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CARBON-13 NMR SPECTROSCOPY OF HETEROCYCLIC COMPOUNDS—IV

A 20 MHz STUDY OF CHEMICAL SHIFTS AND CARBON-PROTON COUPLING CONSTANTS IN A SERIES OF HYDROXY, METHOXY AND GLUCOSYL COUMARINS

N. J. CUSSANS and T. N. HUCKERBY[1]
Department of Chemistry, The University, Bailrigg, Lancaster LA1 4YA, England

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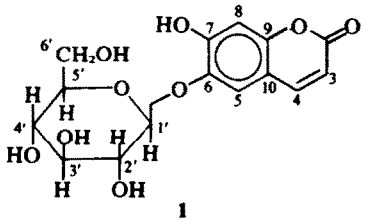
Abstract—Complete assignments of chemical shifts and extensive assignments of carbon-proton coupling constants are presented for all the monohydroxycoumarins except the 5-derivative, together with similar data for 6,7- and 7,8-dihydroxy-, 4- and 7-methoxy-, 7-hydroxy-4-methyl- and 6,7- and 7,8-dihydroxy-4-methyl-coumarins. It is shown that shifts in the polysubstituted molecules can be predicted with high precision from substituent effects evaluated for the more simple derivatives. The approach is extended to some simple glucosyl derivatives, and it is demonstrated that not only the anomeric configuration but also the exact site of the sugar substitution can be determined from ^{13}C data.

INTRODUCTION

There are a large number of natural products based on the coumarin skeleton, and nearly all of these substances possess one or more OH substituents. In contrast to the extensive ^1H NMR studies[2-6] on this class of compounds, there has as yet been no detailed study of the use of ^{13}C NMR as a help in the structural elucidation for naturally occurring coumarins, although when this work was virtually complete a note[7] appeared concerning its use in the assignment of the structure 4,7 - dimethoxy - 5 - methylcoumarin to the fungal metabolite siderin. In the preceding papers[8] we have described in some detail the methods for the complete assignment of chemical shifts and extensive correlations of coupling constants for coumarin and some of its simple derivatives. Here we extend these basic studies to a series of important hydroxy, methoxy and glucosyl coumarins which demonstrate the general approach by which ready identification of complete structures may be achieved. Some derivatives containing a 4-methyl substituent are also included. Contained in the correlations are a range of natural coumarins, including the hydroxy coumarins umbelliferone (7-OH), esculetin (6,7-di-OH), herniarin (7-OCH_3) and daphnetin (7,8-di-OH), and the glucosides skimmin and esculin. Skeletal numbering in the glucosyl coumarins is as shown in **1** for esculin.



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RESULTS AND DISCUSSION

General comments. Chemical shifts and ^{13}C-^1H coupling constants for the hydroxycoumarins and related compounds are summarised in Tables 1–4. Signals from quaternary carbons could be identified on the spectra through the loss of Overhauser enhancement in proton-noise-decoupled experiments, and by the absence of a large one-bond C-H coupling when proton information is retained through gated decoupling experiments. The CO carbon may be easily identified by its characteristic chemical shift. This is confined within an extremely narrow range (1·3 ppm) for all compounds except the 3- and 4-hydroxy derivatives where the signals are displaced very slightly to higher and to lower field positions respectively. The total range, of only 3·7 ppm thus confirms the suggestions from IR and other observations that the potentially tautomeric 3- and 4-hydroxy derivatives do indeed exist in the dominant enol forms as indicated by their names. These CO signals are usually typified by very sharp signals on coupled spectra, and the observed couplings are quite unambigous[8] as indicators of the substitution pattern in the heterocyclic ring.

The other nuclei in the heterocyclic ring also show regular trends in coupling constants. While a C_3H_4 coupling has not been observed in any of the compounds studied, the one-bond interaction C_3H_3 becomes slightly diminished from its typical value of 172 Hz when C_4 is substituted with either a methyl or an oxygen group. Transannular couplings are observed between C_4 and H_5 and between C_5 and H_4 when the latter is present. When a methyl group is present at C_4, both C_3 and C_4 show a quartet splitting of 5–6 Hz.

For those compounds containing no substituent in the benzenoid ring, single-frequency off-resonance proton decoupling experiments provide a ready means of distinguishing between $\alpha(C_5, C_8)$ and $\beta(C_6, C_7)$ carbons.[9] The effect of an OH group when present in the carbocyclic ring, is quite characteristic. The newly formed quaternary carbon moves approx. 30 ppm downfield from the value observed in coumarin itself. Carbons *ortho* and *para* to the substituent both move upfield (by 11–15 and 7–10 ppm respectively), while those which are *meta* appear little affected, but move consistently downfield by 0·3–2·3 ppm. The substituent chemical shifts for the mono-hydroxy coumarins are summarised in Table 5. Replacement of -H by -OH also introduces characteristic

changes in coupling patterns within the benzenoid ring. The one-bond C-H coupling for a carbon *ortho* to the substituent drops by 2–3 Hz, and *meta* couplings *across* an oxygen bonded carbon also drop by this amount. On the contrary, *meta* couplings *involving* the substituted carbon *increase* by 2–3 Hz.

Methoxy coumarins. Table 6 summarises the substituent shifts for 4- and 7-methoxycoumarin. These are essentially similar to the parent hydroxy derivatives. Indeed, with 4-methoxycoumarin the largest change induced on substituting the OH proton is −1·4 ppm (at C_3) and all the other shifts are static or move slightly upfield

Table 1. Parameters for hydroxy and dihydroxy coumarins

	3-Hydroxycoumarin δ	4-Hydroxycoumarin δ	6-Hydroxycoumarin δ
C_2	158.5 (a) C_2H_4 8	162.2 C_2H_3 3	160.1 C_2H_3 4.5 C_2H_4 11
C_3	141.75 C_3-OH(?) 4 -	91.4 - C_3H_3 167.5	116.1 - C_3H_3 172.5 (b)
C_4	115.0 C_4H_4 163.5 C_4H_5 5	165.8 - C_4H_5 4.5 C_4-OH(?) 2.5	143.8 C_4H_4 165.5 C_4H_5 5
C_5	126.3 C_5H_4 4.5 C_5H_5 163 C_5H_7 8 (b)	123.3 C_5H_5 165 C_5H_7 7.5 (b)	112.4 C_5H_4 4 or 4.5 C_5H_5 162 C_5H_7 4.5 or 4
C_6	124.5 C_6H_5 or C_6H_7 2 C_6H_6 163 C_6H_8 6.5	123.7 - C_6H_6 164.5 C_6H_8 7.5	153.7 - - C_6H_8 8 (2., 2.)
C_7	127.5 - C_7H_5 8 C_7H_7 164.5 (b)	132.4 - C_7H_5 8.5 C_7H_7 163 (b)	119.7 - C_7H_5 6 C_7H_7 162
C_8	115.6 C_8H_6 7 C_8H_8 164 (b)	116.3 C_8H_6 7.5 C_8H_8 165 (b)	116.9 - C_8H_8 164.5 (b)
C_9	149.2 (7.5,6.,6.)	153.8 (9.5,8.,2.5)	146.75 (8.,8.,6.,2.)
C_{10}	120.7 (5.,4.5,1)	116.1 (8.,5.5,4.5)	119.1 (8.,5.,1.5)

7-Hydroxycoumarin δ	8-Hydroxycoumarin δ	6,7-Dihydroxy-coumarin δ	7,8-Dihydroxy-coumarin δ
160.7 C_2H_3 4.5 C_2H_4 11.5	160.0 C_2H_3 4.5 C_2H_4 11.5	161.4 C_2H_3 4.5 C_2H_4 11.5	161.1 C_2H_3 5.0 C_2H_4 11.5
111.5 - C_3H_3 172 (b)	116.1 - C_3H_3 172.5 (b)	112.0 - C_3H_3 173 (b)	111.7 - C_3H_3 172.5 (b)
144.3 C_4H_4 164 C_4H_5 4.5	144.5 C_4H_4 165.5 C_4H_5 ca. 3 (c)	144.5 C_4H_4 164 C_4H_5 5 (b)	145.4 C_4H_5 165 C_4H_5 4.5 (b)
129.55 C_5H_4 4 C_5H_5 163	118.4 (d)	112.9 C_5H_4 3.5 C_5H_5 161 (b)	119.4 C_5H_4 4 C_5H_5 165 (b)

Table 1. (*Contd*)

7-Hydroxycoumarin δ	8-Hydroxycoumarin δ	6,7-Dihydroxy-coumarin δ	7,8-Dihydroxy-coumarin δ
113.3 - C_6H_6 163.5 C_6H_8 5	124.4 - C_6H_6 163 - (b)	143.2 C_6-OH(?) 3.5 - C_6H_8 7	113.0 - C_6H_6 162 - (b)
161.6 C_7H_5 11 (3.5,2.)	118.4 (d)	150.6 C_7-OH(?) 3.5 C_7H_5 8	150.0 - C_7H_5 9
102.5 C_8H_6 4.5 C_8H_8 162.5 (b)	144.7 (e)	103.2 - C_8H_8 161.5 (b)	132.6 C_8H_6 5.5 - C_8-OH(?) 1.5
155.7 (10.5,6.,5.)	142.4 (f)	149.1 (10.,6.,5.)	144.2 (8.5 ,6.5)
111.5 (7.5,7.5,5.)	119.7 (g)	111.4 (8.,6.,1.5)	112.7 (8.,8.,2.,2.)

a. For each signal, the chemical shift in ppm from TMS as a secondary reference is given in the top left hand corner. Coupling constants are given in Hz and the nuclei involved are indicated by their type and substitution position number. Unassigned couplings are given in brackets.

b. Contains unresolved coupling(s).

c. Asymmetrically shaped signals suggest presence of second-order character. The small coupling indicated is thus an approximate value only.

d. The signals from C_5 and C_7 were completely overlapped; no measurements of coupling constants were possible.

e. Apparent doublet of doublets, but with second-order 'wings'.

f. Complex multiplet, not well resolved from noise.

g. Complex multiplet, partly overlapped by the signals from C_3 and C_4. Structure not analysed.

Table 2. Parameters for methyl-hydroxy-coumarins

	7-Hydroxy-4-methyl coumarin δ	6,7-Dihydroxy-4-methyl coumarin δ	7,8-Dihydroxy-4-methyl coumarin δ
C_2	160.4 (a) C_2H_3 4.5	160.8 C_2H_3 4.5	160.7 C_2H_3 4.5
C_3	110.4 C_3H_3 170 C_3CH_3 6	110.6 C_3H_3 169 C_3CH_3 5.5	110.5 C_3H_3 170 C_3CH_3 5
C_4	153.2 C_4H_5 4 C_4CH_3 6	153.0 C_4H_5 4 C_4CH_3 6	154.1 (c)
C_5	126.2 C_5H_5 162	109.6 C_5H_5 161 (b)	115.7 C_5H_5 164.5
C_6	112.9 C_6H_6 163.5 C_6H_8 4.5 - (b)	142.8 - C_6H_8 6.5 C_6-OH(?) 4	112.6 C_6H_6 161.5 - -
C_7	161.2 C_7H_5 11 (3.,2.)	150.2 C_7H_5 8 C_7-OH(?) 3.5	149.6 C_7H_5 10 C_7-OH(?) 2
C_8	102.4 C_8H_6 4.5 C_8H_8 162 (b)	102.9 - C_8H_8 161 (b)	132.5 C_8H_6 5 -
C_9	155.0 (10.5,5.) (b)	148.0 (10.,4.5)	143.6 C_9H_5 9
C_{10}	112.2 (d)	111.9 $C_{10}CH_3$ 3.5 (5) (b)	113.25 (c)

Table 2 (*Contd*)

	7-Hydroxy-4-methyl coumarin δ	6,7-Dihydroxy-4-methyl coumarin δ	7,8-Dihydroxy-4-methyl coumarin δ
CH_3	18.0 C-H 12.8 CH_3-H_3 5.5	18.1 C-H 128 CH_3H_3 5.5	18.3 C-H 128.5 CH_3H_3 5

a. For each signal, the chemical shift in ppm from THS as a secondary reference is given in the top left corner. Coupling constants are given in Hz and the nuclei involved are indicated by their type and substitution position number. Unassigned couplings are given in brackets.

b. Contains unresolved coupling(s).

c. Complex multiplet.

d. Complex multiplet; contains a quartet splitting.

Table 3. Parameters for methoxycoumarins

	4-Methoxy coumarin δ	7-Methoxy coumarin δ
C_2	161.7 (a) (b)	160.1 C_2H_3 5 C_2H_4 11.5
C_3	90.0 C_3H_3 169.5	112.3 C_3H_3 172.5
C_4	165.8 - - C_4-OCH_3 4 (c)	143.8 C_4H_4 165 C_4H_5 4.5 -
C_5	122.6 - C_5H_5 165.5 C_4H_7 8 (c)	129.1 C_5H_4 4 C_5H_5 164.5 -
C_6	123.9 C_6H_6 164.5 C_6H_8 7.5	129.1 C_6H_6 164.5 C_6H_8 5
C_7	132.5 C_7H_5 9 C_7H_7 164 - - (c)	162.4 C_7H_5 11 - C_7-OCH_3 4 (?) 3
C_8	116.3 C_8H_6 7.5 C_8H_8 165.5	100.5 C_8H_6 4.5 C_8H_8 164
C_9	152.7 (d)	155.3 (d)
C_{10}	115.1 (d)	112.0 (8.,8.,5.,2.)
OCH_3	56.8	55.6

a. For each signal, the chemical shift in ppm from TMS as a secondary reference is given in the top left corner. Coupling constants are given in Hz and the nuclei involved are indicated by their type and substitution position number. Unassigned couplings are given in brackets.

b. Broad signal; coupling not resolved.

c. Contains unresolved structure.

d. Signal not well resolved from noise.

except for C_6 and C_7. With 7-methoxycoumarin the only significant differences compared with the hydroxy derivative are at C_7 (slight downfield shift) and at the *ortho* sites C_6 and C_8 (upfield shifts of 1–2 ppm). The carbon to which the methoxy group is attached is clearly identified by the presence of a 4 Hz quartet splitting on proton-coupled spectra.

Methyl-hydroxy-, dihydroxy- and methyl-dihydroxy-coumarins. The S.C.S. data summarised in Tables 6 and 7 show that the chemical shifts for these derivatives may be

Table 4. Parameters for glucosylcoumarins-(a) coumarin carbons

	7-β-D-Glucosyl Coumarin δ		6-β-D-Glucosyl-7-hydroxy coumarin δ(b)	δ(c)	7-α-D-Glucosyl-4-methyl coumarin δ		7-β-D-Glucosyl-4-methyl coumarin δ	
C_2	160.3	(a)	160.5	161.4	160.0	(i)	160.3	
	C_2H_3	4.5	C_2H_3	4.5	C_2H_3	4	(j)	
	C_2H_4	11.5	C_2H_4	11.5	-			
C_3	113.2		112.1	112.6	111.7		111.85	
	C_3H_3	173.5	C_3H_3	173.0			C_3H_3	170
	-		-		(k)		C_3CH_3	5
		(d)						(d,l)
C_4	144.2		144.4	145.0	153.3		153.4	
	C_4H_4	165.5	C_4H_4	165			-	
	C_4H_5	4.5	C_4H_5	5	(k)		C_4H_5	4
	-		-				C_4CH_3	6
		(d)						
C_5	129.5		115.0	115.25	126.3		126.4	
	C_5H_4	3.5	C_5H_4	4	-		-	
	C_5H_5	164.5	C_5H_5	165.5	C_5H_5	163.5	C_5H_5	163.5
		(d)		(e)				(d)
C_6	113.8		142.6	143.1	113.9		113.6	
	C_6H_6	165	-				C_6H_6	166
	C_6H_8	4.5	C_6H_8	7	(k)		C_6H_8	4.5
	-		C_6-OH(?)	3.5			-	
		(d)						
C_7	160.3		151.4	151.6	160.0		160.3	
	(f)		C_7H_5	8	(f)		(f)	
			C_7-OH(?)	3.5				
C_8	103.4		103.1	103.3	103.8		103.5	
	C_8H_6	3.5	-		C_8H_6	5	C_8H_6	4
	C_8H_8	165.5	C_8H_8	164	C_8H_8	165	C_8H_8	165.5
		(d)						(d,m)
C_9	155.1		150.5	151.0	154.4		154.5	
	(g)		(10.,6.,5.)		(f)		(10.5,5.)	
C_{10}	113.4		110.8	111.5	?		114.3	
	(7.5,7.5,5.)		(8.,6.)		(n)		(k)	
		(d)		(h)				
CH_3	-		-		18.0		18.2	
					(p)		C-H	129.5
							CH_3-H_3	5
	(b) Sugar Carbons							
C_1'	100.3		102.4	102.8	97.1		100.4	
	C-H	162	C-H	160	C-H	171.5	C-H	161
		(d)		(d)		(i,q)		(d)
C_2'	73.3		73.3	74.0	72.9		73.4	
C_3'	77.2		77.3	77.5	74.1		77.3	
C_4'	69.9		69.9	70.5	69.85		69.9	
C_5'	76.65		76.1	76.5	71.3		76.6	
C_6'	60.9		60.8	61.5	60.7		61.0	
	(triplet)		(triplet)				(triplet)	

[a]For each signal the chemical shift in ppm from TMS as a secondary reference is given in the top left corner. Coupling constants are given in Hz and the nuclei involved are indicated by their type and substitution position number. Unassigned couplings are given in brackets. [b]Chemical shifts for an anhydrous sample (see discussion). [c]Chemical shifts for a hydrated sample (see discussion). [d]Contains unresolved coupling(s). [e]Approximate values; overlapped with C_{10}. [f]Not resolved; overlapped with C_2. [g]Complex multiplet. [h]Approximate values; overlapped with C_5. [i]Spectra are from a 25 mg. sample and couplings quoted are therefore approximate. [j]Structure unclear; overlapped with C_7. [k]Structure not resolved from noise. [l]Overlapped with C_8; [m]J value is approximate. [n]Overlapped with C_3; [o]J value is approximate. [p]Signal not seen. [q]Not measured. [r]Fairly sharp lines.

Table 5. Substituent chemical shifts for the hydroxycoumarins

	3-Hydroxy Coumarin	4-Hydroxy Coumarin	6-Hydroxy Coumarin	7-Hydroxy Coumarin	8-Hydroxy Coumarin
C_2	-1.1 [a]	+2.6	+0.5	+1.1	+0.4
C_3	+25.85	-24.5	+0.2	-4.4	+0.2
C_4	-28.7	+22.1	+0.1	+0.6	+0.8
C_5	-1.8	-4.8	-15.7	+1.45	-9.7
C_6	+0.4	-0.4	+29.6	-10.7	+0.3
C_7	+4.0	+0.9	-11.8	+30.1	-13.1
C_8	-0.3	+0.4	+1.0	-13.4	+28.8
C_9	-4.2	+0.4	-7.65	+2.3	-11.0
C_{10}	+2.2	-2.4	+0.6	-7.0	+1.2

a. All values are expressed as the difference in chemical shift (in ppm) between the derivative and coumarin, both values being from measurements in DMSO.

Table 6. Substituent chemical shifts for methoxy- and methyl-hydroxy-coumarins

	4-Methoxy coumarin		7-Methoxy coumarin		7-Hydroxy-4-methyl coumarin	
					Observed	Predicted
C_2	+2.1 [a]	-0.5 [b]	+0.5 [a]	-0.6 [c]	+0.8 [a]	+0.8 [d]
C_3	-25.9	-1.4	-3.6	+0.8	-5.5	-6.1
C_4	+22.1	0.0	+0.1	+0.5	+9.5	+9.4
C_5	-5.5	-0.7	+1.0	-0.45	-1.9	-1.85
C_6	-0.2	+0.2	-12.1	-1.3	-11.2	-10.8
C_7	+1.0	+0.1	+30.9	+0.8	+29.7	+30.1
C_8	+0.4	0.0	-15.4	-2.0	-13.5	-13.2
C_9	-0.7	-1.1	+1.9	-0.4	+1.6	+1.6
C_{10}	-3.4	-1.0	-6.5	+0.5	-6.3	-6.2

a. Expressed as a chemical shift difference (in ppm) from coumarin in DMSO.
b. Expressed as a chemical shift difference (in ppm) from 4-hydroxy coumarin in DMSO.
c. Expressed as a chemical shift difference (in ppm) from 7-hydroxy coumarin in DMSO.
d. From a combination of SCS parameters for 4-methyl coumarin (in DMSO) and 7-hydroxy coumarin.

Table 7. Substituent chemical shifts for dihydroxy- and dihydroxy-methyl-coumarins

	6,7-Dihydroxy coumarin		6,7-Dihydroxy-4-methyl coumarin		7,8-Dihydroxy coumarin		7,8-Dihydroxy-4-methyl coumarin	
	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted
C_2	+1.8 [a]	+1.6 [b]	+1.2 [a]	+1.3 [c]	+1.5 [a]	+1.5 [b]	+1.1 [a]	+1.2 [c]
C_3	-3.7	-4.2	-5.3	-5.9	-4.2	-4.2	-5.4	-5.3
C_4	+0.8	+0.7	+9.3	+9.5	+1.7	+1.4	+10.4	+10.3
C_5	-15.2	-14.25	-18.5	-17.55	-8.7	-8.25	-12.4	-11.6
C_6	+19.1	+18.9	+18.7	+18.8	-11.1	-10.4	-11.5	-10.9
C_7	+19.1	+18.3	+18.7	+18.3	+18.5	+17.0	+18.1	+16.6
C_8	-12.7	-12.4	-13.0	-12.2	+16.7	+15.4	+16.6	+15.3
C_9	-4.4	-5.35	-5.4	-6.05	-9.2	-9.7	-9.8	-9.4
C_{10}	-7.1	-6.4	-6.6	-5.6	-5.8	-5.8	-5.25	-5.1

a. Expressed as a chemical shift difference (in ppm) from coumarin in DMSO.
b. From a combination of SCS parameters for the two appropriate mono-hydroxy coumarins.
c. From a combination of SCS parameters for 7-hydroxy-4-methyl coumarin and the appropriate mono-hydroxy coumarin.

predicted with some precision by suitable combinations of data from compounds which represent simpler components. With 7-hydroxy-4-methyl coumarin the largest error in prediction is 0·6 ppm, for the two 6,7-dihydroxy derivatives the worst discrepancy is 1·0 ppm and for the two 7,8-dihydroxy compounds the poorest estimate is 1·5 ppm out. These predicted shift changes for the hydroxyl-substituted carbons in the dihydroxy-derivatives are nearly always smaller than the movement actually observed. The discrepancy is more pronounced in the 7,8-disubstituted system, where there are in fact three neighbouring carbons which bear oxygen atoms.

The origin of this difference is unclear, but may partly be due to an *ortho* steric effect.

Glucosyl coumarins. The parameters for four glucosides, two of them natural products, are given in Table 4. Only 25 mg of the α-glucoside was available for study, thus the values given are incomplete and in some instances approximate. In the case of esculin (6 - β - D - glucosyl - 7 - hydroxy - coumarin) peculiarities were observed in the spectra of the commercially available sample, which contained water of crystallisation. Even in the proton - noise - decoupled spectrum all the signals from carbons bearing protons were considerably broadened compared with the quaternary carbon signals. This effect was ascribed to the presence of the water, and was observed over a wide range (10%–40% w/v) of concentrations in dimethylsulphoxide. Spectra from an anhydrous sample were normal in appearance, and showed chemical shifts which were marginally different. Both values have been summarised in Table 4.

Formally, all the parameters are closely related to those observed for the parent hydroxy-compounds. However, a detailed examination of the shift and coupling data reveals subtle information of great structural significance. The signals from the sugar moiety are fairly broad in proton-coupled spectra. This is an artefact from the use of DMSO as solvent, since couplings between carbon and hydroxyl protons are likely to be retained in this medium and they will be relatively small in magnitude. Their chemical shifts are assigned by analogy with those published[10] for methyl α- and β - D - glucosides, and the values for C_2' and C_3' could possibly be interchanged in the α-derivative. Those for C_3' and C_5' in the β-compounds may also be reversed. The C_1' signals at a characteristically low field position are readily distinguished from the nearby aromatic carbon signals by means of a suitable single-frequency off-resonance decoupling experiment which leaves a small residual doublet splitting on all the aromatic carbons having a proton attached. In a proton-coupled experiment these C_1' signals clearly indicate the nature of the anomeric link; the coupling with the attached proton is some 10 Hz larger for the α configuration as has already been observed in some simple glycosidic systems.[11]

In Table 8 are summarised substituent chemical shift data for the glucoside, calculated with respect to the signal positions for the parent aglycones. These data clearly show that for the compounds derived from 7-hydroxy coumarin a characteristic upfield shift (*ca.* 1 ppm) of the oxygen substituted carbon occurs in the glucoside. Similarly, the carbons *ortho* to this site move downfield by *ca.* 1 ppm. If it is postulated that this perturbation is specifically due to the presence of the sugar group at that site, it should be possible to predict from the carbon chemical shift data which of the two hydroxyl groups in esculetin is substituted on formation of esculin. The appropriate S.C.S. values are given in Table 8, and it is clear that the same trend is again evident; C_6 has moved *upfield* while C_5 and C_7 have been shifted *downfield.*

We would therefore suggest that the careful measurement of S.C.S. values between glycosides and aglycones where several possible sites of attachment for the sugar moiety are present may provide a general method for structural determination, the nature of the anomeric link also being provided via C-H coupling data.

EXPERIMENTAL

Carbon spectra were determined at 20 MHz as described previously.[8] Dimethylsulphoxide was employed as common solvent for all samples, and *ca.* 10% d_6-DMSO was added for locking purposes. Solns up to 50% w/v in concentration were used where possible in order to gain maximum sensitivity in proton coupled experiments; there appeared to be little variation of shifts with change in concentration. Spectra were referenced to the solvent carbon signal and converted to δ values from TMS for presentation in the conventional manner. Coupling constants have been quoted to the nearest 0·5 Hz, and chemical shifts to the nearest 0·1 ppm.

The following materials were commercially available and were used without further purification: 4 - hydroxycoumarin; 7 - hydroxycoumarin; 6,7 - dihydroxycoumarin; 7 - hydroxy - 4 - methylcoumarin; 6,7 - dihydroxy - 4 - methylcoumarin; 6 - β - D - glucosyl - 7 - hydroxycoumarin; 7 - α - D - glucosyl- and 7 - β - D - glucosyl - 4 - methylcoumarin. 6 - β - D - glucosyl - 7 - hydroxycoumarin was freed from water of crystallisation by solution in a small quantity of warm MeOH. A ppt quickly formed, and after its recovery by filtration, residual MeOH was removed by heating at 110° for 4 hr.

3-Hydroxycoumarin. Salicylaldehyde and acetyl glycine were reacted in the presence of NaOAc and Ac_2O to give 3-acetamidocoumarin[12] which was then treated with HClaq to afford 3-hydroxycoumarin.[13]

6-Hydroxycoumarin. This was obtained from a Pechmann condensation between hydroquinone and malic acid using conc H_2SO_4 as condensing agent.[14]

8-Hydroxycoumarin. 8-Methoxycoumarin 3-carboxylic acid was prepared from *o*-vanillin and malonic acid and was then demethylated and decarboxylated by boiling in pyridine hydrochloride.[15]

7,8-Dihydroxycoumarin. This material was obtained by reacting pyrogallol and malic acid in conc. H_2SO_4.[16]

7,8-Dihydroxy - 4 - Methylcoumarin. A Pechmann condensation using pyrogallol and ethyl acetoacetate with $SnCl_4$ as condensing agent gave this compound in good yield.[17]

Table 8. Substituent chemical shifts for some glucosyl coumarins

	7-β-D-Glucosyl Coumarin	6-β-D-Glucosyl-7-hydroxy coumarin	7-α-D-Glucosyl-4-methyl coumarin	7-β-D-Glucosyl-4-methyl coumarin
C_2	-0.4 [a]	-0.9 [a]	-0.4 [a]	-0.1 [a]
C_3	+1.7	+0.1	+1.3	+1.45
C_4	-0.1	-0.1	+0.1	+0.2
C_5	-0.05	+2.1	+0.1	+0.2
C_6	+0.5	-0.6	+1.0	+0.7
C_7	-1.3	+0.8	-1.2	-0.9
C_8	+0.9	-0.1	+1.4	+1.1
C_9	-0.6	+1.4	-0.6	-0.5
C_{10}	+1.9	-0.6	-	+2.1

a. Expressed as a chemical shift difference (in ppm) from the appropriate aglycone.

4-Methoxycoumarin. This was prepared from the 4-hydroxy derivative and dimethyl sulphate/potassium carbonate in dry acetone.[18]

7-Methoxycoumarin. This was synthesised as above, using 7-hydroxy coumarin.

7 - β - D - glucosylcoumarin. This was prepared by the general method described by Robertson and Head.[19] Acetobromoglucose[20] and 7-hydroxycoumarin were reacted in the presence of silver oxide, and the tetra-acetyl derivative thus formed was then deacetylated.

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