# Carbon–Carbon Coupling in [90%-$^{13}$C-2]Histidine

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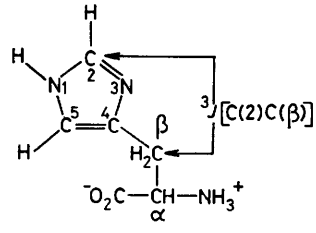
*Summary* The previously unresolved $^3J$(CC) coupling between the imidazole C-2 and the C-$\beta$ carbons of histidine has been observed in [90%-$^{13}$C-2]histidine as a function of pH; comparison of the results with theoretical finite perturbation theory INDO calculations indicates significant hydrogen bonding from the amino to imidazole units at neutral pH.

IN a recent study of [85%-*U*-$^{13}$C]histidine, no resolvable $^{13}$C–$^{13}$C coupling to the ring carbon C(2) was reported.[1] However, long-range carbon–carbon couplings are frequently more easily observed in specifically labelled molecules, as a comparison of the $^{13}$C spectra of [90%-$^{13}$C-1] labelled monosaccharides[2] and [*U*-$^{13}$C]monosaccharides[3] demonstrates. It was expected that the three-bond coupling between the ring carbon C(2) and C($\beta$) should be observable owing to the *trans* orientation of these nuclei. Observation of the C($\beta$) resonances in [90%-$^{13}$C-2]histidine




has confirmed the existence of a $^3J$[C(2)C($\beta$)] coupling. Values of $^3J$[C(2)C($\beta$)] obtained as a function of pH indicate a significant sensitivity to the titration of the imidazole ring (Figure). A small decrease in the coupling co-incident with deprotonation of the amine group also occurs, although the magnitude of this change is close to the spectral resolution. This behaviour of $^3J$[C(2)C($\beta$)] as a function of pH is qualitatively similar to the behaviour of the $^{15}$N-3 and $^{13}$C-2 chemical shifts and the $^1J$[N(3)C(2)] and $^1J$[N(3)-C(4)] couplings as a function of pH, all of which show a significant change co-incident with titration of the imidazole and a smaller change in the opposite direction upon titration of the amino group.[4,5]

Finite-perturbation-theory INDO self-consistent-field molecular orbital calculations have recently been shown to give excellent results for $^3J$(CC) couplings.[6] Numerical results for 4-methylimidazole corresponding to different protonation states and solvent interactions included to model the interaction with the histidine amino group and the $H_2O$ solvent are summarized in the Table. From these

TABLE. Theoretical finite-perturbation-theory INDO carbon–carbon $^3J$[C(2)Me] values in 4-methylimidazole[a]

| Protonation state | Solvent interaction[b] | Coupling constant/Hz |
|---|---|---|
| N-1,N-3 protonated | None | 2·07 |
| N-1 deprotonated | None | 2·06 |
| N-3 deprotonated | None | 6·47 |
| N-3 deprotonated | N-3 --- $H_2O$ | 6·13 |
| N-3 deprotonated | N-3 --- $NH_3$ | 6·23 |
| N-3 deprotonated | N-3 --- $(NH_4)^+$ | 5·02 |

[a] Structural parameters based on the imidazole in S. Martinez-Carrera, *Acta Cryst.*, 1966, **20**, 783. [b] Solvent molecules hydrogen bonded to N-3 with N-3 --- H distance of 1·6 Å and N-3 --- H–N (or O) atoms linear, see G. E. Maciel, J. W. McIver, Jr., N. S. Ostlund, and J. A. Pople, *J. Amer. Chem. Soc.*, 1970, **92**, 1,11.

calculations the following can be concluded. (i) The $^3J$(CC) value obtained for the 4-methylimidazolium ion is in excellent agreement with the analogous low pH value measured in histidine. (ii) Deprotonation of N-1, which is not in the coupling pathway, produces a negligible effect

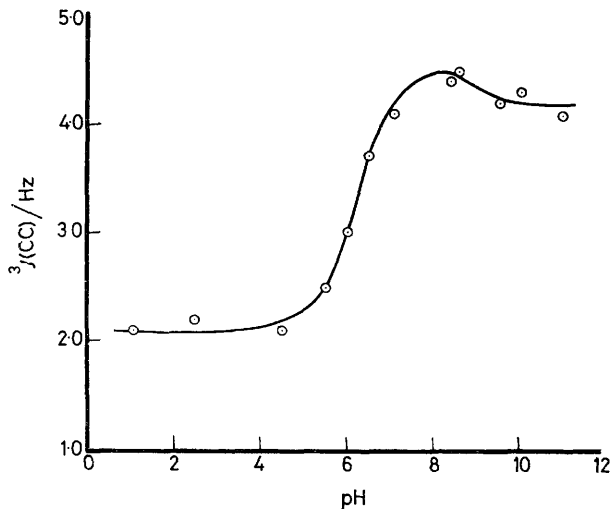



FIGURE. $^3J$[C(2)C($\beta$)] as a function of pH. Spectra were obtained at 0·4 Hz resolution with a 200 Hz sweep width and 512 data points using a Varian XL-100-15 spectrometer interfaced to a Nova 1210 computer for Fourier transform operation. Peak positions were interpolated from the data using a parabolic centroid function and are thus accurate to better than the spectral resolution.

on the $^3J$(CC) coupling. (iii) The theoretical value of 6·47 Hz corresponding to deprotonated N-3 cannot be directly compared with the observed value as this is dependent on the tautomeric equilibrium of the imidazole unit as well as on solvent interactions. {We note, however, that a value of $^3J$(CNCC) of 7·0 Hz has recently been measured in [2-*methyl*-$^{13}$C]thiazoline in $CDCl_3$, which corresponds to a methyl–ring carbon coupling through a non-protonated nitrogen.[7]} (iv) A significant hydrogen bond from the histidine $-NH_3^+$ to the imidazole unit at neutral pH is required to explain the observed coupling. The latter conclusion is deduced as follows. At pH 8·0, the observed coupling constant depends on the tautomeric equilibrium such that $^3J_{obs} = 4{\cdot}3$ Hz $= f[^3J$(CC)-N-1 protonated] + $(1-f)[^3J$(CC)-N-3 protonated], where $f$ is the fractional probability corresponding to the N-1 protonated tautomer. Clearly, $^3J$(CC) for N-3 protonated is 2·1 Hz. Values of 6·1—6·5 Hz for $^3J$(CC) with N-1 protonated give values of $f$ of 55—50%, in very poor agreement with the tautomeric equilibrium deduced using other n.m.r. parameters.[4,8] Alternatively, using a value for $^3J$(CC) with N-1 protonated of 5·0 Hz corresponds to $f$ 76%, in considerably better agreement with the literature values.

We conclude that hydrogen bonding from the amino to the imidazole unit is important in explaining the pH dependence of $^3J$[C(2)C($\beta$)] in histidine. The relatively small change in this parameter which occurs upon deprotonation of the amino group reflects the fact that co-incident with the loss of this interaction the tautomeric equilibrium becomes closer to 50%–50% which produces an opposite effect on this coupling. The theoretical calculation outlined above also predicts a significant effect of charge delocalization on the C(2)–N(3) coupling. However, quantitative comparison of the results with experimental values is less valid since the Fermi contact interaction is not overwhelmingly dominant for these couplings.[9]

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[1] S. Tran-Dinh, S. Fermandjian, E. Sala, R. Mermet-Bouvier, and P. Fromageot, *J. Amer. Chem. Soc.*, 1975, **97**, 1267.
[2] T. E. Walker, R. E. London, T. W. Whaley, R. Barker, and N. A. Matwiyoff, *J. Amer. Chem. Soc.*, 1976, **98**, 5807.
[3] A. S. Perlin and B. Casu, *Tetrahedron Letters*, 1969, **34**, 2921.
[4] F. Blomberg, W. Maurer, and H. Ruterjans, *J. Amer. Chem. Soc.*, 1977, **99**, 8149.
[5] R. E. Wasylishen and G. Tomlinson, *Biochem. J.*, 1975, **147**, 605.
[6] D. Doddrell, I. Burfitt, J. B. Grutzner, and M. Barfield, *J. Amer. Chem. Soc.*, 1974, **96**, 1241; M. Barfield, S. A. Conn, J. L. Marshall, and D. E. Miller, *ibid.*, 1976, **98**, 6253; J. L. Marshall, L. G. Faehl, A. M. Ihrig, and M. Barfield, *ibid.*, p. 3406; V. Wray, *ibid.*, 1978, **100**, 768; R. E. London, T. E. Walker, V. H. Kollman, and N. A. Matwiyoff, *ibid.*, p. 3723.
[7] G. Daub and T. W. Whaley, personal communication.
[8] W. F. Reynolds, I. R. Peat, M. H. Freedman, and J. R. Lyerla, Jr., *J. Amer. Chem. Soc.*, 1973, **95**, 328; R. E. Wasylishen and G. Tomlinson, *Canad. J. Biochem.*, 1977, **55**, 579.
[9] J. M. Schulman and T. Venanzi, *J. Amer. Chem. Soc.*, 1976, **98**, 6739, 4701.