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A METHOD TO DIFFERENTIATE ISOMERIC *C*-GLUCOSYL CHROMONES, ISOFLAVONES AND XANTHONES

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Abstract—6-*C*- and 8-*C*-glucosyl isomeric chromones and isoflavones can be readily distinguished by a study of the NMR signals of their acetates In a similar manner 2-*C*- and 4-*C*-glucosylxanthones can be distinguished

INTRODUCTION

THE NMR signals of the acetates of some naturally occurring and synthetic *C*-glucosyl compounds in $CDCl_3$ have been examined by Gentili and Horowitz [1] They noted that the signals of the 2''-*O*-acetyl and 6''-*O*-acetyl methyl protons of 8-*C*-glucosylflavone acetates were found at higher fields than those of the corresponding 6-*C*-glucosyl compounds (see Table 1). The greater shielding of the 6''-*O*-acetyl in 8-*C*-glucosylflavone acetates is attributed to the acetyl methyl protons lying in the diamagnetic region of the phenyl B ring of the flavone nucleus

The 2''-*O*-acetyl signal of all *C*-glucosyl acetates is found at a higher field due to the shielding by the aromatic A ring to which the glucosyl residue is linked Gentili and Horowitz suggest the extra shielding of this group in 8-*C*-glucosylflavone acetates is due to some shielding by the phenyl B ring

RESULTS AND DISCUSSION

Aloesin (I), a *C*-glucosylchromone, has recently been isolated and its sugar moiety assigned to the 8-position of the chromone nucleus [2] The above hypothesis would predict that the 2''-*O*-acetyl and 6''-*O*-acetyl methyl signals of acetylated 8-*C*-glucosylchromones, which lack a phenyl B ring, would be in the 'normal' range (δ 1 80–1 83) and (δ 2 01–2 04) respectively The 6''-*O*-acetyl signals of aloesin and deacetylaloesin (II) pentaacetates are indeed found to be in the 'normal' range but their respective 2''-*O*-acetyl signals are found at the higher field (see Table 2) Dreiding models show that the 2''-*O*-acetyl methyl group of an acetylated 8-*C*-glucosylchromone is subjected to shielding by the aromatic A ring and the pyrone C ring The 2''-*O*-acetyl methyl group of an acetylated 6-*C*-glucosylchromone is also shielded by the aromatic A ring but not by the pyrone C ring

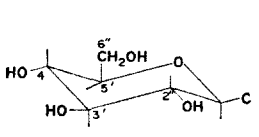
Puerarin (III) hexaacetate,[3] an 8-*C*-glucosylisoflavone gives the following acetate signals, 2''-*O*-acetyl (δ 1 72) and 6''-*O*-acetyl (δ 2 05) Again only the 2''-*O*-acetyl group is found at the higher field Dreiding models show that neither the 2''- nor the 6''-*O*-acetyl methyl groups of puerarin hexaacetate are shielded by the phenyl B ring, which is attached at C-3, but the 2''-*O*-acetyl is shielded by the pyrone C ring.

[1] GENTILI, B and HOROWITZ, R M (1968) *J Org Chem* **33**, 1571
[2] HAYNES, L J and HOLDSWORTH, D K (1970) *J Chem Soc* **C**, 2581
[3] HILLIS, W E and HORN, D H S (1965) *Australian J Chem* **18**, 531

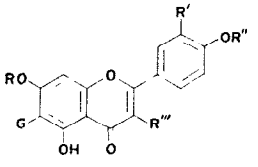
Paniculatin is the first di-*C*-glucosylisoflavone to be recorded and has been shown to be genistein-6,8-di-*C*-glucoside (IV) [4] Its undecaacetate integrates for eight acetyl methyl groups, one at δ 1 75, a second at δ 1 85 and the others at δ 2 05 The geometry of the molecule is such that the sugar acetyl methyl groups are not shielded by the C-3 phenyl B ring The 2''-*O*-acetyl group of the 8-*C*-glucosyl unit is shielded by the pyrone C ring whilst that of the 6-*C*-glucosyl unit is not Their signals are found at the higher field and the 'normal' range respectively Both 6''-*O*-acetyl signals are found in the 'normal' range

TABLE 1 CHEMICAL SHIFT OF ACETYL METHYL PROTONS OF ACETYLATED *C*-GLUCOSYLFLAVONES IN DEUTERIOCHLOROFORM[1]

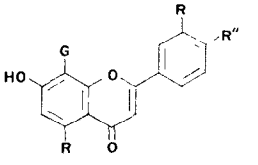
	2''-*O*-acetyl		6 -*O*-acetyl	
	Normal range 1 80–1 83	Higher field 1 70–1 73	Normal range 2 01–2 04	Higher field 1 90–1 95
6-*C*-Glucosylflavones				
Isovitexin heptaacetate	1 83		2 04	
Keyakinin heptaacetate	1 80		2 01	
Isoorientin octaacetate	1 82		2 02	
6-*C*-Glucosyldiosmetin heptaacetate	1 81		2 02	
8-*C*-Glucosylflavones				
Vitexin heptaacetate		1 73		1 91
Baylin hexaacetate		1 72		1 90
7,4'-Dimethylbayintetraacetate		1 70		1 91
Cytisoside hexaacetate		1 73		1 92
Orientin octaacetate		1 72		1 95
8-*C*-glucosyldiosmetin heptaacetate		1 71		1 94



G = *C*-glucosyl



Isovitexin R = H, R = H R'' = H, R''' = H
Keyakinin R = Me, R' = H R'' = H, R''' = OH
Isoorientin R = H R = OH R'' = H, R''' = H
6-*C*-Glucosyl-diosmetin R = H, R' = OH, R'' = Me, R''' = H



Vitexin, R = OH, R = H R' = H
Bayin, R = H, R = H R'' = H
Cytisoside, R = OH R = H R'' = Me
Orientin, R = OH, R = OH, R'' = H
8-*C*-Glucosyl-diosmetin, R = OH, R = OH, R'' = Me

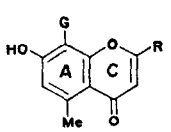
A di-*C*-glycosylflavone has been isolated and shown to be a 6,8-di-*C*-glycosyl derivative of apigenin (V) [5] Its acetate shows three acetyl methyl signals at a higher field (δ 1 77, 1 82 and 1 98) and others below δ 2 02 [6] The first two higher field signals can be assigned to the two 2''-*O*-acetyl groups in the sugar moieties The group which receives slightly extra shielding (δ 1 77) can be assigned to the 8-*C*-glycosyl and the other (δ 1 82) to the 6-*C*-glycosyl The signal δ 1 98 can be assigned to the 6''-*O*-acetyl group of the 8-*C*-glycosyl which is shielded by the B ring of the flavone nucleus It is significant that a similar signal is not found in the spectrum of paniculatin The geometry of the isoflavone molecule is such that the 6''-*O*-

[4] NARAYANAN, V and SESHADRI, T R (1971) *Indian J Chem* **9**, 14
[5] MARKHAM, K R, PORTER, L J and BREHM, B G (1969) *Phytochemistry* **8**, 2193
[6] MARKHAM, K R (1972) personal communication

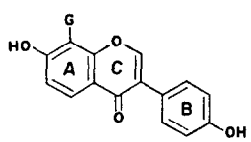
acetyl groups of 6-C and 8-C glucosyl derivatives are not shielded by the B ring and both signals appear in the 'normal' range The signals assigned to the 8-*C*-glycosyl 2''-*O*-acetyl groups of di-*C*-glycosyl flavone and isoflavone were found at a slightly lower field than those of the corresponding group in a mono-*C*-glycosyl compound Examination of models indicates that steric hindrance between the two bulky acetylated sugar units may well reduce the shielding of the 2''-*O*-acetyl group by the aromatic A ring or the pyrone C ring of the molecule

TABLE 2. CHEMICAL SHIFT OF ACETYL METHYL PROTONS OF MISCELLANEOUS *C*-GLUCOSYL COMPOUNDS IN DEUTERIOCHLOROFORM

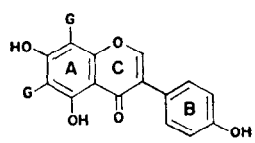
	2''-*O*-acetyl		6''-*O*-acetyl	
C-Glucosyl compound	Normal range 1 80–1 83	Higher field 1 69–1 73	Normal range 2 01–2 05	Higher field 1 90–1 95
Aloesin pentaacetate		1 69	2 01	
Deacetylaloesin pentaacetate		1 70	2 00	
Puerarin hexaacetate		1 72	2 05	
Paniculatin undecaacetate	1 85	1 75	2 05, 2 05	
Hemiphloin heptaacetate	1 83		2 03	
Hemiphloin hexaacetate	1 87		2 05	
Isohemiphloin heptaacetate	1 80		2 00	
Isohemiphloin hexaacetate	1 86		2 01	



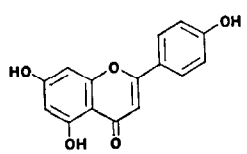
(I) Aloesin, R = CH_2COMe
(II) Deacetylaloesin, R = Me



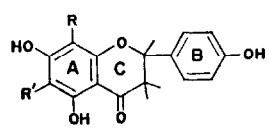
(III) Puerarin



(IV) Paniculatin



(V) Apigenin



(VI) Hemiphloin, R = H, R' = *C*-glucosyl
(VII) Isohemiphloin, R = *C*-glucosyl, R' = H

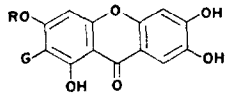
The acetates of hemiphloin (VI), a 6-*C*-glucosylflavanone, and its 8-C isomer isohemophloin (VII) show 2''-*O*-acetyl methyl signals[3] in the 'normal' range These compounds have a dihydropyrone C ring.

The acetates of mangiferin (VIII) and homomangiferin (IX), both 2-*C*-glucosylxanthones, and mangiferin trimethyl ether give signals of the 2''-*O*-acetyl groups in the range δ 1·77–1·79 [7,8] The 2-position of a xanthone is equivalent to the 6-position of a chromone, flavone or isoflavone and models show that the 2''-*O*-acetyl groups are not shielded by the inner

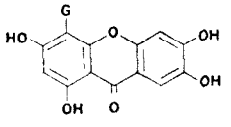
[7] HAYNES, L J and TAYLOR, D R (1966) *J Chem Soc* **19**, 1685
[8] ARITOMI, M and KAWASAKI, T (1970) *Chem Pharm Bull (Tokyo)* **18**, 2224

TABLE 3 CHEMICAL SHIFT OF ACETYL METHYL PROTONS OF *C*-GLUCOSYL XANTHONES IN DEUTERIOCHLOROFORM

C-Glucosylxanthone	2″-*O*-acetyl		6″-*O*-acetyl	
	Normal range 1 77–1 79	Higher field 1 66–1 73	Normal range 2 01–2 05	Higher field
Mangiferin octaacetate	1 79		2 01	
Mangiferin heptaacetate	1 78		2 01	
3,6,7-Trimethoxymangiferin-pentaacetate	1 79		2 02	
3,6,7-Trimethoxymangiferin-tetraacetate	1 77		2 03	
Homomangiferin heptaacetate	1 78		2 02	
Isomangiferin octaacetate		1 73	2 03	
1,3,6,7-Tetramethoxyisomangiferin-tetraacetate		1 66	2 05	



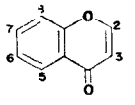
(VIII) Mangiferin R = H
(IX) Homomangiferin, R = Me



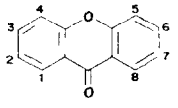
(X) Isomangiferin

TABLE 4 RANGE OF 2″-*O*-ACETYL METHYL PROTON SIGNALS OF ACETYLATED *C*-GLUCOSYL COMPOUNDS

Chromone, isoflavone or flavone	δ 2′-*O*-acetyl	Xanthone	δ 2″-*O*-acetyl
6-*C*-Glucosyl	1 80–1 83 (normal range)	2-*C*-Glucosyl	1 77–1 79 (normal range)
8-*C*-Glucosyl	1 69–1 73 (higher field)	4-*C*-Glucosyl	1 66–1 73 (higher field)



Chromone
Flavone (2-phenyl)
Isoflavone (3-phenyl)



Xanthone

pyrone ring The acetates of isomangiferin (IX), a 4-*C*-glucosylxanthone,[9] and isomangiferin methyl ether show 2″-*O*-acetyl signals at the higher field (see Table 3) The 4-position of a xanthone is equivalent to the 8-position of a chromone, flavone or isoflavone and the geometry of the molecule is such that the 2″-*O*-acetyl methyl group of these compounds are shielded by the pyrone ring

In assigning the position of the *C*-glucosyl group in a molecule, the extra shielding of the

[9] ARITOMI, M and KAWASAKI, T (1970) *Chem Pharm Bull (Tokyo)* **18,** 2327

2″-*O*-acetyl group by the pyrone C ring of certain *C*-glucosyl acetates can be of diagnostic value It is possible to differentiate isomeric chromones, isoflavones and xanthones, as well as flavones, by noting the positions of the 2″-*O*-acetyl NMR signals of their respective acetates (see Table 4)

EXPERIMENTAL

NMR spectra were measured on a Varian A60 spectrometer, with tetramethylsilane as internal standard,

Aloesin pentaacetate ($CDCl_3$, δ) 1 69 (*s*, $C_{2''}$–OAc), 2 01 (*s*, $C_{6''}$–OAc), 2 06 (*s*, $C_{3',4''}$–OAc), 2 27 (*s* C_2–$CH_2COC\underline{H}_3$), 2 40 (*s*, C_7–OAc), 2 79 (*s*, C_5–$C\underline{H}_3$), 6 45 (*s*, C_3–$\underline{H}$), 6 84 (*s*, C_6–$\underline{H}$) and sugar proton signals (*m*) 3 5–5 0 ppm

Deacetylaloesin pentaacetate ($CDCl_3$, δ) 1 70 (*s*, $C_{2''}$–OAc), 2 00 (*s*, $C_{6''}$–OAc), 2 06 (*s*, $C_{3',4''}$–OAc), 2 39 (*s*, C_7–OAc and C_2–$C\underline{H}_3$), 2 80 (*s*, C_5–$C\underline{H}_3$), 6 09 (*s*, C_3–$\underline{H}$), 6 80 (*s*, C_6–$\underline{H}$) and sugar proton signals (*m*) 3 5–5 0 ppm

Acknowledgements—The author wishes to thank Professor L J Haynes (The Open University, England) for helpful discussions, Drs W R Chan, D R Taylor and E J Herbert (University of the West Indies, Jamaica) for measurement of NMR spectra and Dr K R Markham (D S I R , New Zealand) for an NMR spectrum of apigenin 6,8-di-*C*-glycoside