NUCLEAR MAGNETIC RESONANCE SPECTRA AND STRUCTURES OF SOME *C*-GLYCOSYLFLAVONOIDS*

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[*Manuscript received August* 12, 1964]

Summary

From an examination of the nuclear magnetic resonance spectra, optical rotations, and other properties of a number of flavonoid *C*-glycosides, their acetates, and related model compounds, it is concluded that vitexin, bayin, puerarin, and isohemiphloin are *C*-β-D-glucosides with the sugar substituent in the 8-position of the flavonoid nucleus. Hemiphloin and saponaretin are two of the corresponding 6-substituted compounds. In hemiphloin and isohemiphloin the phenyl B ring has the equatorial configuration.

Introduction

Although *C*-glycosylflavonoids or glycoflavonoids occur widely in nature, progress with the elucidation of their structures has been slow and few have been completely worked out. These substances yield very small amounts of sugar when hydrolysed or degraded with ozone. Consequently the nature of the sugar moiety and its location have been largely deduced indirectly; and conclusions have been dependent on several uncertainties, such as isomerization, which can occur during examination of these relatively unstable compounds. Accordingly, a nuclear magnetic resonance study has been made of a number of these substances and related model compounds to provide more direct information about the structures of this class of compound, and in particular, the compounds hemiphloin and isohemiphloin discussed in an earlier communication.[1]

Since this work was started, several spectral correlations of other flavonoid classes have been published,[2–5] and demonstrate the value of this technique. Recently the chemistry of most of the compounds studied (Table 1) has been reviewed,[6] and the structures of vitexin and isovitexin,[7] and orientin and homo-orientin[8] have been related by means of n.m.r. spectroscopy.

* A summary of this work was presented at the Presymposium Meeting of the I.U.P.A.C. Symposium, Tokyo, April 1964. This paper is regarded as Part II of "Nuclear Magnetic Resonance Studies" (Part I, Horn, D. H. S. and Lamberton, J. A., *Chem. & Ind.*, 1963, 691) and Part V of "The Chemistry of Eucalypt Kinos" (Part IV, Hillis, W. E., and Carle, A., *Aust. J. Chem.*, 1963, **16**, 147).

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[1] Hillis, W. E., and Carle, A., *Aust. J. Chem.*, 1963, **16**, 147.

[2] Massicot, J., and Marthé, J.-P., *Bull. Soc. Chim. Fr.*, 1962, 1962.

[3] Massicot, J., Marthé, J.-P., and Heitz, S., *Bull. Soc. Chim. Fr.*, 1963, 2712.

[4] Batterham, T. J., and Highet, R. J., *Aust. J. Chem.*, 1964, **17**, 428.

[5] Clark-Lewis, J. W., Jackman, L. M., and Spotswood, T. M., *Aust. J. Chem.*, 1964, **17**, 632.

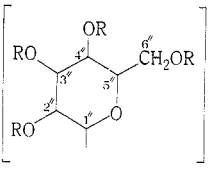
[6] Haynes, L. J., *Adv. Carbohyd. Chem.*, 1963, **18**, 227.

[7] Horowitz, R. M., and Gentili, B., *Chem. & Ind.*, 1964, 498.

[8] Koeppen, B. H., *Z. Naturf.*, 1964, **19b**, 173.

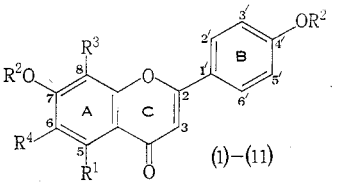
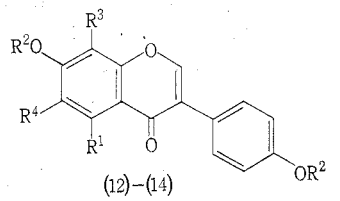
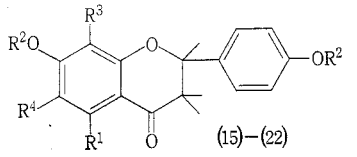
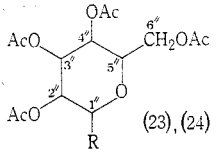
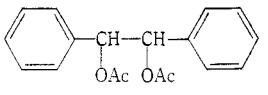
TABLE 1

FLAVONOID FORMULAE



= G when R = H

= GAc when R = Ac

	No.	Compound	Substituents			
			R^1	R^2	R^3	R^4
 (1)–(11)	1	Vitexin	OH	H	G	H
	2	Vitexin tetraacetate	OH	H	GAc	H
	3	Vitexin heptaacetate	OAc	Ac	GAc	H
	4	Saponaretin	OH	H	H	G
	5	Saponaretin hexaacetate	OAc	Ac	H	GAc
	6	Apigenin	OH	H	H	H
	7	Apigenin triacetate	OAc	Ac	H	H
	8	Bayin	H	H	G	H
	9	Bayin hexaacetate	H	Ac	GAc	H
	10	7,4′-Dimethoxybayin tetraacetate	H	Me	GAc	H
	11	4′-Methoxy-7-acetoxy-flavone	H	Me+Ac	H	H
 (12)–(14)	12	Puerarin	H	H	G	H
	13	Puerarin hexaacetate	H	Ac	GAc	H
	14	Daidzein diacetate	H	Ac	H	H
 (15)–(22)	15	Isohemiphloin	OH	H	G	H
	16	Isohemiphloin hexaacetate	OH	Ac	GAc	H
	17	Isohemiphloin heptaacetate	OAc	Ac	GAc	H
	18	Hemiphloin	OH	H	H	G
	19	Hemiphloin hexaacetate	OH	Ac	H	GAc
	20	Hemiphloin heptaacetate	OAc	Ac	H	GAc
	21	Naringenin	OH	H	H	H
	22	Naringenin triacetate	OAc	Ac	H	H
 (23), (24)	23	See at left, R = AcO				
	24	See at left, R = Ph				
(25) 	25	See at left				

The present study is concerned chiefly with *C*-glycosylflavonoid acetates, which can be prepared under mild conditions, and are easily soluble in deuterochloroform, unlike the parent compounds which can only be examined satisfactorily in deuterodimethylsulphoxide or deuteropyridine. However, as the acetates are of much higher molecular weight, there is some loss of sensitivity, but, as will be seen later, the positions of the signals from protons of acetyl groups provide useful information.

TABLE 2

CHEMICAL SHIFTS OF PROTONS OF FLAVONOID ACETATES

Shifts (δ, p.p.m.) measured with a Varian A60 instrument with $CDCl_3$ as solvent and tetramethylsilane as internal reference (δ 0·00)

Proton Position	Vit-Ac_4 (2)*	Vit-Ac_7 (3)	Sap-Ac_6 (5)	Apig-Ac_3 (7)	Bay-Ac_6 (9)	MeBay-Ac_4 (10)	MeAc-Flav (11)	Puer-Ac_6 (13)	Daid-Ac_2 (14)
H at									
2								8·18	7·96
3	6·62	6·70	6·69	6·61[3,7]	6·81	6·72	6·73[3]		
5					8·25	8·22	8·16	8·30	8·27
6	6·40	6·83		6·86	7·15	7·02	7·16	7·20	7·16
8			6·94	7·35			7·40		7·29
2′,6′	7·80	8·15	7·96	7·87	8·05	8·05	7·85	7·63	7·55
3′,5′	7·05	7·42	7·42	7·26	7·36	7·06	7·02	7·20	7·13
OMe or OAc at									
5		2·42		2·42[3]					
7		2·42	2·47	2·32	2·45	4·00	2·35	2·43	2·30
4′		2·37	2·36	2·32	2·37	3·92	3·87	2·32	2·26
2″	1·67	1·72	1·85		1·72	1·70		1·72	
3″	2·09	2·10	2·10		2·10	2·10		2·07	
4″	2·09	2·02	2·10		2·02	2·01		2·07	
6″	2·03	1·92	2·05		1·90	1·91		2·05	
OH at									
5	12·90		13·57						

* Number of compound in Table 1.

As deductions about the structures of the parent compounds are open to the criticism that rearrangement may take place on acetylation, perhaps most serious in the case of flavanones, a study has also been made of the free compounds in a number of different solvents to check deductions made from spectra of acetyl derivatives. The data obtained are summarized in Tables 2–4.

RESULTS AND DISCUSSION

(*a*) *Assignment of the Aromatic Acetyls*

The aromatic and aliphatic acetyl signals of *C*-glycosylflavonoid acetates fall conveniently in different ranges of the n.m.r. spectra, the former between δ 2·30 and 2·45 and the latter between δ 1·67 and 2·09. Massicot *et al.*[3] have shown that the signals of the 4′- and 7-acetoxyl protons in simple flavone acetates are in the range

of δ 2·30–2·35, while those of 5-acetoxyl protons are at δ 2·45. In the spectra of *C*-glycosylflavonyl acetates such as bayin acetate (No. 9, Table 2) which have 4′- and 7-acetyl groups but lack a 5-acetyl group, the signal found at δ 2·37 is assigned to the 4′-acetyl, and that at δ 2·45 to the 7-acetyl, since the latter only is likely to be shifted by the presence of the sugar ring.

TABLE 3

CHEMICAL SHIFTS OF PROTONS OF FLAVONE AND OTHER ACETATES

Shifts (δ, p.p.m.) measured in $CDCl_3$ except where otherwise indicated

Proton Position	Isoh-Ac_5 (16)†	Isoh-Ac_7 (17)	Hemi-Ac_6 (19)	Hemi-Ac_7 (20)	Nar-Ac_3 (22)	Glu-Ac_5 (23)	Phglu-Ac_5 (24)	Phglu-Ac_5* (24)
H at								
2	‡		‡		5·46			
3	*c.* 2·8		*c.* 3·0					
6	6·33	6·56			6·57			
8			6·37	6·79	6·83			
Ph							7·32	‡
2′,6′	7·50	7·52	7·50	7·42	7·50			
3′,5′	7·27	7·15	7·30	7·29	7·18			
1″	*c.* 5·3	*c.* 5·4	‡	‡		5·80	4·36	4·72
2″,3″,4″§	*c.* 5·3	*c.* 5·2	*c.* 5·0	*c.* 5·0		5·20	5·25	5·70
5″§	*c.* 3·9	*c.* 3·9	*c.* 3·8	*c.* 3·8		3·85	3·83	4·20
6″§	4·25	4·20	*c.* 4·3	‡		4·21	4·24	4·48
OMe or OAc at								
5		2·37		2·41	2·38			
7	2·36	2·38	2·41	2·45	2·30			
4′	2·32	2·32	2·33	2·30	2·31			
1″						2·12		
2″	1·86	1·80	1·87	1·83		2·04	1·78	1·78
3″	2·05	2·03	2·08	2·05		2·09	2·04	2·06
4″	2·05	2·03	2·06	2·05		2·04	2·03	2·02
6″	2·01	2·00	2·05	2·03		2·02	1·98	1·99
OH at								
5	11·2		12·58					

* In pyridine. † Number of compound in Table 1. ‡ Not distinct. § Centre of multiplets.

(*b*) *Assignment of the Aliphatic Acetyls*

The spectra of penta-*O*-acetyl-β-D-glucopyranoside (No. 23, Table 3) and 2″,3″,4″,6″-tetra-*O*-acetyl-β-D-glucopyranosylbenzene (No. 24, Table 3, I) show broad similarities. However, the signal of one of the aliphatic acetyls in the latter is at higher field (0·26 p.p.m.) than any of those of pentaacetyl glucopyranoside. This higher field signal (at 1·78 p.p.m.) is assigned to the 2″-acetyl, since it can be expected to be influenced by the magnetic anisotropy of the phenyl ring.[9] A similar diamagnetic shift occurs in the acetylenic proton of 1-ethynyl-*cis*-2-*o*-tolylcyclohexanol (II; p. 536).[10]

[9] Johnson, C. E., and Bovey, F. A., *J. Chem. Phys.*, 1958, **29**, 1012.

[10] Huitric, A. C., Stavropoulos, W. S., and Nist, B. J., *J. Org. Chem.*, 1963, **28**, 1539.

The conclusion to be drawn is that the 2″-acetyl group prevents coplanarity of the aromatic and pyranosyl rings, so that the molecule exists chiefly in two identical conformations with the planes of the two rings roughly perpendicular to each other, with the 2″-acetyl group over the plane of the phenyl ring in the diamagnetic region of its field.

TABLE 4

CHEMICAL SHIFTS (δ, P.P.M.) OF FLAVONOID PROTONS

Numbers in parentheses refer to compound numbers in Table 1

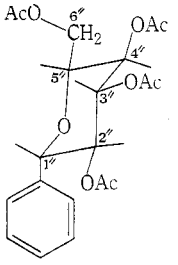
Proton Position	Vit. (1)[a]	Sap. (4)[a]	Sap. (4)[b]	"Isov."[f] (4)[b]	Apig. (6)[b]	Bay. (8)[a]	Puer. (12)[a]	Isoh. (14)[a]
H at								
2							8·05	5·36
3 or 3*ax*	6·60	6·62	6·78	6·77	6·76	6·57		—
3*eq*								—
5						7·93[g]	8·05[g]	
6	6·31[e]				6·25	6·98[h]	7·00[h]	5·97
8		6·58	6·61	6·56	6·52			
2′,6′	7·94	7·87	7·90	7·95	7·94	7·93	7·36	7·34
3′,5′	6·98	7·00	6·98	6·96	6·98	6·98	6·98	6·83
1″	4·91	4·88	4·73	4·67		*c.* 5·1	5·11	4·63
OH at								
5	13·13	13·60	13·60					12·20
7	10·80	10·00	10·00					

Proton Position	Hemi. (17)[a]	Nar. (20)[a]	Hemi. (17)[b]	Isoh. (14)[c]	Hemi. (17)[c]	Nar. (20)[c]	Nar. (20)[d]	Hemi. (17)[d]
H at								
2	5·33	5·30	5·39	5·57	5·38	5·48	5·23	5·06
3 or 3*ax*	*c.* 2·6	*c.* 2·8	—	*c.* 2·9	2·97	2·87	*c.* 2·8	—
3*eq*	*c.* 3·1	*c.* 3·0	—	*c.* 3·0	3·31	3·33	*c.* 3·0	—
5								
6		5·93		6·41		6·43	6·01	
8	6·02	5·93	6·01		6·31	6·34	6·01	5·90
2′,6′	7·27	7·26	7·30					
3′,5′	6·89		6·89					
1″	4·80		4·75	5·57	5·67			5·04
OH at								
5	12·80	12·10[i]	12·75					
7		10·00						

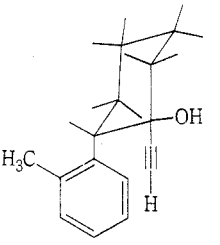
[a] In deuterochloroform/deuterodimethylsulphoxide 4 : 1. [b] In deuterodimethylsulphoxide. [c] In pyridine. [d] In deuterochloroform/pyridine 9 : 1. [e] Ref. 7 has 6·29 and ref. 4 6·28. [f] See ref. 7. [g] 8·02 in formononetin, see ref. 4. [h] 6·97 in formononetin. [i] 12·20 on dilution with deuterochloroform.

The diamagnetic shift is not observed with the isomeric compound (III), in which the acetylenic group is axially oriented. It is therefore concluded that a diamagnetic shift of the signal from an acetyl group of a *C*-glycosylflavonoid acetate

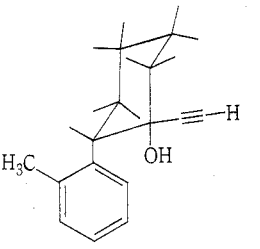
is evidence for its equatorial orientation. The shift was found in the spectra of all the *C*-glycosylflavonoid acetates examined and they are considered to have an equatorial 2″-acetyl group. This is further supported by the observation that the signal (identified as described below) of the 1″-position proton in these compounds, where resolved,



(I)

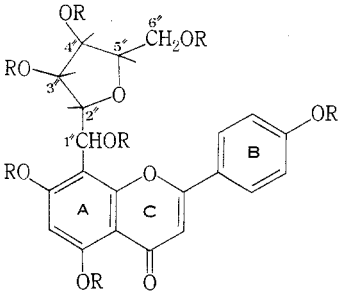


(II)



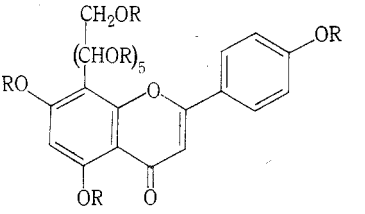
(III)

appeared as a doublet with the coupling (J *c.* 9 c/s) expected if the 1″- and 2″-protons occupy axial positions.[11–13] Thus it seems likely that these *C*-glycosylflavonoids are all β-D-glucopyranosyl derivatives.



(IV) R=H

(VI) R=Ac



(V) R=H

(VII) R=Ac

As the 2″-position acetyl only is expected to be significantly affected by the anisotropy of the aromatic ring, the next acetyl signal (δ, 1·98) in the spectrum of tetra-*O*-acetyl-β-D-glucopyranosylbenzene (No. 24, Table 3) is assigned to the 6″-acetyl group, as in penta-*O*-acetylglucopyranoside (No. 23, Table 3).[14] In most of the *C*-glycosylflavonoid acetates examined, a similar signal, separated from the others at higher field, was observed and is tentatively assigned to the 6″-acetyl group.

Since the structure (IV) had been proposed for vitexin,[15,16] it was at first considered that the signal of the 1″-acetyl in vitexin acetate (VI) might exhibit the observed diamagnetic shift if it was forced by steric interaction of the adjacent

[11] Karplus, M., *J. Chem. Phys.*, 1959, **30**, 11.

[12] Lemieux, R. U., Kullig, R. K., Bernstein, H. J., and Schneider, W. G., *J. Amer. Chem. Soc.*, 1958, **80**, 6098.

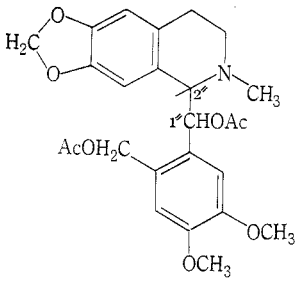
[13] Lemieux, R. U., Stevens, J. D., and Fraser, R. R., *Canad. J. Chem.*, 1962, **40**, 1955.

[14] Slomp, G., unpublished data.

[15] Evans, W. H., McGookin, A., Jurd, L., Robertson, A., and Williamson, W. R. N., *J. Chem. Soc.*, 1957, 3510.

[16] Briggs, L. H., and Cambie, R. C., *Tetrahedron*, 1958, **3**, 269.

acetyls into a conformation in which it was oriented over the aromatic A ring. To test this view, the diacetate of hydrastine diol (VIII), recently prepared by Ohta *et al.*[17] was chosen as a model, because its structure is similar to that proposed for vitexin acetate (VI), in being essentially a secondary carbinol acetate with two bulky substituent groups, one aromatic and the other aliphatic. However, the signal of the 1″-acetoxy group of hydrastine diol diacetate appeared at δ 1·97, that is without the pronounced diamagnetic shift observed in all the *C*-glycosylflavonoid acetates examined. The coupling constant of the spin–spin interaction between 1″- and



(VIII)

2″-protons of (VIII) was found to be quite large (*J* 8 c/s) and indicates that a staggered conformation with the 1″- and 2″-protons in a *trans* configuration is the most stable and populated.[18] Thus the signal of the 1″-proton in structure (VI) could be expected to show a similar large coupling of the same order as the diaxial coupling of the 1″- and 2″-protons of a glucopyranosyl ring.

An open chain structure (V) has been suggested for saponaretin[19] but is excluded because none of the acetyls of the corresponding acetate (VII) could show the observed diamagnetic shift. This is clear from the spectrum of dihydrobenzoin diacetate (No. 25, Table 1) in which the signals of the acetyls are at δ 2·00 instead of about δ 1·85. Also saponaretin afforded 5-hydroxysaponaretin hexaacetate instead of the octaacetate, which would be expected if saponaretin had an open chain structure.

In tetra-*O*-acetyl-β-D-glucopyransoylbenzene (No. 24, Table 3, I) the signal of the protons of the sugar nucleus are grouped in four multiplets, centering at roughly δ 4·36, 5·25, 4·24, and 3·83. The three latter signals are assigned by comparison with those of penta-*O*-acetyl-β-D-glucopyranoside (No. 23, Table 3), to the 2″-, 3″-, and 4″-position, 6″-position, and 5″-position protons respectively.[14,20] Similar multiplets in the same ranges occurred in the spectra of all of the *C*-glycosylflavonoids examined.

In some instances, the acetyl signals were not sharp at the normal operating temperature of about 40° and this is attributed to isomerism due to hindered rotation, since sharp signals were obtained at higher temperatures. A study of the rotational isomerism found in some of the acetyl derivatives will be reported in a following paper.

[17] Ohta, M. Tani, H., and Morozumi, S., *Tetrahedron Letters*, 1963, No. 13, 859.
[18] Freeman, R., and Pachler, K., *Mol. Phys.*, 1962, **5**, 85.
[19] Seikel, M. K., and Geissman, T. A., *Archiv. Biochem. Biophys.*, 1957, **71**, 17.
[20] Coxon, B., and Fletcher, H. G., *Chem. & Ind.*, 1964, 663.

(c) Chemical Shift of the 1″-Position Proton

The doublet (J 9 c/s), due to the 1″-anomeric proton, is moved by 1·4 p.p.m. to higher field when the 1″-acetyl of pentaacetylglucopyranoside is replaced by the phenyl group. The signal of the 1″-position proton of No. 24 was detected by integration, at δ 4·36, in the same range as the multiplet of the 6″-proton, centered at δ 4·24. The signal of the 1″-proton of 2″,3″,4″,6″-tetra-*O*-acetylglucopyranosylcyanide[20] has been found to occur in a similar position. The two sets of signals are more clearly distinguished in pyridine solution and are then respectively at δ 4·72 and δ 4·50. The doublet of the 1″-position protons of the free *C*-glycosylflavanones falls in a similar range (δ 4·63–5·11) and at a somewhat higher range (δ 5·33–5·45) in the corresponding acetates. The signal is very sensitive to solvent changes and in the study of bayin hexaacetate it was found to vary with temperature.

(d) Position of Attachment of the Sugar Ring

It has been shown fairly conclusively that the sugar residues in puerarin and vitexin[6] are attached at the 8-positions. Perhaps this is less certain in the latter case because of the possibility of rearrangements during degradative studies.

In the spectra of saponaretin and vitexin, the signal from the proton of the A ring of saponaretin is at a much lower field position than that of vitexin. It is concluded that in saponaretin the 6-position carries the substituent, since Massicot *et al.*[3] have assigned the downfield doublet of 5,7-dihydroxyflavones to the 8-position proton.[2] Horowitz and Gentili[7] have similarly related vitexin and isovitexin by a comparison of the positions of the signals of the aromatic A ring protons in these compounds with those of apigenin, which has both 6- and 8-position protons.

Since the chemical shift positions in substituted and unsubstituted compounds are very similar, the sugar substituent, like a methyl group,[2] has very little effect on the position of the chemical shift of the meta protons.

It has been suggested that isovitexin and saponaretin are identical, and the chemical shift values reported for isovitexin are included in Table 4 with those of apigenin for comparison. A comparison of the chemical shifts of the acetate peaks of acetyl derivatives and of optical rotations of saponaretin and isovitexin should establish their relationship.

It was not possible to establish unequivocally the relationship of vitexin and saponaretin by examining the chemical shift positions of the A ring protons in the spectra of their acetates, because the 5-hydroxy group in the latter was not acetylated. However, when an allowance of +0·48 p.p.m. is made for this[3] the corrected value of δ 7·43 for the 8-position proton (No. 5, Table 2) matches reasonably well with that of δ 7·35 reported for the 8-position proton of apigenin triacetate.[3]

Bayin has been obtained from vitexin under mild conditions and thus can be expected to have the sugar attached in the same position.[21] In the n.m.r. spectra of bayin and puerarin and their corresponding acetates there are present two doublets (J 10 c/s) which can be assigned only to the 5- and 6-position protons (see Tables 2

[21] Eade, R. A., Salasoo, I., and Simes, J. J. H., *Chem. & Ind.*, 1962, 1720.

and 4). The signal of the 6-position proton is at lower field in the spectra of these 5-deoxy compounds [cf. 7-acetoxy-4-methoxy-flavone[3] and daidzein diacetate (No. 14, Table 2)] than in those of the corresponding 5-hydroxy compounds (e.g. vitexin No. 1, Table 4).

(*e*) *The Structure of Hemiphloin and Isohemiphloin*

In an earlier communication[1] hemiphloin was tentatively formulated as 2,3-dihydrosaponaretin. A correlation of the shift positions of hemiphloin and isohemiphloin acetates (Table 3) with those of naringenin triacetate show that hemiphloin is 6-substituted like saponaretin and that isohemiphloin is 8-substituted like vitexin.

The position of the signal due to the 2″-acetoxy group varies between narrow limits of δ 1·67–1·72 for the 8-substituted flavone acetates and is markedly different in the 6-substituted saponaretin acetate (δ 1·85). Contrary to expectation a similar difference was not found in the 6-/8-position flavanone isomers. Apparently as the orientations of the phenyl B ring in the flavanone and flavone series are different there will also be differences in the angles between the planes of the sugar and flavonoid nuclei in the most stable conformations and consequently the amounts of deshielding of the 2″-acetyl group.

The signals of the 6- and 8-position protons of naringenin are not resolved in dimethylsulphoxide nor in mixtures of dimethylsulphoxide or pyridine with deuterochloroform. However, they are resolved in pure pyridine, which is thus the solvent of choice for studying ring isomerism of the free flavanones. In this case the higher field proton (δ 6·34) in naringenin (No. 20[c], Table 4) is assigned to the 8-position proton from the correlation of the shift positions in hemiphloin and isohemiphloin heptaacetates.

The chemical shift of the 1″-proton, which appears as a doublet (*J* *c.* 9 c/s) following diaxial coupling with the 2″-proton, shows considerable variation with solvent (see Table 4). By comparison, the signal of the 2-position proton (a quartet, X part of the ABX pattern resulting from the coupling between the 2-proton and the two 3-protons), which is in the same range, varies much less and can be distinguished most readily from it by using pyridine or dimethyl sulphoxide as solvent. The latter is less satisfactory because the chemical shift of the 1″-proton in this solvent is in the range of the other sugar-ring protons.

Hemiphloin and saponaretin have consistently higher R_F values in different solvents than their isomers, and this supports the view that they are of similar structure. In addition, hemiphloin gave a positive Gibbs test for a proton *para* to the 5-hydroxy group.[22]

(*f*) *The Chemical Shift of the Hydrogen-Bonded 5-Hydroxyl Group*

As expected the chemical shift of this proton is lower in flavones than in the corresponding flavanones. Also it is significantly lower (0·30–0·60 p.p.m.) in hemiphloin and saponaretin than in isohemiphloin and vitexin respectively (see Table 4). A similar difference is also to be seen in the partially acetylated derivatives and it is

[22] King, F. E., King, T. J., and Manning, L. C., *J. Chem. Soc.*, 1957, 563.

possible that steric interaction of the 6-position substituent in hemiphloin and saponaretin contribute to the strength of the hydrogen bond.

(*g*) *6-/8-Position Isomerization of* C-*Glycosylflavonoids*

When a *C*-glycosylflavonoid with a 5-hydroxy group, e.g. vitexin or hemiphloin, is heated with acid for several hours it gives, in addition to decomposition products, mixtures containing equal amounts of this compound with its respective isomer, saponaretin or isohemiphloin. Under identical conditions, puerarin and bayin afforded only minor amounts (about 10%) of a compound with chromatographic properties which might be expected for an isomer. As the latter cannot undergo the Wessely–Moser rearrangement because of the absence of a 5-hydroxy group, the main rearrangement taking place on boiling vitexin or hemiphloin with acid involves the A and C rings, and not the sugar ring.

TABLE 5

ROTATIONS OF *C*-GLYCOSYLFLAVONOIDS AND THEIR ACETATES

C-Glycosylflavonoid	Rotation $[\alpha]_D$	Rotation of Acetates $[\alpha]_D$
Vitexin	−14·5° (pyridine)[15]	hepta- −73·2° (acetone)[15]
	−14·3° (pyridine)[16]	−75·7° (ethanol)[16]
		penta- −4·4° (acetone)[15]
Saponaretin	+16·2° (ethanol)[16,23]	
	0° (ethanol)[24]	
Bayin	−1·0° (ethanol)[21]	
Orientin	+18·4° (pyridine)[25]	octa- −54·4° (acetone)[25]
Homoorientin	+30·8° (pyridine)[25]	octa- −27·4° (acetone)[25]
Isohemiphloin	−12·1° (acetone/water)	hepta- −85·0° (acetone)
Hemiphloin	+41·0° (acetone/water)	hepta- −35·6° (acetone)

(*h*) *Stereochemistry*

The calculated coupling constants ($J_{2ax,3eq}$ 2·8, $J_{2ax,3ax}$ 12·8, and $J_{3eq,3ax}$ 17·0,* independent of solvent) for both naringenin and hemiphloin is consistent with equatorial orientation of the B ring.[5,27,28] In isohemiphloin the 3-position protons were nearly equivalent and the coupling constants could not be determined.

Since the naturally occurring flavanones are all laevorotatory and have the same stereochemistry[29] at C 2, it is likely that all the naturally occurring *C*-glycosylflavonoids have the same configuration at this centre.

* This geminal coupling constant may be opposite in sign to the other vicinal coupling constants.[26]

[23] Perkin, A. G., *J. Chem. Soc.*, 1898, **73**, 1019.

[24] Nakaoki, T., *J. Pharm. Soc. Japan*, 1944, **64**, 304.

[25] Koeppen, B. H., Smit, C. J. R., and Roux, D. G., *Biochem. J.*, 1962, **83**, 507.

[26] Freeman, R., McLaughlan, J. I., and Pachler, K. G. R., *Mol. Phys.*, 1962, **5**, 321.

[27] Clark-Lewis, J. W., and Jackman, L. M., *Proc. Chem. Soc.*, 1961, 165.

[28] Horn, D. H. S., and Lamberton, J. A., *Chem. & Ind.*, 1963, 691.

[29] Whalley, W. B., "The Chemistry of Flavonoid Compounds." (Ed. T. A. Geissman.) p. 451. (Pergamon Press: Oxford, 1962.)

A correlation of the optical rotations of the free compounds and their acetates with the position of attachment of their sugar ring (see Table 5), shows that in each case the 6-substituted isomer has the more positive rotation. This observation may prove useful in structural assigmnents, if care is taken to avoid racemization during isolation.

EXPERIMENTAL

The paper and adsorption chromatographic techniques have been described previously.[1] The Gibbs reaction was carried out under the prescribed conditions[22] and the observed maximum wavelength (580 mμ) reached maximum intensity in about 40 min with hemiphloin. During the first 7 min, isohemiphloin had the strongest absorption in this region, but thereafter the relative absorption in this region rapidly decreased and a brown-coloured solution was obtained.

Dehydrogenation of Hemiphloin

Hemiphloin (700 mg) was dehydrogenated with iodine as previously reported.[1] The saponaretin produced could not be purified completely by several chromatographic separations and was acetylated with acetic anhydride and pyridine at room temperature. Recrystallization from a mixture of methanol and water afforded saponaretin hexaacetate (17 mg, m.p. 169–171, lit.[24] 165–166°).

Reaction of C-Glycosylflavonoids with Acid

The *C*-glycosylflavonoid (5 mg) was heated with 6% hydrochloric acid in a sealed tube at 100° for 5 hr and chromatographed. The relative amounts of the components were estimated visually from spot area and intensity of colour. Vitexin afforded a mixture of vitexin and saponaretin in a ratio of 3 : 1 (and 1 : 1 after 2 days). Hemiphloin gave a mixture of hemiphloin and isohemiphloin in a ratio of 3 : 5. Bayin and puerarin gave about 10% of another substance with similar colour reactions but with higher R_F values in B.A.W.

Saponaretin

Contrary to earlier reports[24] the hydrolysis of saponarin (saponaretin 7-glucoside) could not be effected with an active β-glucosidase. Saponarin (98 mg, isolated from *Saponaria officinalis* by Dr. H. G. C. King), was sealed in a glass ampoule with 2N HCl and methanol (1 : 1; 37 ml) and heated at 98° for 1·5 hr with constant mixing. Saponaretin, the main product, was purified by streaking onto washed No. 3 Whatman papers, and developing with 6% acetic acid. The main band formed was extracted with methanol and the pale yellow solid remaining after removal of the solvent was dissolved in the minimum amount of cold methanol, filtered, and concentrated in vacuum to yield saponaretin (51 mg). It could not be induced to crystallize and had a m.p. range of 190–220°, decomp. 223° (lit.[21] 225–226°). The R_F values in B.A.W., 6% acetic acid, and phenol/water, and the ultraviolet absorption spectra[1] of this compound were identical with those of authentic saponaretin. Chromatographic examination did not reveal visible impurities.

Isohemiphloin

A mixture of hemiphloin and isohemiphloin (5·0 g) was separated on a 30 in. by 1·8 in. diameter polyamide column by developing with water, and the eluates examined. Chromatographic examination in 6% acetic acid showed that isohemiphloin was eluted first but accompanied by traces of hemiphloin. A small amount of this mixture was further purified using No. 3 Whatman paper and 6% acetic acid. Isohemiphloin could not be crystallized and had $[\alpha]_D^{20}$ −12·1 (*c*, 0·62 in acetone/water, 1 : 1).

Acetylation

Acetates were generally prepared by dissolving the flavonoid in several times the theoretical quantity of acetic anhydride and adding a few drops of pyridine. After standing at room temperature for a day the reaction mixture was poured into ice-water and the insoluble material crystallized from aqueous alcohol.

Hemiphloin hexaacetate was prepared by dissolving hemiphloin in a small amount of pyridine and adding several times the theoretical quantity of acetic anhydride.

Acetates

The following acetates were prepared: Vitexin heptaacetate, m.p. 256–258°; saponaretin hexaacetate, m.p. 169–171°; bayin hexaacetate, m.p. 130–133°; hemiphloin hexaacetate, m.p. 144–145°; hemiphloin heptaacetate, m.p. 146–147°; isohemiphloin heptaacetate, m.p. 223–224°; puerarin hexaacetate, m.p. 133–135°; naringenin triacetate, m.p. 87–89°.

2″,3″,4″,6″-*Tetra*-O-*acetyl*-β-D-*glucopyranosylbenzene*

Tetraacetyl-β-D-glucopyranosyl chloride, benzene, and anhydrous aluminium chloride were made to react and the product purified according to the method of Hurd and Bonner.[30] A product with m.p. 157–159° was obtained.

Acknowledgments

We thank Professors T. Nakaoki, V. Venkateswarlu, S. Shibata, and particularly Drs. R. A. Eade, H. G. C. King, and M. Ohta for generous gifts of samples.

[30] Hurd, C. D., and Bonner, W. A., *J. Amer. Chem. Soc.*, 1945, **67**, 1664.