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^{13}C NMR OF FLAVONOIDS—II

FLAVONOIDS OTHER THEN FLAVONE AND FLAVONOL AGLYCONES

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Abstract—^{13}C NMR spectra for a number of dihydroflavones, dihydroflavonols, flavans, chalcones, flavonols, an isoflavone and several glycosides in DMSO-d_6 solvent are reported and analysed. The use of established substitution additivity rules and ^{13}C–^{1}H coupling constants together with suitable model compounds has permitted near complete, self-consistent interpretations of these spectra. Sites of C-methylation and glycosylation are readily determined as also is the interglycosidic linkage within the disaccharide of flavonoid O-diglycosides. Stereochemical effects are observed in the spectra of the dihydro-flavonoids and some flavonol 3-O-glycosides.

Our first study in the present series[1] dealt with the interpretation of the ^{13}C-NMR spectra of a range of hydroxylated flavones and flavonols. These interpretations were based partly on the ^{13}C-NMR spectra of cinnamic acids and acetophenones and partly on an earlier detailed study of the spectrum of flavone itself. It was concluded that established aromatic substitution additivity rules hold well for structural variations within these flavonoids as long as the variations do not involve the C-3, 4 and 5 positions (for flavonol numbering see structure **13**).

Since the completion of this work several further investigations involving ^{13}C–NMR of flavonoids have appeared. A series of monomethoxy flavones has been studied by Kingsbury and Looker[2] with the object of supplying background data for the interpretation of the spectra of $CDCl_3$-soluble permethylated flavone aglycones. The use of two-, three- and four-bond spin–spin coupling between ^{13}C and hydrogen-bonded hydroxyl protons (identified by deuterium exchange) has been shown to aid the assignment of the ^{13}C–NMR spectrum of 5,7,4′-trihydroxydihydroflavone.[3] In particular, the distinction of C-6 and C-8 signals in the spectra of 5-hydroxyflavonoids is possible by this means, C-6 appearing as a pair of broad triplets and C-8 as a pair of doublets.† Rotenoids, derivatives of dihydro-isoflavones, and related compounds, have also been the subject of recent ^{13}C–NMR studies.[4]

The object of the present paper is to extend the range of flavonoids so far studied and to provide comparable data on all major flavonoid groups, including glycosides, relating to the one basic solvent system, DMSO-d_6, a solvent which is sufficiently polar to dissolve both aglycones and glycosides.

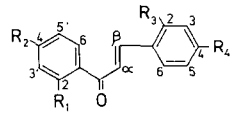
RESULTS AND DISCUSSION

Flavonoid aglycone types

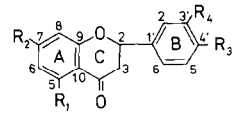
Chalcones. The spectrum of 2′,4,4′-trihydroxychalcone (isoliquiritigenin, **1**) exhibits a total of 13 signals (see Table 1), two of which are of high intensity and which by analogy with apigenin[1] (5,7,4′-trihydroxyflavone) are assigned to C-2, 6 and C-3, 5. Of the remaining 11 signals, C-1 and C-4 were assigned on the basis of the apigenin spectrum and the remainder on the basis of the spectrum of 2,4-dihydroxyacetophenone.[1] Of the hydroxylated carbons, C-4 at 159.9 ppm was clearly distinguished in the proton coupled spectrum by its appearance as a multiplet. By comparison, both C-2′ and C-4′ appeared as doublets and were not distinguishable from one another. The previously obtained spectrum of 4-hydroxycinnamic acid[1] provided a good model for the assignment of the distinctively chalcone carbons, C-α and C-β, to the signals at 117.8 and 143.8 ppm, $J(C\text{-}\alpha/C\text{-}\beta(H)) = 4.6$ Hz.

Fourteen signals were detected in the spectrum of 2,2′-dihydroxychalcone **2**, but again one signal (121.0 ppm) is very much more intense than the rest and is considered to represent two super-imposed signals. The assignments presented for this spectrum (Table 1) are based in general on assignments previously established[1] for 2-hydroxyacetophenone (for the A-ring carbons), isoliquiritigenin **1** and methyl benzoate[5] (for the cinnamyl carbons). Two groups of signals, 130.1, 131.2 and 133.7 ppm and, 121.0, 121.4 and 122.2 ppm, require further comment. In the first group, the signal at 131.2 ppm is assigned to C-6′ by comparison with isoliquiritigenin (allowing for the effect of the 5-OH). The other two signals were assigned to C-4 and C-6 on the basis of the spectrum of methyl benzoate[5] after adjustment for the introduction of a 2-OH (thus C-4 = 132.2 + 1.4 and C-6 = 128.2 + 1.4 ppm). Of the second group of signals only one could be assigned. The group represents C-1′, 1, 3 and 5 (by a process of elimination), and C-1′ is ascribed

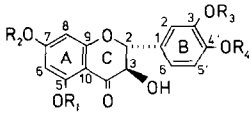
†In Part I of the present series of papers, assignment of signals to C-6 and 8 of flavones and flavonols was made on the basis of the relative positions of the 6- and 8-proton signals in PMR spectra. As a result of further studies utilizing the coupling of each with the C-5 hydroxyl proton, it is now clear that some of our earlier assignments should be reversed. Those affected are flavones and flavonols containing 5-hydroxyl groups. Thus, the introduction of a 5-OH produces a 13.8–14.6 ppm upfield shift in C-6 and a 10.1–11.2 ppm upfield shift in C-8. Hence C-8 is showing the larger than expected shift.



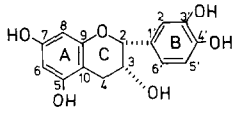
1 $R_1 = R_2 = R_4 = OH$, $R_3 = H$
2 $R_1 = R_3 = OH$, $R_2 = R_4 = H$
3 $R_1 = OH$, $R_2 = R_3 = R_4 = H$



4 $R_1 = R_2 = R_3 = OH$, $R_4 = H$
5 $R_1 = R_3 = OH$, $R_2 = O$-neohesperidosyl, $R_4 = H$
6 $R_1 = R_3 = OH$, $R_2 = O$-rutinosyl, $R_4 = OCH_3$
7 $R_1 = R_2 = CH_3$, $R_3 = OCH_3$, $R_4 = H$



8 $R_1 = R_2 = R_3 = R_4 = H$
9 $R_1 = R_2 = R_3 = R_4 = CH_3$



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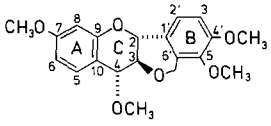

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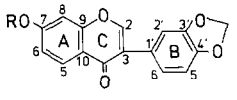
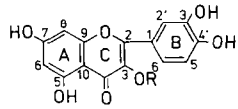


12 R = rutinosyl



13 R = H
14 R = rutinosyl
15 R = rhamnosyl

to the signal at 122.2 ppm on the basis of the spectrum of 2'-hydroxychalcone **3**. This compound lacks B-ring hydroxylation and thus C-1' is the only carbon atom to produce a signal in the 121–122 ppm region. C-1' here appears at 122.2 ppm indicating that the signal at 122.2 in 2,2'-dihydroxychalcone is also due to this carbon.

The sample of 2'-hydroxychalcone **3**, although somewhat impure, provided a spectrum in which most signals could be clearly detected. The analysis presented here is based entirely on comparison with the spectra of flavone[6] (for B-ring assignments)† and 2,2'-dihydroxychalcone (for A-ring and cinnamyl side-chain assignments). It is of interest that loss of the 2-hydroxyl group from the B-ring appears to cause a downfield shift of both C-α and C-β rather than the "para-" and "meta-" effects that might have been expected.

Dihydroflavones (flavanones). The spectrum of 5,7,4'-trihydroxyflavanone (naringenin **4**), has been studied in part previously[3] and assignments were made for the A-ring carbons using ^{13}C–^{1}H spin–spin coupling to the 5-hydroxyl group as an assignment aid. This coupling was encountered in our own work and provides a useful method for the distinction of the C-6 and C-8 signals and also the C-5 and C-9 signals. A-ring assignments made on empirical grounds in the present study are in accord with the published work. The B-ring carbons, C-2',6' and C-3',5' are clearly visible as intense signals at 127.6 and 115.1 ppm respectively, and C-4' at 157.4 ppm is, as expected, close to the C-1 resonance of phenol.[5] By elimination therefore, the low intensity signal at 128.9 ppm is ascribed to C-1'.

The signals at 78.2 and 42.0 ppm represent C-2 and C-3 respectively, C-2 being assigned the lower field signal because it is oxygenated. These assignments are confirmed by ^{13}C–^{1}H coupling (low resolution) which transformed the C-2 signal to a doublet ($J = 149.5$ Hz) and the C-3 signal to a triplet ($J < 149$ Hz, undetermined due to DMSO signal interference).

The spectrum of the naringenin 7-O-rhamnoglucoside, naringin **5** approximates closely to that of naringenin with regard to the carbon atoms of the flavonoid nucleus. Two new signals appear in the 95–104 ppm region however, due to C-1'' and C-1''' (of glucose and rhamnose respectively) and C-7 has shifted from 166.4 to 164.9 ppm due to glycosylation. Of the signals in the 95–104 ppm region, the low intensity signal at 103.5 ppm is assigned to the quaternary C-10, and those at 96.5 and 95.4 ppm are assigned to C-6 and C-8 respectively on the basis that glycosylation at the 7-OH should effect C-6 and C-8 equally. The remaining signals at 100.4 and 98.0 ppm thus relate to the sugar C-1 carbons.

Analysis of the spectrum of the hesperetin 7-O-rhamnoglucoside, hesperidin **6**, follows to some extent from the naringin assignments. The pattern of signals representing the B-ring carbons is however altered by the presence of the 3'-methoxyl. The new C-3' signal appears close to that of C-4', but additivity rules[5] predict that C-4' would be the least shielded. Of the other B-ring carbons, C-1' (lacking an attached proton) appears slightly downfield from that in naringin, C-5' is identifiable at 112.7 ppm by its lack of *meta*-^{13}C–^{1}H coupling,[1] and C-2' and C-6' are represented by signals at 114.3 and 117.8 ppm which are ascribed on the basis of established additivity rules. In this spectrum the C-3 signal is obscured by the DMSO resonances and the 3'-methoxyl, which appears as a quartet ($J = 144.2$ Hz) in the undecoupled spectrum, is visible at 56.0 ppm.

The only other dihydroflavone studied is 5,7-dimethyl-4'-methoxyflavanone **7**, the spectrum of which is quite different from those discussed above, particularly with respect to the A-ring carbons. Tentative assignments of signals to B- and C-ring carbons can be made by comparison with the naringenin spectrum, allowing for the effect of methylation of the 4'-OH. Assignments to

†Although the spectrum of flavone was recorded with $CDCl_3$ as solvent, previous work[1] has established that solvent effects are minimal on B-ring carbon resonances when the B-ring is unsubstituted.

Table 1. [13]C-NMR spectra of flavonoids

Compound‡	Carbon number and shift (in ppm from TMS)																	
	2	3	4	5	6	7	8	9	10	1′	2′	3′	4′	5′	6′	Other		
Flavones, Flavonols																C–Me	O–Me	CH_2
Flavone[6]	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			
Apigenin[1]†	165.5	104.3	183.2	158.7	100.3	164.9	95.6	162.0	105.1	122.7	129.8	117.3	161.8	117.3	129.8			
Quercetin[1] (13)†	146.9	135.5	175.8	156.2	98.2	163.9	93.3	160.7	103.1	122.1	115.3[a]	145.0	147.6	115.6[a]	120.0			
Quercitrin (15)	156.4[a]	134.4	177.7	157.0[a]	98.6	164.0	93.5	161.2	104.2	130.3	115.4[b]	145.1	148.3	115.8[b]	121.0			
Rutin (14)	156.4[a]	133.6	177.4	156.6[a]	98.8	164.0	93.6	161.2	105.2	121.6	115.3[b]	144.6	148.3	116.5[b]	121.6			
Dihydroflavones																		
Naringenin (4)	78.2	42.0	195.6	163.3	95.8	166.4	94.9	162.7	101.9	128.9	127.6	115.1	157.4	115.1	127.6			
Naringin (5)	78.6	42.0	196.7	162.9[a]	96.5	164.9	95.4	162.7[a]	103.5	128.7	128.0	115.3	157.7	115.3	128.0			
Hesperidin (6)	78.4	42	196.7	163.0	96.7	165.2	95.8	162.5	103.5	131.2	114.3	146.7	148.1	112.7	117.8		56.0	
5,7-Dimethyl-4′ methoxy-	77.5	44.8	192.0	140.4	125.3	145.1	115.6	162.0	126.2	131.0	127.4	113.1	159.3	113.1	127.4	20.7, 21.3	55.0	
Dihydroflavonols																		
Taxifolin (8)	83.1	71.7	197.1	163.3	96.1	166.8	95.1	162.5	100.6	128.1	115.3	144.9[a]	145.7[a]	115.3	119.2			
Tetramethyl Taxifolin (9)	83.6	72.5	189.7	163.7	93.8[a]	165.7	93.1[a]	161.7	103.7	129.9	112.5	148.9[b]	149.5[b]	112.3	120.6		55.9, 55.6	
Flavans																		
(−)-Epicatechin (10)	78.1	65.1	28.0	156.4[a]	95.6	156.3[a]	94.5	155.7[a]	98.8	130.7	118.1	144.4[b]	144.5[b]	115.0	118.1			
Tetramethyl Peltagynol (11)	77.5	71.6	68.0	129.0	110.1	159.6	100.7	154.3	118.5	127.3[a]	108.1	148.2[b]	149.1[b]	108.1	124.6[a]		56.1, 55.9, 55.2	67.0
Isoflavone																		
Pseudobaptisin (12)§	153.7	123.4[a]	174.5	126.9	115.6	161.4	103.9	156.9	118.7	125.6[a]	109.3	147.0	147.0	107.9	122.2			100.9
Chalcones‖	β	α		2′	3′	4′	5′	6′	1′	1	2	3	4	5	6			
Isoliquiritigenin (1)	143.8	117.8	191.4	164.6[a]	102.6	165.4[a]	107.9	132.3	113.2	125.8	130.6	115.8	159.9	115.8	130.6			
2,2-Dihydroxy- (2)	141.8	117.3	195.0	162.7	118.7	137.3	120.4	131.2	122.2	121.0[a]	158.2	120.4[a]	133.7	121.0[a]	130.1			
2′-Hydroxy- (3)	145.5	118.3[a]	194.7	161.7	118.6[a]	137.2	120.2	131.0[b]	122.2	136.8?	126.9	129.3	131.5[b]	129.3	126.9			

†Assignments for C-6 and C-8 are reversed relative to those reported by us in ref. 1 (see comments in text).

‡All spectra were determined for DMSO-d_6 solutions at 95° except for those of flavone ($CDCl_3$), apigenin (3:5, D_2O:DMSO), and chalcones **2** and **3** (2:7, D_2O:DMSO).

§Additional signals at 129.9 and 113.7 ppm were attributed to B-ring carbons of a daidzein 7-O-rh-glu impurity.

‖Note different numbering system for chalcones.

[a,b]Assignments bearing the same superscript in any one spectrum may be reversed.

A-ring carbons follow from the spectrum of flavone, allowing for the substitution effects of the 5- and 7-methyl groups. Assignments made on these bases were fully confirmed by examination of the high resolution proton coupled spectrum. Thus, C-5 and C-7.each appeared as a quartet (J = 6.1 Hz) due to the attached methyl group, and C-6 and C-8 each appeared as doublets (J = 159 and J = 164 Hz respectively) of quartets (both J = 5.3 Hz) due to ^{1}H and methyl coupling.

At high resolution, the C-2′,6′ signal was resolved into a pair (J = 159.5 Hz) of double doublets (J = 5.3 Hz, J = 4.6 Hz), the double doublet reflecting ^{13}C–^{1}H coupling with protons at both C-2 and C-3′,5′. In contrast, the C-3′,5′ signal appears as a pair (J = 160.6 Hz) of doublets (J = 5.3 Hz), coupling with the C-2 proton being undetectable. The methyl carbon resonances at 21.3 and 20.7 ppm can also be assigned by the extent of their coupling to ring protons. Hence, the 7-methyl is represented by the quartet of triplets (J = 126.7, 4.6 Hz, centred at 20.7 ppm) due to coupling with protons at both C-6 and C-8, whereas the 5-methyl relates to the quartet of doublets (J = 128.2, 5.3 Hz) centred at 21.3 ppm.

It is evident from the above that the site of C-methylation in flavonoids is readily determined by ^{13}C-NMR spectroscopy. Not only is the point of attachment obvious by the appearance of its signal as a quartet in the proton coupled spectrum, but also the ortho-related carbons are apparent by their presence as doublets of quartets when they possess an attached proton, or quartets when they do not. In addition, a study of the methyl carbon resonance coupling can give valuable information about the point of attachment, in particular the number of ortho-related hydrogens is immediately evident.

Dihydroflavonols. Dihydroflavonols differ from flavanones in that they possess a hydroxyl at C-3. Their ^{13}C-NMR spectra are therefore similar except for the C-ring carbon resonances. In the spectrum of 5,7,3′,4′-tetrahydroxydihydroflavonol ((+)-taxifolin **8**) for example, the A-ring carbons have almost the same chemical shifts as do the same carbons in naringenin. This is also true for the B-ring carbons if allowance is made for the influence of the 3′-hydroxyl group. In the C-ring however, addition of an hydroxyl at C-3 would be expected to produce a downfield shift of about 7 ppm in C-2[7] and this can only be approximated to if C-2 is ascribed the 83.1 ppm signal. C-3 thus appears at 71.7 ppm and has shifted only 30 ppm on introduction of the 3-hydroxyl, considerably less than would have been expected had the adjacent carbonyl been absent.[7] These assignments, however, were confirmed by the coupling observed in the proton coupled spectrum of the 5,7,3′,4′-tetramethyl ether derivative **9**. In this spectrum, the C-3 signal appears essentially as a double (J = 146.5 Hz) doublet (J = 6.1 Hz) whereas C-2 is represented by a pair (J = 148.0 Hz) of lower intensity multiplets due to coupling with protons at C-3, C-2′ and C-6′.

Preliminary assignments of other signals in taxifolin tetramethyl ether followed from the spectrum of (+)-taxifolin itself, after allowing for methylation effects previously defined for 3′,4′-dihydroxyflavonol. Confirmatory evidence from the proton coupled spectrum included (a) the presence of C-6 and C-8 each as pairs (J = 163.3 and 161.7 Hz) of doublets (J = 4.6 Hz), (b) the appearance of C-6′ as a pair (J = 161.7 Hz) of double doublets (J = 7.6 and 3.8 Hz) and (c) the distinction of the C-5′ signal from that of C-2′ by its appearance as a doublet (J = 158.7 Hz) lacking meta-^{1}H coupling. It is also of interest to note in this spectrum, the upfield shift of 7.4 ppm in C-4 (relative to taxifolin) brought about presumably through loss of H-bonding to the 5-hydroxyl group.

Flavan derivatives. (−)-Epicatechin **10**, in terms of its oxidation pattern, may be regarded as taxifolin lacking the 4-keto function. This carbonyl has far reaching effects in the A- and C-rings as can be seen from the chemical shifts of the oxygenated carbons. While C-3′ and 4′ appear in much the same position as they did in the spectrum of taxifolin, carbons 5, 7 and 9 are represented by a close assemblage of signals at about 156 ppm, well upfield from their previous positions. The meta-related C-6 and C-8 however, are barely affected by the loss of the 4-keto function. Of the ring C carbons, C-4 appears as the highest field signal (28.0 ppm) which is readily identified as C-4 by its conversion into a triplet (J = 131.2 Hz) in the low resolution proton coupled spectrum. The signals at 78.1 and 65.1 ppm clearly relate to C-2 and C-3, and are assigned on the assumption that the chemical shift of C-3 should approximate to that of C-1 in cyclohexanol. This resonance is quoted at 69.8 ppm,[8] but when allowance is made for the substituent effects of the C-2 linked oxygen and phenyl group, a chemical shift nearer 67 ppm would be predicted. The low resolution undecoupled spectrum was of no assistance here, exhibiting doublets (J = 141.9 Hz, 78.1 ppm; J = 145.0 Hz, 65.1 ppm) for both carbons. However, the allocation of the 65 ppm signal to C-3 is consistent with assignments previously established independently.[9]

As mentioned above, most B-ring carbons in (−)-epicatechin have chemical shifts similar to their equivalents in the dihydroflavonols. C-5′ is readily distinguished at 115.0 ppm by its lack of meta-^{1}H coupling and the only other signals in the region, the two at 118.1 ppm, must relate to C-2′ and C-6′. The C-2′ signal has thus unexpectedly moved 3 ppm downfield from its position in the spectrum of (+)-taxifolin. This possibly reflects the different stereochemistry in ring C. In (−)-epicatechin the bulky substituents at C-2 and C-3 are oriented in such a way (see **10**) that physical interaction of the C-2′ proton with the 3-hydroxyl group is likely. Such interaction is not possible with (+)-taxifolin **8**. Further studies with other stereochemical isomers however are needed to verify this point.

Peltagynol tetramethyl ether **11** is the second member of the flavan group. Its spectrum exhibits 4 signals due to oxygenated carbons, and the assignments are based on those previously derived for tetramethyl-taxifolin (B-ring) and (−)-epicatechin (A-ring, using substitution rules). The four signals, 77.5, 71.6, 68.0 and 67.0 ppm relate to the carbons in the two central rings and are assigned as follows. The signal at 67.0 ppm is clearly defined as that of the methylene group since it appears as a triplet (J = 143.4 Hz) in the low resolution proton coupled spectrum whereas the other three all appear as doublets. Further, the same carbon in rotenone is known to resonate at 66.3 ppm.[4] The signal at 77.5 ppm is assigned to C-2 since the structural changes in this compound relative to epicatechin would not be expected to influence this carbon to any extent. Distinction of the remaining two signals is based on the combined predicted effects[10] of orthomethoxylation and derivatization on the shift of C-3 in epicatechin. Thus the signal at 71.6 ppm is tentatively ascribed to C-3 leaving that at 68.0 ppm for C-4. Of the other high field signals, that at 108.1 ppm is outstandingly intense and appears as a

doublet (J = 157.2 Hz) in the undecoupled spectrum. This is assigned to C-2′ and C-5′. The signals either side of this (110.1 and 100.7 ppm) represent C-6 and C-8 and are allotted using standard substituent effect data. Four signals remain unassigned. The one at 129.0 ppm relates to C-5 as the only remaining carbon with an attached proton, while the others all represent quaternary carbons, i.e. C-10, 1′ and 6′. Of these three, C-10 would be expected to appear at highest field, i.e. 118.5 ppm, because of its ortho/para relationship to the –OR substituents (versus meta/para for C-1′ and C-6′).

Isoflavones. Only one isoflavone is included in the present study, the diglycoside, pseudobaptisin **12**. The spectrum reveals five signals in the region expected for oxygenated aromatic carbon atoms, and proton coupling indicates that all but that at 153.7 ppm represent quaternary carbons. Their assignments follow from those previously made for related flavones,[1] the signal at 147.0 ppm apparently representing both C-3′ and C-4′. The same two carbons in the related model compound isosafrole, resonate at 146.1 and 147.6 ppm. The signal at 153.7 ppm appears in the proton coupled spectrum, as a doublet with the remarkably large coupling constant of 200 Hz. Such a coupling constant is typical of C-2 and 6 carbons in γ-pyrones (and like compounds)[11] and thus defines the 153.7 ppm signal as that due to C-2 in pseudobaptisin.

Another novel structural feature of pseudobaptisin is the methylene-dioxy group and this carbon, as in isosafrole, is represented by a signal at 100.9 ppm. This is superimposed on the glucose and rhamnose C-1 signals at 100.5 and 100.7 ppm but its presence is confirmed by the appearance of a triplet (J = *ca.* 175 Hz) centred at 100.8 ppm in the proton coupled spectrum.

Other signals in the pseudobaptisin spectrum are assigned primarily on the basis of proton coupling and by the use of established substitution rules. Thus the signals of quaternary carbons (C-1′, 3, 10), and carbons with attached protons, with (C-6,6′,2′,8) and without (C-5,5′) meta-related protons were distinguished. Actual assignments for signals of A- and C-ring carbons are based on the spectrum of flavone, allowance being made for substituent effects due to the 7-OR and 3-O-phenyl groups. Allocations of signals to B-ring carbons followed from the spectrum of isosafrole in which the equivalents of C-1′, 2′, 5′ and 6′ resonate at 132.1, 105.1, 107.9 and 119.7 ppm respectively. The pseudobaptisin C-1′ signal at 125.6 (or 123.4) ppm is at considerably higher field than that of isosafrole, presumably because the presence of the heterocyclic oxygen and 4-keto function largely negate the electron withdrawing effect[5] of the double bond on the C-1′ position.

Flavonoid glycosides

Sugar carbons. Several flavonoid glycosides were included in the present study and the regions of the ^{13}C-NMR spectra relating to the sugar carbon atoms are discussed here. The prime objective of this study was to assess the usefulness of ^{13}C-NMR spectroscopy for the determination of the interglycosidic linkage in diglycosides, a determination which is often very difficult to carry out by other means. Four rhamnoglucoside derivatives were available for this work, two of which (hesperidin, **6** and rutin **14**) are rutinosides, i.e. rhamno (1→6) glucosides, one (naringin **5**) a neohesperidoside, i.e. rhamno (1→2) glucoside, and one, pseudobaptisin **12**, in which the linkage was not known with certainty.[12]

The spectra of rhamnose and glucose methyl glycosides have been studied previously by Gorin and Mazurek[13] and their assignments were used here as a guide for the interpretation of the flavonoid glycoside spectra (Table 2). However, it was not possible by this means to distinguish, with confidence, glucose C-3 from glucose C-5, or glucose C-4 from rhamnose C-2 and C-3. Nevertheless a comparison of the spectra in Table 2 does enable the immediate distinction of rutinosides from neohesperidosides. When the spectra of rutin and hesperidin are compared with those of the individual sugars, it is evident that the major difference is in the glucose C-6 signal which has moved downfield by an average of 4.8 ppm. In the spectrum of the neohesperidoside naringin, however, the glucose C-6 signal remains unaffected whereas the glucose C-2 signal has moved downfield by 2.5 ppm. It is thus clear that the linkage point of rhamnose to glucose in these rhamnoglucosides is readily determined by this means. Using these criteria it is evident that the

Table 2. ^{13}C-NMR spectra of glycosides (sugar carbons)

Compound	Solvent	Carbon resonances (ppm from TMS)†										
		C-1^{R+G}	C-2^{G}	C-3^{G}	C-4^{G}	C-5^{G}	C-6^{G}	C-2^{R}	C-3^{R}	C-4^{R}	C-5^{R}	C-6^{R}
Methyl-β-D-glucopyranoside[13]	D_2O	104.3	74.2	76.9	70.8	76.9	61.9					
Methyl-α-L-rhamnopyranoside[13]	D_2O	101.9						71.0	71.3	73.1	69.4	17.7
Quercetin 3-O-rutinoside (Rutin)	DMSO/D_2O	103.4, 102.0	75.3	77.4[a]	71.1[b]	76.6[a]	68.7	71.5[b]	71.7[b]	73.2	69.7	18.6
	DMSO-d_6	101.5, 100.7	74.2	76.8[a]	70.4[b]	76.1[a]	67.1	70.4[b]	70.8[b]	72.2	68.2	17.5
Quercetin 3-O-rhamnoside (Quercitrin)	DMSO-d_6	101.9						70.4	70.6	71.5	70.1	17.3
Hesperetin 7-O-rutinoside (Hesperidin, **6**)	DMSO-d_6	100.7, 99.8	73.3	76.6[a]	69.9[b]	75.8[a]	66.4	70.6[b]	71.0[b]	72.4	68.6	18.2
Naringenin 7-O-neohesperidoside (Naringin **5**)	DMSO-d_6	100.4, 98.0	76.7[a]	77.2[a]	70.1[b]	77.0[a]	60.8	70.4[b]	70.7[b]	72.1	68.2	17.8
Pseudobaptisin (**12**	DMSO-d_6	100.5, 100.7	73.2	76.7[a]	70.2[b]	75.9[a]	66.7	70.4[b]	71.0[b]	72.3	68.4	17.7

†R refers to rhamnose, G to glucose.

[a,b]Assignments bearing the same superscript in any one spectrum may be reversed.

disaccharide in pseudobaptisin is rutinose as had been previously proposed with less certainty on the basis of the ^{1}H-NMR spectrum.[12]

Effect of glycosylation on the aglycone spectrum. From the results obtained in the present study, it is evident that glycosylation of a flavonoid hydroxyl group generally induces shifts in the resonances of the flavonoid carbons similar to those observed in other phenolics.[14] Thus, when the 7-hydroxyl group is glycosylated in naringenin **4**, or hesperetin, C-7 shifts upfield by 1.5–1.8 ppm whereas the ortho-related C-6 and C-8 shift downfield by 0.7–0.9 and 0.5–0.9 ppm respectively. The only other resonance to be significantly affected is that of the para-related C-10 which moves downfield by 1.6 ppm. These shifts are all analogous to those recently reported for 7-O-glucosyl-coumarin[14] and thus could well be of more general applicability.

More distinctive perhaps, is the effect of glycosylation of the 3-hydroxyl group in quercetin **13**. In this case the effect on the C-3 signal is an upfield shift of 1.1–1.9 ppm, similar to that observed for 7-O-glycosylation above. The effect on the "ortho"-related C-2 is much larger than anticipated with a downfield shift of 9.5 ppm being observed. In this regard it is of interest that the introduction of a 3-hydroxyl group into the flavone nucleus also causes a larger than anticipated "ortho"-shift in the C-2 signal.[1] These large shifts are more characteristic of olefins than of aromatic systems[15] and it is likely here that they reflect the semi-olefinic character of the flavonol C-2,3 double bond.

The particular case of quercitrin and rutin. It has been previously recorded[16] that Band-I values in the UV/visible absorption spectra of quercitrin **15** and rutin **14** are significantly different.† This observation is unexpected in view of the fact that both compounds are 3-O-glycosides of the same aglycone, quercetin **13**, and to our knowledge no reason has ever been put forward to account for these spectral differences. A comparison of the ^{13}C-NMR spectra of these two glycosides (Table 1) reveals a close similarity in the shifts of all signals but that of C-1′. In the spectrum of rutin, C-1′ appears at 121.6 ppm,‡ the expected position for C-1′ in 3′,4′-dioxygenated flavonols,[1] whereas in the quercitrin spectrum C-1′ occurs at 130.3 ppm. This chemical shift is more like that of C-1′ in the dihydroflavones and dihydroflavonols (see Table 1) in which there is no conjugation between the B- and C-rings. Significantly, in these two flavonoid groups, the lack of cross conjugation is evidenced only in the chemical shift value of C-1′ and not in the shifts of the other B-ring carbons. Thus, these results suggest that π-orbital overlap between rings B and C in quercitrin is considerably less than in rutin. This is probably due to steric hindrance imposed on the B-ring by the α-linked[17] rhamnose at position 3 in quercitrin. Such hindrance would not be expected with rutin in which the primary sugar, glucose, in β-linked to the 3-hydroxyl. This explanation is supported both by molecular models and by the observation that the UV/visible absorption spectrum of quercetin 3-O-β-galactoside[14] is near identical with that of rutin. Further, the chromophore recognized as being primarily responsible for the Band-I absorption in flavonoids is the cinnamoyl chromophore,[16] and it is this chromophore that would be affected by any change in the degree of B/C-ring conjugation.

†These spectra, remeasured in the present study, showed Band-I absorption for rutin at 358 nm and for quercitrin at 348 nm (MeOH). On the addition of NaOMe, the rutin absorption shifted to 409 nm and that of quercitrin to 392 nm.

‡When DMSO-d_6/D_2O was used as solvent, the C-1′ signal appeared at 122.4 ppm, distinct from that of C-6′ at 121.7 ppm.

EXPERIMENTAL

The ^{13}C magnetic resonance spectra were recorded on a JEOL P-100 Fourier transform spectrometer operating at 25.15 MHz. Spectral widths were 5000 Hz. 16 K data points were used except in a few cases where, for technical reasons, only 8 K 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 dimethyl-sulphoxide (DMSO) solvent was used as lock signal. Spectra were recorded with few exceptions (see Table 1 footnote) on samples of about 100 mg in DMSO-d_6 at 95° in 10 mm tubes. Sample sources were as follows: (−)-epicatechin, rutin, quercitrin, quercetin and dihydroquercetin (Fluka AG, purum); hesperidin (Light and Co.); naringin (Eastman-Kodak) and by hydrolysis, naringenin; pseudobaptisin (ex. *Baptisia lectontei*); isoliquiritigenin (ex. Dr. E. Wong, Applied Biochem. Division, D.S.I.R., Palmerston North, New Zealand); other samples were either synthesized or prepared by methylation of the parent compound. All samples were recrystallized before use. UV-visible absorption spectra were determined in methanol on a Beckman Acta-V spectrophotometer. Shift reagents used are as described in Ref. 16.

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