MAGNETIC RESONANCE IN CHEMISTRY
Magn. Reson. Chem. 2007; **45**: 835–845
Published online in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/mrc.2054

MRC

A predictive tool for assessing ^{13}C NMR chemical shifts of flavonoids

Darcy C. Burns, David A. Ellis and Raymond E. March*

Department of Chemistry, Trent University, 1600 West Bank Drive, Peterborough, ON K9J 7B8, Canada

Received 7 May 2007; Revised 15 June 2007; Accepted 18 June 2007

Herein are presented the ^{1}H and ^{13}C NMR data for seven monohydroxyflavones (3-, 5-, 6-, 7-, 2′-, 3′-, and 4′-hydroxyflavone), five dihydroxyflavones (3,2′-, 3,3′-, 3,4′-, 3,6-, 2′,3′-dihydroxyflavone), a trihydroxyflavone (apigenin; 5,7,4′-trihydroxyflavone), a tetrahydroxyflavone (luteolin; 5,7,3′,4′-tetrahydroxyflavone), and three glycosylated hydroxyflavones (orientin; luteolin-6C-β-D-glucoside, homoorientin; luteolin-8C-β-D-glucoside, vitexin; apigenin-8C-β-D-glucoside). When these NMR spectra are compared, it is possible to assess the impact of flavone modification and to elucidate detailed structural and electronic information for these flavonoids. A simple predictive tool for assigning flavonoid ^{13}C chemical shifts, which is based on the cumulative differences between the monohydroxyflavones and flavone ^{13}C chemical shifts, is demonstrated. The tool can be used to accurately predict ^{13}C flavonoid chemical shifts and it is expected to be useful for rapid assessment of flavonoid ^{13}C NMR spectra and for assigning substitution patterns in newly isolated flavonoids. Copyright © 2007 John Wiley & Sons, Ltd.

Supplementary electronic material for this paper is available in Wiley InterScience at http://www.interscience.wiley.com/jpages/0749-1581/suppmat/

KEYWORDS: ^{13}C nuclear magnetic resonance spectroscopy; flavonoids; monohydroxyflavones; polyhydroxyflavones; flavonoid glycosides; chemical shift prediction

INTRODUCTION

The ubiquitous class of phytochemicals known as the flavonoids[1] are synthesized by plants for protection against pathogens and herbivores; thus flavonoids are found primarily in petals, the foliage of trees and bushes, and are distributed widely in the edible parts of plants. The chemistry and biochemistry of the flavonoids (i.e. flavones, flavanones, flavonols, and isoflavones) were reviewed extensively in 2005.[2] The structure of flavone is that of a C_{15} phenylbenzopyrone skeleton where two benzene rings (A and B) are linked through a γ-pyrone ring (C) in the middle as shown in Fig. 1.

Flavonoids and isoflavonoids may be of ecotoxicological importance because they are present in the heartwood of tree species used for wood pulp[3,4] and are found in a variety of fruits and vegetables. Flavonoids are of environmental significance because several flavonoid aglycons (flavonoid glycosides that have lost the sugar or glycan moiety) are known to be biologically active[5] while some isoflavones are also phytoestrogens[6,7] that have radical scavenger[8,9] and anticarcinogenic[10,11] activities. Because of their antioxidant and anticancer properties, flavonoids present in foodstuffs, herbal medicines[12] and preventative therapeutics[10] have received much attention recently.

*Correspondence to: Raymond E. March, Department of Chemistry, Trent University, 1600 West Bank Drive, Peterborough, ON K9J 7B8, Canada. E-mail: rmarch@trentu.ca

A range of flavonoids have been extracted from plants and subsequently characterized by ^{13}C and ^{1}H nuclear magnetic resonance (NMR) spectroscopy.[13–18] NMR spectroscopy has also been combined with fast atom bombardment/tandem mass spectrometry (FAB/MS/MS);[19] with high-performance liquid chromatography (HPLC)-MS for the structural characterization of flavonoids and flavonoid-*O*-glycosides;[20,21] with HPLC-UV (ultra-violet absorbance)-SPE (solid phase extraction)-MS for the identification of flavonoids taxifolin, aromadendrin, eriodictyol, naringenin, and apigenin present in Greek Oregano;[22] HPLC-MS for the identification of quercitin and quercetin glycosides in Hypericum perforatum L.;[20] HPLC-MS for the identification of quercetin glycosides in apple peel;[21] and with HPLC-UV-MS for the on-line structural investigation of isoflavones and isoflavanones.[23] In addition, ^{13}C NMR spectra have been obtained for a number of glycosylated flavonoids,[16,24,25] and both ^{13}C and ^{1}H NMR spectra have been reported for acylated derivatives of apigenin-7-*O*-glucoside.[26]

In NMR spectroscopy, the magnetic field experienced at the nucleus of an atom is less than that of the applied magnetic field because electrons around the nucleus shield it from the applied field; the difference between the applied magnetic field and the field at the nucleus is termed the *nuclear shielding*. The chemical shift that is defined as the nuclear shielding/applied magnetic field is a function of the nucleus and its environment; it is measured in relation to a reference compound. For two bonded or adjacent carbon

atoms, the chemical shifts will reflect the environment of both atoms and is indicative of the order (and thus the strength) of the connecting bond. The environment of a ^{13}C nucleus can also be affected in an inductive, through-space (field effect), or resonance-based manner by substituents that are bonded to its carbon framework (skeleton). These effects, which become apparent when ^{13}C substituent chemical shift (SCS) analyses are performed,[27] cause the associated chemical shifts to vary in a systematic and predictable manner.

Recently a method has been described for the estimation of ^{13}C-chemical shifts of carbon atoms in ions that also relies upon systematic variation of chemical shifts in response to differential hydroxyl substitution; such chemical shifts are described as pseudo-NMR chemical shifts.[28] The method involves computation of atomic charges for each carbon atom in neutral, protonated, and deprotonated molecules and relating atomic charges for molecules with experimental ^{13}C chemical shifts for carbon atoms. The relationship between atomic charges and experimental ^{13}C chemical shifts, together with computed atomic charges for carbon atoms in protonated and deprotonated molecules was applied to obtain pseudo-NMR ^{13}C chemical shifts for carbon atoms in protonated and deprotonated molecules. The pseudo-NMR ^{13}C chemical shifts for carbon atoms in ions were applied, in turn, to the rationalization of the relative propensities for competing cross-ring cleavage and ring opening fragmentations observed in product ion mass spectra of protonated and deprotonated monohydroxyflavone molecules. For convenience, the pseudo-NMR ^{13}C chemical shifts in protonated molecules were expressed in relation to pseudo-NMR ^{13}C chemical shifts in protonated flavone, while those for deprotonated molecules were expressed relative to NMR ^{13}C chemical shifts in flavone.

In the present study, a similar approach has been taken whereby the monohydroxy-flavone ^{13}C chemical shifts have been assigned and compared to those of flavone for the development of a predictive tool for the ^{13}C NMR of polyhydroxylated flavonols and their glycosylated analogs. This tool, which relies on the cumulative chemical shift differences between a test set of shifts (i.e. the monohydroxyflavonols) and a baseline set of shifts (i.e. flavone), has been used to predict polyhydroxyl flavonoid chemical shifts. The predicted ^{13}C chemical shifts were then compared to the measured ^{13}C chemical shifts of the polyhydroxyflavones listed above and to previously reported polyhydroxyflavone ^{13}C chemical shifts as a test for accuracy in this approach.

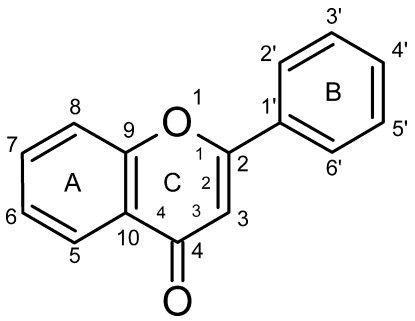

Figure 1. Structure of flavone and numbering scheme for flavone.

The ^{1}H and ^{13}C NMR data of flavone, the monohydroxyflavonols (3-, 5-, 6-, 7-, 2′-, 3′-and 4′-hydroxyflavone), selected polyhydroxylated flavonoids (3,2′-, 3,3′-, 3,4′-, 2′,3′-, and 3,6-dihydroxyflavone, apigenin (5,7,4′-trihydroxyflavone) luteolin (5,7,3′,4′-tetrahydroxyflavone), and glycosylated flavonoids (luteolin-*8C*-*β*-D-glucoside (orientin), luteolin-*6C*-*β*-D-glucoside (homoorientin), and apigenin-*8C*-*β*-D-glucoside (vitexin)), for which the structures are shown in Fig. 2, are presented here along with an assessment of this simple approach for predicting flavonoid ^{13}C chemical shifts.

EXPERIMENTAL

Flavone, 3-, 5-, 6-, and 7-hydroxyflavone were purchased from Sigma–Aldrich Canada Ltd., Oakville, ON. Apigenin (5,7,4′-trihydroxyflavone), luteolin (5,7,3′,4′-tetrahydroxy flavone), luteolin-8C-*β*-D-glucoside (orientin), luteolin-6C-*β*-D-glucoside (homoorientin), 2′-, 3′- and 4′-hydroxyflavone, as well as 3,2′-, 3,3′-, 3,4′, 2′,3′-, and 3,6-dihydroxyflavone were all purchased from Indofine Chemical Company Inc., Hillsborough, New Jersey. Apigenin-8C-*β*-D-glucoside (vitexin) was purchased from Carl Roth GmbH and Co., Karlsruhe, Germany. d_6-Dimethyl-sulphoxide (DMSO) and d_6-acetone were both purchased from Sigma–Aldrich Canada, Oakville,

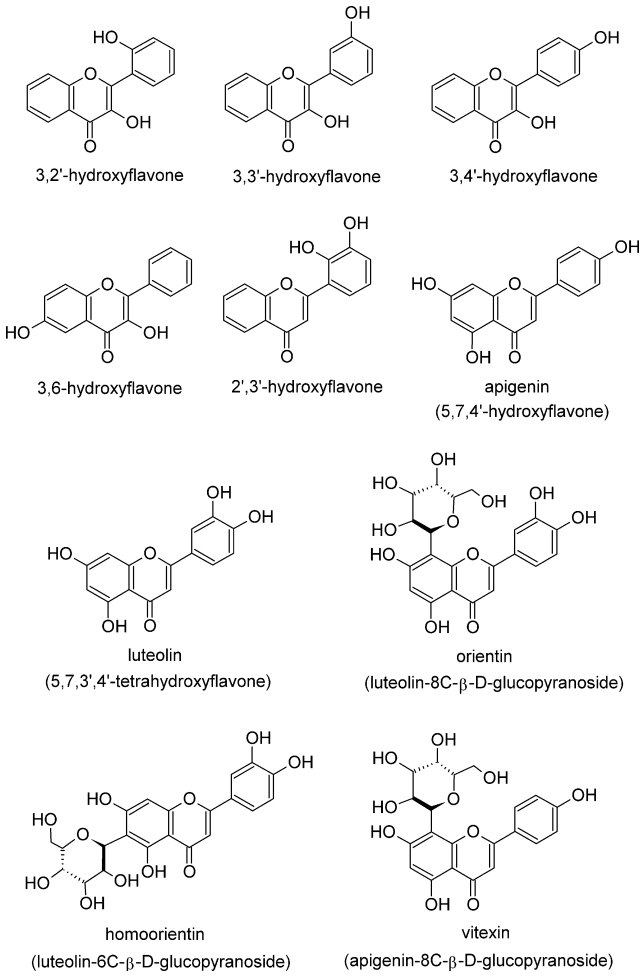

Figure 2. Structures of the compounds for which NMR ^{13}C and ^{1}H spectra were observed; the structures of monohydroxyflavones are not shown.

DOI: 10.1002/mrc

ON. All chemicals were used as supplied, without further purification.

The flavonoid NMR samples were prepared by dissolving 5 mg in 650 µl d_6-DMSO (unless otherwise noted) and were analyzed at 25 °C using 1D ^1H NMR, gCOSY,[29,30] ROESY,[31] ^{13}C–^1H gHSQC[32,33] and ^{13}C–^1H gHMBC[34,35] spectroscopy. All NMR experiments were performed using a Varian Unity spectrometer (Varian Inc., Palo Alto, USA) operating at 499.837 MHz for ^1H and 125.696 MHz for ^{13}C and equipped with a 5 mm variable temperature indirect–detection probe for improved ^1H sensitivity. The detailed acquisition parameters for these experiments can be found in the supplementary material. Processing was performed using the VnmrJ software as supplied by the spectrometer manufacturer. Chemical shifts are reported in ppm and referenced to residual proton and carbon solvent signals (δ 2.50 for proton and δ 39.52 for carbon).

RESULTS AND DISCUSSION

Flavonoid chemical shift assignments were determined from $^1H/^1H$ coupling information, $^1H/^1H$ COSY connectivities, ^1H–^{13}C HSQC single-bond connectivities, and long-range ^1H–^{13}C HMBC connectivities. For flavone, ROEs between H3 and H2′/H6′ and long-range HMBC crosspeaks between C1′ and H3 as well as H2′/H6′ and C2 were extremely useful in establishing a link between the A-ring and B-ring spin systems. The A-ring and C-ring spin-systems were connected using HMBC crosspeaks between H5 to C10 and H3 to C10. The ^{13}C NMR chemical shifts are shown in Tables 1–5 and the ^1H chemical shifts are shown in Tables S1–S3. Since the chemical shifts of flavone (Tables 1 and S1) can be used as base-line peaks for comparison against those of the hydroxylated and glycosylated flavones, they have been further characterized as follows (Fig. 3). Flavone ^1H chemical shifts (Table S1) for the A-, B-, and C-rings are, on the whole, sensibly constant at 7.66 ± 0.11 ppm; H5, H2′, and H3′ are shifted upfield by approximately 0.34 ppm whereas H3 is shifted downfield by 0.60 ppm. Flavone C-ring ^{13}C chemical shifts (Table 1) are affected by the O1 ether, and C4 carbonyl and are, therefore, clearly separated in the ^{13}C NMR spectrum (162.6, 107.0, and 177.1 ppm for C2–C4, respectively); flavone B-ring chemical shifts (C1′–C6′) are similar to those of a singly-substituted benzene ring (126.4–131.7 ppm, respectively); flavone A-ring chemical shifts (C5–C10) are also affected by the presence of the O1 ether and C4 carbonyl and resonate between 118.7 and 125.3 ppm (C5, C6, C8, C10), at 134.3 ppm (C7), and at 155.7 ppm (C9).

For the monohydroxylated species, ^{13}C and ^1H chemical shifts (Tables 1 and S1, respectively) are affected by inductive withdrawal, donation of electron density, and lone-pair resonance contribution into the ring structures. The effect of single-site hydroxylation on the ^1H and ^{13}C NMR spectra of flavone can be assessed quickly and easily by comparing the mono-hydroxyflavone ^{13}C and ^1H chemical shifts to those of the base-line compound so as to obtain $\Delta\delta = \delta_{\text{hydroxyflavone}} - \delta_{\text{flavone}}$ for each atom. C-ring hydroxylation in 3-hydroxyflavone perturbs the ^1H and ^{13}C chemical shifts throughout the entire molecule so that changes in electrostatics, which are brought about by hydroxylation, are localized to the hydroxylated ring and the adjacent ring. In general, A-ring (5-, 6-, and 7-hydroxyflavones) and B-ring (2′-, 3′-, and 4′-hydroxyflavones) hydroxylation does not result in long-range chemical shift perturbations: A-ring hydroxylation has no effect on B-ring chemical shifts and B-ring hydroxylation has no effect on A-ring chemical shifts (^1H: $\Delta\delta \leq \pm 0.06$ ppm, ^{13}C: $\Delta\delta \leq \pm 0.6$ ppm).[36] The sum of all ^{13}C chemical shift deviations, $\Sigma\Delta\delta$ is negative for the hydroxylated rings (excluding the C-ring), indicating that the hydroxyl moiety has the capacity to push electron density into the flavone framework.

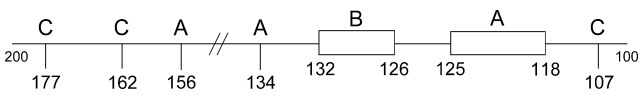

Figure 3. A depiction of the ^{13}C NMR spectrum of flavone showing the ^{13}C chemical shift ranges for nuclei within the A-ring, B-ring, and C-ring (spectrum not to scale). The ranges for these shifts are either labeled below the ring assignment, or are outlined as a box.

The structural and electronic characteristics of the mono-hydroxylated flavones can be distinguished by examining $\Delta\delta$ at each nucleus. For the A- and B-ring hydroxylated species, C_{ipso} nuclei become deshielded in comparison to flavone, resulting in the downfield shift of these ^{13}C resonances ($\Delta\delta_{ipso} = 28.5$ to 30.1 ppm). Interestingly, a low field C5 (and 5OH; δ 12.66 ppm) resonance, thought to arise from intramolecular hydrogen bonding between the C5-hydroxyl group and C-ring carbonyl oxygen,[16,22,24] causes $\Delta\delta_{ipso}$ to be greater for 5-hydroxyflavone ($\Delta\delta_{ipso} = 35.1$ ppm) than for the other A- and B-ring monohydroxyflavones. Both the $H_{ortho/para}$ and $C_{ortho/para}$ nuclei are shielded with respect to flavone as a result of increased electron density supplied by resonance-based lone pair electron distribution into the A- or B-rings (^1H: $\Delta\delta_{ortho}$ −0.52 to −0.80 ppm; $\Delta\delta_{para}$ −0.43 to −0.59 ppm, ^{13}C : $\Delta\delta_{ortho}$ −10.2 to −16.8 ppm; $\Delta\delta_{para}$ −6.4 to −11.1 ppm). The $^1H_{meta}$ and $^{13}C_{meta}$ chemical shifts respond to A- and B-ring hydroxylation in an opposite fashion: $^1H_{meta}$ chemical shifts are slightly shielded ($\Delta\delta_{meta}$ −0.15 to −0.21 ppm) and $^{13}C_{meta}$ chemical shifts are slightly deshielded ($\Delta\delta_{meta}$ 0.3 to 2.1 ppm). Variations in H_{meta} and C_{meta} electron density and, therefore, chemical shift arise exclusively as a result of inductive effects and one can presume that additional electron density is pushed onto the ^1H nuclei and away from the carbon framework. Indeed, semiempirical calculations performed on the monohydroxyflavones reveal greater electron density on the ring protons in comparison to the carbon framework.[28]

The effect of A-ring and B-ring hydroxylation on C-ring chemical shifts is varied and specific to each monohydroxyflavone. Electron density can flow into the C-ring as a result of C6-, C7-, C3′-, and C4′-hydroxylation, where the C2, C3, and C4 resonances are predominantly shifted upfield relative to those of flavone (^{13}C $\Sigma\Delta\delta_{C-ring}$: −0.3 to −2.3 ppm). Electron density may also be removed from the C-ring in response to hydroxylation, as observed for 5-hydroxyflavone ($\Sigma\Delta\delta_{C-ring} = 6.3$ ppm). In this case, hydrogen bonding between OH5 and the C4 carbonyl oxygen can once again be invoked to explain the deshielding that

DOI: 10.1002/mrc

MRC

Table 1. ^{13}C chemical shifts (ppm) for flavone, and selected monohydroxyflavones; in parentheses are given the chemical shift differences δ (flavonoid)-δ (flavone). Samples were dissolved in d_6-DMSO (30.3 mM) and acquired at 25 °C using a Varian Unity INOVA spectrometer operating at 125.696 MHz equipped with a 5 mm variable temperature indirect-detection probe. ^{13}C chemical shift assignments were determined from known ^1H chemical shifts and gHSQC, and gHMBC spectra. Samples were internally referenced to residual d_6-DMSO (^1H; 2.50 ppm, ^{13}C; 39.52 ppm). Underlined values correspond to sites of hydroxylation. $\Sigma\Delta\delta$ is the sum of all chemical shift perturbations within a ring, where $\Delta\delta$ is the difference in chemical shift between a hydroxyflavone ^{13}C nucleus and the corresponding flavone nucleus ($\Delta\delta = \delta_{hydroxyflavone} - \delta_{flavone}$). Negative $\Sigma\Delta\delta$ values correspond to additional shielding within a monohydroxyflavone ring, while positive $\Sigma\Delta\delta$ values correspond to additional deshielding within a monohydroxyflavone ring

Ring A carbon	**flavone** δ (ppm)	**3-OH** δ (ppm)	**5-OH** δ (ppm)	**6-OH** δ (ppm)	**7-OH** δ (ppm)	**2′-OH** δ (ppm)	**3′-OH** δ (ppm)	**4′-OH** δ (ppm)
5	124.7	124.8(0.1)	159.8(35.1)	107.9(−16.8)	126.5(1.8)	124.6(−0.1)	124.7(0)	124.6(−0.1)
6	125.3	124.5(−0.8)	110.9(−14.4)	154.8(29.5)	115.1(−10.2)	125.2(−0.1)	125.4(0.1)	125.3(0)
7	134.3	133.7(−0.6)	136.0(1.7)	123.1(−11.2)	162.8(28.5)	134.1(−0.2)	134.2(−0.1)	134.0(−0.3)
8	118.7	118.4(−0.3)	107.6(−11.1)	119.8(1.1)	102.6(−16.1)	118.5(−0.2)	118.4(−0.3)	118.4(−0.3)
9	155.7	154.6(−1.1)	156.0(0.3)	149.3(−6.4)	157.5(1.8)	155.8(0.1)	155.5(−0.2)	155.5(−0.2)
10	123.3	121.2(−2.1)	110.2(−13.1)	124.1(0.8)	116.2(−7.1)	123.1(−0.2)	123.3(0)	123.3(0)
$\Sigma\Delta\delta$	–	−4.8	−1.5	−3.0	−1.3	−0.7	−0.5	−0.9
Ring C								
2	162.6	145.2(−17.4)	164.1(1.5)	162.1(−0.5)	161.9(−0.7)	160.7(−1.9)	162.6(0)	162.9(0.3)
3	107.0	139.1(32.1)	105.7(−1.3)	105.8(−1.2)	106.7(−0.3)	111.0(4.0)	106.8(−0.2)	104.8(−2.2)
4	177.1	173.1(−4.0)	183.2(6.1)	177.0(−0.1)	176.4(−0.7)	177.2(0.1)	177.0(−0.1)	176.7(−0.4)
$\Sigma\Delta\delta$	–	10.7	6.3	−1.8	−1.7	2.2	−0.3	−2.3
Ring B								
1′	131.0	131.3(0.3)	130.6(−0.4)	131.3(0.3)	131.3(0.3)	117.6(−13.4)	132.3(1.3)	121.5(−9.5)
2′	126.4	127.6(1.2)	126.6(0.2)	126.1(−0.3)	126.1(−0.3)	156.5(30.1)	112.8(−13.6)	128.2(1.8)
3′	129.1	128.6(−0.5)	129.2(0.1)	129.1(0)	129.1(0)	117.0(−12.1)	157.8(28.7)	115.8(−13.3)
4′	131.7	129.6(−2.1)	132.3(0.6)	131.6(−0.1)	131.5(−0.2)	132.4(0.7)	118.7(−13.0)	160.9(29.2)
5′	129.1	128.6(−0.5)	129.2(0.1)	129.1(0)	129.1(0)	119.4(−9.7)	130.1(1.0)	115.8(−13.3)
6′	126.4	127.6(1.2)	126.6(0.2)	126.1(−0.3)	126.1(−0.3)	128.5(2.1)	117.1(−9.3)	128.2(1.8)
$\Sigma\Delta\delta$	–	−0.4	0.8	−0.4	−0.5	−2.3	−4.9	−3.3

Note: 8-Hydroxyflavone ^{13}C chemical shifts as reported by Ares *et al.*[36] ^{13}C NMR (d_6-DMSO, 75 MHz) δ 107.03, 114.6, 119.8, 125.1, 125.7, 126.8, 129.5, 131.7, 132.1, 145.8, 147.4, 162.5, 177.7.

occurs at C4 ($\Delta\delta = 6.1$ ppm). The loss of electron density at C4 is of consequence to the remaining nuclei within the 5-hydroxyflavone C-ring and primarily elicits adjustment of the C2–C3 bond electronics. Here, electron density is donated from C2 and accumulates at C3, so that these ^{13}C resonances are shifted downfield and upfield, respectively. The H3 resonance of 5-hydroxyflavone responds in like, and also shifts to a higher field with respect to the unmodified flavone H3 resonance.

When chemical shift perturbations are examined for each nucleus within the C-ring of 2′-hydroxyflavone, it is found that H3 is deshielded and that C2 and C3 have become alternately shielded and deshielded ($\Delta\delta_{C2} = -1.9$ ppm; $\Delta\delta_{C3} = 4.0$ ppm). This trend is indicative of additional factors affecting the ^1H and ^{13}C chemical shifts that cannot be explained by through-bond resonance and inductive effects alone. Deshielding of the H3 proton and C3 carbon is most likely caused by through-space interactions with the electron-rich 2′-hydroxyl group, and has likewise been observed for the 2-hydroxybiphenyl analog.[37] Decreased shielding at C3 (and increased shielding at C2) may alternatively be the consequence of interactions between OH2′ and the O1 lone pair electrons (i.e. a weak ether-hydroxyl hydrogen bond), which would impact their ability to delocalize into the C-ring. Thus, although the B-ring may be in rapid rotation around the O1–C2–C1′–C2′ torsion angle, there may be at least two low-energy orientations, where the B-ring hydroxyl group is near O1 of the C-ring, or H3 of the C-ring. This observation is in agreement with data presented by Meyer, who has observed two low-energy conformations for 2′- and 3′-hydroxyflavone in an *ab initio* conformational analysis performed at the HF/6–31G(d) level in a continuum solvent.[38] Indeed, H3 T_1 relaxation time constants are much larger for 2′-hydroxyflavone (Table S4), 2′,3′-, and 3,3′-dihydroxyflavone (Table S5) *versus* the remaining flavonoids and may also reflect a tendency for these flavonoids to adopt more than one low-energy conformation in solution.

While the effects of flavone A- and B-ring hydroxylation are localized and do not extend throughout the entire molecule, C-ring hydroxylation (3-hydroxyflavone) perturbs the ^{13}C and ^1H chemical shifts within each ring of the flavone structure. The largest chemical shift differences are observed within the C-ring (C2, C3, C4) and portions of the A-ring (C9, C10) and occur in response to inductive withdrawal of electron density at C_{ipso} and π-back donation of electrons into the OH–C3 bond through resonance. Here, inductive withdrawal of electron density by the

DOI: 10.1002/mrc

Table 2. ^{13}C chemical shifts (ppm) for flavone, 3,2′-, 3,3′-, 3,4′-, 2′,3′-, and 3,6-dihydroxyflavone. Underlined values correspond to sites of hydroxylation. Experimental conditions as for Table 1

Ring A position	**Flavone**[a] δ (ppm)	**3,2′-OH** δ (ppm)	**3,3′-OH** δ (ppm)	**3,4′-OH**[a] δ (ppm)	**2′,3′-OH** δ (ppm)	**3,6-OH**[a] δ (ppm)
5	124.7	124.7	124.7	124.6	124.7	106.7
6	125.3	124.3	124.5	124.4	125.3	154.0
7	134.3	133.2	133.6	133.3	134.1	123.3
8	118.7	118.2	118.2	118.3	118.5	119.8
9	155.7	155.1	154.5	154.4	155.9	148.6
10	123.3	122.0	121.2	121.3	123.3	122.0
$\Sigma\,\Delta\delta$ (ppm)	–	−4.5	−5.3	−5.7	−0.2	−7.6
Ring C						
2	162.6	147.6	145.2	145.9	161.2	144.8
3	107.0	138.9	139.0	137.7	111.1	138.2
4	177.1	172.6	172.9	172.4	177.2	172.5
$\Sigma\,\Delta\delta$ (ppm)	–	12.4	10.4	9.3	2.8	8.8
Ring B						
1′	131.0	118.0	132.3	122.0	118.2	131.4
2′	126.4	155.3	114.3	129.5	145.3	127.5
3′	129.1	116.3	157.1	115.4	145.9	128.4
4′	131.7	131.3	116.8	159.1	117.7	129.5
5′	129.1	118.6	129.3	115.4	119.2	128.4
6′	126.4	130.7	118.2	129.5	118.6	127.5
$\Sigma\,\Delta\delta$ (ppm)	–	−2.8	−5.7	−2.8	−8.8	−1.0

[a] H2′, H6′ and H3′, H5′ exhibit chemical shift equivalence.

3-hydroxy group not only shifts the C3 resonance downfield ($\Delta\delta = 32.1$ ppm), but places increased electron density at C2, causing it to become shielded and resonate at a much higher field than that of flavone ($\Delta\delta = -17.4$ ppm), or the remaining hydroxyflavones ($\Delta\delta_{aver} = -17.2 \pm 0.1$ ppm). The C4 resonance is shifted upfield ($\Delta\delta = -4.0$ ppm) as electron density is drawn from the C4 carbonyl oxygen towards an electropositive C3 nucleus. Electron density is also donated into the A-ring from O1 in response to charge–charge repulsion between O1 and C2 so that the C9 and C10 ^{13}C chemical shifts resonate upfield. The B-ring chemical shifts of 3-hydroxyflavone are only mildly influenced by C-ring hydroxylation, and generally become more shielded in the distal portion of the ring (C3′, C4′, C5′) and deshielded in the proximal portion of the ring (C1′, C2′, C6′).

^{13}C and ^{1}H NMR spectra have also been recorded and the chemical shifts assigned for 3,2′-dihydroxyflavone, 3,3′-dihydroxyflavone, 3,4′-dihydroxyflavone, 2′,3′-dihydroxy flavone, 3,6-dihydroxyflavone (Tables 2 and S2, respectively), 5,7,4′-trihydroxyflavone (apigenin), and 5,7,3′,4′-tetrahydroxyflavone (luteolin) (Tables 3 and S3, respectively). The ^{1}H and ^{13}C chemical shifts of these flavonoids respond to differential hydroxylation in a manner that is similar to the monohydroxyflavones and are dictated by the site of hydroxyl substitution on the ring. For example, ^{13}C chemical shifts (and their differences from flavone) vary in response to inductive and resonance effects arising from each additional hydroxyl group, where C_{ipso} is greatly deshielded, and $C_{ortho/para}$ are shielded. B-ring hydroxylation has little to no effect on the A-ring chemical shifts, and the OH5 chemical shift resonates at high frequency for apigenin and luteolin; indicating that the hydrogen bond to C4 carbonyl is retained in these flavonoids. Finally, chemical shift perturbations caused by the addition of each hydroxyl derivative are cumulative at each nucleus so that differences in ^{1}H or ^{13}C chemical shift from flavone ($\Delta\delta$) can be predicted.

It has been observed that, for the monohydroxyflavones, ^{13}C chemical shift differences can be used to assess the impact of hydroxylation with regards to the modification of the flavone electronics and structure. These shift differences may be measured for the polyhydroxyflavones [Eqn (1)] or may be predicted by summing the chemical shift differences between the constituent monohydroxyflavones and flavone [Eqn (2)]:

$$\Delta\delta_{measured} = (\delta_{phf} - \delta_{flavone}) \quad (1)$$

$$\Delta\delta_{predicted} = \Sigma[(\delta_{mhf1} - \delta_{flavone}) + (\delta_{mhf2} - \delta_{flavone})\ldots] \quad (2)$$

$$\Delta = \Delta\delta_{measured} - \Delta\delta_{predicted} \quad (3)$$

where 'mhf1' and 'mhf2' are monohydroxyflavones and 'phf' is the corresponding polyhydroxyflavone.

For illustration, let us consider the value of Δ from Eqn (3) for C2 from 3,2′-dihydroxyflavone. According to Eqn (1) and using Table 2, $\Delta\delta_{measured} = 147.6 - 162.6 = -15.0$ ppm as shown in Table 4. In accordance with Eqn (2), $\Delta\delta_{predicted}$(C2 from 3,2′-dihydroxyflavone) = Σ [(^{13}C shift for C2 in 3-hydroxyflavone − ^{13}C shift for C2 in flavone) + (^{13}C shift for C2 in 2′-hydroxyflavone − ^{13}C shift for C2 in flavone)]. Using the appropriate values given in Table 1, $\Delta\delta_{predicted}$(C2 from 3,2′-dihydroxyflavone) = Σ[(145.2 − 162.6) + (160.7 − 162.6)] = [(−17.4) + (−1.9)] = −19.3 ppm. Thus $\Delta = -15.0 - (-19.3) = 4.3$ ppm, as shown in Table 4.

DOI: 10.1002/mrc

Table 3. ^{13}C chemical shifts (ppm) for each of the rings within selected polyhydroxyflavones (apigenin and luteolin) and three glycoside flavonoids (vitexin, orientin, and homoorientin). Underlined values correspond to sites of hydroxylation and emboldened values correspond to sites of glycosylation. The published ^{13}C NMR chemical shifts of luteolin, vitexin, and orientin are presented here for comparative purposes. Experimental conditions as for Table 1

Ring A position	Apigenin[a] δ (ppm)	Luteolin δ (ppm)	Luteolin[b] δ (ppm)	Vitexin[a] (apigenin-8C-β-D-glc) δ (ppm)	Vitexin[a,c] (apigenin -8C-β-D-glc) δ (ppm)	Orientin (luteolin -8C-β-D-glc) δ (ppm)	Orientin[c] (luteolin -8C-β-D-glc) δ (ppm)	Homoorientin (luteolin -6C-β-D-glc) δ (ppm)
5	161.2	161.4	161.8	160.2	155.6[d]	160.3	160.5	160.6
6	98.7	98.7	98.7	97.9	98.5	97.9	98.3	**108.9**
7	163.9	164.1	164.8	162.2	162.3	162.5	162.8	163.1
8	93.8	93.6	93.6	**104.3**	**104.6**	**104.5**	**104.6**	93.2
9	157.1	157.2	158.0	155.7	160.3[d]	155.9	156.0	156.1
10	103.5	103.6	103.9	103.8	104.1	104.0	104.0	103.4
$\Sigma\Delta\delta$ (ppm)	−3.8	−3.4	–	2.1	–	3.1	–	3.3
Ring C								
2	163.6	163.8	163.9	163.5	165.0	164.1	164.2	163.6
3	102.6	102.8	102.5	102.2	102.5	102.3	102.4	102.6
4	181.6	181.6	182.5	181.8	182.7	182.0	182.0	181.7
$\Sigma\Delta\delta$ (ppm)	1.1	1.5	–	0.8		1.7	–	1.2
Ring B								
1′	121.0	121.4	123.3	121.3	122.1	122.0	122.0	121.4
2′	128.3	113.1	N/A	128.7	129.0	113.9	114.1	113.1
3′	115.8	145.6	N/A	115.5	115.0	145.9	146.0	145.8
4′	161.0	149.6	N/A	160.8	161.3	149.7	149.9	149.7
5′	115.8	115.8	N/A	115.5	115.0	115.5	115.8	115.7
6′	128.3	118.8	N/A	128.7	129.0	119.2	119.4	118.7
$\Sigma\Delta\delta$ (ppm)	−3.5	−9.4	–	−3.2	–	−7.5	–	−9.3
Glc								
1″	–	–	–	73.2	73.9	73.3	73.5	72.8
2″	–	–	–	70.6	71.0	70.6	70.9	69.9
3″	–	–	–	78.4	79.0	78.6	78.9	78.7
4″	–	–	–	70.3	70.2	70.5	70.8	70.4
5″	–	–	–	81.5	81.3	81.7	82.0	81.4
6″	–	–	–	61.0	61.4	61.4	61.8	61.2

[a] H2′, H6′ and H3′, H5′ exhibit chemical shift equivalence.
[b] ^{13}C NMR (d_6-DMSO, 125 MHz) from Wawer and Zielinska.[16]
[c] ^{13}C NMR (d_6-DMSO, 125 MHz) from Zhou *et al*.[24]
[d] Vitexin C5 and C9 are assigned incorrectly (the assignments should be switched so that C5 = 160.3 ppm and C9 = 155.6 ppm): correction of these assignments is an example of the utility of the predictive tool presented here.

When the difference between $\Delta\delta_{measured}$ and $\Delta\delta_{predicted}$ is calculated [Δ; Eqn (3)], it is immediately apparent how polyhydroxylation affects the chemical shifts and, therefore, the electrostatics of flavone. The results of these calculations (Table 4) indicate three possible scenarios for the polyhydroxyflavones. First, when multiple hydroxyl moieties are isolated on discrete rings so that they only influence flavone electronics locally (long-range effects do not overlap), the predicted chemical shift differences match very closely with the measured values ($\Delta \leq \pm 0.3$ ppm). One such example is 3,3′-dihydroxyflavone, where the effects of 3- and 3′-hydroxyl substitution are confined to the C- and B-rings, respectively. In the second scenario, discrepancies between the measured and predicted chemical shift differences occur so that the influence of individual hydroxyl substituents on flavone electronics is either depressed or enhanced. Thus, differential multiple hydroxyl substitutions can act either in a positively or negatively cooperative manner to impact flavone electronics. Such variations are minimal when the hydroxyl substituents are confined to separate rings (3,4′-dihydroxyflavone, 3,6-dihydroxyflavone; $\Delta \leq \pm 1.3$ ppm) and are amplified when they are located on the same ring (A-ring: apigenin, luteolin; $\Delta \leq \pm 2.7$ ppm). For example, the differences between measured and predicted chemical shift deviations from flavone are amplified for apigenin and luteolin, where C6 (which is *ortho* to OH5 and OH7) is shielded

DOI: 10.1002/mrc

Table 4. The ^{13}C chemical shifts of flavone, apigenin, luteolin, selected dihydroxyflavones and flavone for each nucleus within rings A, B, and C. $\Delta\delta_{measured}$ is the chemical shift difference between dihydroxyflavone and flavone at each nucleus. $\Delta\delta_{predicted}$ is the predicted chemical shift difference between dihydroxyflavone and flavone at each nucleus according to Eqn (2). Cells that are underlined indicate a hydroxylation site

		Ring A						Ring C			Ring B					
Flavonoid	^{13}C	**5**	**6**	**7**	**8**	**9**	**10**	**2**	**3**	**4**	**1′**	**2′**	**3′**	**4′**	**5′**	**6′**
Flavone	δ (ppm)	124.7	125.3	134.3	118.7	155.7	123.3	162.6	107.0	177.1	131.0	126.4	129.1	131.7	129.1	126.4
3,2′-OH	δ (ppm)	124.7	124.3	133.2	118.2	155.1	122.0	147.6	138.9	172.6	118.0	155.3	116.3	131.3	118.6	130.7
	$\Delta\delta_{measured}$	0.0	−1.0	−1.1	−0.5	−0.6	−1.3	−15.0	31.9	−4.5	−13.0	28.9	−12.8	−0.4	−10.5	4.3
	$\Delta\delta_{predicted}$	0.0	−0.9	−0.8	−0.5	−1.0	−2.3	−19.3	36.1	−3.9	−13.1	31.3	−12.6	−1.4	−10.2	3.3
	$\Delta_{2',3}$	0.0	−0.1	**−0.3**	0.0	**0.4**	**1.0**	**4.3**	**−4.2**	**−0.6**	0.1	**−2.4**	−0.2	**1.0**	**−0.3**	**1.0**
3,3′-OH	δ (ppm)	124.7	124.5	133.6	118.2	154.5	121.2	145.2	139.0	172.9	132.3	114.3	157.1	116.8	129.3	118.2
	$\Delta\delta_{measured}$	0.0	−0.8	−0.7	−0.5	−1.2	−2.1	−17.4	32.0	−4.2	1.3	−12.1	28.0	−14.9	0.2	−8.2
	$\Delta\delta_{predicted}$	0.1	−0.7	−0.7	−0.6	−1.3	−2.1	−17.4	31.9	−4.1	1.6	−12.4	28.2	−15.1	0.5	−8.1
	$\Delta_{3',3}$	−0.1	−0.1	0.0	0.1	0.1	0.0	0.0	0.1	−0.1	**−0.3**	**0.3**	−0.2	0.2	**−0.3**	−0.1
3,4′-OH	δ (ppm)	124.6	124.4	133.3	118.3	154.4	121.3	145.9	137.7	172.4	122.0	129.5	115.4	159.1	115.4	129.5
	$\Delta\delta_{measured}$	−0.1	−0.9	−1.0	−0.4	−1.3	−2.0	−16.7	30.7	−4.7	−9.0	3.1	−13.7	27.4	−13.7	3.1
	$\Delta\delta_{predicted}$	0.0	−0.8	−0.9	−0.6	−1.3	−2.1	−17.1	29.9	−4.4	−9.2	3.0	−13.8	27.1	−13.8	3.0
	$\Delta_{4',3}$	−0.1	−0.1	−0.1	0.2	0.0	0.1	**0.4**	**0.8**	**−0.3**	0.2	0.1	0.1	**0.3**	0.1	0.1
3,6-OH	δ (ppm)	106.7	154.0	123.3	119.8	148.6	122.0	144.8	138.2	172.5	131.4	127.5	128.4	129.5	128.4	127.5
	$\Delta\delta_{measured}$	−18.0	28.7	−11.0	1.1	−7.1	−1.3	−17.8	31.2	−4.6	0.4	1.1	−0.7	−2.2	−0.7	1.1
	$\Delta\delta_{predicted}$	−16.7	28.7	−11.8	0.8	−7.5	−1.3	−17.9	30.9	−4.1	0.6	0.9	−0.5	−2.2	−0.5	0.9
	$\Delta_{6,3}$	**−1.3**	0.0	**0.8**	**0.3**	**0.4**	0.0	0.1	**0.3**	**−0.5**	−0.2	0.2	−0.2	0.0	−0.2	0.2
2′,3′-OH	δ (ppm)	124.7	125.3	134.1	118.5	155.9	123.3	161.2	111.1	177.2	118.2	145.3	145.9	117.7	119.2	118.6
	$\Delta\delta_{measured}$	0.0	0.0	−0.2	−0.2	0.2	0.0	−1.4	4.1	0.1	−12.8	18.9	16.8	−14.0	−9.9	−7.8
	$\Delta\delta_{predicted}$	−0.1	0.0	−0.3	−0.5	−0.1	−0.2	−1.9	3.8	0.0	−12.1	16.5	16.6	−12.3	−8.7	−7.2
	$\Delta_{3',2'}$	0.1	0.0	0.1	**0.3**	**0.3**	0.2	**0.5**	**0.3**	0.1	**−0.7**	**2.4**	0.2	**−1.7**	**−1.2**	**−0.6**
Apigenin	δ (ppm)	161.2	98.7	163.9	93.8	157.1	103.5	163.6	102.6	181.6	121.0	128.3	115.8	161.0	115.8	128.3
(5,7,4′-OH)	$\Delta\delta_{measured}$	36.5	−26.6	29.6	−24.9	1.4	−19.8	1.0	−4.4	4.5	−10.0	1.9	−13.3	29.3	−13.3	1.9
	$\Delta\delta_{predicted}$	36.8	−24.6	29.9	−27.5	1.9	−20.2	1.1	−3.8	5.0	−9.6	1.7	−13.2	29.6	−13.2	1.7
	Δ_a	**−0.3**	**−2.0**	**−0.3**	**2.6**	**−0.5**	**0.4**	−0.1	**−0.6**	**−0.5**	**−0.4**	0.2	−0.1	**−0.3**	−0.1	0.2
Luteolin	δ (ppm)	161.4	98.7	164.1	93.6	157.2	103.6	163.8	102.8	181.6	121.4	113.1	145.6	149.6	115.8	118.8
(5,7,3′,4′-OH)	$\Delta\delta_{measured}$	36.7	−26.6	29.8	−25.1	1.5	−19.7	1.2	−4.2	4.5	−9.6	−13.3	16.5	17.9	−13.3	−7.6
	$\Delta\delta_{predicted}$	36.8	−24.5	29.8	−27.8	1.7	−20.2	1.1	−4.0	4.9	−8.3	−11.9	15.5	16.6	−12.2	−7.6
	Δ_1	−0.1	**−2.1**	0.0	**2.7**	−0.2	**0.5**	0.1	−0.2	**−0.4**	**−1.3**	**−1.4**	**1.0**	**1.3**	**−1.1**	0.0

to a much greater extent than expected, and C8 is deshielded with respect to the predicted value (*para*/*ortho* to OH5/OH7).

In the third and final scenario, differences between measured and predicted $\Delta\delta$ values (Table 4) may occur as a result of direct interaction between the hydroxyl substituents in combination with electronic interactions (2′,3′-dihydroxyflavone, 3,2′-dihydroxyflavone, luteolin). In these cases, Δ can be large for the nuclei that are directly affected ($\Delta \leq \pm 4.3$ ppm) and extend towards the chemical shifts of the remaining nuclei within the hydroxylated rings. Both 2′,3′-dihydroxyflavone and luteolin contain 1,2-diols that are capable of hydrogen-bonding in an intramolecular fashion so that the effect on B-ring electrostatics is not that of two hydroxyl groups acting independently, but of a hydrogen-bonded 1,2-diol. In these cases, electron density is reallocated within the B-ring so that the ^{13}C nucleus at one hydroxylation site becomes deshielded (2′,3′-dihydroxyflavone: $\Delta = 2.4$ ppm, luteolin: $\Delta = 1.3$ ppm) and the remaining nuclei shielded ($\Delta = -0.6$ to -1.7 ppm). Hydrogen-bonding has also been observed between the two hydroxyl groups of 3,2′-dihydroxyflavone, and has profound consequences for the C-ring and B-ring electrostatics. In this case, the C-ring predicted chemical shift differences (to flavone) tend to be overestimated resulting in larger Δ values for C2, C3, C4, C9, and C10 (4.3, −4.2, −0.6, 0.4, and 1.0 ppm, respectively). The B-ring predicted chemical shift values behave similarly, resulting in negative Δ values for C2′ and C5′ (−2.4 ppm and −0.3 ppm, respectively), and positive Δ values for C4′ (1.0 ppm), and C6′ (1.0 ppm).

The ^{13}C and ^{1}H NMR spectra of orientin, homoorientin, and vitexin are shown in Tables 3 and S3, respectively; the data were analyzed so that the effect of glycosylation on flavonoid structure and electrostatics might be investigated. Only one set of glycosyl chemical shifts is observed in the ^{1}H and ^{13}C spectra of these flavonoids. The H1″-anomeric proton exhibits large coupling constants to H2″ ($^{3}J_{H1''H2''}$: 9.7–9.8 Hz), the H4″ proton in these spectra is coupled to 4″-OH and H5″, and there is no evidence of a 5″-OH resonance. Thus, according to the Karplus relationship,[39] chemical shifts, and coupling patterns (spin systems), the sugar unit exists exclusively as a β-linked pyranoside in these flavonoids as shown in Fig. 2. This assessment is in agreement with

DOI: 10.1002/mrc

Table 5. The ^{13}C chemical shifts of flavone, vitexin, orientin, and homoorientin for each nucleus within rings A, B, and C. $\Delta\delta_{flavonoid}$ is the measured chemical shift difference between a flavonoid and flavone at each nucleus. Cells that are underlined indicate a hydroxylation site and cells that are emboldened indicate a glycosylation site. $\Delta_v = (\Delta\delta_{vitexin} - \Delta\delta_{apigenin})$; $\Delta_o = (\Delta\delta_{orientin} - \Delta\delta_{luteolin})$; $\Delta_{ho} = (\Delta\delta_{homorientin} - \Delta\delta_{luteolin})$

		Ring A						Ring C			Ring B					
Flavonoid	^{13}C	**5**	**6**	**7**	**8**	**9**	**10**	**2**	**3**	**4**	**1′**	**2′**	**3′**	**4′**	**5′**	**6′**
Flavone	δ (ppm)	124.7	125.3	134.3	118.7	155.7	123.3	162.6	107.0	177.1	131.0	126.4	129.1	131.7	129.1	126.4
Vitexin	δ (ppm)	160.2	97.9	162.2	**104.3**	155.7	103.8	163.5	102.2	181.8	121.3	128.7	115.5	160.8	115.5	128.7
(apigenin-8C-**β-D-glc**)	$\Delta\delta_{vitexin}$	35.5	−27.4	27.9	−14.4	0.0	−19.5	0.9	−4.8	4.7	−9.7	2.3	−13.6	29.1	−13.6	2.3
	$\Delta\delta_{apigenin}$[a]	36.5	−26.6	29.6	−24.9	1.4	−19.8	1.0	−4.4	4.5	−10.0	1.9	−13.3	29.3	−13.3	1.9
	Δ_v	**−1.0**	**−0.8**	**−1.7**	**10.5**	**−1.4**	**0.3**	−0.1	**−0.4**	0.2	**0.3**	**0.4**	**−0.3**	−0.2	**−0.3**	**0.4**
Orientin	δ (ppm)	160.3	97.9	162.5	**104.5**	155.9	104.0	164.1	102.3	182.0	122.0	113.9	145.9	149.7	115.5	119.2
(luteolin-8C-**β-D-glc**)	$\Delta\delta_{orientin}$	35.6	−27.4	28.2	−14.2	0.2	−19.3	1.5	−4.7	4.9	−9.0	−12.5	16.8	18.0	−13.6	−7.2
	$\Delta\delta_{luteolin}$[a]	36.7	−26.6	29.8	−25.1	1.5	−19.7	1.2	−4.2	4.5	−9.6	−13.3	16.5	17.9	−13.3	−7.6
	Δ_o	**−1.1**	**−0.8**	**−1.6**	**10.9**	**−1.3**	**0.4**	**0.3**	**−0.5**	**0.4**	**0.6**	**0.8**	**0.3**	0.1	**−0.3**	**0.4**
Homoorientin	δ (ppm)	160.6	**108.9**	163.1	93.2	156.1	103.4	163.6	102.6	181.7	121.4	113.1	145.8	149.7	115.7	118.7
(luteolin-6C-β-D-glc)	$\Delta\delta_{homoorientin}$	35.9	−16.4	28.8	−25.5	0.4	−19.9	1.0	−4.4	4.6	−9.6	−13.3	16.7	18.0	−13.4	−7.7
	$\Delta\delta_{luteolin}$[a]	36.7	−26.6	29.8	−25.1	1.5	−19.7	1.2	−4.2	4.5	−9.6	−13.3	16.5	17.9	−13.3	−7.6
	Δ_{ho}	**−0.8**	**10.2**	**−1.0**	**−0.4**	**−1.1**	−0.2	−0.2	−0.2	0.1	0.0	0.0	0.2	0.1	−0.1	−0.1

[a] Same as $\Delta\delta_{measured}$ in Table 4.

those of Zhou *et al.*,[24] Xie *et al.*[25] and Kumarasamy *et al.*[40] who have reported ^1H NMR data for vitexin, orientin, and homoorientin, respectively.

The glycosylated polyhydroxyflavone NMR spectra were analyzed by comparing their ^{13}C chemical shifts to flavone [Eqn (4)]. These chemical shift differences, $\Delta\delta_g$, were then compared against the nonglycosylated analogs, $\Delta\delta_{phf}$ [Eqns (5), (6)], to reveal the impact of glycosylation on polyhydroxyflavone electrostatics (Table 5):

$$\Delta\delta_g = \delta_{\text{glycosylated polyhydroxyflavone}} - \delta_{\text{flavone}} \quad (4)$$

$$\Delta\delta_{phf} = \delta_{\text{polyhydroxyflavone}} - \delta_{\text{flavone}} \quad (5)$$

$$\Delta_g = \Delta\delta_g - \Delta\delta_{phf} \quad (6)$$

where 'g' and 'phf' have identical hydroxyl-substitution patterns.

Many of the nuclei within vitexin, orientin, and homoorientin yield small Δ_g values, particularly those of the B- and C-rings ($\Delta_g \leq 0.2$ ppm). In these situations, the factors that influence the chemical shifts, electronics, and structure for each compound and its aglycan analog are equivalent and their local structures and electrostatics are likely similar. For example, slight ^1H chemical shift differences (obtained as for the ^{13}C chemical shift differences) between the OH5 resonance positions of vitexin and apigenin suggest that this hydroxyl group remains hydrogen-bonded to the C4 carbonyl in vitexin. Extrapolating, one can assume that the overriding influences of polyhydroxyl substitution on flavone electrostatics, such as electron withdrawal at C_{ipso} and heavy shielding at $C_{ortho/para}$, would also be expected to occur in the glycosylated analogs. Hence, one can presume that the structure and electronics of the B- and C-rings of homoorientin and its C6-glucoside analog luteolin are similar because their ^{13}C chemical shifts are equivalent. Thus, the B- and C-rings of homoorientin are not at all influenced by glycosylation.

Vitexin, orientin, and homoorientin are all examples of A-ring glycosylated flavonoids and it is these resonances that are affected to the greatest extent. Overall, glycosylation is expected to contribute in an additive way to the chemical shift positions (and electrostatics) of ^{13}C and ^1H nuclei in much the same manner as additional hydroxyl substituents affect the electrostatics of each monohydroxyflavone. In each of the glycosylated flavonoids, the ^{13}C resonances at the site of substitution are shifted downfield significantly (C_{ipso}; $\Delta = 10.2 - 10.9$ ppm). The remaining A-ring carbon nuclei and C-ring C9 nucleus are shielded with respect to the nonglycosylated analogs ($\Delta_g = -0.4$ to -1.7 ppm). Thus, additional electron density is drawn into the A-ring in response to electron withdrawal at the site of glucopyranose substitution. Interestingly, the B-ring ^{13}C and ^1H chemical shifts of orientin and vitexin are influenced by glycosylation even though none of the glycosylated flavonoids are expected to participate in long-range scalar interactions. These chemical shift differences are not apparent in the C6-substituted homoorientin NMR spectra and must, therefore, be indicative of indirect and/or direct through-space interactions between the C8-substituted glycosyl subunit and rings B and C. Hydrogen-bonding between the glycosyl unit and B ring is not possible for vitexin so that additional deshielding of the H2′/H6′, C1′, and C2′/C6′ nuclei may occur either as a result of their proximity to the glucopyranose ring or as a result of interaction with O1 on the C-ring.

The degree and breadth of the effect of glycosylation on B-ring resonances is more pronounced in orientin (than in vitexin and homoorientin), where the glycosyl ring hydroxyl groups may also have the opportunity to participate in hydrogen-bonding with OH3′. Here the H2′, H6′, OH4′ (Table S3), C1′, C2′, C3′, and C6′ (Table 3) resonances shift downfield and the OH3′, and H3′ resonances shift upfield with respect to those of luteolin. The pronounced influence of the C8-glycosyl substitution could be the combination

DOI: 10.1002/mrc

Table 6. The ^{13}C chemical shifts of flavone, 5,4′-, 6,4′-, and 7,4′-dihydroxyflavone (Ibrahim and Abul-Hajj),[41] 2′,4′- and 3′,4′-dihydroxyflavone (Moon *et al.*)[42] 7,3′- and 7,3-dihydroxyflavone (Park *et al.*)[43] for each nucleus within rings A, B, and C. $\Delta\delta_{measured}$ is the chemical shift difference between dihydroxyflavone and flavone at each nucleus. $\Delta\delta_{predicted}$ is the predicted chemical shift difference between dihydroxyflavone and flavone at each nucleus according to Eqn (2). Cells that are underlined indicate a hydroxylation site

		Ring A						Ring C			Ring B					
Flavonoid	^{13}C	**5**	**6**	**7**	**8**	**9**	**10**	**2**	**3**	**4**	**1′**	**2′**	**3′**	**4′**	**5′**	**6′**
Flavone	δ (ppm)	124.7	125.3	134.3	118.7	155.7	123.3	162.6	107.0	177.1	131.0	126.4	129.1	131.7	129.1	126.4
5,4′-OH	δ (ppm)	159.4	110.6	135.7	107.4	155.8	109.9	164.7	103.3	182.9	120.9	128.8	116.0	161.5	116.0	128.8
	$\Delta\delta_{measured}$	34.7	−14.7	1.4	−11.3	0.1	−13.4	2.1	−3.7	5.8	−10.1	2.4	−13.1	29.8	−13.1	2.4
	$\Delta\delta_{predicted}$	35.0	−14.4	1.4	−11.4	0.1	−13.1	1.8	−3.5	5.7	−9.9	2.0	−13.2	29.8	−13.2	2.0
	$\Delta_{4',5}$	**−0.3**	**−0.3**	0.0	0.1	0.0	**−0.3**	**0.3**	−00.2	0.1	−0.2	**0.4**	0.1	0.0	0.1	**0.4**
6,4′-OH	δ (ppm)	103.8	149.2	122.7	119.9	154.7	124.2	162.7	107.5	176.8	121.7	128.2	115.9	160.9	115.9	128.2
	$\Delta\delta_{measured}$	−20.9	23.9	−11.6	1.2	−1.0	0.9	0.1	0.5	−0.3	−9.3	1.8	−13.2	29.2	−13.2	1.8
	$\Delta\delta_{predicted}$	−16.9	29.5	−11.5	0.8	−6.6	0.8	−0.2	−3.4	−0.5	−9.2	1.5	−13.3	29.1	−13.3	1.5
	$\Delta_{4',6}$	**−4.0**	**−5.6**	−0.1	**0.4**	**5.6**	0.1	**0.3**	**3.9**	0.2	−0.1	**0.3**	0.1	0.1	0.1	**0.3**
7,4′-OH	δ ppm)	126.4	114.9	162.5	102.5	157.4	116.1	162.7	104.5	176.2	121.9	128.1	115.9	160.7	115.9	128.1
	$\Delta\delta_{measured}$	1.7	−10.4	28.2	−16.2	1.7	−7.2	0.1	−2.5	−0.9	−9.1	1.7	−13.2	29.0	−13.2	1.7
	$\Delta\delta_{predicted}$	1.7	−10.2	28.2	−16.4	1.6	−7.1	−0.4	−2.5	−1.1	−9.2	1.5	−13.3	29.0	−13.3	1.5
	$\Delta_{4',7}$	0.0	−0.2	0.0	0.2	0.1	−0.1	**0.5**	0.0	0.2	0.1	0.2	0.1	0.0	0.1	0.2
2′,4′-OH	δ (ppm)	124.6	125.0	133.8	118.3	155.7	123.2	161.1	109.0	177.2	109.0	158.6	103.3	161.5	108.0	129.8
	$\Delta\delta_{measured}$	−0.1	−0.3	−0.5	−0.4	0.0	−0.1	−1.5	2.0	0.1	−22.0	32.2	−25.8	29.8	−21.1	3.4
	$\Delta\delta_{predicted}$	−0.2	−0.1	−0.5	−0.5	−0.1	−0.2	−1.6	1.8	−0.3	−22.9	31.9	−25.4	29.9	−23.0	3.9
	$\Delta_{2',4'}$	0.1	−0.2	0.0	0.1	0.1	0.1	0.1	0.2	**0.4**	**0.9**	**0.3**	**−0.4**	−0.1	**1.9**	**−0.5**
3′,4′-OH	δ (ppm)	124.8	125.3	134.1	118.3	155.6	123.3	163.3	104.9	176.9	122	113.4	145.8	149.5	116.0	118.9
	$\Delta\delta_{measured}$	0.1	0.0	−0.2	−0.4	−0.1	0.0	0.7	−2.1	−0.2	−9.0	−13.0	16.7	17.8	−13.1	−7.5
	$\Delta\delta_{predicted}$	−0.1	0.1	−0.4	−0.6	−0.4	0.0	0.3	−2.4	−0.5	−8.2	−11.8	15.4	16.2	−12.3	−7.5
	$\Delta_{3',4'}$	0.2	−0.1	0.2	0.2	**0.3**	0.0	**0.4**	**0.3**	**0.3**	**−0.8**	**−1.2**	**1.3**	**1.6**	**−0.8**	0.0
7,3′-OH	δ (ppm)	126.6	115.1	162.8	102.5	157.5	116.2	162.1	106.6	176.4	132.6	112.6	157.9	118.6	130.2	117.0
	$\Delta\delta_{measured}$	1.9	−10.2	28.5	−16.2	1.8	−7.1	−0.5	−0.4	−0.7	1.6	−13.8	28.8	−13.1	1.1	−9.4
	$\Delta\delta_{predicted}$	1.8	−10.1	28.4	−16.4	1.6	−7.1	−0.7	−0.5	−0.8	1.6	−13.9	28.7	−13.2	1.0	−9.6
	$\Delta_{3',7}$	0.1	−0.1	0.1	0.2	0.2	0.0	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.2
3,7-OH	δ (ppm)	126.6	115.0	162.6	102.1	156.6	114.3	144.2	138.5	172.4	131.5	127.4	128.5	129.6	128.5	127.4
	$\Delta\delta_{measured}$	1.9	−10.3	28.3	−16.6	0.9	−9	−18.4	31.5	−4.7	0.5	1	−0.6	−2.1	−0.6	1
	$\Delta\delta_{predicted}$	1.9	−11	27.9	−16.4	0.7	−9.2	−18.1	31.8	−4.7	0.6	0.9	−0.5	−2.3	−0.5	0.9
	$\Delta_{3,7}$	0	**0.7**	**0.4**	−0.2	0.2	0.2	**−0.3**	**−0.3**	0	−0.1	0.1	−0.1	0.2	−0.1	0.1

of indirect effects such as diamagnetic anisotropy provided from the pyranose ring and direct effects such as hydrogen-bonding between glucopyranoside hydroxyl groups and OH3′ so that B-ring electrostatics are perturbed.

Long-range glycosyl-B-ring interactions are also evident from an examination of the ROESY spectra of vitexin and orientin (Table S6). For vitexin, weak ROEs are observed between H2″ and H2′/H6′ and between H4″ and H2′/H6′. As well, ROEs are observed between the H6β″ proton of the glycosyl unit and both the H2′/H6′ resonance and the H3′/H5′ resonance on the B-ring. Many more ROEs can be observed in the analogous ROESY spectrum of orientin, where longer-range interactions between the glycosyl unit and B-ring are apparent. These include 6 ROEs to H2′, 6 ROEs to H5′, and 5 ROEs to H6′. Long-range ROEs to B-ring nuclei are not evident in the C6-substituted homoorientin ROESY spectrum.

^{1}H and ^{13}C NMR data have been recorded for flavone, selected monohydroxy-, dihydroxy-, trihydroxy-, and tetrahydroxyflavones, as well as three glycosylated flavones (luteolin-6C-β-D-glucoside, luteolin-8C-β-D-glucoside, and apigenin-8C-β-D-glucoside). The ^{13}C chemical shifts of flavone can be grouped to the A-, B-, or C-rings (Fig. 3) and perturbations in these shifts upon hydroxylation and glycosylation are readily identifiable. For the hydroxylated flavones, the ^{13}C NMR assignments and number of hydroxyl substituents can be estimated based simply on inspection and comparison of the ^{13}C chemical shifts relative to those of flavone. There should be at least one $\Delta\delta$ value above +27.4 ppm for each hydroxyl group and two $\Delta\delta$ values above 16.5 ppm for each 1,2-diol. Because the measured polyhydroxyflavone chemical shift differences and predicted values are in close agreement and because the measured chemical shift differences will *only* match closely to one set of predicted chemical shift differences (even 3,2′-dihydroxy-flavone), the ^{13}C assignments of the polyhydroxyflavones may be immediately identified without the need for a rigourous gHMBC-based assignment strategy. The number and location of flavone glycosyl substituents may also be predicted based solely on a ^{13}C chemical shift

DOI: 10.1002/mrc

MRC

Table 7. The ^{13}C chemical shifts of 3,6,3′-, 3,7,3′-, 3,6,4′-, and 3,7,4′-trihydroxyflavone reported by Kim *et al.*[14] and of flavone for each nucleus within rings A, B, and C. $\Delta\delta_{measured}$ is the chemical shift difference between trihydroxyflavone and flavone at each nucleus. $\Delta\delta_{predicted}$ is the predicted chemical shift difference between a trihydroxyflavone and flavone ^{13}C nucleus according to Eqn (2). Cells that are underlined indicate a hydroxylation site

		Ring A						Ring C			Ring B					
Flavonoid	^{13}C	**5**	**6**	**7**	**8**	**9**	**10**	**2**	**3**	**4**	**1′**	**2′**	**3′**	**4′**	**5′**	**6′**
Flavone	δ (ppm)	124.7	125.3	134.3	118.7	155.7	123.3	162.6	107.0	177.1	131.0	126.4	129.1	131.7	129.1	126.4
3,6,3′-OH	δ (ppm)	106.7	154.0	123.3	119.6	148.5	121.9	144.9	138.3	172.5	132.5	114.4	157.2	116.8	129.4	118.3
	$\Delta\delta_{measured}$	−18.0	28.7	−11.0	0.9	−7.2	−1.4	−17.7	31.3	−4.6	1.5	−12.0	28.1	−14.9	0.3	−8.1
	$\Delta\delta_{predicted}$	−16.7	28.8	−11.9	0.5	−7.7	−1.3	−17.9	30.7	−4.2	1.9	−12.7	28.2	−15.2	0.5	−8.4
	$\Delta_{3',3,6}$	**−1.3**	−0.1	**0.9**	**0.4**	**0.5**	−0.1	0.2	**0.6**	**−0.4**	**−0.4**	**0.7**	−0.1	**0.3**	−0.2	**0.3**
3,7,3′-OH	δ (ppm)	126.5	114.8	162.5	101.8	156.4	114.1	144.1	138.3	172.2	132.5	114.2	157.4	116.6	129.4	118.1
	$\Delta\delta_{measured}$	1.8	−10.5	28.2	−16.9	0.7	−9.2	−18.5	31.3	−4.9	1.5	−12.2	28.3	−15.1	0.3	−8.3
	$\Delta\delta_{predicted}$	1.9	−10.9	27.8	−16.7	0.5	−9.2	−18.1	31.6	−4.8	1.9	−12.7	28.2	−15.3	0.5	−8.4
	$\Delta_{3',3,7}$	−0.1	**0.4**	**0.4**	−0.2	0.2	0.0	**−0.4**	**−0.3**	−0.1	**−0.4**	**0.5**	0.1	0.2	−0.2	0.1
3,6,4′-OH	δ (ppm)	106.8	154.0	122.8	119.5	148.4	122.1	145.8	137.2	172.1	122.2	129.0	115.4	159.0	115.4	129.0
	$\Delta\delta_{measured}$	−17.9	28.7	−11.5	0.8	−7.3	−1.2	−16.8	30.2	−5.0	−8.8	2.6	−13.7	27.3	−13.7	2.6
	$\Delta\delta_{predicted}$	−16.8	28.7	−12.1	0.5	−7.7	−1.3	−17.6	28.7	−4.5	−8.9	2.7	−13.8	27.0	−13.8	2.7
	$\Delta_{4',3,6}$	**−1.1**	0.0	**0.6**	**0.3**	**0.4**	0.1	**0.8**	**1.5**	**−0.5**	0.1	−0.1	0.1	**0.3**	0.1	−0.1
3,7,4′-OH	δ (ppm)	126.4	114.8	162.5	101.8	156.4	114.1	145.0	137.1	172.0	112.1	129.2	115.3	158.7	115.3	129.2
	$\Delta\delta_{measured}$	1.7	−10.5	28.2	−16.9	0.7	−9.2	−17.6	30.1	−5.1	−18.9	2.8	−13.8	27.0	−13.8	2.8
	$\Delta\delta_{predicted}$	1.8	−11	27.6	−16.7	0.5	−9.2	−17.8	29.6	−5.1	−8.9	2.7	−13.8	26.9	−13.8	2.7
	$\Delta_{4',3,7}$	−0.1	**0.5**	**0.6**	−0.2	0.2	0.0	0.2	**0.5**	0.0	**−10.0**	0.1	0.0	0.1	0.0	0.1

analysis since glycosylation was found to deshield the ^{13}C nuclei at the site of substitution (causing a 10.2 ppm upfield shift) without significantly affecting the remaining chemical shifts.

The accuracy of this tool was assessed by comparing the reported ^{13}C chemical shifts of several di- and trihydroxylated flavones[15,41–43] to their predicted values (Tables 6 and 7, respectively). The measured and predicted ^{13}C chemical shifts of the dihydroxyflavones were in close agreement ($\leq \pm 0.4$ ppm), possibly because the hydroxyl substituents were unable to jointly influence the electronics at any particular ^{13}C nucleus. The predicted ^{13}C chemical shifts differed from the reported ^{13}C chemical shifts when two or more hydroxyl substituents were able either to participate in hydrogen bonding with one another (i.e. 3′,4′-dihydroxyflavone, Table 6) or to affect strongly the environment of a single ^{13}C nucleus. This latter behavior was most apparent when two hydroxyl groups occupied positions that were *meta* to one another on ring A or ring B. Thus, a 5,7-dihydroxy substitution pattern, as in apigenin and luteolin, can be recognized by an average deviation of −2.1 ppm at C6 and of 2.7 ppm at C8 from the predicted value (Table 4) and a 2′,4′-dihydroxy substitution pattern could be recognized by deviations of 0.9 ppm at C1′, −0.4 ppm at C3′, and 1.9 ppm at C5′ from the predicted values (Table 6). Chemical shift deviations of similar magnitude were consistent amongst each of the dihydroxyflavones suggesting that these could be treated as correction factors for the predicted values, although this approach was not taken in the current study. The reported and predicted chemical shifts of 3,7,3′-, and 3,7,4′-trihydroxyflavone (Table 7) were consistent and exhibited differences of >0.4 ppm at only four of thirty ^{13}C nuclei, while those of 3,6,3′-, and 3,6,4′-trihydroxyflavone were not inconsistent with those observed for 3,6-dihydroxyflavone. Thus, on the whole, the predicted and measured chemical shifts of the trihydroxyflavones are in good agreement.

From Table 6, it is proposed that a deviation of >4 ppm between the measured and predicted ^{13}C chemical shifts indicates either an incorrect assignment or an incorrectly reported ^{13}C chemical shift. In the case of 6,4′-dihydroxyflavone, there are four chemical shifts whose predicted and reported values differ by more than ±3.9 ppm: $\Delta C3 = 3.9$ ppm; $\Delta C5 = -4.0$ ppm; $\Delta C6 = -5.6$ ppm; and $\Delta C9 = 5.6$ ppm (Table 6). When the assignments of C3 and C5 are switched for one another and those of C6 and C9 are switched similarly, then the chemical shift deviations of these nuclei fall within $< \pm 0.3$ ppm. Thus it is suggested that the reported chemical shifts for C3, C5, C6, and C9 in 6,4′-dihydroxyflavone are incorrect. For 3,7,4′-trihydroxyflavone, there is a deviation (Δ) of −10.0 ppm between the reported and predicted C1′ ^{13}C chemical shifts (Table 7). It is proposed that the reported C1′ chemical shift (112.1 ppm) should be given as 121.1 ppm, which is in line with similarly substituted flavones (Tables 1 and 2) and would yield a more reasonable chemical shift deviation of −1.0 ppm.

CONCLUSIONS

Overall, the polyhydroxy- and glycosylated flavone chemical shifts and, by proxy, their structure and electronics are *fully predictable*. The power of such a tool is apparent for predicting the NMR of flavonoids and identifying unknown flavonoids from novel sources. For example, a recent search using SciFinder[44] revealed that ^{13}C NMR spectra of only 11 of 28 commercially available dihydroxyflavones have been recorded and analyzed. Using the strategy proposed

DOI: 10.1002/mrc

here, ^{13}C NMR data for the remaining 17 flavonoids can be predicted. The ^{13}C chemical shifts can also be predicted for polyhydroxylated flavonoids whose NMR have not been determined, for rapid assessment of unassigned flavonoids NMR spectra and for verifying previously reported ^{13}C chemical shifts of flavonoids. The prediction of these values is of importance because it allows quick and facile assessment of flavonoid structure and electronics, which can be important for determining their behavior in a laboratory setting, (i.e. mass spectrometric fragmentation patterns, UV-VIS maxima, etc.), environmental setting, and *in vivo* setting.

Supplementary material

Supplementary electronic material for this paper is available in Wiley InterScience at: http://www.interscience.wiley.com/jpages/0749-1581/suppmat/

Acknowledgements

The authors acknowledge the financial support from each of the Natural Sciences and Engineering Research Council of Canada (Discovery Grants Program), the Canada Foundation for Innovation, the Ontario Research & Development Challenge Fund, and Trent University.

REFERENCES

1. Bohm BA. *Introduction to the Flavonoids*. Harwood Academic Publishers: Australia, 1998.
2. Anderson OM, Markham KR (eds). *Flavonoids: Chemistry, Biochemistry and Applications*. CRC Press: Boca Raton, 2005.
3. Sjostrom E. *Wood Chemistry Fundamentals and Applications*. Academic Press: New York and London, 1981.
4. Bate-Smith EC. *Wood Extractives and their Significance to the Pulp and Paper Industries*. Hillis WE (ed). Academic Press: New York and London, 1962; 133.
5. Harborne JB, Grayer RJ. *The Flavonoids: Advances in Research since 1986*. Harborne JB (ed). Chapman and Hall: London, 1994; 589.
6. Beck V, Unterrieder E, Krenn L, Kubelka W, Jungbauer A. *J. Steroid Biochem. Mol. Biol.* 2003; **84**: 259.
7. Boue SM, Wiese TE, Nehls S, Burow ME, Elliott S, Carter-Wientjes CH, Shih BY, McLachlan JA, Cleveland TE. *J. Agric. Food Chem.* 2003; **51**: 2193.
8. Shirley BW. *Trends Plant Sci.* 1996; **1**: 377.
9. Fritz KL, Seppanen CM, Kurzer MS, Csallany AS. *Nutr. Res.* 2003; **23**: 479.
10. Dixon RA, Steel CL. *Trends Plant Sci.* 1999; **4**: 394.
11. Gerhauser C, Klimo K, Heiss E, Neumann I, Gamal-Eldeen A, Knauft J, Liu GY, Sitthimonchai S, Frank N. *Mutat. Res.* 2003; **523**: 163.
12. Packer L, Rimach G, Virgili F. *Free Radic. Biol. Med.* 1999; **27**: 704.
13. Blasko G, Xun L, Cordell GA. *J. Nat. Prod.* 1988; **51**: 60.
14. Kim H, Moon BH, Ahn JH, Lim Y. *Magn. Reson. Chem.* 2006; **44**: 188.
15. Aksnes DW, Standnes A, Andersen OM. *Magn. Reson. Chem.* 1996; **24**: 820.
16. Wawer I, Zielinska A. *Magn. Reson. Chem.* 2001; **39**: 374.
17. Budzianowski J, Morozowska M, Wesolowska M. *Phytochemistry* 2005; **66**: 1033.
18. Biekofsky RR, Buschi CA, Pomilio AB. *Magn. Reson. Chem.* 1991; **29**: 569.
19. Borges C, Martinho P, Martins A, Rauter AP, Ferreira MAA. *Rapid Commun. Mass Spectrom.* 2001; **15**: 1760.
20. Hansen SH, Jensen AG, Cornett C, Bjørnsdottir I, Taylor S, Wright B, Wilson ID. *Anal. Chem.* 1999; **71**: 5235.
21. Lommen A, Godejohann M, Venema DP, Hollman PCH, Spraul M. *Anal. Chem.* 2000; **72**: 1793.
22. Exarchou V, Godejohann M, van Beek TA, Gerothanassis IP, Vervoort J. *Anal. Chem.* 2003; **75**: 6288.
23. Wolfender J-L, Ndjoko K, Hostettmann K. *J. Chromatogr. A.* 2003; **1000**: 437.
24. Zhou X, Peng JY, Fan GR, Wu YT. *J. Chromatogr. A.* 2005; **1092**: 216.
25. Xie C, Veitch NC, Houghton PJ, Simmonds MS. *Chem. Pharm. Bull* 2003; **51**: 1204.
26. Švehlíková V, Bennett RN, Mellon FA, Needs PW, Piacente S, Kroom PA, Bao Y. *Phytochemistry* 2004; **65**: 2323.
27. Ewing DF. *Magn. Reson. Chem.* 1979; **12**: 499.
28. Burns DC, Ellis DA, Li H, Lewars EG, March RE. *Rapid Commun. Mass Spectrom.* 2007; **21**: 437.
29. von Kienlin M, Moonen CTW, van der Toorn A, Vanzijl PCM. *J. Magn. Reson.* 1991; **93**: 423.
30. Hurd RE. *J. Magn. Reson.* 1990; **87**: 422.
31. Hwang TL, Shaka AJ. *J. Am. Chem. Soc.* 1992; **114**: 3157.
32. Bodenhausen G, Ruben DJ. *Chem. Phys. Lett.* 1980; **69**: 185.
33. Kay LE, Keifer P, Saarinen T. *J. Am. Chem. Soc.* 1992; **114**: 10663.
34. Bax A, Summers MF. *J. Am. Chem. Soc.* 1986; **108**: 2093.
35. Willker W, Leibfritz D, Kerssebaum R, Bermel W. *Magn. Reson. Chem.* 1993; **31**: 287.
36. Ares JJ, Outt PE, Randall JL, Murray P, Weisshaar PS, O'Brien LM, Ems BL, Kakodkar SV, Kelm GR, Kershaw WC, Werchowski KM, Parkinson A. *J. Med. Chem.* 1995; **38**: 4937. The ^1H NMR and ^{13}C chemical shifts of 8-Hydroxyflavone have been reported by Ares, *et al*. ^1H NMR (d_6-DMSO, 300 MHz) δ 7.01 (1H, s), 7.21 (2H, m), 7.45 (1H, m), 7.58 (3H, m), 8.11 (2H, m), 10.49 (1H, s, OH); ^{13}C NMR (d_6-DMSO, 75 MHz) δ 107.03, 114.6, 119.8, 125.1, 125.7, 126.8, 129.5, 131.7, 132.1, 145.8, 147.4, 162.5, 177.7.
37. Okamoto K. *Org. Lett.* 2004; **6**: 1987.
38. Meyer M. *Int. J. Quantum. Chem.* 2000; **76**: 724.
39. Karplus M. *J. Am. Chem. Soc.* 1963; **85**: 2870.
40. Kumarasamy Y, Byres M, Cox PJ, Delazar A, Jaspars M, Nahar L, Shoeb M, Sarker SD. *Chem. Nat. Compd.* 2004; **40**: 122.
41. Ibrahim A-L, Abul-Hajj YJ. *J. Nat. Prod.* 1990; **53**: 1471.
42. Moon B-H, Lee Y, Ahn J-H, Lim Y. *Magn. Reson. Chem.* 2006; **44**: 99.
43. Park Y, Lee Y-U, Kim H, Lee Y, Yoon Y-A, Moon B, Chong Y, Ahn J-H, Shim Y-H, Lim Y. *Bull. Korean Chem. Soc.* 2006; **27**: 1537.
44. https://scifinder.cas.org [accessed 2007].