titr.	3·14		12·19
(6)	H-2	1·76 ± 0·02 (0·07)	8·84 ± 0·32 (0·20)	[c]
	F-4	2·05 ± 0·05	8·92 ± 0·10	[c]

[a] Subscripts defined by equations (2)—(5). [b] Apparent pK values based on ^{1}H n.m.r. data (in D_2O) were reduced to the above pK values by the quantities in parentheses (see Experimental section). [c] Not determined.

The pK_1 value of 2-fluoroimidazole, 2·40,* is lower than that of 2-bromoimidazole, 3·85,[13] and that of 4-fluoroimidazole, 2·44, is correspondingly lower than that of 4-bromoimidazole, 3·88.[13] For both fluoro-isomers, the trend indicates the inductive effect of the halogen atom to be considerably more important than any resonance effect due to backbonding by an electron pair on fluorine. These results are more consistent with pK data for the isomeric halogenopyridines than for the halogenoanilines. The importance of the inductive effect is seen again in pK_4 values: 2-fluoroimidazole, 10·45, 2-bromoimidazole, 11·03;[13] 4-fluoroimidazole, 11·92, 4-bromoimidazole, 12·32.[13] On the basis of these and other literature pK values, electronegative substituents at C-2 appear consistently more effective than those at C-4 in lowering pK_4.† This phenomenon may be due either to the fact that the C-2 substituent operates inductively on both nitrogen atoms or that a more symmetrical anion (with more favourable resonance stabilization) is formed from the 2-substituted imidazole. On the basis of the limited data available, no clear pattern is discernible with respect to pK_1.

N.m.r. titrations of 2-fluorohistidine (3) show three transitions, that at lowest pH being attributed to dissociation of the imidazolium ion (pK_1). Through its σ-inductive and field effects, the positively charged side chain ‡ in histidine reduces pK_1 from 7·08 in imidazole itself to 6·0 in the amino-acid. Assuming the side chain of 2-fluorohistidine to have a conformation similar to that of histidine (in the same charge state) and a comparable effect on pK_1, the fluorinated amino-acid should show a pK_1 of 1·32. The value derived from the ^{1}H n.m.r. titration, 1·22, is reasonably consistent with the assumptions of similar conformation and similar electrostatic effects. The significantly lower value of 0·92 derived from ^{19}F spectra apparently results from the fact that the first transition (Figure 2B) represents not only the titration of the imidazolium ring but the response of the fluorine atom to the titration of the side-chain carboxy-group. On the basis of the inductive effect of fluorine, we estimate pK_2 for the carboxy-group in (3) as 1·12 (in histidine, pK_2 = 1·82), clearly too close to pK_1 to permit any resolution of the composite titration curve. The ^{1}H signal is far less sensitive than that of ^{19}F to charge variation in the side chain; accordingly, the pK_1 value derived from ^{1}H spectra may be considered the more reliable in such a case. The second transition for (3), at pK 8·88, corresponds to titration of the side-chain amino group (pK_3 = 9·17 in histidine). This modest reduction in basicity of 0·29 units is probably due to electron withdrawal by fluorine. The third transition, at pK 10·75, is attributable to pK_4, and is comparable to the same transition for (1) at pK 10·45. It seems reasonable that the amino and carboxylate groups in the side chain of (3) should retard loss of a proton from the ring by a combination of hydrogen bonding and electrostatic effects.

The histidine–imidazole comparison predicts a pK_1 value for 4-fluorohistidine of 1·36; however, ^{1}H and ^{19}F titrations provide the higher values of 1·76 and 2·05, respectively. If the effect of the side chain on pK_1 were primarily σ-inductive, the values for (3) and (6) ought to show better agreement. The divergence in results reveals the significance of field effects on pK_1 and suggests that the principal conformation of 4-fluorohistidine may differ from those of 2-fluorohistidine and histidine. Again, we consider the value based on ^{1}H titration to be the more reliable. The value of pK_3 for 4-fluorohistidine agrees with that obtained for 2-fluorohistidine, 8·88. As in the case of (4), no attempt was made to obtain pK_4 (above pH 11·5) by n.m.r. titration.

It is evident from the Δ values of Tables 1 and 2 that the fluorine resonance signal is considerably more sensitive than the proton signal to changes in the state of ionization of the imidazole ring or side chain, as well as to variation in the chemical nature of the side chain.§ The canonical forms for 5-alkylimidazoles suggest that C-4 may be considered comparable to the *ortho*-position of a monosubstituted benzene ring and C-2 comparable to the *para*-position. In toluene, both the *o*- and *p*-

* Except for pK_1 values for (3) and (6), pK values cited are averages of those derived from ^{1}H and ^{19}F n.m.r. data.

† On the basis of the limited data available, a fair correlation of pK data with σ_m has been shown for 2-substituted imidazoles and with σ^* for 4-substituted imidazoles.[14]

‡ In the usual biochemical terminology, side chain refers to a group replacing a hydrogen atom of glycine. In the present case, side chain refers to any substituent at C-5 of the imidazole ring.

§ ^{13}C N.m.r. signals have also been found to be much more sensitive than ^{1}H signals to these variations (refs. 4*a* and *b*).

[13] G. B. Barlin, *J. Chem. Soc.* (*B*), 1967, 641.

[14] M. Charton, *J. Org. Chem.*, 1965, **30**, 3346; T. B. Paiva, M. Tominaga, and A. C. M. Paiva, *J. Medicin. Chem.*, 1970, **13**, 689.

hydrogen signals are displaced upfield (relative to benzene) in keeping with the $-\sigma$ value for the methyl group; the data of Table 1 show the same direction of displacement in the 5-alkylimidazoles (8) and (9) relative to imidazole (7), with a somewhat greater upfield shift at H-4 than at H-2 regardless of the charge state of the imidazole ring. While entirely parallel results are found in the H-4 signals of 2-fluoroimidazoles, the H-2 signals of 4-fluoroimidazoles are displaced to lower field by alkyl groups at C-5 (Table 1). The ^{19}F signals are influenced by a 5-alkyl group in exactly the opposite way: downfield displacement in the 2-fluoro-series and upfield in the 4-fluoro-series (Table 2). In fluorotoluenes, the methyl group displaces an *o*- or *p*-fluorine signal upfield (relative to fluorobenzene), as expected on electronic grounds. We propose, therefore, that while substituents at C-4 and -5 are coupled through the double bond by the usual combination of inductive and resonance effects, C-2 is coupled to C-4 or -5 primarily *via* the inductive effect.*

The fluorine atom, by virtue of its strong electronegativity, might be expected to exert a strong deshielding effect on ring hydrogens. The data show, however, that introduction of a fluorine atom at C-2 effects an upfield displacement on H-4 while a fluorine atom at C-4 has the same effect on H-2, as well as on H-5. Similar results are found with fluorobenzene, in which the fluorine atom shifts the *o*-proton 0·30 and the *p*-proton 0·22 p.p.m. upfield, relative to benzene.[15] While the inductive effect of fluorine does, in fact, operate in the expected direction, this effect must be counteracted and overwhelmed by the shielding effect of magnetic anisotropy of the fluorine atom,[16] resulting in a net reversal of field direction.

Protonation of the imidazole ring of histidine (or of other imidazoles) results in a deshielding of the ring protons, H-2 shifting downfield 1·12 and H-4 0·55 p.p.m.[2] Similar downfield shifts are observed in the fluorinated imidazoles, *ca.* 1·2 for H-2 and 0·45—0·57 p.p.m. for H-4. The two-fold effect on H-2 may result from its exposure to a full positive charge, half exerted by each nitrogen atom, while H-4 is adjacent to only one nitrogen atom.† Ring protonation should produce a similar effect on ^{19}F n.m.r. signals and, indeed, a downfield shift of 3·5—4·5 p.p.m. is seen for the 2-fluoroimidazoles. In the 4-fluoroimidazoles, however, ring protonation causes an *upfield* shift (3·7—5·5 p.p.m.).

This contrasting behaviour has been observed previously in the protonation of 2- and 3-fluoropyridine,[17] and was attributed to the shielding effect of magnetic anisotropy. Detailed ^{13}C n.m.r. studies of five- and six-membered heteroaromatic rings also reveal anomalous shift directions,[4a,18] while ^{1}H spectra generally follow the direction expected for increased or decreased availability of a nitrogen lone pair. Mathematical analysis of the ^{13}C cases has shown that gradual deshielding of a ring carbon atom can lead to the expected downfield shift, followed by a reversal to higher field.[18a] This complex situation arises, apparently, from the different ways in which changes in electronegativity at nitrogen influence adjacent σ and π electron densities.‡ The conversion of imidazoles or fluoroimidazoles to their anions produces the expected upfield shift in ^{1}H signals, the result of enhanced shielding by the extra lone pair on nitrogen. Although the 4-fluoroimidazoles follow the 'normal' direction by showing a ^{19}F upfield shift (4·1—6·8 p.p.m.) upon anion formation, the 2-fluoroimidazoles are now anomalous in exhibiting a downfield shift (1·5—3·1 p.p.m.). Any attempt to interpret these contradictory and puzzling results would be beyond the scope of the present work and probably premature.

Table 2 shows that the magnitude of the upfield displacement (Δ_1) of the ^{19}F signal at C-2, as a result of the first transition ($ImH^+ \longrightarrow Im$), is significantly greater (*ca.* 0·8 p.p.m.) for (3) than for (1) or (2). This effect may be attributed to the simultaneous conversion of CO_2H to CO_2^- and the attendant reduction in σ-electron withdrawal, or to the field effect of the carboxylate negative charge on fluorine, or both. In the 4-fluoroimidazole cases, the same transition shows a displacement *ca.* 1·6 p.p.m. less downfield (and thus upfield) for (6) relative to (4) or (5). It is noteworthy, but perhaps coincidental, that quite similar differences are seen in the later transition $NH_3^+ \longrightarrow NH_2$: 0·6 upfield for (3) and 1·7 p.p.m. upfield for (6). The apparently greater shielding effect at F-4 than at F-2 may stem from the fact that, regardless of their conformations, the side-chain functional groups are always closer to F-4 than to F-2. Efforts to interpret variation in ^{19}F signals in other series have already shown that the superposition of electronic effects, field effects, and magnetic anisotropy can sometimes lead to almost incomprehensible complexity.[16] The apparent complexities of σ and π electron transmission in the imidazole ring serve only to compound the difficulties.

The present work was undertaken to provide a basis for correlation of ^{19}F n.m.r. shifts with changes in the charge state of the fluoroimidazole ring and of its environment. Our results indicate that ^{19}F signals can be used to evaluate p*K* data in complex molecules containing fluorohistidine. In principle, the direction and magnitude of signal displacement should also provide useful

* Our studies of various other properties of substituted imidazoles lead to the same conclusion; the evidence and arguments will be presented in later publications.

† Similarly, the proton signal for the methyl group in 2-methylimidazole is shifted 0·31 p.p.m. downfield by ring protonation, while that of 4-methylimidazole is shifted 0·18 p.p.m. downfield.

‡ Anomalous shifts in the ^{13}C n.m.r. spectra of nonaromatic systems suggest that inductive and field effects may oppose each other.[19]

[15] H. Spiesecke and W. G. Schneider, *J. Chem. Phys.*, 1961, **35**, 731.

[16] J. W. Emsley, J. Feeney, and L. H. Sutliffe, 'High Resolution Nuclear Magnetic Resonance,' Pergamon, New York, 1966, ch. 11; J. W. Emsley and L. Phillips, *Progr. Nuclear Magnetic Resonance Spectroscopy*, 1971, **7**, 1.

[17] C. S. Giam and J. L. Lyle, *J. Amer. Chem. Soc.*, 1973, **95**, 3235.

[18] R. J. Pugmire and D. M. Grant, *J. Amer. Chem. Soc.*, 1968, **90** (*a*) 697; (*b*) 4232.

[19] W. J. Horsley and H. Sternlicht, *J. Amer. Chem. Soc.*, 1968, **90**, 3738; F. R. N. Gurd, P. J. Lawson, D. W. Cochran, and E. Wenkert, *J. Biol. Chem.*, 1971, **246**, 3725.

information about electronic or geometrical changes in the field. In the case of the fluorinated amino-acid, inductive and field effects could not be separated with full confidence because of the inadequate length of the side chain.* In the case of a polypeptide or protein matrix, however, detection and identification of field variations should prove clearer; hopefully, further investigation will support this optimism.

* The pK values of a series of ω-imidazolylalkanoic acids are being investigated and will be reported separately.

[4/2460 *Received, 25th November,* 1974]