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CIRCULAR DICHROISM OF C-GLYCOSYLFLAVONES

A CHIROPTICAL METHOD FOR DIFFERENTIATING 6-C-, 8-C- AND 6,8-DI-C-β-GLYCOSYL ISOMERS[1a]

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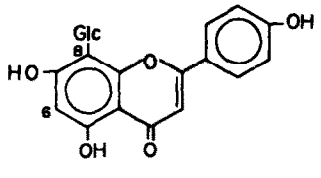
Abstract—Examination of a wide variety of C-glycosylflavones has shown that the sign of the CD band at 250–275 nm is diagnostic of the point of attachment of the glycosyl residue to the phenolic moiety. A positive CD band at 250–275 nm indicates a 6-C-linkage, as in isovitexin, whereas a negative CD band in this region indicates an 8-C- linkage, as in vitexin. 6,8-Di-C-β-glycosylflavones generally show two CD bands at 250–275 nm, a positive one at 263–275 nm and a negative one at 250–262 nm. Preliminary studies on C-α-glycosylflavones indicate that both 6-C- and 8-C- isomers show a negative CD band at 250–275 nm. The chiroptical properties of C-β-glycosylflavones are explained by proposing a quadrant rule based upon the substituted benzoyl chromophore present in these molecules.

C-Glycosylflavones are compounds in which the glycosyl residue is attached to the aglycone by a C–C bond. The first crystalline C-glycosyl compound was isolated in 1851 but until the 1950's little progress had been made in establishing structures for these compounds.[2] Since then a large number of C-glycosylflavones have been isolated from various plant sources, including dicotyledons, monocotyledons, ferns, mosses and green algae. A recurring problem in the structure determination of isomeric C-glycosylflavones, such as vitexin (1) and isovitexin (2), is assignment of the position of attachment of the glycosyl residue to the flavone. Methods that have been used for this purpose include PMR spectra of acetyl derivatives[3], mass spectra[4] and R_F values.[3] These methods, however, require the preparation of derivatives of the parent compound, or may require that both isomers be on hand for comparison. We have found that CD measurements can be used to differentiate 6- and 8-C-β-glycosylflavones and we propose a benzoyl sector rule to account for the differing chiroptical properties.



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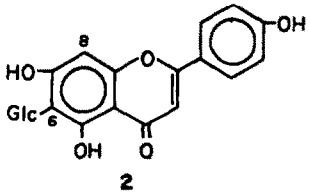

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RESULTS

C-Glycosylflavones show absorption maxima in three regions of the UV spectrum. These maxima occur at 300–340 nm, at 250–275 nm and near 215 nm with extinction coefficients of 15,000–25,000. At least one CD band was observed in each of the three absorption regions. Examination of over 40 compounds (Chart 1, Table 1) has shown that although CD bands at high and low wavelength are of little value in providing structural information, the sign of the CD band at 250–275 nm is determined by the position of the C-β-glycosyl residue on the flavone. A positive CD band at 250–275 nm indicates that the glycosyl residue is linked to C-6 of the flavone, whereas a negative band indicates that it is linked to C-8. These conclusions refer to β-D-glycopyranosyl derivatives, which are almost always the naturally occurring isomers, and to α-L-arabinopyranosyl derivatives, which are structurally similar to β-D-glycopyranosyl derivatives.[a] Limited studies on C-α-L-glycosylflavones, where the glycosyl residue is attached to the flavone by an axial bond, have shown no correlation between CD and structure. In a previous report, we noted[7] that 6,8-di-C-glycosylflavones showed CD bands at high and low wavelength of magnitude similar to those of monosubstituted C-glycosylflavones, while the CD band(s) at 250–275 nm was greatly reduced. Thus, a cancelling effect was observed upon disubstitution. Our subsequent studies have shown that the magnitude of the CD bands at 250–275 nm of di-C-glycosylflavones may be as large as those of mono-C-glycosylflavones. The di-C-glycosylflavones are usually characterized by *two* CD bands of opposite sign at 250–275 nm, whereas the monosubstituted isomers have only one CD band in this range.[b] All subsequent discussion of CD bands in this paper refers to the 250–275 nm region.

[a]The sign of a CD band at 274–280 nm has been found to depend upon the anomeric configuration for some aryl α- and β-D-glucopyranosides[5] and -D-glucopyranosiduronic acids[5]. Also, CD bands at 245–275 nm in phenyl α- and β-D-glucopyranosides have been found[6] to be positive and negative, respectively.

[b]For related CD studies on flavone glycosides, see Ref. 8.

Table 1. Circular dichroism[a] of C-glycosylflavones

Compound	[θ]285–355	[θ]<250	[θ] 250–275
8-SUBSTITUTED FLAVONES			
Vitexin (1)[b]	4150(340) 6460(302)	-6340(232) 19600(217)	-15600(272)
Vitexin heptaacetate (3)[b]	6300(315)	-18700(228) 9690(215)	-18000(261)
(3) in 60% aq. dioxane	-8240(298)	-16400(232) 19400(218)	-13700(258)
(3) in chloroform	-490(342) 770(318) -8250(294)	'''	-20600(250)
6-Bromovitexin (5)[b]	6500(325) 6430(304)	9860(215)	-9930(272)
Cytisoside (6)[c]	5160(307)	-16500(233) 20100(218)	-21300(269)
Scoparine (7)[c]	4700(342)	5240(242) -5240(232)	-17500(266)
5,7,4'-Tri-O-methylvitexin (8)[b]	9730(335) -7590(286)	-10300(236) 28100(215)	-13000(260)
Hepta-O-methylvitexin (13)[b]	16300(333) -9760(285)	-20400(233) 29100(217)	-20400(263)
Orientin octaacetate (14)[b]	2590(340)	-7590(232)	-16700(265)
8-C-Hesperidosylacacetin (21)[b]	2120(347) 5820(302)	2120(242) -5950(232)	-11000(272)
2"-O-Xylosylvitexin (22)[b]	3030(335) 6710(303)	-7720(234) 29100(219)	-20200(273)
2"-O-p-Hydroxybenzoylvitexin (23)[b]	370(355) -8180(314)	5810(222) -16500(213)	-45200(269)
8-C-Galactosylapigenin (28)[c]	4900(305)	-3860(233)	-8920(270)
8-C-Rhamnosylapigenin (30)[c]	-6170(313)	-14700(230)	-12700(265)
Hexa-O-methyl-8-C-rhamnosylapigenin (31)[c]	2290(337) 2210(285)	---	-5670(260)
6-SUBSTITUTED FLAVONES			
Isovitexin (2)[b]	1490(343) -630(320)	-11800(225)	8730(265)
Isovitexin Hepta-acetate(4)[b]	10300(335) -7560(308)	-39200(212)	24300(258)
(4) in 60% aq. dioxane	12400(335) -4720(309)	-41100(218)	26400(255)
Isocytisoside (9)[c]	1950(350) -290(315)	-4270(234)	7890(271)
Isoscoparine (10)[c]	1130(362)	-4630(235)	6320(270)
6-C-Glucosylchrysin (11)[c]	2080(340)	-2910(237)	7080(272)
5,7,4'-Tri-O-methylisovitexin (12)[b]	4830(335) -6210(303)	-12100(218)	15100(263)
5,7,4'-Tri-O-methylisovitexin tetraacetate (15)[b]	8140(337) -7710(307)	-20600(218)	25700(258)
Hepta-O-methylisovitexin (16)[b]	7270(335) -9430(303)	-14100(220)	21000(263)
Isoorientin octaacetate (17)[b]	10100(335) -2170(308)	...	26800(252)
6-C-Cellobiosylacacetin (18)[c]	2160(355) -840(315) 750(300)	-5630(235)	5160(270)
6-C-Rutinosylacacetin (19)[c]	6170(335) 4500(295)	-10800(235)	11800(270)
6-C-Hesperidosylacacetin (20)[b]	1620(360) -2060(317)	-2160(232)	6920(273)
6-C-Galactosylapigenin (24)[c]	1810(352) -1810(317)	-9250(231)	14300(270)
6-C-Xylosylluteolin (25)[c]	1730(360) 1230(340)	-5430(233)	3950(273)
6-C-Xylosylchrysin (26)[c]	1510(335)	-2720(242)	5300(275)
6-C-Arabinosylapigenin (27)[c]	1530(360) -4120(318)	-8350(235)	20700(269)
6-C-Arabinosylapigenin hexaacetate (29)[c]	9880(331) 10500(278)	...	20200(257)
Hexa-O-methyl-6-C-rhamnosylapigenin (32)[c]	-5050(335) 8420(305)	...	-13600(268)

Table 1 (*Contd*)

6,8-DISUBSTITUTED FLAVONES

Compound	[θ]297–372	[θ] < 250	[θ]250–275
6,8-Di-C-glucosylapigenin (33)[c]	9400(303)	-15800(232)	4980(273) -8020(250)
6,8-Di-C-glucosylluteolin (34)[c]	5050(352) 5630(297)	...	5140(275) -2520(262)
Dodeca-O-methyl-6,8-di-C-glucosylluteolin (35)[b]	24300(345) -20000(309)	2930(247) -17200(234)	3110(263) -1100(255)
6-C-Xylosyl-8-C-glucosylapigenin (36)[c]	8240(302)	...	5890(273) -6520(250)
6-C-Xylosyl-8-C-glucosylluteolin (37)[c]	4540(297)	-13000(228)	5890(275) -1510(258)
6-C-Glucosyl-8-C-arabinosylapigenin (38)[c]	12500(322) 17100(303)	...	2540(275) -16900(250)
6-C-Arabinosyl-8-C-glucosylapigenin (39)[c]	3570(345) -700(322) -9030(301)	...	17300(272) -5650(250)sh
5,7,3',4'-Tetra-O-methyl-6,8-di-C-glucosylluteolin (40)[b] octaacetate	6860(343) -4750(307)	2850(246) -28500(208)	-2300(270)
6-C-Glucosyl-8-C-rhamnosylapigenin (41)[c]	2080(372) -700(327) 1880(303)	-9900(234)	3760(271) -2670(250)sh
6-C-Rhamnosyl-8-C-glucosylapigenin (42)[c]	9570(330)	-8800(234)	-29700(271)
Nonaperdeuteromethyl-6,8-di-C-rhamnosylapigenin (43)[c]	7530(345) -14400(310)	19400(229)	7530(275) -2510(257)

O-GLYCOSYLFLAVONES

Compound	[θ]300–385	[θ]< 250	[θ]250–275
Gossypin (44)[b]	5700(375) 5820(330) 3600(300)	24300(238)	-35200(261)
Gossypitrin (45)[b]	-2270(385) 1070(320)	...	-6410(263)

[a] Spectra obtained in methanol at 27° unless otherwise noted.

[b] Sample from the Fruit and Vegetable Chemistry Laboratory, Pasadena, CA.

[c] Sample from the Université Claude Bernard, Villeurbanne, France.

Mono-C-glycosylflavones

The CD bands of vitexin heptaacetate, **3**, and isovitexin heptaacetate, **4**, differ in magnitude but not in sign from those of the parent flavones, **1** and **2**, respectively. The CD band of 6-bromovitexin, **5**, is about two-thirds the magnitude of the CD band of **1**, but the sign is negative for both of these bands. Thus, a 6-C substituent does not greatly affect the CD of an 8-C-glucosylflavone when the substituent lies in the plane of the A-ring. Various B-ring substituents at positions 3' and 4' have little effect on the CD band as shown by the 8-C-glucosylflavones **6**, **7**, and **8** and the 6-C-glucosylflavones **9**, **10**, **11** and **12**.

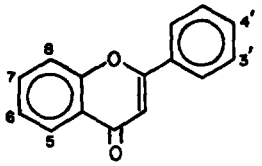
Forming a methyl or acetyl derivative of the glucosyl moiety in 8-C-isomers, (e.g. **3**, **13** and **14**) has little effect on the magnitude of the CD band relative to **1**, whereas modification of the carbohydrate in 6-C-glucosylflavones (**4**, **15**, **16** and **17**) increases the CD band two or three fold over that of **2**. This increase may be due to rotational isomerism of the carbohydrate about the C-6, C-1″ bond (see below). Substitution of another carbohydrate residue on the glucosyl residue of either 6-C(**18**, **19** and **20**) or 8-C-glucosylflavones (**21**) does not markedly affect the CD.

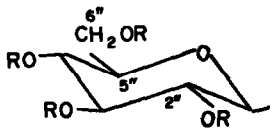
The effect of different C-2″ substituents upon the CD is illustrated by 2″-O-β-D-xylosylvitexin (**22**) and 2″-O-*p*-hydroxybenzoylvitexin (**23**). The CD of **22** differs little from that of **1**, whereas the CD band of **23** is nearly three times as large as that of **1**. This enhancement of the CD band may be due to an additional contribution from the *p*-hydroxybenzoyl chromophore, an alteration of the carbohydrate conformation, or from electronic interaction of the benzoyl chromophore at C-2″ with the benzoyl chromophore of the A-ring.[c]

Several glycosyl residues, other than β-D-glucosyl were examined as 6- or 8-substituents in *C*-glycosylflavones. 6-C-β-D-Galactopyranosyl (**24**), 6-C-β-D-xylopyranosyl (**25**) and (**26**) and 6-C-α-L-arabinopyranosyl (**27**) derivatives of various flavones all gave a positive CD band (Fig. 1) although the intensity of the band varied widely among this group of compounds. 8-C-β-D-Galactosylapigenin (**28**) gave a negative CD band which was roughly one-half that of **1**. Acetylation of the arabinosyl derivative **27**, to yield **29**, did not appreciably alter the CD compared to the effect that acetylation had on **2**. CD studies on underivatized (**30**) and permethylated (**31** and **32**) C-α-L-rhamnosylapigenins have shown that both C-6 and C-8 isomers exhibit a negative CD band at 250–275 nm. Thus, to judge from

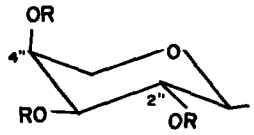
[c] The study of such interactions between two benzoate chromophores has resulted in the "dibenzoate chirality rule" of Harada and Nakanishi.[9] However, **23** does not exhibit the split CD bands (couplet) characteristic of interacting benzoyl groups.

Chart 1. Flavone substitution patterns

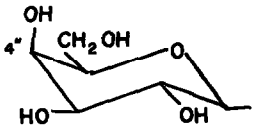




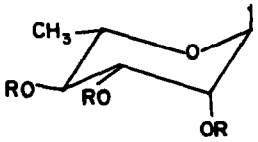
Glc, R = H
Ac_4Glc, R = Ac
Me_4Glc, R = Me



Ara, R = H
Ac_3Ara, R = Ac



Gal



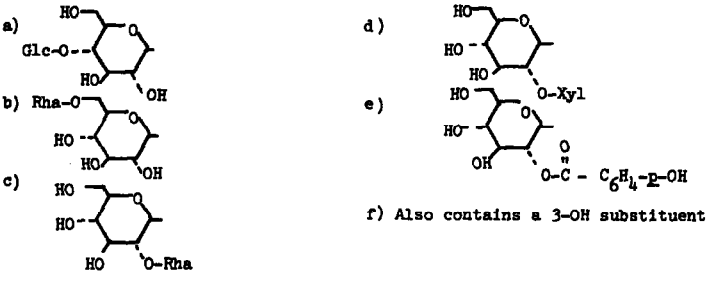
Rha, R = H
Me_3Rha, R = Me
$(CD_3)_3$Rha, R = CD_3

Xyl

Compound	5	6	7	8	3'	4'
1	OH	'''	OH	Glc	'''	OH
2	OH	Glc	OH	'''	'''	OH
3	OAc	'''	OAc	Ac_4Glc	'''	OAc
4	OAc	Ac_4Glc	OAc	'''	'''	OAc
5	OH	Br	OH	Glc	'''	OH
6	OH	'''	OH	Glc	'''	OMe
7	OH	'''	OH	Glc	OMe	OH
8	OMe	'''	OMe	Glc	'''	OMe
9	OH	Glc	OH	'''	'''	OMe
10	OH	Glc	OH	'''	OMe	OH
11	OH	Glc	OH	'''	'''	'''
12	OMe	Glc	OMe	'''	'''	OMe
13	OMe	'''	OMe	Me_4Glc	'''	OMe
14	OAc	'''	OAc	Ac_4Glc	OAc	OAc
15	OMe	Ac_4Glc	OMe	'''	'''	OMe
16	OMe	Me_4Glc	OMe	'''	'''	OMe
17	OAc	Ac_4Glc	OAc	'''	OAc	OAc
18	OH	a	OH	'''	'''	OMe
19	OH	b	OH	'''	'''	OMe
20	OH	c	OH	'''	'''	OMe
21	OH	'''	OH	c	'''	OMe
22	OH	'''	OH	d	'''	OH
23	OH	'''	OH	e	'''	OH

Chart 1 (*Contd*)

	5	6	7	8	3'	4'
24	OH	Gal	OH	'''	'''	OH
25	OH	Xyl	OH	'''	OH	OH
26	OH	Xyl	OH	'''	'''	'''
27	OH	Ara	OH	'''	'''	OH
28	OH	'''	OH	Gal	'''	OH
29	OAc	Ac_3Ara	OAc	'''	'''	OAc
30	OH	'''	OH	Rha	'''	OH
31	OMe	'''	OMe	Me_3Rha	'''	OMe
32	OMe	Me_3Rha	OMe	'''	'''	OMe
33	OH	Glc	OH	Glc	'''	OH
34	OH	Glc	OH	Glc	OH	OH
35	OMe	Me_4Glc	OMe	Me_4Glc	OMe	OMe
36	OH	Xyl	OH	Glc	'''	OH
37	OH	Xyl	OH	Glc	OH	OH
38	OH	Glc	OH	Ara	'''	OH
39	OH	Ara	OH	Glc	'''	OH
40	OMe	Ac_4Glc	OMe	Ac_4Glc	OMe	OMe
41	OH	Glc	OH	Rha	'''	OH
42	OH	Rha	OH	Glc	'''	OH
43	OCD_3	$(CD_3)_3$Rha	OCD_3	$(CD_3)_3$Rha	'''	OCD_3
44[f]	OH	'''	OH	O-Glc	OH	OH
45[f]	OH	'''	O-Glc	OH	OH	OH



f) Also contains a 3-OH substituent

these three examples, there is no change in sign with position of substitution if the glycosyl residue exists in the 1C conformation and is axially substituted at the 1-position.

Di-C-glycosylflavones.

All 6,8-di-C-glycosylflavones (33–43), except the luteolin derivative 40, and the rhamnosyl-containing compound 42, exhibited two CD bands at 250–275 nm, a positive CD band at 263–275 nm and a negative CD band at 250–262 nm (Fig. 2). The CD spectra of arabinosyl-containing diglycosyl compounds 38 and 39 were noteworthy since the arabinosyl residue gave rise to larger CD bands than the glucosyl residue. The negative lower wavelength band dominated the CD of the 8-C-arabinosyl compound 38, whereas the positive higher wavelength band dominated the CD of the 6-C-arabinosyl isomer 39. These findings may prove helpful in structural elucidation of di-C-glycosylflavones containing arabinose and glucose.

Micellaneous glycosylflavones

Examination of two flavanol glycosides where the carbohydrate is bonded to a phenolic oxygen atom has shown that the 8-β-D-glucoside 44 gave a CD band 5–6 times greater than that of the 7-β-D-glucoside 45. CD measurements have been used recently to distinguish[10] isomeric 7-O- and 4'-O-β-glycosylisoflavones and 3-O-β-glycosylflavones.

Variable temperature studies

Variable temperature CD curves of vitexin heptaacetate (3) and isovitexin heptaacetate (4) are shown in Figs. 3 and 4, respectively.

The intensity of each CD band in the two isomers increases upon lowering the temperature. In particular, the 250–275 nm CD band increases in magnitude about threefold in 3 and nearly twofold in 4. At low temperature (~−130 to −140°), most of the molecules of the isomeric C-glycosylflavones are probably frozen in a single preferred conformation. Since the 250–275 nm CD

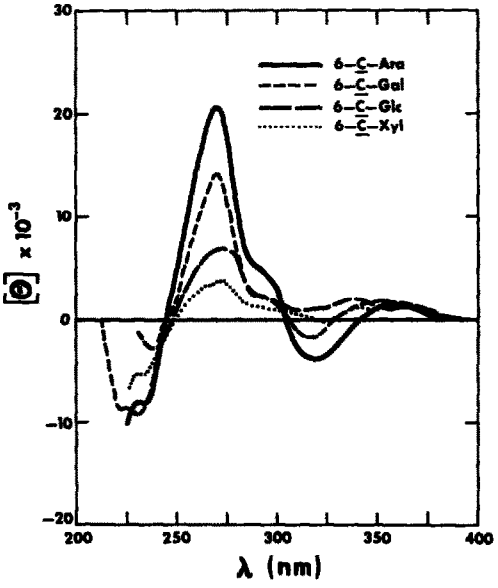


Fig. 1. Effect of different carbohydrates upon the CD of 6-C-glycosylflavones. CD spectra of 6-C-arabinopyranosylapigenin (27) (——), 6-C-galactopyranosylapigenin (24) (– – –), 6-C-glucopyranosylchrysin (11) (——), 6-C-xylopyranosylluteolin (25) (·····).

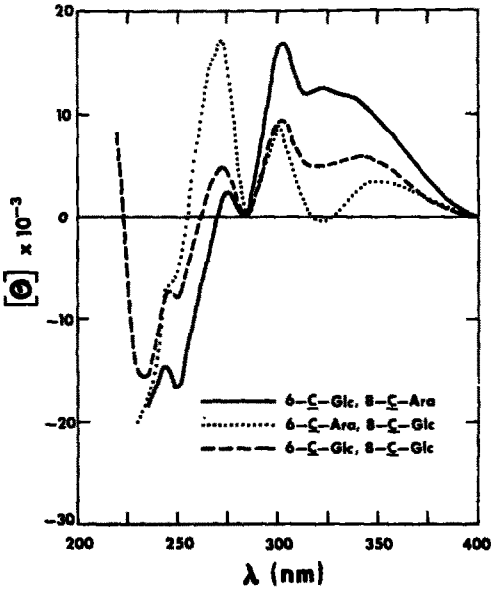


Fig. 2. Effect of different carbohydrates upon the CD of 6,8-di-C-glycosylflavones. CD spectra of 6-C-arabinopyranosyl-8-C-glucopyranosylapigenin (39) (·····), 6-C-glucopyranosyl-8-C-arabinopyranosylapigenin (38) (——), 6,8-di-C-glucopyranosylapigenin (33) (– – –).

bands of 3 and 4 changed only in magnitude but not in sign upon warming to 20°, the predominance of one preferred conformation is indicated in the temperature range studied.

DISCUSSION

A probable explanation for the dissimilar chiroptical properties of 6-C- and 8-C-glycosylflavones is that the carbohydrate occurs on opposite sides of a symmetry plane of the substituted benzoyl chromophore. The ab-

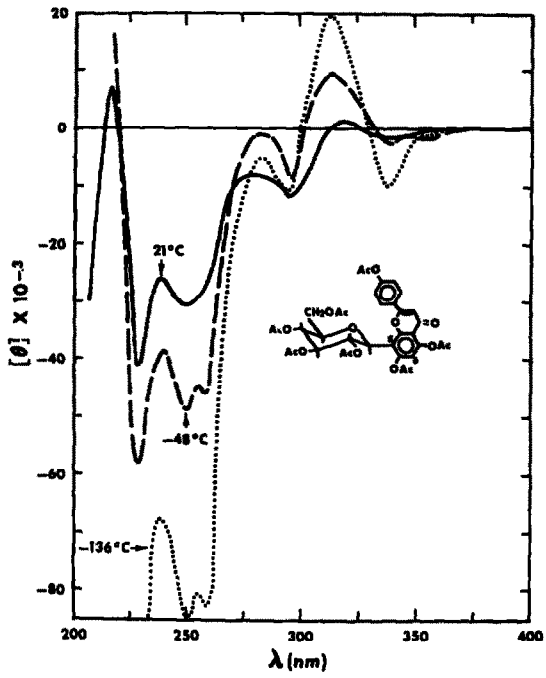


Fig. 3. Effect of temperature upon the CD of vitexin heptaacetate (3). CD spectra at 21° (——), −48° (– – –) and −136° (·····) in 2:1 methanol–ethanol.

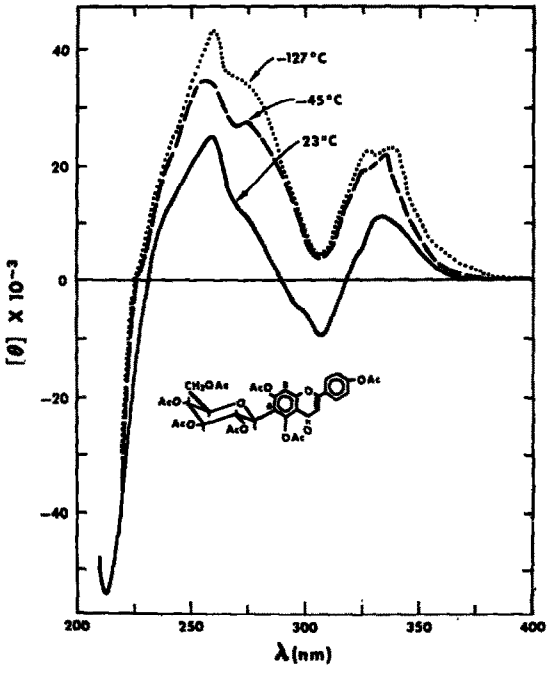
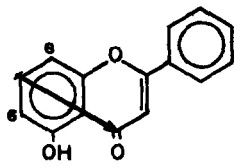
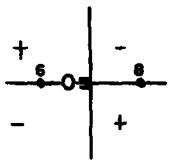


Fig. 4. Effect of temperature upon the CD of isovitexin heptaacetate (4). CD spectra at 23° (——), −45° (– – –) and −127° (·····) in 2:1 methanol–ethanol.

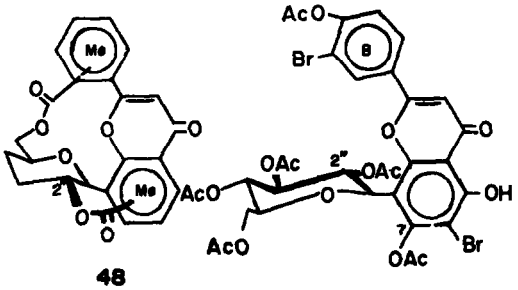
sorption band at 230–260 nm in substituted acetophenones has been tentatively assigned[11] as an intramolecular charge-transfer transition in which the transition moment is directed[12] from the *para* position of the acetophenone ring toward the CO group, as in **46**. To define a quadrant, we take as symmetry planes of the benzoyl chromophere the plane of the aromatic A-ring



46



47



48

49

and a plane perpendicular to the A-ring and containing the transition moment.[d] Viewing the flavone from the CO end of the transition moment, the sector diagram shown in **47** is obtained. The sign of the CD band related to the charge-transfer transition should be determined by whichever sector or sectors are predominantly occupied by the asymmetric substituent or perturbing group, which is the carbohydrate group in a C-glycosylflavone.

The sign of the rotational perturbation due to a group lying in a particular sector can be deduced empirically by determining the preferred conformation of the C-glycosylflavone and then relating the observed CD band to the sector contribution given by this conformation. Two lines of evidence define the preferred conformation of C-glycosylflavones. First, pmr studies[3,13,14] have shown **48** to be the most likely conformation of vitexin heptaacetate (**3**) because the 2″-O-acetyl group appears to be shielded by the A-ring and the 6″-O-acetyl group by the B-ring. In **48**, most of the atoms of the carbohydrate, particularly those nearest the benzoyl chromophore, lie in the upper right quadrant. Second, an X-ray crystallographic study[15] of 3′,6-dibromo-2″,3″,4′,4″,6″,7-hexaacetylvitexin has shown that the plane of the pyranosyl ring is approximately perpendicular to the benzopyrone ring with the 2″-acetoxy group located over the heterocyclic ring, approximately halfway between the 7-acetoxy group and the B-ring, as in **49**. In both conformations, the glycosyl residue of **48** and **49** predominantly occupies the upper right quadrant. Both 6-C- and 8-C-glycosylflavones have been shown by PMR to exhibit rotational isomerism with the 6-C-isomers more heavily weighted toward one conformer than the 8-C-isomers.

Since all the 8-C-β-glycosylflavones examined gave negative CD bands at 250–275 nm,[e] the upper right quadrant may be assigned a negative sign.

The remaining sectors are assigned alternating signs, resulting in the signed sector diagram **47**. Thus, the oppositely signed 250–275 nm CD band of the 6-C substituted flavones would be due to the major conformer predominantly occupying the upper left quadrant. Further support for these conclusions is found in the CD spectra of the 6,8-di-C-glycosyl derivatives. These compounds apparently have a C-6 substituent primarily in one sector and a C-8 substituent primarily in another sector of opposite sign, resulting in the presence of two CD bands of opposite sign at 250–275 nm. Consequently, as a result of overlapping of oppositely signed adjacent CD bands,[16] the CD bands in di-C-glycosylflavones are often much weaker than those in the mono glycosides.

Our proposal for C-glycosylflavones is somewhat similar to the benzoate sector rule[17] developed by Harada and Nakanishi, who divided the inherently symmetric benzoate chromophore into eight sectors and experimentally assigned signs to these sectors. The signs of the benzoyl sectors for C-glycosylflavones are the same as those of the inner sectors of Harada and Nakanishi's benzoates.[17]

Acetylation or methylation of the carbohydrate hydroxyls increased the CD band two to three-fold in 6-C-glucosylflavones. Thus, derivatized 6-C-glucosylflavones (**1**) may possess conformations in which the glycosyl residue occupies the upper left quadrant to a greater extent than it does in the parent compounds or the increased CD may result from (2) an increased polarizability of the perturbing group due to a bulkier substituent or (3) from altered electronic properties resulting in different perturbations on the aromatic chromophore transitions. No independent evidence exists on these points at present.

The CD of 6-C-galactopyranosylapigenin (**24**) is enhanced relative to that of 6-C-glucopyranosylapigenin (isovitexin) (**2**). Models show that equatorial but not axial C-4″ substituents lie near the plane of the benzoyl chromophore, regardless of conformation. Apparently a conformer is present in **24** in which the galactosyl residue is coplanar with the flavone such that the axial 4″-hydroxyl projects into the upper left quadrant. Similarly, arabinosyl derivatives **27** and **29** probably owe their strongly positive CD bands to conformations which project the hydroxyl and acetoxyl groups at C-2″ and C-4″ into the upper left quadrant. The CD bands of xylosyl derivatives **25** and **26** are weak relative to those of **2**, **27** and **29**. This is probably due to the fact that (1) compounds **25** and **26** contain equatorial rather than axial C-4″ hydroxy groups and (2) loss of the hydroxymethyl group at C-5″ probably allows the glycosyl group greater conformational mobility so that the upper left quadrant is less populated. Loss of the perturbing effect of this group at C-5″ may be less important than loss of the bias it exerts upon the conformational equilibrium.

While the CD spectra of 8-C-rhamnosylflavones are consistent with those of all other 8-C-glycosyl compounds examined herein, even with a 6-C-glucosyl present in the molecule (see **41**), the presence of a 6-C-rhamnosyl on a flavone results in a strong negative CD band at 250–275 nm. These observations suggest that 6-C-rhamnosylflavones adopt a preferred conformation in which the axially linked rhamnose predominantly occupies the lower left quadrant in **47**.

CD results on O-glycosylflavones **44** and **45** are consistent with our benzoyl sector rule. The observation that the 8-glucoside **44** shows a stronger CD band than the 7-glucoside **45** suggests that the 7-substituent lies

[d] The transition moment for the 230–260 nm band of benzoic acid is tilted 6.5° toward the carbonyl group from the axis in question.[12]

[e] In order to compare the chiroptical properties of **3** with the PMR data obtained[3] in chloroform, we have measured the CD of **3** in chloroform and observed a negative CD band at 250 nm (Table 1).

near a symmetry plane of the chromophore and thus results in a lesser contribution to the CD.

These findings show clearly than CD provides a useful,[18,19] sensitive method for determining whether C-β-glycosylflavones contain 6-C-, 8-C- or 6,8-di-C-glycosyl residues (see Experimental).

EXPERIMENTAL

The CD data recorded in Table 1 were obtained with the aid of a Cary 60 spectropolarimeter equipped with a 6003 circular dichrometer accessory calibrated as previously described.[20] Samples of C-glycosylflavones (0.1–0.5 mg) were weighed on a microanalytical balance and dissolved in 0.1–3.0 ml of solvent. Generally, CD measurements were performed using a 2 mm cell (2.3 ml volume), but, for samples possessing weak CD, either a 1 cm cell was used which required 0.6 ml of solution or a 1 mm cell was used which required 0.1 ml of solution. Measurements were obtained using the full range setting of either 0.02 or 0.04 θ.

The variable temp. CD data recorded in Figs. 3 and 4 were obtained on a Jobin-Yvon Dichrograph Mark III. The low temperature data were corrected for solvent contraction by use of data reported[21] for methanol-glycerol.

The acetyl derivative (29), of the arabinopyranosylapigenin (27), was prepared by allowing 27 to stand in acetic anhydride–pyridine on the steam bath for 1 hr followed by evaporation of the reagents under vacuum.

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