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CARBON-13 NMR STUDIES OF FLAVONOIDS—III

NATURALLY OCCURRING FLAVONOID GLYCOSIDES AND THEIR ACYLATED DERIVATIVES

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Abstract—^{13}C NMR spectra for a variety of flavonoid glycosides are presented and analysed. Evidence is presented which demonstrates that ^{13}C NMR spectroscopy is a valuable technique for distinguishing the sites of methylation, glycosylation and acylation in flavonoid glycosides, and in some cases the nature and sites of specific sugars and acyl groups. Shifts observed in the spectrum on derivization of the 5-OH group are unusual. The ring size and C-1 configuration in glycosidic sugars are also evident from the spectra. Structural assignments are made for several glycosides.

The usefulness of ^{13}C NMR spectroscopy for flavonoid aglycone structure analysis is well established. Published work includes studies of flavones,[1-6] flavonols,[1-3] dihydroflavonoids,[2,3,6,7] chalcones,[2,6,8] flavans,[2] isoflavones,[2,6,11] biflavonoids[10] and aurones,[6] together with occasional flavonoid glycosides.[2,9,13] There is presently good agreement between different workers on the assignment of signals, especially since the assignments for C-6 and C-8, and C-5 and C-9 in 5-hydroxyflavonoids have been clarified as a result of more intensive studies.[2,7,11,12]

Because flavonoid glycosides are more important than aglycones in nature, we initiated a systematic study of this class of natural products. In the present communication, the ^{13}C NMR spectra of a wide range of naturally occurring flavonoid O-glycosides and some acylated derivatives are presented and analysed to assess the usefulness of this technique for glycoside structure elucidation. Comparability with previous work in this series[1,2] has been maintained through the use of hexadeuterio-dimethylsulphoxide as solvent.

RESULTS AND DISCUSSION

The glycoside spectra studied here are presented in Tables 1 and 2. Table 1 contains a listing of the signals associated with the aglycone portion of each molecule. Assignments were made primarily by analogy with previous studies.[1-7] For reference purposes several of these aglycones and their methylated derivatives are included in the table. One difficulty arising with the flavonol 3-, and 3,7-O-glycosides is the near equivalence of the C-2 and C-9 signals, and no attempt has been made to differentiate between them in this study.

It has recently been shown that the oxygenated carbons C-5 and C-9 each give signals which have distinctive ^{13}C–^{1}H coupling in 5-hydroxylated flavonoids.[7,11,12] Accordingly, C-5 has now been assigned the lower field signal (*ca.* 161 ppm) and C-9 the higher (*ca.* 157 ppm), representing a reversal of some earlier assignments.[1-3] These current assignments appear to be inconsistent with substitution effects observed on methylation of the 5-OH.[3] However, the 5-OH is strongly H-bonded to the 4-keto function in these compounds and for this reason the signal of the adjacent carbon would not necessarily obey "normal"[14] substitution additivity rules. Indeed in a comparable case with 2-hydroxyacetophenone, it has been shown that these rules are not applicable to the C-2 signal which moves upfield on methylation of the 2-OH[15] rather than downfield as predicted by the substitution rules.

Table 2 contains a listing of the signals associated with the carbohydrate portion of the flavonoid glycosides. For the monoglycosides, assignments were based on those previously made for monosaccharides and methyl glycosides.[16,17] The spectra of the di- and tri-glycosides were resolved either by "best fit" matching to appropriate monosaccharide spectra, or where possible, through a comparative study of the simpler mono- or di-glycosides that constitute part of the structure. Thus for example, assignments in the spectrum of kaempferol 7-O-glucoside-3-O-rutinoside were deduced by reference to the spectra of kaempferol 7-O-glucoside, kaempferol 3,7-di-O-glucoside and kaempferol 3-O-rutinoside. Glucose C-1 signals in the spectra of the 3-O-sophorosides and 7-O-neohesperidosides were assigned on the basis of previous work on various glucobiosides.[18,19] In some of the more complex spectra accurate assignments could not be made for a number of signals with similar chemical shifts. This limitation however, has not affected the conclusions reached in this communication.

Table 1. ^{13}C NMR spectra of flavonoid glycosides

	FLAVONOID*	C-2	C-3	C-4	C-5	C-6	C-7	C-8
1a.	Apigenin**	165.5	104.3	183.2	162.0	100.3	164.9	99.6
1b.	A - 7-OMe	164.1	103.0	181.7	161.2[a]	97.8	165.1	92.6
1c.	A - 7-Oglu(2-1)apiose	164.4	103.3	181.9	161.3[a]	98.8	162.8	95.0
3	Luteolin++	165.1	103.9	182.6	161.6	99.9	164.3	94.9
3a.	L-3'-O-glu	164.1	103.4[a]	181.7	161.4	99.0	163.5	94.1
3b.	L-7-O-glu	164.5	103.2	181.6	161.1	99.7	162.9	94.9
3c.	L-7,3'-di-O-glu	164.0	103.6	181.8	161.1	99.6	163.0	95.0
3d.	L-5-O-glu	162.3	105.7	176.6	158.3[a]	104.3	161.3	98.1
2.	Kaempferol	146.8	135.6	175.9	160.7	98.2	163.9	93.5
2a.	K-3-O-glu	156.3	133	177.4	161.1	98.7	164.1	93.6
2b.	K-3-O-glu (2-1)glu	156.4	133.1	177.5	161.2	98.6	164.0	93.6
2c.	K-3-O-glu (6-1) rha**	156.6[a]	133.5	177.5	161.3	98.8	164.2	93.8
2d.	K-7-O-glu (2-1) rha	147.9	135.9	176.1	160.4	98.8	162.4	94.4
2e.	K-7-O-glu 3-O-glu	156.1[a]	133.8	177.7	160.9	99.6	163.0	94.8
2f.	K-7-O-glu 3-O-glu (O-Ac)	156.0[a]	133.6	177.5	160.8	99.5	162.9	94.7
2g.	K-7-O-glu 3-O-glu(2-1)glu(O-sinapoyl)	155.8	133.4	177.4	160.8	99.4	162.8	94.5
2h.	K-7-O-glu 3-O-glu(2-1)glu	155.9	133.3	177.5	160.9	99.4	162.8	94.6
2i.	K-7-O-rha 3-O-glu	156.0[a]	133.8	177.6	160.9	99.4	161.8	94.5
2j.	K-7-O-rha 3-O-glu (OAc)	156.0[a]	133.6	177.5	160.9	99.5	161.8	94.6
2k.	K-7-O-rha 3-O-glu (6-1)rha	156.0[a]	133.7	177.6	160.9	99.4	161.7	94.6
2l.	K-7-O-glu	147.9	136.0	176.1	160.5	99.2	162.9	94.8
2m.	K-7-O-glu 3-O-glu (6-1)rha	156.0[a]	133.7	177.6	160.9	99.7	162.9	94.9
4.	Quercetin	146.9	135.5	175.8	160.7	98.2	163.9	93.3
4a.	Q-3-O-glu	156.5	133.7	177.6	161.3	98.8	164.2	93.6
4b.	Q-3-O-gal	156.3	133.8	177.5	161.2	98.6	164.0	93.4
4c.	Q-3-O-gal (6"-O-galloyl)	156.3[a]	133.7	177.4	161.2	98.8	164.1	93.6
4d.	Q-3-O-arab	156.4[a]	133.5	177.8	161.2	98.7	164.1	93.5
4e.	Q-3-O-arab	156.3	134.0	177.6	161.2	98.7	164.1	93.5
4f.	Q-3-O-rha	156.4[a]	134.4	177.7	161.2	98.6	164.0	93.5
4g.	Q-4'-O-glu	147.0[a]	136.5	176.3	161.0	98.7	164.3	93.9
4h.	Q-3'-OMe 3-O-gal	156.2[a]	133.4	177.5	161.3	98.8	164.2	93.7
4i.	Q-3'-OMe 3-O-glu (6-1)rha	156.2[a]	133.3	177.3	161.2	98.7	164.0	93.7
4j.	Q-4'-OMe 7-O-glu (6-1)rha	147.3	136.3	176.1	160.4	98.9	162.8	94.5
4k.	Q-7-O-glu	147.9	135.9	175.9	160.3	98.9	162.7	94.5
4m.	Myricetin 3-O-gal	156.2	133.9	177.4	161.2	98.6	164.0	93.3
4b.	M-3-O-rha	156.5[a]	134.5	177.8	161.4	98.7	164.1	93.6
5.	Tricetin	164.2	103.2	181.6	161.6	99.0	164.2	93.9
5a.	Tricetin 3',4',5'-OMe	164.2[b]	103.9[a]	181.7	161.4	99.0	163.0[b]	94.2

*Chemical shifts are expressed in ppm from TMS: nv = not visible; spectrum of low intensity. All spectra were
[a,b,c]Assignments bearing the same superscript in any one spectrum may be reversed.
+Sugar abbreviations: glu (glucose), gal (galactose), rha (rhamnose), xyl (xylose), arab (arabinose).
++Solvent:— DMSO-d_6: D_2O (approx 2:1). Data ex Ref. 1.
**An unknown kaempferol 3-O-diglycoside from *Carthamus tinctoria* (Compositae) flowers also gave this

and related compounds (aromatic region)*

C-9	C-10	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'	CH_3O	SOURCE
158.7	105.1	122.7	129.8	117.3	161.8	117.3	129.8		
157.2	104.7	121.3	128.2	116.0	161.1[a]	116.0	128.2	55.8	Synthetic(apigenin)[33]
157.0	105.6	121.3	128.5	116.1	161.2[a]	116.1	128.5		Petroselinum crispum[34]
158.2	104.8	123.1	114.4	146.0	149.8	117.0	120.1		Reseda luteola[26]
157.4	103.9[a]	121.1	115.3	145.6	150.9	116.6	121.1		Reseda luteola[26]
156.9	105.5	121.6	113.7	145.7	149.7	116.1	119.0		Metasequoia glyptostroboides[31]
157.0	105.5	121.7	115.3	145.7	151.1	116.5	122.2		Reseda luteola[26]
158.4[a]	108.5	121.8	113.2	145.6	149.0	116.0	118.3		Galega officinalis[23]
156.2	103.1	121.7	129.5	115.4	159.2	115.4	129.5		
156.3	104.1	121.0	130.7	115.0	159.8	115.0	130.7		Equisetum silvaticum[27]
156.4	104.2	121.1	130.6	115.2	159.7	115.2	130.6		Galanthus glycoside[35] (+ emulsin)
156.9[a]	104.2	121.1	130.9	115.2	159.9	115.2	130.9		Ginkgo biloba[36**]
155.9	104.9	121.6	129.5	115.5	159.4	115.5	129.5		Synthetic (naringin)[37]
157.0[a]	105.9	120.9	130.9	115.2	160.1	115.2	130.9		Equisetum telmateja[28]
157.1[a]	105.7	120.7	130.6	115.0	160.0	115.0	130.6		Equisetum telmateja[28]
155.8	105.9	121.0	130.6	115.1	159.9	115.1	130.6		Brassica napus[22]
155.9	105.8	120.8	130.7	115.2	159.9	115.2	130.7		Equisetum hyemale[30]
156.8[a]	105.8	120.9	130.7	115.0	160.0	115.0	130.7		Equisetum silvaticum[27]
157.2[a]	105.7	120.7	130.7	115.0	160.1	115.1	130.7		Equisetum telmateja[28]
157.1[a]	105.8	120.8	130.7	115.1	159.9	115.1	130.7		Equisetum silvaticum[27]
156.0	105.0	121.7	129.6	115.6	159.4	115.6	129.6		Equisetum telmateja[28]
157.2[a]	105.8	120.8	130.7	115.7	159.9	115.7	130.7		Equisetum palustre[32]
156.2	103.1	122.1	115.3[a]	145.0	147.6	115.6[a]	120.0		
156.5	104.2	121.4	115.3[a]	144.8	148.5	116.5[a]	121.6		Equisetum silvaticum[27]
156.3	104.0	121.3	115.2[a]	144.7	148.3	116.2[a]	121.8		Arctostaphylos uva-ursi[21]
156.5[a]	104.0	121.2[b]	115.2[c]	144.7	148.4	116.2[c]	121.8[b]		" " "
156.8[a]	104.1	121.1[b]	115.6[c]	145.0	148.4	115.8[c]	121.6[b]		" " "
156.3	104.1	121.2	115.4[a]	144.9	148.4	116.1[a]	121.7		" " "
157.0[a]	104.2	121.0	115.4[b]	145.1	148.3	115.8[b]	121.0		Metasequoia glyptostroboides[31]
156.7	103.5	125.8	115.7	147.0[a]	146.4[a]	117.0	120.0		Allium cepa[29]
156.4[a]	104.2	121.2[b]	113.9[c]	149.5	147.1	115.3[c]	122.1[b]	56.2	Cereus grandiflorus[29]
156.4[a]	104.1	121.2[b]	113.9[c]	149.5	147.0	115.3[c]	122.4[b]	56.0	Cereus grandiflorus[29]
155.8	104.8	123.4	115.2	146.3	149.6	112.4	119.9	55.8	Synthetic(hesperidin)
155.7	104.7	121.9	115.5[a]	145.0	147.9	115.4[a]	120.1		Synthetic[38]
156.2	104.0	120.2	108.8	145.3	136.6	145.3	108.8		Arctostaphylos uva-ursi[21]
157.4[a]	104.2	119.8	108.3	145.8	136.5	145.8	108.3		Metasequoia glyptostroboides[31]
157.5	104.0	120.9	106.0	146.5	137.9	146.5	106.0		Synthetic[39]
157.4	104.8[a]	125.9	104.8	153.2	141.4	153.2	104.8	60.2 56.5	Synthetic[39]

determined for DMSO-d_6 solutions at 95° unless otherwise stated.

spectrum and is accordingly identified.

Table 2. ^{13}C NMR spectra of flavonoid

FLAVONOID GLYCOSIDE*	++	GLUCOSE C-1	C-2	C-3	C-4	C-5	C-6
1c. A-7-O-glu(2-1)apiose		99.7	76.8	76.6[a]	70.2	77.2[a]	60.9
3b. L-7-O-glu		100.4	73.3	76.6[a]	70.0	77.3[a]	61.0
3a. L-3'-O-glu		102.4	73.4	76.2[a]	70.3	77.4[a]	61.2
3c. L-7,3'-O-glu	3'	102.4	73.4	76.2[a]	70.4	77.4[a]	61.2
" " "	7	100.2	73.3	76.6[b]	69.8	77.2[b]	60.8
3d. L-5-O-glu		104.3	73.6	75.8[a]	70.1	77.4[a]	61.1
2a. K-3-O-glu		101.4	74.2	76.5[a]	70.1	77.2[a]	61.0
2b. K-3-O-glu(2-1)glu	3	98.6	82.0	76.6[a]	70.0[b]	76.6[a]	61.0[c]
" " "	2"	103.6	74.3	76.7[a]	70.5[b]	76.7[a]	61.4[c]
2c. K-3-O-glu(6-1)rha		101.5	74.2	76.5[a]	70.1	75.8[a]	66.9
2d. K-7-O-glu(2-1)rha		98.4	77.3[a]	77.1[a]	70.1[b]	76.8[a]	60.9
3l. K-7-O-glu		100.5	73.4	76.7[a]	70.1	77.3[a]	61.2
2e. K-7-O-glu 3-O-glu	7	100.3	73.3	76.6[a]	70.0[b]	77.3[a]	61.0
" " "	3	101.3	74.4	76.6[a]	70.2[b]	77.4[a]	61.0
2m. K-7-O-glu 3-O-glu(6-1)rha	7	100.7	73.2	76.6[a]	70.0[b]	77.2[a]	61.0
" " "	3	101.4	74.2	76.6[a]	70.2[b]	76.0[a]	67.0
2f. K-7-O-glu 3-O-glu(O-Ac)	7	100.3	73.2	76.5[a]	70.0	77.1[a]	60.9
" " "	3	101.3	74.1	76.4[a]	70.0	74.1	62.8
2g. K-7-O-glu 3-Oglu(2-1)glu(O-sinapoyl)	7	100.4	73.2	76.7[a]	70.0[b]	77.0[a]	61.2[c]
" " "	3	97.7	80.0	76.7[a]	70.4[b]	77.0[a]	61.0[c]
	2"	99.1	74.6[d]	74.1[d]	70.7[b]	76.1[a]	61.0[c]
2h. K-7-O-glu 3-O-glu(2-1)glu	7	100.3	73.2[d]	76.4[a]	69.9[b]	77.2[a]	60.9[c]
" " "	3	98.4	82.1	76.6[a]	69.9[b]	77.2[a]	61.0[c]
" " "	2"	103.6	74.3	76.6[a]	70.3[b]	76.6[a]	61.3[c]
2l. K-7-O-rha 3-O-glu		101.5	74.3	76.5[a]	70.0[b]	77.1[a]	61.1
2j. K-7-O-rha 3-O-glu(O-Ac)		101.3	74.1	76.4	70.0[a]	74.1	62.9
2k. K-7-O-rha 3-O-glu(6-1)rha	7						
" " "	3	101.4	74.2	76.6	70.2[a]	76.0	66.9
" " "	6"						
4a. Q-3-O-glu		101.4	74.3	76.8[a]	70.3	77.5[a]	61.3
4l. Q-3'-OMe 3-O-glu(6-1)rha		101.5	74.4	76.7	70.2[a]	76.1	67.0
4g. Q-4'-O-glu		102.2	73.7	76.4[a]	70.5	77.5[a]	61.4
4b. Q-3-O-gal							
4c. Q-3-O-gal(6"-O-galloyl)							
4h. Q-3'- OMe 3-O-gal							
4d. Q-3-O-arab							
4e. Q-3-O-arab							
4f. Q-3-O-rha							
4k. Q-7-O-glu		100.3	73.2	76.5[a]	69.9	77.2[a]	60.9
4j. Q-4'-OMe 7-O-glu(6-1)rha		100.3[a]	73.2	76.4	69.8[b]	75.8	66.2
6b. M-3-O-rha							
6a. M-3-O-gal							

**Chemical shifts are expressed in ppm from TMS. All spectra were determined for DMSO-d_6 solutions at 95°.
*When re-run at 21° the methyl resonance appeared at 17.9 ppm.
[a,b,c,d]Assignments bearing the same superscript in any one spectrum may be reversed.
+Abbreviations: A (apigenin), L (luteolin), K (kaempferol), Q (quercetin), M (myricetin), glu (glucose), gal
++This column designates glycosylation site in compounds where the same sugar is present at two different

glycosides (sugar region)[a,a]

RHAMNOSE						OTHER SUGARS					
C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-5	C-6
						109.0	76.5[a]	79.1	74.0	64.4	
100.6	70.3[b]	70.7[b]	72.0	68.1	17.4						
100.5	70.5[b]	70.8[b]	72.2	68.3	20.9						
100.3	70.4[b]	70.8[b]	72.1	68.1	17.5						
98.9	70.3[b]	70.6[b]	71.8	70.0[b]	17.5						
98.7	70.0[a]	70.5[a]	71.8	69.8[a]	17.7						
98.9	70.0[a]	70.6[a]	71.9	69.8[a]	17.5						
100.6	70.3[a]	70.8[a]	72.1	68.1	17.5						
100.8	70.4[a]	70.8[a]	72.1	68.2	17.6						
						102.3	71.3	73.4	68.0	75.8	60.8
						102.4	71.2	73.1	68.0	72.7	62.3
						102.0	71.5	73.4	68.1	75.9	60.4
						108.1	82.1	77.2	86.2	61.0	
						101.8	71.7	70.8	65.9	64.1	
101.9	70.4[a]	70.6[a]	71.5	70.1[a]	17.3						
100.5[a]	70.3[b]	70.9[b]	72.2	68.2	17.6						
102.1	70.5[b]	70.7[b]	71.6	70.1[a]	21.4[a]						
						102.5	71.4	73.4	68.1	75.9	60.1

(galactose), rha (rhamnose), arab (arabinose).
sites.

Most of the glycosides studied are natural products (Table 1) and although the points of attachment to the aglycone and the sequence of sugars are firmly established in the majority of cases, the ring size of each sugar, the anomeric form of each sugar and the interglycosidic linkages between sugars are generally unknown or poorly defined. For example the β-anomeric form of the 7-O-glucosides was recognized by its lability to emulsin and conversly, sophorosides were detected by their stability to this enzyme. The contribution that ^{13}C NMR spectroscopy can make in these and other problem areas will now be considered.

1. *The effects of glycosylation on the aglycone spectrum.* The effects of glycosylation on the ^{13}C NMR spectra of flavonoid aglycones are summarised in Table 3. These data represent averaged values of shifts observed in all relevant models studied and are compared in the table with similar shifts observed on methylation of the same OH function.

The effects of glycosylation of the 7-OH on the C-7 signal is a 1.4 ppm upfield shift which is in agreement with our earlier observations.[2] This is accompanied by significant downfield shifts of the signals of the *ortho*- and *para*-related carbons, C-6, 8 and 10, the effect being more noticeable with C-10. Glycosylation with rhamnose appears to have a more marked effect on the C-7 signal than does glycosylation with other sugars, the average upfield shift being of the order of 2.35 ppm. This difference is of diagnostic value.

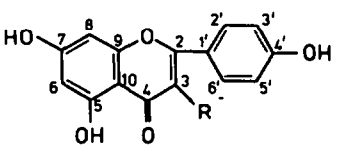
The same general pattern of signal shifts is also observed on glycosylation at C-3, 3′ or 4′. Glycosylation at C-3 however does produce a larger than expected "ortho"-effect on the C-2 signal thus confirming previous observations.[2] Rhamnosylation of the 3-OH, as with the 7-OH, appears to produce a different shift at C-3 than do other sugars.† Thus the C-3 signal is seen to shift upfield by about 1.05 ppm on rhamnosylation of the 3-OH compared with 2.1 ppm for glycosylation with other sugars. Since only one example each of glycosylation at the 4′- and 3′-OH's was available for study, the figures presented must be treated as preliminary. However, the general trend of shifts observed is the same as that discussed for the 7-OH, the signal of the *para*-related carbon again showing the greatest shift. Indeed, this shift together with those of the *ortho*-related carbons may well be a more reliable guide to the site of glycosylation than the shift of the oxygenated carbon itself (e.g. see shifts quoted for 3′-O-glycosylation, Table 3).

Glycosylation of the 5-OH group clearly represents a special case. As a result of glycosylation the 5-hydroxyl-4-keto H-bond is broken and this has a profound effect on the electron density distribution in the molecule. Thus although the shift observed in the C-5 signal is similar to that expected from a study of glycosylation effects at other sites, as also are the shifts of the *ortho* (C-6, 10)- and *para* (C-8)-related carbons, a marked effect is also noticed in the signals of the C-ring carbons due to the release (in luteolin) of the 4-keto function from H-bonding. Also, the C-2 and C-4 signals move sharply upfield and that of C-3 downfield, the reverse effect to that observed when H-bonding is introduced, for example by hydroxylation of flavone at C-5.[1]

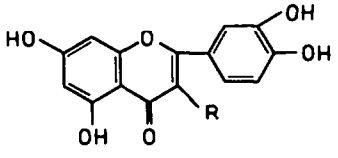
In the vicinity of the OH group, the effect of methylation of that OH group appears to be the reverse of glycosylation (Table 3). Thus, whereas glycosylation produces an upfield shift in the signal of the oxygenated carbon, methylation produces an approximately equivalent downfield shift. Likewise the shifts observed in the signals of the *ortho*-related carbons tend to be upfield rather than downfield as on glycosylation. This reversal is not observed at the *para*-position however. Methylation of the 5-OH is the exception; here, as with glycosylation methylation produces an upfield shift of the C-5 signal. Shifts in the C-ring too mirror those observed on glycosylation, thus confirming that release of the CO from H-bonding is the primary cause of these shifts. In the A-ring, shifts observed are also all in the same direction as those for glycosylation, except for C-6.

2. *Interglycosidic linkages.* It has been established that glycosylation of sugar hydroxyls produces a sizeable downfield shift in the resonance of the hydroxylated carbon.[18] Likewise, this has been observed in a preliminary study[2] of flavonoid O-diglycosides. For example in flavonoid rutinosides (rhamnosyl (1–6) glucosides) the glucose C-6 signal was found to appear 4.5–5.2 ppm downfield from that of glucose itself, and in a flavanone neohesperidoside (rhamnosyl (1–2) glucoside), the C-2 signal appeared about 2.6 ppm downfield from that of C-2 in glucose. The present study includes five rutinosides (**2c,k,m, 4i,j**) and generally confirms the above, although the average downfield shift is 5.8 ppm. It is also noted that the glucose C-5 signal undergoes an upfield shift of about 1.4 ppm. The single neohesperidoside studied (**2d**) is also consistent with previous work, exhibiting a 3.9 ppm downfield shift of the glucose C-2 signal and a 2.1 ppm upfield shift of the glucose C-1 signal due to rhamnosylation. Apiose appears to have much the same effect on the glucose spectrum as does rhamnose (*cf.* **1c**). When glucose is the glycosylating sugar as in sophorosides (e.g. **2b** and **2h**) however, a much larger downfield shift, of the order of 8 ppm, is evident in the C-2 signal. Shifts of this size appear to be typical of β-glucosylation generally, in disaccharides[18,19] and oligosaccharides.[19] In **2b** and **2h** glucosylation at C-2 is also detectable in the glucose C-1 signal which shifts upfield by about 2.9 ppm. The shift differences observed above

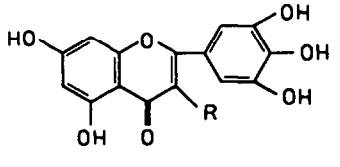
†The previously expressed opinion[2] that 3-O-rhamnosylation of quercetin may cause a large shift in the C-1′ signal is not supported however. The 130.3 ppm signal formerly attributed to C-1′ was in fact the C-3′, 5′ signal of a small kaempferol 3-O-glycoside impurity. A proton-coupled spectrum has revealed the C-1′ signal at 121 ppm, hidden beneath the combined C-2′, 5′ signals.



I; R = H, Apigenin
II; R = OH, Kaempferol



III; R = H, Luteolin
IV; R = OH, Quercetin



V; R = H, Tricetin
VI; R = OH, Myricetin

Table 3. The effect of hydroxyl derivatization on the ^{13}C NMR spectra of flavonoids

Site of Glycosylation or Methylation	Observed Shifts in Carbon Signals+														
	2	3	4	5	6	7	8	9	10	1'	2'	3'	4'	5'	6'
7-O-glycose					+0.75	-1.35	+1.05		+1.7						
7-O-rhamnose					+0.75	-2.35	+1.0		+1.7						
7-O-methyl[3]					-0.8	+1.4	-1.4		+1.0						
3-O-glycose	+9.2	-2.1	+1.6	+0.4					+1.0	-0.8	+1.1	-0.3	+0.7	-0.4	+1.45
3-O-rhamnose	+10.3	-1.05	+1.95	+0.6					+1.1						
5-O-glucose	-2.8	+2.2	-6.0	-2.7	+4.4	-3.0	+3.2	+1.4	+4.3	-1.3	-1.2	-0.4	-0.8	-1.0	-1.8
5-O-methyl[3]	-3.7	+4.3	-6.1	-1.6	-2.4	-1.3	+1.2	+1.7	+2.3	+0.2	-0.7	-0.2	-0.5	-0.2	-0.7
3'-O-glucose	-0.5	+0.4									+1.6	0	+1.4	+0.4	+3.2
3'-O-methyl											-3.0	+4.7	-1.7	-0.8	-0.4
4'-O-glucose	+0.1	+1.0								+3.7	+0.4	+2.0	-1.2	+1.4	0
4'-O-methyl										+2.0	-0.4	+1.3	+1.7	-3.5	-0.3

+ Shift values quoted represent averaged values where this was possible with available data. Only consistent, sizeable, shifts are listed. Shifts quoted are relative to the equivalent (or nearest equivalent) compound lacking derivatization at the site, apigenin and luteolin aglycone data being avoided where possible to avoid solvent effect complications. The data for the 3-O-methyl and 7-O-methyl flavonoids were obtained from ref. 3. after transposing C-5 and C-9 values (see text).

could have considerable diagnostic value for the structure elucidation of flavonoid polyglycosides.

3. *Site of acylation in glycosides.* The effects on the ^{13}C NMR spectrum resulting from acylating the C-3 OH of glucose have been recorded.[20] This study involved acylation with acetic acid, monochloroacetic acid and trichloroacetic acid, and it was found that as the electron withdrawing power of the acylating function increased, so also did the extent to which the C-3 signal moved downfield on acylation. The downfield shift of C-3 was accompanied by upfield shifts of the C-4 and C-5 signals which in contrast, were not appreciably affected by the change in acyl groups. The series of compounds under study in the present work includes glycosides acylated with acetyl **2f**, **2j**, galloyl **4c** and sinapoyl **2g** functions. Of these, for only one compound, **4c**, are there published data supporting the location of the acylation site. In this case, ^1H NMR spectroscopic data were cited in favour of acylation at the 6″-hydroxyl.[21]

Examination of the ^{13}C NMR spectra of these compounds (Table 2) clearly reveals the sites of acylation. The spectra of the two acetates, **2f** and **2j**, differ from the spectra of the non-acetylated equivalents, **2e** and **2i**, chiefly in the resonance values of glucose C-5 and C-6. In both cases, the C-6 signal has moved downfield by 1.8 ppm and the signal of the adjacent C-5, upfield by about 3 ppm, thus defining the acylation site as glucose C-6. The position of attachment of gallic acid to the 6-position of galactose in **4c** is evidenced by similar shifts of the C-5 and C-6 signals. The identity of the galloyl group itself was evident from signals at 165.4 (CO_2), 145.4 (C-3, 5), 138.4 (C-4), 119.5 (C-1) and 108.8 (C-2, 6) ppm.

The acylation site in compound **2g**, the monosinapoyl derivative of kaempferol 7-O-glucoside-3-O-sophoroside, is considerably more difficult to determine because of the complexity of the spectrum. In this, superimposed on the complex spectrum of the unacylated equivalent, **2h**, are the sinapoyl signals at 165.4 (CO_2), 148.1 (C-3, 5), 144.2 (C-β), 138.6 (C-4), 124.8 (C-1), 115.6 (C-α) and 106.5 (C-2, 6) ppm , and the presence of the acyl function has altered the chemical shifts of a number of sugar carbons. However, it has been established by other means that the sinapoyl residue is attached to the sophorose moiety,[22] and by eliminating the signals associated with the aglycone, the sinapoyl residue and the 7-linked glucose, from the total spectrum, the problem is simplified. Using a "best fit" technique, the remaining signals can be ascribed to sophorose carbons by comparison with the spectra of compounds **2b** and **2h**.

The results of this procedure are presented in Table 2. On this basis it is proposed that the sinapoyl function is located on the C-2 OH group of the terminal glucose in the sophorose. Although the downfield shift of this C-2 carbon is only about 0.3 ppm, the upfield shifts of the adjacent carbons C-1 and C-3 are sizeable, viz. 4.5 ppm and 2–3 ppm respectively, and are comparable in size with those observed for the acetyl and galloyl groups above.

4. *Ring size and* C-1 *configuration in glycosidic sugars.* Methyl glycofuranosides and methyl glycopyranosides of the same sugar have been found to be readily distinguishable from their ^{13}C NMR spectra.[16,17] By using the established criteria for this distinction it is evident that with the exception of the quercetin 3-O-arabinoside, **4d**, all sugars in the glycosides studied are in the pyranose form. The spectrum of **4d**, displays signals in the sugar carbon region which match well with those recorded for methyl α-L-arabinofuranoside[17] (e.g. C-1 at 108.1 ppm and C-4 at 86.2 ppm) but which are quite distinct from those of the β-L-arabinofuranoside[17] and the two pyranosides.[16] Compound **4d** is therefore considered to be quercetin 3-O-α-L-arabinofuranoside, a formulation which has previously been given the name avicularin.[23] The same glycoside has been isolated together with another quercetin 3-O-arabinoside, **4e**, from a number of sources including *Arctostaphylos uva-ursi*[21] and *Taxodium distichum*.[23,28] The spectrum of **4e** is certainly not that of an arabinofuranoside, nor does it match closely with published data for methyl arabinopyranosides.[16,24] However, in a number of respects (m.p., R_f, IR) **4e** is identical with quercetin 3-O-α-L-arabinopyranoside (guaijaverin[23]), and the ^{13}C NMR spectrum could be interpreted as supporting this structure since variable conformational equilibria in pentose aldopyranosides are known to give rise to large discrepancies in reported signal values.[25]

With regard to the configuration of the glycosidic bond in the glycosides studied, it is clear from both the constancy of the signals for any one sugar (Table 2) and from the good agreement with published results for methyl glycosides,[16] that the "normal" configuration occurs in each case. That is, glucosides and galactosides are all β-linked and rhamnosides (and probably both arabinosides) are all α-linked. Generally such information is only obtained through treatment of glycosides with a variety of enzymes. ^{13}C NMR offers a most satisfactory, unequivocal method for this determination when sufficient sample is available.

EXPERIMENTAL

The ^{13}C NMR spectra were recorded at LaTrobe University on a Jeol P-100 Fourier transform spectrometer operating at 25.15 MHz. Spectral widths were 5000 Hz. 8K data points were used in most cases. Proton coupled spectra were obtained using an electronic gating system which permits the retention of the nuclear Overhauser enhancement. The deuterium signal of the deuterio-dimethyl-sulphoxide (DMSO-d_6) solvent was used as a lock signal. The normal pulse time of 3.6 sec was lengthened to 6.7 sec for some samples (using a 90° pulse) in which signals such as C-2 and C-10 had been adversely affected by the short relaxation time. Spectra were recorded on samples of 12–100 mg in DMSO-d_6 (1.25 cm^3) at 95° unless otherwise stated. Sample sources are detailed in Table 1.

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