Proton–Coupled ^{13}C Nuclear Magnetic Resonance Spectra Involving ^{13}C–^{1}H Spin–Spin Coupling to Hydroxyl-protons, a Complementary Assignment Aid

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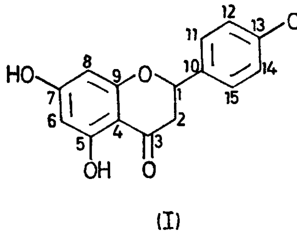
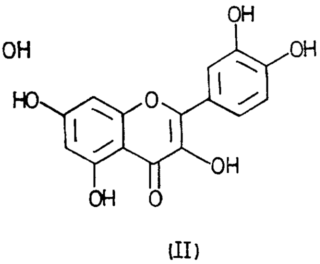
Summary Two-, three- and four-bond spin–spin coupling between ^{13}C and hydrogen-bonded hydroxy-protons, identified by deuterium exchange, is shown to aid the assignment of ^{13}C n.m.r. spectra in a series of 5-hydroxyflavones and flavonones.

Although single resonance spectra have been used in a number of ^{13}C studies, spin–spin coupling involving hydrogen-bonded hydroxy-protons has not been characterized as yet. In the molecules chosen it is demonstrated that closely spaced lines can unequivocally be assigned whereas all presently known alternative assignment aids almost certainly would fail.



(I)



(II)

The proton-coupled ^{13}C n.m.r. spectra of 5-hydroxyflavonones and 5-hydroxyflavones were found to exhibit spin–spin coupling between the aromatic carbons and the chelated hydroxy-proton [C(5)-OH] and thus permitted identification of these carbon atoms.[1] Among the sp^2 carbons in (I), C(4), C(6), and C(8) are expected to resonate at highest field. The proton-coupled ^{13}C spectrum in the region between 90 and 105 p.p.m. (rel. to Me_4Si) in Figure (a) reveals two methine and one quaternary carbon resonance. The two CH multiplets assignable to C(6) and C(8) differ in their fine structure. The doublet of triplets originates from C(6) and is due to vicinal coupling to C(8)-H and the hydrogen-bonded C(5)-hydroxy-proton. The designation of this coupling is corroborated by the spectrum in Figure (b) in which the triplets have changed into doublets after addition of D_2O. The coupling constant ^{3}J[C(6)–C(5)OH] is $4{\cdot}5 \pm 1$ Hz. Likewise the quartet centred at 101·5 p.p.m. pertaining to C(4) collapses into a triplet upon deuterium exchange {^{3}J[C(4)–C(5)OH] $4{\cdot}5 \pm 1$ Hz}. The three remaining quaternary carbons C(5), C(7), and C(9) are expected to be the least shielded among the aromatic carbon resonances in (I) and (II) and thus are assigned to the three signals between 162 and 167 p.p.m.

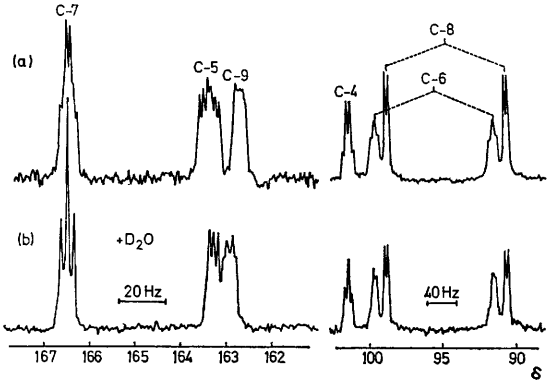


Figure. 20 MHz proton-coupled ^{13}C spectra of (I) in $(CD_3)_2SO$. The high-field region was recorded at a digital resolution of 1 Hz/point, the low field region at 0·5 Hz/point. Experimental conditions for (a) and (b) were identical except that for (b) 5% D_2O had been added.

Comparison between Figures (a) and (b) clearly shows that all three carbons are coupled to C(5)-OH. The multiplet at 166·5 p.p.m., which changes from a doublet of overlapping triplets into a simple triplet after addition of D_2O, is assigned to C(7). The smaller of the two coupling constants is attributed to ^{4}J[C(7)–C(5)OH] ($1{\cdot}5 \pm 0{\cdot}5$ Hz), and the larger arises from equal coupling to the two geminal protons C(6)-H and C(8)-H {^{2}J[C(7)–C(6)H = ^{2}J[C(7)–C(8)H] = $3{\cdot}0 \pm 0{\cdot}5$ Hz}. The correctness of this assignment was confirmed by comparison of the corresponding geminal coupling constants of C(4) to protons C(3)-H and C(5)-H in

2,4,6-trihydroxyacetophenone {^2J[C(4)–C(3)H] 3·2 Hz} which served as a model compound. The symmetric six-line multiplet at 163·3 p.p.m. which simplifies to a three-line pattern in Figure (b) is assigned to C(5) with ^2J[C(5)–OH] 5·0 ± 0·5 Hz. Since protons C(6)-H and C(8)-H were found to be accidentally degenerate, the spin system involving C(5) is of the type AA'MX, which may be second-order in principle. However, in 2,4,6-trihydroxyacetophenone a first-order triplet was observed for C(2) and C(6) {^2J[C(2)–C(3)H = ^4J[C(2)–C(5)H] = 1·5 Hz}; hence the corresponding spin system in (I) should equally be first-order. It is believed that the somewhat distorted intensities of the C(5) multiplet arise from an overlaying impurity signal which could be detected in the proton noise-decoupled spectrum. ^4J[C(9)–C(5)OH] could not be determined because of insufficient resolution of the C(9) signal.

The geminal and vicinal coupling constants involving the hydroxy-proton C(5)-OH in (I) are thus comparable in magnitude to those found in toluene between ring carbons and the methyl protons[2] {^2J[C(1)–Me] 6·0 Hz; ^3J[C(2)–Me 4·25 Hz}.

A comparison of the chemical shifts of C(4), C(5), and C(6) in the protio and deuterio isotopomers revealed small upfield shifts for the latter (−0·06, −0·39, and −0·07 p.p.m.). The value found for the geminal isotope effect is considerably larger than those reported earlier.[3]

It is noteworthy that in the spectrum of (II) no resolved splittings could be detected for those carbons expected to exhibit coupling to the C(5)-hydroxy-proton. This behaviour points to an increased exchange rate for the hydrogen-bonded proton, presumably arising from a less stable hydrogen bond owing to competition between C(2)-OH and C(5)-OH.

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[1] H. Fuhrer, *Helv. Chim. Acta*, 1973, **56**, 2377; in this paper broadenings of some signals in the single-resonance ^{13}C n.m.r. spectrum of rifamycin-S were observed, which could be ascribed to spin-spin coupling to hydroxy-protons.

[2] F. J. Weigert, J. Husar, and J. D. Roberts, *J. Org. Chem.*, 1973, **38**, 1313.

[3] D. H. Hunter, A. L. Johnson, J. B. Stothers, A. Nickon, J. L. Lambert, and D. F. Covey, *J. Amer. Chem. Soc.*, 1972, **94**, 8582; J. Feeney, P. Partington, and G. C. K. Roberts, *J. Magnetic Resonance*, 1974, **13**, 268.