Studies on circular dichroism. XII.* Chiroptical properties of some flavone and isoflavone glycosides

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Ultraviolet and CD spectra of twelve isoflavone and five flavone glycosides were measured. The saccharide moiety was bound to the aglycone by a β-glycosidic bond in positions 7 or 4′, and 3, respectively. The CD spectra of 7-glycosyloxyisoflavones revealed negative, those of 4′-glycosyloxyisoflavones positive Cotton effects at about 330 nm. The spectra of flavone glycosides showed a characteristic intense positive chiroptical band at 250 nm and a negative one at 350 nm. The intensities of Cotton effects are lower in isoflavone than those in flavone glycosides.

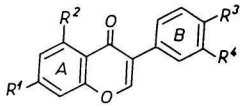
Были измерены УФ и КД спектры двенадцати изофлавоных гликозидов, в которых сахаридная составляющая связана с агликоном β-гликозидной связью в положении 7 или же 4′ УФ и КД спектры измерялись также и для пяти флавоновых гликозидов с сахаридом в положении 3. На КД спектрах 7-гликозилоксиизофлавонов в области приблизительно 330 *нм* наблюдались отрицательные эффекты Коттона, а на спектрах флавоновых гликозидов можно было наблюдать характеристическую интенсивную положительную хироптическую полосу ири 250 *нм*, а в длинноволновой ири 350 *нм* отрицательную. Интенсивность эффектов Коттона изофлавоновых гликозидов меньше, чем флавоновых гликозидов.

The most frequent saccharide moiety of naturally occurring isoflavone and flavone glycosides was reported to be D-glucose, rutinose (6-*O*-α-L-rhamnosyl-D-glucose), neohesperidose (2-*O*-α-L-rhamnosyl-D-glucose), sophorose (2-*O*-β-D-glucosyl-D-glucose), and 2-*O*-apiosyl-D-glucose [2]. The saccharide portion can be attached to the aglycone at various positions in flavone glycosides; in isoflavone glycosides in positions 7 or 4′ by a β-glycosidic bond [3]. Isoflavone cellobiosides were so far only synthesized [4—7]. The site of attachment of the glycoside was determined either by chemical degradation [8—11], or using ^{1}H-PMR spectrometry [12, 2]. More recently, chiral methods (ORD, CD) were employed to elucidate the structure of flavone glycosides [13—19]. The locus of the glycoside to aglycone attachment in

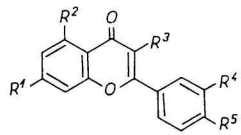
* For Part XI see Ref. [1].

four flavone glycosides was determined by means of CD spectra [17]. It has been found that the circular dichroitic method is a sensitive tool for structure elucidation of *C*-glycosylflavones, where the saccharide moiety is bound in position 6 and/or 8 [18].

The u.v. spectra of flavones showed two characteristic maxima due to the absorption of a cinnamoyl (300—380 nm) and a benzoyl (240—280 nm) system of the molecule [20]. The substitution of the flavone or isoflavone skeleton by a hydroxyl, methoxyl, *O*-acetyl, or *O*-glycosyl in various positions did not considerably influence the character of those two intense absorption bands [2]. This paper refers to the study of twelve isoflavone glycosides *I*—*XII** having the saccharide moiety



	R^1	R^2	R^3	R^4
I	OH	OH	OGl	H
II	OAc	OAc	$OGl(Ac)_4$	H
III	OH	OH	ONe	H
IV	OAc	OAc	$ONe(Ac)_6$	H
V	OGl	H	H	H
VI	$OGl(Ac)_4$	H	H	H
VII	OGl	H	OCH_3	H
VIII	$OGl(Ac)_4$	H	OCH_3	H
IX	OCb	H	H	H
X	OCb	H	OCH_3	H
XI	$OCb(Ac)_7$	H	OCH_3	H
XII	$OGl(Ac)_4$	H	OCH_2O	



	R^1	R^2	R^3	R^4	R^5
XIII	OH	OH	ORu	OH	OH
XIV	OCH_3	OCH_3	ORu	OCH_3	OCH_3
XV	OH	OH	OSo	H	OH
XVI	OCH_3	OCH_3	OSo	H	OCH_3
XVII	OH	OH	ORu	H	OH

Gl = β-D-glucosyl; $Gl(Ac)_4$ = tetra-*O*-acetyl-β-D-glucosyl; Ne = β-neohesperidosyl; $Ne(Ac)_6$ = hexa-*O*-acetyl-β-neohesperidosyl; Cb = β-cellobiosyl; $Cb(Ac)_7$ = hepta-*O*-acetyl-β-cellobiosyl; Ru = β-rutinosyl; So = β-sophorosyl.

Scheme 1

* In our previous paper [19] isoflavone glycosides were measured using a JASCO ORD/UV-5 spectropolarimeter adapted for CD measurement at a 1×10^{-3} sensitivity.

bound to the aglycone in position 7 or 4′, and five flavone glycosides *XIII*—*XVII* with the saccharide attached to C-3 (Scheme 1).

The first bands in the u.v. spectra (Table 1), occurring in the 291—350 nm region, are less intensive than those in the 242—264 nm region. The second band of isoflavone glycosides *V*, *VI*, *IX* without substitution of ring *B* lay at about 251 nm; the close maxima at 295—303 nm were due to the cinnamoyl residue. The substitution of ring *B* by a methoxyl shifted the second band bathochromically by 12 nm and overlapped the band at 295 nm. Consequently, a broad shoulder at about 303 nm appeared in the u.v. spectra of compounds *VII*, *VIII*, *X*, *XI*. The dioxymethylene substitution of the *B* ring in positions 3′ and 4′ in compound *XII* lowered the intensity of the second absorption band, whilst the first band was hypsochromically shifted. Substitution of the hydroxyl group at C-5 by an acetyl group resulted in a hypsochromic shift of the second band up to 10 nm and of the first band up to 20 nm (compounds *II*, *IV*) when compared with the unacetylated counterparts (compounds *I*, *III*). Flavone glycosides having the saccharide component in position C-3 displayed a further absorption band in the long-wave region. Rutine (*XIII*), sophoroflavonoside (*XV*), and campherolrutinoside (*XVII*) revealed the above-mentioned bands at about 350 nm, whereas their methoxy derivatives *XIV*, *XVI* had the bands hypsochromically shifted by 15—20 nm. A similar shift could be observed also with the second absorption band in the 258—263 nm region. As seen, the substitution of the ring *A* or *B* did not only influence the benzoyl or cinnamoyl system but it was also associated with shifts of the first or second absorption band. Hence it follows that a partial conjugation of rings *A* and *B* took place through the γ-pyrone ring.

The sign of Cotton effects of isoflavone glycosides *I*—*XII* in the long-wave region (311—345 nm; Table 1), was characteristic of the attachment of the saccharide moiety to the aglycone. Positive Cotton effects were found with substances *I*—*IV*, where the saccharide portion was bound in position C-4′, and negative ones with compounds *V*—*XII* with saccharide attached to C-7. These Cotton effects might be due to the forbidden $n-\pi^*$ transitions, which were not recorded in the u.v. spectra, since they were overlapped by the neighbouring strong $\pi-\pi^*$ transitions. However, the quantum-mechanic calculations confirmed the existence of those bands in the 330—350 nm region [21]. In the absorption region of benzoyl chromophore (250—264 nm) the chiroptic bands also differed in signs. The Cotton effects of 4′-isoflavone glycosides were found to be negative and those of 7-isoflavone glycosides positive. It could be assumed that positive chiroptic bands (*V* and *VII*) appeared in the absorption region of the benzoyl system similarly as in acetylated analogues *VI* and *VIII*. The rotation power of those chiroptic bands is weak throughout the measurable region, since the chiral centre is remote from the chromophore, and free rotation of the saccharide moiety around the β-glycosidic bond is possible.

A quite intense positive chiroptic band ($\Delta\varepsilon = 3.7—6.6$) was observed in the absorption region of the second band (248—251 nm). In the long-wave region (332—374 nm) negative Cotton effects were determined; they were of different intensity depending of the bulkiness of the saccharide. Substances *XIII*, *XIV*, and *XVII*, where the respective saccharide was 6-*O*-α-L-rhamnosyl-D-glucose, revealed a weaker Cotton effect than substances *XV* and *XVI*, having 2-*O*-β-D-glucosyl-D-glucose in their molecule. Methylated derivatives *XIV* and *XVI* had a stronger rotation power of both those chiroptic bands when compared with substances *XIII* and *XV*

Table 1

Ultraviolet and CD data of glycosides

No.	UV		CD		No.	UV		CD	
	λ_{max}	(log ε)	λ_{max}	($\Delta\varepsilon$)		λ_{max}	(log ε)	λ_{max}	($\Delta\varepsilon$)
I	262	(4.56)	265	(−1.50)	*X*	303 sh	3.84	281	(−0.75)
	320 sh	(3.89)	276	(−1.32)				306	(+0.10)
			344	(+0.02)				325	(−0.23)
								332 sh	(−0.19)
II	253	(4.53)	245	(−1.11)					
	300 sh	(3.75)	283	(−0.72)	*XI*	263	(4.51)	265	(+0.44)
			320	(−0.10)		300 sh	(3.94)	282	(−0.17)
			340	(+0.05)				295 sh	(+0.10)
								304	(+0.17)
III	262	(4.44)	265	(−0.86)				325	(−0.24)
	320 sh	(3.49)	375	(−0.64)				335 sh	(−0.17)
			343	(+0.05)					
					XII	264	(4.00)	262	(+0.22)
IV	253	(4.50)	245	(−1.12)		291	(3.80)	276	(−0.32)
	300 sh	(3.77)	280	(−0.54)				295	(+0.16)
			320	(−0.11)				303	(+0.32)
			345	(+)				327	(−0.19)
								337	(−0.20)
V	251	(4.45)	275	(−0.93)					
	294	(4.45)	297 sh	(+0.19)					
	304	(3.93)	304	(+0.29)	*XIII*	254	(4.29)	252	(+3.76)
			321	(−0.23)		260 sh	(4.26)	273	(−0.44)
			333 sh	(−0.13)		300 sh	(3.87)	292	(−1.11)
						350	(4.26)	310	(+0.22)
VI	250	(4.48)	252	(+0.44)				335	(−0.44)
	294	(3.89)	275	(−0.63)					
	303	(3.91)	296 sh	(+0.34)	*XIV*	245	(4.40)	248	(+6.60)
			304	(+0.48)		258 sh	(4.26)	266	(−4.22)
			322	(−0.29)		300 sh	(4.08)	298	(−1.65)
			335	(−0.15)		335	(4.31)	325	(+0.39)
								355	(−0.66)
VII	263	(4.50)	279	(−0.63)					
	302 sh	(3.82)	299	(+0.04)	*XV*	242 sh	(4.15)	249	(+3.73)
			305	(+0.16)		263	(4.36)	263 sh	(+2.79)
			324	(−0.25)		298 sh	(4.09)	307	(+1.05)
			334	(−0.09)		348	(4.27)	331	(−1.81)
								374	(−1.05)
VIII	263	(4.50)	262	(+0.58)					
	303 sh	(3.89)	280	(−0.23)	*XVI*	252 sh	(4.19)	250	(+5.78)
			295 sh	(+0.12)		258	(4.23)	263 sh	(+1.35)
			304	(+0.19)		300 sh	(4.03)	294	(−1.09)
			325	(−0.29)		329	(4.16)	330 sh	(−2.12)
			335	(−0.21)				346	(−3.92)
IX	253	(4.39)	272	(−1.27)					
	296	(3.81)	293	(+0.22)	*XVII*	243 sh	(4.12)	251	(+5.76)
	304	(3.85)	308 sh	(+0.11)		263	(4.32)	268	(−1.34)
			320	(−0.22)		300 sh	(4.05)	281	(+0.42)
						350	(4.26)	307	(+1.05)
X	263	4.46	250	(+0.42)				332	(−0.35)

sh — shoulder.

The chiroptical behaviour of isorhamnetine-3-β-D-glucoside [17] was in agreement with that of rutine (*XIII*) or rutine tetramethylether (*XIV*), which differed both in the saccharide portion and substituent at C-3′, or C-5, C-7, and C-4′, respectively.

It has been found that there is no difference in chiroptic bands of acetylated and unacetylated saccharide component of isoflavone glycosides. A substitution of the monosaccharide (D-glucosyl) for a disaccharide (neohesperidosyl, cellobiosyl) did also not alter the character of Cotton effects. The site of attachment of the saccharide to the aglycone determined the sign of the Cotton effect both in the long-wave region and in that corresponding to the benzoyl absorption. Thus, 7-glycosyloxyisoflavones exhibited negative and positive Cotton effects in the 311—345 nm and 250—264 nm regions, respectively; 4′-glycosyloxyisoflavones displayed opposite Cotton effects. Flavone glycosides with the saccharide bound in position C-3 showed characteristic intense positive Cotton effects in the 248—251 nm region and negative ones in the long-wave region (332—334 nm). The difference between the spectra of 7-glycosyloxyisoflavones and 3-glycosyloxyflavones has been found in the intensity of the long-wave chiroptic bands. 3-Glycosyloxyflavones have more abundant chiroptic bands due to the hindered rotation around the β-glycosidic bond.

Experimental

Ultraviolet spectra were measured using a JASCO ORD/UV-5 spectropolarimeter. The CD curves were registered with a JOUAN dichrograph model 185 at room temperature in dioxan (concentration 0.5—1.0 mg/ml) in 10—1 mm cells. The sensitivity of the CD spectrometer was 1×10^{-5} units of the differential absorption per 1 mm of the record. Flavone glycosides were isolated from natural sources, isoflavones were synthesized in a usual way. The purity of compounds was checked by determination of their melting points and specific rotations.

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References

1. Sticzay, T., Peciar, C., Bystrický, S., and Kučár, Š., *Zborník prednášok, II. konferencia organických chemikov* v Smoleniciach, 1973 — *Pokroky v chémii karbonylových zlúčenín.* (Proceedings of the 2nd Conference of Organic Chemists — Progress in the Chemistry of Carbonyl Compounds, Smolenice, 1973.) Edičné stredisko SVŠT. (Publishing Department of the Slovak Technical University.) Bratislava, 1973.
2. Mabry, T. J., Markham, K. R., and Thomas, M. B., *The Systematic Identification of Flavonoids.* Springer-Verlag, Berlin, 1970.
3. Wong, E., *Fortschr. Chem. Org. Naturst.* ***28***, 1 (1970).
4. Wagner, H., Hörhammer, L., Böhringer, W., and Farkas, L., *Chem. Ber.* ***100***, 107 (1967).
5. Wagner, H., Hörhammer, L., Budweg, W., Major, A., and Farkas, L., *Chem. Ber.* ***102***, 3006 (1969).

6. Lévai, A. and Bognár, R., *Magy. Kém. Foly.* ***79***, 182 (1973).
7. Lévai, A. and Bognár, R., *Acta Chim.* (Budapest) ***79***, 191 (1973).
8. Zemplén, G. and Bognár, R., *Ber.* ***75***, 482 (1942).
9. Zemplén, G., Bognár, R., and Farkas, L., *Ber.* ***76***, 267 (1943).
10. Krishnamurti, M. and Seshadri, T. R., *J. Sci. Ind. Res.* ***13B***, 1 (1954).
11. Malhotra, A., Murti, V. V. S., and Seshadri, T. R., *Tetrahedron* ***23***, 409 (1967).
12. Markham, K. R., Mabry, T. J., and Swift, W. T., *Phytochemistry* ***7***, 803 (1968).
13. Gaffield, W. and Waiss, A. C., Jr., *Chem. Commun.* ***1968***, 29.
14. Markham, K. R. and Mabry, T. J., *Tetrahedron* ***24***, 823 (1968).
15. Gaffield, W., *Tetrahedron* ***26***, 4093 (1970).
16. Aurnhammer, G., Wagner, H., Hörhammer, L., and Farkas, L., *Chem. Ber.* ***103***, 3667 (1970).
17. Voelter, W., Oster, O., Jung, G., and Breitmaier, E., *Chimia* ***25***, 26 (1971).
18. Gaffield, W. and Horowitz, R. M., *Chem. Commun.* ***1972***, 648.
19. Lévai, A., Bognár, R., Peciar, C., Bystrický, S., and Sticzay, T., *Acta Chim. Acad. Sci. Hung.* ***79***, 365 (1973).
20. Jurd, L., in *The Chemistry of Flavonoid Compounds.* (T. A. Geissman, Editor.) Pp. 107—155. Pergamon Press, Oxford, 1962.
21. Dinya, Z. and Lévai, A., unpublished results.

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