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# CARBON-13 NMR STUDIES OF FLAVONOIDS—I
## FLAVONES AND FLAVONOLS

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**Abstract**—$^{13}$C-NMR spectra of hydroxylated flavones and flavonols are presented for the first time. Analyses of the spectra are derived by consideration of a series of acetophenones, cinnamic acids, flavones and flavonols of increasing oxygenation pattern. The accepted substitution additivity rules have been shown to hold for these compounds except in cases where structural modifications involve the C-3,4 and 5 positions.

### INTRODUCTION

The occurrence of flavonoids in the plant kingdom is widespread. They have been isolated from representatives of all major groups of green plants ranging from the green algae to the angiosperms.[1] Flavonoid structure information has proven to be of great value in studies of plant taxonomy, phylogeny and hybridization.[1,2] To date, this structure information has been obtained largely by the use of UV and PMR spectroscopy[3] and to some extent by M.S.[4] The use of $^{13}$C-NMR spectroscopy for the determination of flavonoid structure, has not as yet been reported although a detailed CMR study of flavone itself has recently appeared.[5]† We report here a study of the CMR spectra of a range of hydroxylated flavones and flavonols, with the object of providing background data and conclusions for use in future structural studies of naturally occurring flavonoids.

The solvents used in the present study, DMSO-$d_6$ and DMSO-$d_6$/$D_2O$, were chosen because of their ability to dissolve a wide range of phenols, flavonoids, glycosides and sugars. Substituent effect data available for such solvent systems is limited however, and for this reason reference to substituent effects throughout the following discussion refers to data compiled by Levy and Nelson[6] for solutions in $CCl_4$. The limited data available on shifts in DMSO[7] suggest that apart from the effect at the site attachment, the shifts are comparable with those referred to above.

### RESULTS AND DISCUSSION

1. *Acetophenones and cinnamic acids*

As a preliminary to the flavonoid work, a study of several model acetophenones and cinnamic acids with relevant oxygenation patterns was undertaken using the same solvent system originally chosen for the flavonoids. The results are presented in Table 1, in which the carbon numbering relates for comparison purposes to the equivalent carbon in the flavone nucleus (Fig. 1).

The proton noise decoupled $^{13}$C-NMR spectra of the chosen acetophenones all contained a low field singlet in the 205–208 ppm region attributed[8] to the CO carbon, and a high field singlet between 27 and 35 ppm due to the Me carbon.

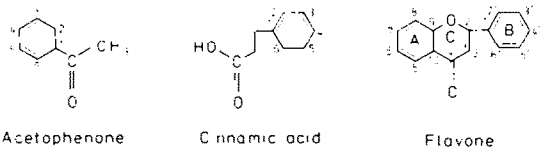


Fig. 1. Numbering systems used for acetophenones, cinnamic acids and flavonoids.

In the symetrically substituted, 2,6-dihydroxyacetophenone only four signals remain unassigned. The signal at 162·46 ppm is assigned to the two shielded oxygen substituted carbons, C-2 and C-6, and the highest field signal (108·70 ppm) to the C-3 and C-5 carbons *ortho* to these. Carbon atom C-4 appears at lower field (137·82 ppm) as expected for a carbon *meta* to the hydroxylation site. The quaternary carbon C-1 at 111·67 ppm is readily identifiable by its low intensity signal (caused by longer relaxation time and lower nuclear Overhauser enhancement[6]).

The assignments for the other acetophenones follow from the above. For example in 2,4,6-trihydroxyacetophenone the introduction of the 4-OH group causes C-4 to move downfield by 27·6 ppm, C-2/C-6, to move downfield by 2·8 ppm and C-1 and C-3/C-5 to move upfield by 6·0 ppm and 12·6 ppm respectively. All of these chemical shifts are of the magnitude expected by application of substituent effects data. In 2,4-dihydroxyacetophenone the signal at 103·67 ppm is ascribed to C-3 rather than to C-5, since C-3 is influenced by two *ortho* OH groups and C-5 only one *ortho* and one *para*. Similarly with 2-hydroxyacetophenone, C-3 is considered to be represented by the signal at 116·65 ppm and C-5 by that at 118·95 ppm.

The assignments made above for each of the acetophenones are in general agreement with those previously published by Dhami and Stothers[9] although actual chemical shift values do vary somewhat presumably due to solvent effects.

The spectra of the cinnamic acids exhibit the carbonyl

†The work of C. A. Kingsbury and J. H. Looker, *J. Org. Chem.* **40**, 1120 (1975) which reports the $^{13}$C spectra of some methoxyflavones, has just come to our notice.

Table 1. $^{13}$C-NMR spectra of flavones and related compounds

| COMPOUND | CARBON NUMBER* and SHIFT (in ppm from TMS) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1' | 2' | 3' | 4' | 5' | 6' | METHYL | |
| Acetophenones: | | | | (2) | (3) | (4) | (5) | (6) | (1) | | | | | | | C-Me | C-Me |
| 2-OH | | | 207.35 | 161.91 | 116.65 | 138.25 | 118.95 | 132.91 | 121.14 | | | | | | | 28.26 | |
| 2,4-OH | | | 205.11 | 165.00 | 103.67 | 165.61 | 109.67 | 135.21 | 114.34 | | | | | | | 27.34 | |
| 2,6-OH | | | 207.35 | 162.46 | 108.70 | 137.82 | 108.70 | 162.46 | 111.67 | | | | | | | 34.38 | |
| 2,4,6-OH | | | 204.99 | 165.25 | 96.08 | 165.49 | 96.08 | 165.25 | 105.67 | | | | | | | 33.60 | |
| Cinnamic Acids: | | | | | | | | | | (1) | (2) | (3) | (4) | (5) | (6) | | |
| 4-OH | 146.68 | 115.98 | 170.65 | | | | | | | 126.90 | 131.70 | 117.26 | 160.33 | 117.26 | 131.70 | | |
| 4-OH; 3-OMe | 146.92$^{hm}$ | 116.22$^{h+}$ | 170.65 | | | | | | | 127.39 | 112.28$^{hm}$ | 149.11 | †149.78 | 116.83$^{h+}$ | 124.23$^{hm}$ | | 57.01 |
| 4-OH; 3,5-OMe | 147.23$^{hm}$ | 116.65$^{h}$ | 170.59$^{m}$ | | | | | | | 126.30 | 107.0$^{hm}$ | 149.17 | 138.79$^{o}$ | 149.17 | 107.0$^{hm}$ | | 57.38 |
| Flavones: | | | | | | | | | | | | | | | | | |
| Flavone[5] | 163.2 | 107.6 | 178.4 | 125.7 | 125.2 | 133.7 | 118.1 | 156.3 | 124.0 | 131.8 | 126.3 | 129.0 | 131.6 | 129.0 | 126.3 | | |
| 5-OH‡ | 164.07 | 105.61$^{h}$ | 182.90 | 155.85 | 107.22$^{hm}$ | 135.61$^{ho}$ | 110.83$^{hm}$ | 159.82 | 110.13 | 130.54 | 126.39$^{hm}$ | 128.91$^{hm}$ | 131.97$^{hm}$ | 128.91$^{hm}$ | 126.39$^{hm}$ | | |
| 5,4'-OH | 165.46 | 103.94$^{h}$ | 183.45 | 156.42 | 108.04$^{hm}$ | 136.19$^{ho}$ | 111.45$^{hm}$ | 160.00 | 110.49 | 121.90 | 129.18$^{hm}$ | 116.71$^{ho}$ | 161.67 | 116.71$^{ho}$ | 129.18$^{hm}$ | | |
| 5-OH; 4'-OMe‡ | 164.10 | 104.00 | 182.57 | 155.75 | 106.97 | 135.19 | 110.58 | 159.73 | 109.91 | 119.26 | 128.18 | 114.50 | 163.37 | 114.50 | 128.18 | | 55.37 |
| 5,7,4'-OH | 165.49 | 104.33 | 183.18 | 158.67 | 95.57 | 164.93 | 100.27 | 162.03 | 105.06 | 122.72 | 129.79 | 117.32 | 161.79 | 117.32 | 129.79 | | |
| 5,7,3',4'-OH | 165.07 | 103.94$^{h}$ | 182.63 | 158.24 | 94.9 | 164.34 | 99.91 | 161.56 | 104.82 | 123.06 | 114.38$^{hm}$ | 145.95 | 149.84 | 117.05$^{h}$ | 120.14$^{hm}$ | | |
| 3',4'-OH | 164.16 | 105.37$^{h}$ | 177.93 | 125.66$^{hm}$ | 125.08$^{hm}$ | 134.43$^{hm}$ | 118.44$^{hm}$ | 156.12 | 123.57 | 122.87 | 113.98$^{hm}$ | 145.80 | 149.54 | 116.62$^{h}$ | 119.47$^{hm}$ | | |
| 3',4'-OMe‡ | 162.64 | 105.79 | 176.65 | 125.03 | 124.60 | 135.64 | 118.11 | 155.60 | 123.75† | 123.33† | 110.52 | 149.35 | 152.26 | 112.46 | 119.99 | | 55.92,55.16 |
| 5-OH; 3',4'-OMe‡ | 164.07 | 104.24$^{h}$ | 182.60 | 155.73 | 106.94$^{hm}$ | 135.10$^{ho}$ | 110.58$^{hm}$ | 159.76 | 109.92 | 122.93 | 110.58$^{hm}$ | 149.29 | 152.66 | 112.37$^{h}$ | 120.26$^{hm}$ | | 56.07,55.80 |
| Flavonols: | | | | | | | | | | | | | | | | | |
| 3',4'-OH‡ | 147.69 | 138.13 | 173.83 | 125.45$^{hm}$ | 125.45$^{hm}$ | 134.52$^{hm}$ | 118.83$^{hm}$ | 155.45 | 121.53† | 123.42† | 116.32$^{hm}$ | 145.44 | 148.08 | 116.71$^{h}$ | 121.72$^{hm}$ | | |
| 3',4'-OMe | 145.35 | 137.82 | 172.35 | †124.03$^{hm}$ | †124.45$^{hm}$ | 133.01$^{hm}$ | 117.92$^{hm}$ | 154.39 | 121.11† | 123.78† | 112.35$^{hm}$ | 148.75 | 150.72 | 112.22$^{h}$ | 121.51$^{hm}$ | | 56.04,55.74 |
| 5,7,3',4'-OH | 148.32† | 136.73 | 176.71 | 157.48 | 94.99 | 164.52 | 99.60 | 161.56 | 104.39 | 123.75 | 116.47 | 145.71 | 148.02† | 116.95 | 122.05 | | |
| 5,7,2',4'-OH | 149.63 | 136.49 | 176.71 | 156.94 | 94.99$^{hm}$ | 164.10 | 99.57$^{hm}$ | 161.31 | 104.97 | 111.19 | 158.09 | 104.97$^{hm}$ | 160.97 | 109.25$^{hm}$ | 132.31$^{h}$ | | |

*The numbering system is that of flavone. Acetophenone and cinnamic acid numbering systems appear in parentheses below.
†Assignments may be reversed.
‡Spectrum measured in hexadeutero-dimethylsulphoxide. Others were measured in DMSO-$d_6$:$D_2O$ (approx. 2:1) except for flavone itself which was measured in deuterochloroform.
$^{h}$Indicates $^{13}$C–$^{1}$H coupling of 155–170 Hz.
$^{m}$Indicates $^{13}$C–$^{1}$H coupling of 6–8 Hz (coupling of 1–3 Hz may also be present but not indicated).
$^{o}$Indicates $^{13}$C–$^{1}$H coupling of 1–3 Hz.

carbon signal at about 170·6 ppm and the O-methyl carbon (where present) at about 57 ppm. In the spectrum of the symetrically substituted 4-hydroxycinnamic acid the two intense signals at 131·70 and 117·26 ppm are assigned to the two pairs of equivalent carbons, C-2/C-6 and C-3/C-5 respectively. The C-1 carbon being *para*-related to the OH would be expected to appear between the *ortho*- and *meta*-related carbons and it is assigned the 126·90 ppm signal. The remaining signals at 146·68 and 115·98 ppm are ascribed to the vinyl carbons C-2 and C-3 (flavone numbering) respectively on the basis of assignments previously made for 4-methoxystyrene[10] and methoxycoumarin.[11] The chemical shifts of these vinyl protons are affected very little by further OMe substitution as found in the other two cinnamic acid samples.

Other assignments in the cinnamic acid spectra follow from the above after consideration of the relevant shift data.[12] Confirmation of the assignments for 4-hydroxy-3-methoxycinnamic acid and 4-hydroxy-3,5-dimethoxycinnamic acid was obtained from a study of the $^{13}C$–$^{1}H$ couplings. Thus in the spectrum of the latter the signals at 147·23, 116·65 and 107·0 ppm show coupling consistent with the presence of an attached proton (J = *ca.* 158 Hz), and the signals at 170·59, 147·23, 138·79, 126·30 and 107 ppm exhibit coupling (J = *ca.* 7 Hz) associated with a *meta*-related proton. It was not possible by this means, however, to distinguish the C-3 and C-5 signals from each other in the spectrum of 4-hydroxy-3-methoxycinnamic acid.

2. *Flavones*

The first series of flavonoids to be considered will be the 5-hydroxy-, 5,4′-dihydroxy-, 5,7,4′-trihydroxy- and 5,7,3′,4′-tetrahydroxy-flavone series.

The simplest member of the above series is 5-hydroxyflavone, the spectrum of which is largely interpretable from the assignments established above for 2,6-dihydroxyacetophenone and from those previously published for flavone itself[5] (reproduced in Table 1). Thus assignments in 5-hydroxyflavone for C-4, 7 and 10 follow from the former, and those for C-1′, 2′, 3′, 4′, 5′, 6′ and C-3 from the latter. Proton coupling data is consistent with these assignments (Table 1). The signals at 110·83 and 107·22 ppm are the only unassigned signals in the region in which C-6 and C-8 should occur (*cf.* 2,6-dihydroxyacetophenone); and, since the *ortho* and *para* effects of the strongly H-bonded OH at C-5 have not been previously established, these two signals are assigned on the basis of the now well established[3,13] assignments for the 6- and 8-protons (of 5-hydroxyflavonoids) in proton NMR spectra. Thus, C-6 is considered to be represented by the higher field signal and C-8 by the lower field signal.† The oxygenated carbons were assigned to signals in the following manner. In flavone the C-2 signal appears at 163·2 ppm and the introduction of a 5-hydroxyl would not be expected to affect the electron density appreciably at this site. Thus the C-2 signal is considered to be that at 164·07 ppm. Carbon 9 however, which appears at 156·3 ppm in flavone, should shift downfield on the introduction of a *meta*-OH at C-5 and is thus assigned the 159·82 ppm signal. The signal at 155·85 ppm is therefore ascribed to C-5.

†In a related compound, the dihydroxanthone morellin, W. von Philipsborn (*Pure Appl. Chem.* **40**, 159 (1974)) has, without comment, assigned the higher field signal to the carbon equivalent to C-6.

Much of the spectrum of 5,4′-dihydroxyflavone (Fig. 2) closely approximates that of 5-hydroxyflavone. Changes observed occur mainly in the B-ring carbons. The new oxygenated carbon, C-4′, appears as a singlet at 161·67 ppm and the carbons *ortho*- (C-3′,5′) *meta*- (C-2′, 6′) and *para*- (C-1′) to it are shifted −12·2, +2·8 and −8·6 ppm respectively in accord with accepted substitution effects. The C-2 signal exhibits a reduced *meta*- effect. $^{13}C$-$^{1}H$ coupling confirmed many of these assignments (Table 1). The spectrum 5-hydroxy-4′-methoxyflavone shows much the same pattern of signals, and the assignments tabulated were deduced directly from those of 5,4′-dihydroxyflavone.

Assignments of signals in the spectrum of the commonly encountered natural product, apigenin (5,7,4′-trihydroxyflavone Fig. 2), follow to some extent from those established for 5,4-dihydroxyflavone above. This is so for all B- and C-ring carbons except for C-3, which is not immediately distinguishable from C-10. By analogy these two carbons are represented by the signals at 104·33 and 105·06 ppm, and the former, being the less intense is mostly likely[6] to represent the quaternary carbon C-10. This is confirmed by a comparison of C-10 (flavone numbering) chemical shifts in the acetophenones and the flavones. The difference in the C-10 chemical shifts of 2,6-dihydroxyacetophenone and the equivalently oxygenated flavone, 5-hydroxyflavone, is 1·5 ppm and when this is applied to the C-10 chemical shift for 2,4,6-trihydroxyacetophenone, the calculated value of C-10 in apigenin is 104·2 ppm. Thus for apigenin the signal at 104·33 ppm is ascribed to C-10 and that at 105·06 to C-3.

Introduction of a 7-OH group into 5,4′-dihydroxyflavone would be expected to cause the signals of *ortho*-related C-6 and C-8 to move upfield by about 13 ppm.

The only signals in this range are those at 100·27 and 95·57 ppm and these are assigned to C-8 and C-6 respectively. Shifts are also expected in the signals of the oxygenated carbons C-5 and C-9, but not in C-4′ and C-2. Accordingly C-2 and C-4′ are assigned the signals at 165·49 and 161·79 ppm respectively, and C-5 and C-9, both of which would be expected to move downfield by about the same amount are assigned the signals 158·67 and 162·03 ppm respectively. The only remaining low field signal, 164·93 ppm is thus ascribed to C-7.

Assignments for 5,7,3′,4′-tetrahydroxyflavone (luteolin) may be deduced from a comparison with the spectra of 5,7,4′-trihydroxyflavone, for the A- and C-ring carbons, and 3′,4′-dihydroxyflavone (see below), for the B-ring carbons. Agreement is remarkably good, and the only ambiguous assignments, those for C-3 (103·94 ppm) and C-10 (104·82 ppm) were confirmed by the 168 Hz $^{13}C$–$^{1}H$ coupling observed in the former.

3′,4′-Dihydroxyflavone is the first member of the second series of flavones studied, viz. 3′,4′-dihydroxy-3′,4′-dimethoxy- and 5-hydroxy-3′,4′-dimethoxyflavone. In the spectrum of 3′,4′-dihydroxyflavone assignments for carbons 3–10 inclusive, which would be relatively unaffected by the oxidation pattern in the B-ring, follow directly from those established[5] for flavone itself. The chemical shift of C-2 also would not be expected to change appreciably since in a comparable case with toluenes it has been established[14,15] that *meta*- and *para*-oxygenation has only a minor effect on the position of the methyl carbon resonance. Thus C-2 is assigned the 164·16 ppm signal. The remaining two low field signals therefore relate to C-3′ and C-4′. That at

149·54 ppm is ascribed to C-4′ on the basis that this signal should appear approx. 13 ppm upfield from that in 5,4′-dihydroxyflavone due to the presence of the 3′-OH. The other signal at 145·80 ppm is therefore allocated to C-3′. Of the other B-ring carbons, C-1′ is represented by a low intensity signal at 122·87 ppm which exhibits no coupling due to an attached proton. The C-5′ signal is distinguishable at 116·62 ppm by its lack of *meta*-proton coupling, leaving the 119·47 and 113·98 ppm signals to be assigned to C-6′ and C-2′. Since these carbons are identical in 4′-hydroxyflavones and the introduction of a 3′-OH would be expected to shift the *ortho*-related C-2′ further upfield than the *para*-related C-6′, C-2′ is ascribed to the 113·98 ppm signal and C-6′ to the 119·47 ppm signal.

Interpretation of the spectrum of 3′,4′-dimethoxyflavone follows directly from the assignments for the dihydroxyflavone, with the exception of the signals of the quaternary carbons (C-1′ and C-10) at 123·33 and 123·75 ppm which remain unassigned. The introduction of a 5-OH group into this system, as in 5-hydroxy-3′,4′-dimethoxyflavone, affects as expected only the A- and C-ring carbon resonances. Carbon shifts for 5-hydroxy-3′,4′-dimethoxyflavone are readily assigned by reference to the spectra of the closely related 5-hydroxyflavone (for A- and C-ring carbons) and 3′,4′-dihydroxyflavone (for B-ring carbons). The agreement is excellent and although only 14 signals are visible in the spectrum, one (at 110·58 ppm) is very intense and is considered to represent both C-8 and C-2′. A study of the proton coupled spectrum did not provide additional evidence on this point, both carbons apparently possessing similar proton coupling constants. The pattern of $^{13}C$–$^{1}H$ coupling observed however did confirm a number of other assignments (Table 1).

3. *Flavonols (3-hydroxyflavones)*

The simplest of the flavonols, 3′,4′-dihydroxyflavonol gave a spectrum containing only thirteen signals. However, one of these, the very intense signal at 125·45 ppm, almost certainly represents the two C atoms C-5 and C-6 (by analogy with the spectrum of flavone). The other missing signal is one of the low intensity signals associated with the quaternary carbons, C-1′ and C-10, one being visible at 123·42 ppm. The missing signal was shown to be hidden under that of C-6′ at 121·72 ppm by a study of the proton coupled spectrum in which the C-6′ signal is removed from this region by coupling. The visible signal at 123·42 ppm is tentatively assigned to C-1′ since in the spectrum of 5,7,3′,4′-tetrahydroxyflavonol where C-10 is well removed from this region, C-1′ is found at 123·75 ppm.

Comparison of this spectrum with that of 3′,4′-dihydroxyflavone permits the assignment of most signals. Two, however are completely new (147·69 and 138·13 ppm) and these must represent C-2 and C-3 both of which would be expected to shift drastically on the introduction of an hydroxyl at C-3. On the basis of established average substituent effects the C-2 signal should shift upfield from 164·16 to about 151 ppm and the C-3 signal downfield from 105·37 to about 132 ppm. Thus the higher field signal (147·69 ppm) is considered to represent C-2 and the other (138·13 ppm) to represent C-3, assignments which are supported by the presence of a well defined singlet at 138·13 ppm in the proton coupled spectrum. It is interesting to note that both shifts are considerably greater than expected.

The only other carbons notably affected by the introduction of the 3-OH group are C-2′ and C-6′. These two carbons plus C-5′ are represented by signals at 121·72, 116·71 and 116·32 ppm (that at 118·83 ppm being assigned to C-8 by analogy with 3′,4′-dihydroxyflavone). The C-5′ signal is readily identified as that at 116·71 ppm by its lack of *meta*-proton coupling in the proton coupled spectrum. The higher field signal of the remaining two must therefore be due to C-6′ which has undergone a 2·3 ppm downfield shift on the introduction of the 3-OH (as also has the C-2′ signal).

Assignments in the spectrum of 3′,4′-dimethoxyflavonol parallel those above for the dihydroxyflavonol and most were confirmed by a study of $^{13}C$–$^{1}H$ coupling (Table 1). Notable differences between the two spectra however are found in the B-ring carbon shifts. For example, as with the equivalent flavones, downfield shifts of the substituted carbons C-3′ and C-4′ (of about 3 ppm) were observed together with upfield shifts of the *ortho*-related C-2′ and C-6′ carbons (of about 4 ppm). Although these shifts are in the directions expected, that of the *ortho*-related carbons is markedly greater than predicted. This effect is probably due to steric interference between the neighbouring methoxyl groups which is known to result in deviations from the established additivity rules and generally in an enhancement of the predicted effect.[12,16]

The spectrum of quercetin (5,7,3′4′-tetrahydroxyflavonol, Fig. 2) is essentially a combination of the spectra of 3′4′-dihydroxyflavonol and 5,7,3′4′-tetrahydroxyflavone, the B- and C-ring carbons relating primarily to the former and the A-ring carbons to the latter. The major differences between this spectrum and that of 5,7,3′,4′-tetrahydroxyflavone (i.e. caused by the introduction of the C-3 hydroxyl) are the same as discussed above for the 3′,4′-dihydroxyflavone/flavonol pair. That is, the C-3 signal moved downfield by 33 ppm, the C-2 signal moved upfield by 17 ppm and the C-2′ and C-6′ signals were again shifted downfield by 2 ppm.

Only fourteen signals are visible in the spectrum of morin (5,7,2′,4′-tetrahydroxyflavonol). The missing signal is however visible as a low intensity triplet at 104·97 ppm in the proton coupled spectrum (in which the C-3′ signal is removed from that region by proton coupling). This signal is ascribed to C-10 by analogy with quercetin (above).

Assignments for the A-ring carbons in morin were made by direct comparison with the quercetin spectrum. C-3 and C-4 were also assigned in this way, and C-2 which would be expected to show a "*meta*-type" downfield shift of about 1·5 ppm on the introduction of an OH at C-2′, was assigned the signal at 149·63 ppm. Of the B-ring carbons, C-3′, being *ortho* to both OH groups should be at highest field and is therefore assigned the signal at 104·97 ppm. Using apigenin as a model, C-3′ in the presence of an OH at C-2′ would be expected to appear at about 104 ppm. C-5′, by the same reasoning, should appear at about 109 ppm and the signal at 109·25 ppm, rather than that at 111·19 ppm is assigned to C-5′ on the basis of the hydrogen coupling (Table 1). The 111·19 ppm signal which appears as a low intensity triplet in the proton coupled spectrum is ascribed to C-1′. This leaves the 132·31 ppm signal to be allocated to C-6′ and this shift is close to the 133 ppm calculated from apigenin for C-6′ after allowing for the introduction of hydroxyls at C-3 and C-2′. The lack of *meta*-proton coupling of this signal in the proton coupled spectrum confirms this assignment.

Of the oxygenated carbons in morin, C-2 and C-3 have already been discussed and C-5, 7 and 9 were assigned by analogy with quercetin. The remaining signals at 160·97

and 158·09 ppm were assigned to C-4′ and C-2′ respectively on the basis of theoretical chemical shifts calculated from the apigenin spectrum. Thus C-2′ in apigenin after allowance is made for the introduction of the 3- and 2′-hydroxyls should appear at about 158 ppm and C-4′ at about 161·5 ppm.

## CONCLUSION

This study of the $^{13}$C–NMR spectra of related series of acetophenones, cinnamic acids, flavones and flavonols has demonstrated that in the main, the additivity principles previously established for simple aromatic systems[6,7] are a reliable guide to the interpretation of flavone and flavonol $^{13}$C–NMR spectra. It is apparent however that these rules are not strictly applicable when changes in molecular structure involve the H-bonded 5- or 3-OH functions or OMe groups *ortho*-related to one another. In the case of the introduction of a 3-OH for example, the effects are surprisingly far-reaching. Not only is C-3 shifted downfield by a larger than expected 33 ppm, but C-2′ and C-6′ are shifted downfield by 2–2·3 ppm and C-4′, upfield by about 1·5–2 ppm. The "*ortho*"-effect of the 3-OH on C-2 is also large at about −17 ppm. In the cases studied in which the effect of an introduced 5-OH group can be gauged, the downfield shift of C-5 is of the expected order (in DMSO solvent[7]) of 30 ppm but the upfield shift of the *ortho*-related carbon (C-6) at about 18 ppm is much larger than expected.

The effects of introducing a 3- or 5-OH group on the shift of the CO carbon, C-4, are also of interest. The CO carbon resonance in flavonoids lacking these groups appears at about 178 ppm. However the presence of a 5-OH shifts this down to near 183 ppm and a 3-OH shifts it up to around 173 ppm. When both 3- and 5-OH groups are present the signal occurs at about 177 ppm. H-bonding is thought to be a major factor in determining the downfield CO carbon shift in systems of this type,[9,17] but clearly in the flavonols the *ortho*-substituent effect of the 3-OH group is over-riding this factor to produce a nett upfield shift.

It is thus concluded that if the special situations outlined above are taken into consideration, then $^{13}$C–NMR data are of significant predictive value in the structure identification of new or unknown flavones and flavonols. This is especially so in view of the fact that, as in UV spectroscopy,[3] the A- and B-rings are largely independent of one another, in that changes in the oxidation pattern of one are not reflected in the spectral characteristics of the other.

The current work has also highlighted the value of studying $^{13}$C–$^{1}$H coupling as an aid to interpreting the spectra of this group of compounds. For example, the distinction of the C-3 signal (J = *ca.* 168 Hz) from those of the C-6 and C-8 (J = *ca.* 168 Hz; $J_{meta}$ = *ca.* 7 Hz) in 5- and/or 7-oxygenated flavones is made very simple by this means. In a similar way in the B-ring, C-5′, which in the commonly encountered 3′,4′-dioxygenated flavonoids lacks a *meta*-related proton, is readily distinguishable from the C-2′ and C-6′ carbons which have similar chemical shifts. *ortho*- and *para*-Couplings, although considerably smaller than the *meta*, are also of value in certain situations as also is the lack of major coupling in the signals of the oxygenated carbons.

Solvent effects have not proven to be a significant problem with this group of compounds. The carbon atoms in unsubstituted A- and B-rings, in particular, appear to be little affected by solvent changes. This is evidenced by the generally good agreement observed between the chemical shift values of B-ring carbons in flavone (measured in $CDCl_3$) and 5-hydroxyflavone (measured in DMSO-$d_6$), and of A-ring carbons carbons in flavone ($CDCl_3$), 3′,4′-dimethoxyflavone (DMSO-$d_6$) and 3′,4′-dihydroxyflavone (DMSO-$d_6$/$D_2O$). Even in hydroxylated flavonoids the chemical shifts of A- and B-ring carbons were not markedly affected by changing the solvent system from DMSO-$d_6$/$D_2O$ to DMSO-$d_6$. For example, the A-ring carbon shifts in the spectrum of 5,4′-dihydroxyflavone compare well with those in 5-hydroxyflavone as also do the B-ring carbon shifts of 5,7,3′,4′-tetrahydroxyflavonol with those of 3′,4′-dihydroxyflavonol.

## EXPERIMENTAL

The $^{13}$C NMR were recorded on a JEOL P-100 Fourier transform spectrometer operating at 25·15 MHz. Spectral widths were 5000 Hz. 16K data points were used except in a few cases where, for technical reasons, only 8K points were available. Proton-coupled spectra were obtained using an electronic gating system which allowed the retention of the nuclear Overhauser enhancement. The deuterium signal of the dimethylsulphoxide (DMSO) solvent was used as lock signal.

Solns were mainly 1 molar in 10 mm tubes, however some sample sizes were as low as 18 mg and necessitated the use of more dilute soln.† The solvents used were DMSO-$d_6$ or DMSO-$d_6$ + $D_2O$ (Table 1). Some of the compounds were not sufficiently soluble at the normal probe temp and for this reason all spectra were recorded at 95°.

All flavonoid samples but apigenin, quercetin and morin which were obtained from Fluka AG., were synthesized at Chemistry Division, D.S.I.R., and all were recrystallized from methanol for the NMR studies. Sample size was generally about 100 mg but ranged from 18 to 250 mg.

†To test for concentration-dependent shift effects, some of the more readily available compounds were recorded at lower concentrations, but no significant chemical shift changes were observed.

## REFERENCES


[1]J. B. Harborne, *The Flavonoids* (Edited by J. B. Harborne, T. J. Mabry and H. Mabry), Chap. 19. Chapman & Hall, London (1975).
[2]T. Swain, *The Flavonoids* (Edited by J. B. Harborne, T. J. Mabry and H. Mabry), Chap. 20, Chapman & Hall, London (1975).
[3]T. J. Mabry, K. R. Markham and M. B. Thomas, *The Systematic Identification of Flavonoids*. Springer-Verlag, Berlin (1970).
[4]T. J. Mabry and K. R. Markham, *The Flavonoids* (Edited by J. B. Harborne, T. J. Mabry and H. Mabry), Chap. 3, Chapman & Hall, London (1975).
[5]P. Joseph-Nathan, J. Mares, Ma. C. Hernandez and J. N. Schoolery, *J. Magnetic Resonance* **16**, 447 (1974).
[6]G. C. Levy and G. L. Nelson, *Carbon-13 Nuclear Magnetic Resonance for Organic Chemists*, pp. 80–81. Wiley, New York (1972).
[7]G. E. Maciel and R. V. James, *J. Am. Chem. Soc.* **86**, 3893 (1964).
[8]J. B. Stothers, *Carbon-13 NMR Spectroscopy*, Vol. 24 of *Organic Chemistry, A series of Monographs*, p. 287. Academic Press, New York (1972).
[9]K. S. Dhami and J. B. Stothers, *Can. J. Chem.* **43**, 479 (1965).
[10]Ref. 6, p. 66.
[11]R. D. Lapper, *Tetrahedron Letters* No. 49–50, 4293 (1974).
[12]K. S. Dhami and J. B. Stothers, *Can. J. Chem.* **44**, 2855 (1966).
[13]J. Massicot and J-P Marthe, *Bull. Soc. Chim. Fr.* 1962 (1962).
[14]P. C. Lauterbur, *Ann. N.Y. Acad. Sci.* **70**, 841 (1958).
[15]Ref. 8, p. 204.
[16]P. C. Lauterbur, *J. Am. Chem. Soc.* **83**, 1846 (1961); Ref. 8, p. 202.
[17]J. B. Stothers and P. C. Lauterbur, *Can. J. Chem.* **42**, 1563 (1964).