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PLANT POLYPHENOLS. X. FLAVONE AND AURONE GLYCOSIDES OF ANTIRRHINUM*

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Abstract—Five flavones present in flowers of *Antirrhinum majus* have been identified, namely: apigenin 7,4′-diglucuronide, luteolin 7-glucuronide, chrysoeriol 7-glucuronide, kampferol 3-glucoside and kampferol 3,7-diglucoside. A new aurone, bracteatin 6-glucoside, has also been found. These results, together with those of earlier studies, show that each class of flavonoid in *A. majus* has its own glycosidic pattern. The occurrence of these and other flavonoids in species of *Antirrhinum* and other genera of the Scrophulariaceae has been briefly surveyed. Delphinidin 3,5-diglucoside has been identified in flowers of *Antirrhinum cornutum* and luteolin 7-glucuronide and a luteolin 7-glucosylglucuronide have been found in leaves of *Digitalis purpurea*.

INTRODUCTION

THE FLOWER colour of *Antirrhinum majus* has been the object of much genetical work.[1] Nevertheless, the structures of many of the pigments, particularly with regard to their glycosylation pattern, are yet unknown. This is because the pigments occur as rather complex mixtures of glycosides which are difficult to separate and are unusually resistant to acid hydrolysis. The object of this work was, therefore, to determine the glycosidic patterns of those flavonoids which had not been fully characterized, an aim which has some immediate genetical interest, because Fincham[2] has found that one of the genes controlling anthocyanin concentration in *A. majus* also affects the glycosidic pattern of the flavonols.

Only two flavones (quercetin 3-glucoside[3] and apigenin 7-glucuronide[4]) have previously been completely characterized in *Antirrhinum*. In the present work, five more flavones have been identified. The aglycone of one of them has not previously been found in this plant, although it occurs in all MM genotypes.† The only aurone previously found in *A. majus* is aureusin (aureusidin 6-glucoside[3]); the other major aurone constituent has now been characterized.

RESULTS

Antirrhinum majus

A composite two-dimensional chromatogram of fresh petal extracts of colour mutants of *Antirrhinum majus* (Fig. 1) shows the flavones, flavonols, anthocyanins and aurones that are variously present. There appear to be only ten major flavone and flavonol derivatives, although Geissman and Jorgensen[5] record nineteen; these workers used dried petals so that

* Part 9: J. B. HARBORNE, *Phytochemistry* **2**, 85 (1963).

† Gene symbolism used in this paper is that of Geissman, Jorgensen and Johnson.[5]

[1] J. B. HARBORNE, *Chemistry of the Flavonoid Compounds* (Edited by T. A. GEISSMAN), p. 593. Pergamon Press, London (1962).

[2] J. R. S. FINCHAM, *53rd Ann. Rep. John Innes Inst.*, p. 20 (1962).

[3] E. C. JORGENSEN and T. A. GEISSMAN, *Arch. Biochem. Biophys.* **54**, 72 (1955).

[4] M. K. SEIKEL, *J. Am. Chem. Soc.* **77**, 5685 (1955).

[5] T. A. GEISSMAN, E. C. JORGENSEN and B. L. JOHNSON, *Arch. Biochem. Biophys.* **49**, 368 (1954).

some of their components may have been artifacts. The five flavones studied in the present work are F2, F3, F6, F8 and F10 and they were isolated by chromatographic methods from appropriate stocks (Table 4). The properties of these five compounds are shown in Table 1. F2, F3 and F10 were all very resistant to acid or β-glucosidase hydrolysis, and could only be hydrolysed satisfactory with β-glucuronidase. F2 and F3, which occur only in MM stocks and which appeared from earlier studies to be luteolin glycosides, were separable only with some difficulty in butanol–ethanol–water. F2 was identified as chrysoeriol 7-glucuronide; the aglycone, chrysoeriol (5,7,4′-trihydroxy-3′-methoxyflavone), has been found previously in *Eriodictyon glutinosum*[6] and in the lemon *Citrus*,[7] but is a rare compound. It was probably

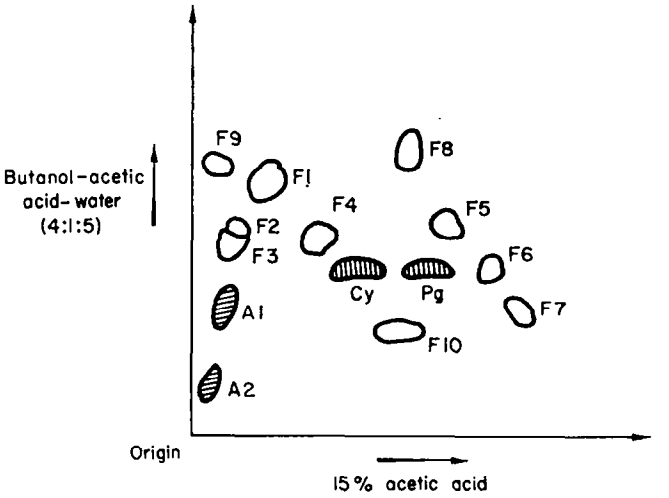


FIG. 1. TWO-DIMENSIONAL CHROMATOGRAM OF *A. majus* FLAVONOIDS

Key:

Previously identified	*Now identified*
Cy = cyanidin 3-rutinoside	F2 = chrysoeriol 7-glucuronide
Pg = pelargonidin 3-rutinoside	F3 = luteolin 7-glucuronide
A1 = aureusidin 6-glucoside	F6 = kampferol 3,7-diglucoside
F1 = apigenin 7-glucuronide	F8 = kampferol 3-glucoside
F4 = quercetin 3-glucoside	F10 = apigenin 7,4′-diglucuronide
	A2 = bracteatin 6-glucoside

Unidentified
F5, F7, F9

Note: The positions of flavanone glycosides and cinnamic acid esters are not shown.

overlooked in earlier studies of *Antirrhinum* because it has the same R_F value as apigenin in some solvents (e.g. Forestal and phenol) and as luteolin in other solvents (e.g. butanol–acetic acid–water). The associated substance, F3, gave luteolin and glucuronic acid on hydrolysis and was identified as the 7-glucuronide.

The third flavone, F10, is present in all non-albino plants and corresponds to Jorgensen's and Geissman's component "A–B–C".[3] It is particularly resistant to acid hydrolysis but by hydrolysis with β-glucuronidase readily gives apigenin and glucuronic acid in the ratio of 1 : 2. Its spectral and other properties indicate that it has substituents in the 7- and 4′-positions,

[6] F. B. POWER and F. TUTIN, *Proc. Am. Pharm. Mfrs. Assoc.* **54**, 352 (1960).
[7] R. M. HOROWITZ and B. GENTILI, *J. Org. Chem.* **25**, 2183 (1960).

TABLE 1. PROPERTIES OF FLAVONE AND FLAVONOL GLYCOSIDES

(a) *R_F values and fluorescence*

Pigment	Colours in u.v. light: Alone	Colours in u.v. light: Ammonia†	R_F values in *: BAW	H_2O	BEW	PhOH
F10	Dull dark brown	Dark brown	0·12	0·53	0·10	0·10
F3 and D1		Bright yellow–green	0·24	0·12	0·14	0·17
F2		Bright green	0·29	0·13	0·17	0·46
D2		Bright yellow–green	0·22	0·33	0·11	0·00
F6	Dull ochre	Bright yellow–green	0·28	0·57	0·41	0·44
F8		Yellow–green	0·68	0·13	0·73	0·74

(b) *Spectral properties*: λ_{max} in 95% EtOH (mμ)

Pigment	Alone: Band I	Alone: Band II	with 2 N NaOH: Band II	with 5% $AlCl_3$: Band II	Satd. with NaOAc: Band I	Satd. with NaOAc–H_3BO_3: Band II
F10	273	320	373	332	275	320
F3 and D1	255	353	407	357,373	255	377
F2	253,269	351	395	351	254,269	351
D2	256	353	403	368,387	256	376
F6	264	351	393	346,396	264	351
F8	267	351	403	348,388	277	351

(c) *Hydrolysis products*

Pigment	Products and their ratios	Identified as the
F10	Apigenin (1·0) and glucuronic acid (2·11)†	7,4′-Diglucuronide
F3 and D1	Luteolin (1·0) and glucuronic acid (0·93)†	7-Glucuronide
F2	Chrysoeriol (1·0) and glucuronic acid (0·79)†	7-Glucuronide
D2	Luteolin, glucose and glucuronic acid†	7-Glucosylglucuronide
F6	Kampferol, kampferol 7-glucoside, kampferol 3-glucoside and glucose‡	3,7-Diglucoside
F8	Kampferol and glucose‡	3-Glucoside

* Whatman No. 1 paper using solvent systems: BAW, n-butanol–acetic acid–water (4:1:5); BEW, n-butanol–ethanol–water (4:1:2·2). R_F values of apigenin 7-glucuronide in these solvents were, 0·43, 0·13, 0·68 and 0·37 respectively.

† Hydrolysis by mouse-liver β-glucuronidase (1 hr in acetate buffer, pH 4·9 at 37°C).

‡ Acid or β-glucosidase hydrolysis.

and this was confirmed by methylation and hydrolysis, the product of which was apigenin 5-methyl ether. F10 is, therefore, apigenin 7,4′-diglucuronide which is a new glycosidic combination; it is worth noting however that the related apigenin 7-glucuronide occurs in *A. majus*[4] and in several other plants.

Plants with magenta flowers (genotype PM) are pigmented by cyanidin 3-rutinoside[8] and the main accompanying flavonol is quercetin 3-glucoside.[3] The anthocyanin of pink (P-mm)

[8] R. SCOTT-MONCRIEFF, *Biochem. J.* **24**, 753 (1930).

flowers is pelargonidin 3-rutinoside[9] which is accompanied by F6 and F8, now identified as kampferol 3-glucoside and 3,7-diglucoside respectively. Thus, on acid hydrolysis, F6 gives kampferol, glucose and kampferol 7-glucoside and, on treatment with β-glucosidase, it is hydrolysed completely to kampferol and glucose, although kampferol 3-glucoside can be detected as an intermediate. Identification was confirmed by direct comparison with authentic material, isolated from petals of *Paeonia albiflora*.[10] By similar procedures, F8 was identified as kampferol 3-glucoside, a pigment which is fairly widely distributed in plants (see [11]).

TABLE 2. PROPERTIES OF THE AURONE GLUCOSIDE, A2

(a) *R_F values*

Pigment*	R_F value in†			
	BAW	PAW	PhOH	Forestal
A2	0·06	0·20	0·04	0·34
Bractein (bracteatin 4-glucoside)	0·27	0·39	0·07	0·49
Aureusin (aureusidin 6-glucoside)	0·22	0·39	0·18	0·52
Cernuoside (aureusidin 4-glucoside)	0·48	0·58	0·29	0·48
A2 aglycone	0·32	0·29	0·03	0·24
Bracteatin	0·32	0·29	0·03	0·24
Aureusidin	0·61	0·50	0·18	0·38

(b) *Spectral properties*: λ_{max} in 95% EtOH (mμ)

Pigment	Alone		with 2N NaOH	with 5% $AlCl_3$	with NaOAc—H_3BO_3
	Band I	Band II	Band II	Band II	Band II
A2	263	322,408	367,470	415,478	340,452
Bractein	259	330,409	decomp.	335,394	—,438
A2 aglycone‡	262	327,404	decomp.	409,465	338,430
Bracteatin‡	260	327,403	decomp.	409,463	340,433

* Colours of all aurones on paper: in visible light, yellow; in u.v. light, yellow-green; and in u.v. + ammonia, orange–red.

† R_Fs measured on Whatman No. 1 paper. PAW is n-propanol–acetic acid–water (1:1:1) and Forestal is acetic acid–hydrochloric acid–water (30:3:10).

‡ Methyl ethers both had λ_{max} at 325 and 388 mμ and the same R_F values in all solvent systems. Acetates both had λ_{max} at 246, 309 and 373 mμ.

An aurone, A2, of low R_F in BAW, accompanies aureusin in all non-albino forms and is the only other major aurone pigment in *Antirrhinum*. A2 corresponds to the compound "Ad G_1", of Jorgensen and Geissman,[3] but the other three aurones reported by these workers could not be found. A2 was assumed[3] to be a glycoside of aureusidin; however, on acid or enzymic hydrolysis, it gives glucose and an aglycone which, from its R_F values (Table 2), clearly has one more hydroxyl group in its structure than aureusidin. From a comparison of its spectral properties with those of a number of natural and synthetic aurones (cf. Geissman

[9] J. B. HARBORNE and H. S. A. SHERRATT, *Biochem. J.* **65**, 23 (1957).

[10] K. EGGER, *Z. Naturforsch.* **16b**, 430 (1961).

[11] S. HATTORI, *Chemistry of the Flavonoid Compounds* (Edited by T. A. GEISSMAN), p. 317. Pergamon Press, London (1962).

and Harborne[12]), the most likely structure for this aglycone appeared to be 4,6,3′,4′,5′-pentahydroxyaurone (bracteatin), a compound recently isolated as the 4-glucoside (bractein) from flower petals of *Helichrysum bracteatum*.[13] A direct comparison of the A2 aglycone and bracteatin showed them to be identical (see Table 2). That A2 is the 6-glucoside of bracteatin is apparent from the following observations: (1) it is hydrolysed by β-glucosidase as rapidly as aureusin is and is therefore presumably a simple glucoside; (2) its spectral properties indicate that the glucose is not attached either to the 4-hydroxyl group or to any of the B-ring hydroxyls; and (3) its R_F values are related to those of the isomeric 4-glucoside (bractein) in exactly the same way as the R_Fs of the 6- and 4-glucosides of aureusidin are related (Table 2).

TABLE 3. FLAVONES OF *Antirrhinum*

Series	Species	Anthocyanins			Aurones		Flavones				
		Pg 3RG	Cy 3RG	Dp 3G5G	A1	A2	F1	F10	F2	F3	others*
Section Saerorhinum											
Axilliflora	*cornutum*	−	−	+	−	−	−	−	−	−	—
Section Antirrhinum											
Sempervirentia	*sempervirens*	−	+	−	+	+	+	−	+	+	F11
Glutinosa	*hispanicum*	−	+	−	+	+	+	−	−	+	F12
Meonantha	*meonanthum*	−	+	−	+	+	+	−	+	+	F11
Sicula	*siculum*	−	+	−	+	+	+	−	+	+	F11
Majore	*majus*†	−	+	−	+	+	+	−	+	+	F11
unclassified											
—	*orontium*	−	+	−	−	−	+	−	−	+	F13
—	*speciosum*	+	−	−	−	−	+	−	−	+	F13

* R_F values of unidentified flavones present, in BAW and 15% acetic acid, are for F11: 0·41, 0·27; F12: 0·59, 0·41; F13: 0·34, 0·47.

† Representatives of four subspecies examined, namely *A. tortuosom*, *latifolium*, *linkianum* and *majus* (the latter includes the cultivated snapdragon).

Antirrhinum *species*

The results of a survey of the flavonoids of *Antirrhinum* are presented in Table 3. The species are arranged according to the classification of Rothmaler[14] and genetical colour variants of *A. majus* are excluded. One anthocyanin, not previously found in the genus, was isolated from *A. cornutum* and identified as delphinidin 3,5-diglucoside. Three flavones, not present in *A. majus*, were found in some of the other species but these were not further investigated.

The following points of systematic interest may be noted. (1) Species of the section Antirrhinum are chemically similar to each other: the two aurone glucosides, and apigenin, luteolin and chrysoeriol glucuronides are consistently present. (2) *Antirrhinum cornutum*, the only species in the other section of the genus (Saerorhinum) so far available for examination, is quite distinct. It has a different anthocyanin (both the aglycone and the sugars are distinctive), and lacks both aureusidin and flavone glycosides. (3) *Antirrhinum orontium* and *A. speciosum*,

[12] T. A. GEISSMAN and J. B. HARBORNE, *J. Am. Chem. Soc.* **78**, 833 (1956).
[13] R. HÄNSEL, L. LANGHAMMER and A. G. ALBRECHT, *Tetrahedron Letters*, 599 (1962).
[14] W. ROTHMALER, *Feddes Rep. Spec. Nov.*, **136–7**, 1 (1956–7).

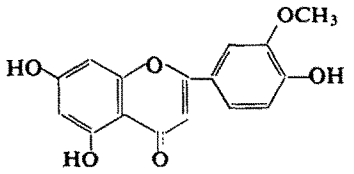
two other morphologically distinct species (which, curiously, do not appear in Rothmaler's classification) are also distinctive in their flower pigments.

Digitalis purpurea

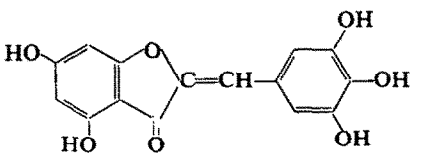
The leaves of *Digitalis purpurea* are reported to contain luteolin 7-glucoside[15]; on re-investigation, two luteolin glycosides D1 and D2, neither of which correspond to the 7-glucoside were isolated (Table 1). Both were extremely resistant to acid hydrolysis. D1, however, proved to be identical with the 7-glucuronide isolated from *Antirrhinum majus*. The second substance, D2, from spectral evidence, was also a 7-glycoside and, on hydrolysis with β-glucuronidase, gave luteolin, glucose and glucuronic acid. Since it was not hydrolysed by β-glucosidase, D2 must be luteolin 7-glucosylglucuronide. It is worth noting that a quercetin 3-glucosylglucuronide has recently been found in *Nelumbo nucifera*[16] and it is possible that both this and the *Digitalis* pigment have the same disaccharide unit. Glucuronides are a relatively uncommon class of flavonoid glycoside and the occurrence of flavone glucuronides (rather than glucosides) in both *Antirrhinum* and *Digitalis* suggests that the family Scrophulariaceae may be a relatively rich source of such substances. Glucuronides have also been chromatographically identified in leaves of several other *Digitalis* species.

DISCUSSION

The discovery of two flavonoid aglycones, chrysoeriol (I) and bracteatin (II), hitherto not recognized in *Antirrhinum majus* is of biogenetic interest, since both compounds are significantly different in structure from the eight pigment aglycones previously found,[5] all



(I) Chrysoeriol



(II) Bracteatin

of which have hydroxylation patterns based on apigenin (5,7,4′-trihydroxyflavone) or luteolin (5,7,3′,4′-tetrahydroxyflavone). Chrysoeriol is of special interest, because it is the first methylated flavonoid to be found here. The only other methylated phenol previously isolated from this source is the cinnamic acid related to chrysoeriol, i.e. ferulic or 3-methoxy-4-hydroxycinnamic acid,[17] a result which suggests that the cinnamic acids are more closely related biogenetically to the flavones than they are to the other classes of flavonoid.

The presence of 4,6,3′,4′,5′-pentahydroxyaurone (bracteatin) in *A. majus* fits in with the related discovery of delphinidin 3,5-diglucoside in *A. cornutum* and these results together demonstrate for the first time that this genus has the ability of producing pigments with the 3′,4′,5′-trihydroxylation pattern. The fact that bracteatin is the only pigment in *A. majus* with this hydroxylation pattern confirms the conclusion drawn earlier by Jorgensen and Geissman[3] that the pathway of aurone synthesis diverges at a relatively early stage from the pathway to flavones and other flavonoids.

[15] G. HUKUTI, *J. pharm. Soc. Japan*, **56**, 569 (1936).
[16] T. NAKOOKI, N. MORITA, Y. NAGATA and H. OGURI, *Yakugaku zasshi*, **81**, 1158 (1961).
[17] J. B. HARBORNE and J. J. CORNER, *Arch. Biochem. Biophys.* **92**, 192 (1961).

A general summary of all the flavonoid glycosides that have been found in *A. majus* is given in Table 4: it is clear that all five classes of pigment have their own distinctive glycosidic patterns. Even the anthocyanidins and flavonols are normally present in different glycosidic forms. Thus quercetin 3-rutinoside, which corresponds in structure to the major *Antirrhinum* anthocyanin, cyanidin 3-rutinoside, is absent from most genotypes. It is only found in plants which are doubly recessive at the Pallilda locus, the primary function of which is the control of anthocyanin concentration.[2]

TABLE 4. GLYCOSIDIC PATTERN OF FLAVONOIDS IN *Antirrhinum Majus*

Flavonoid class	Pigment	Present in genotypes	Reference
Anthocyanidin	Cyanidin 3-rutinoside	P–M–	8
	Pelargonidin 3-rutinoside	P–mm	9
Flavonol	Quercetin 3-glucoside	P–M–	3
	Quercetin 3-rutinoside	P–M–pal*	2
	Kampferol 3-glucoside	P–mm	This paper
	Kampferol 3,7-diglucoside	P–mm	This paper
Flavone	Apigenin 7-glucuronide	All non-albinos	4
	Apigenin 7,4′-diglucuronide	All non-albinos	This paper
	Luteolin 7-glucuronide	–M–	This paper
	Chrysoeriol 7-glucuronide	–M–	This paper
Aurone	Aureusidin 6-glucoside	All non-albinos	3
	Bracteatin 6-glucoside	All non-albinos	This paper
Flavanone	Naringenin 7-glucoside	P–m–	4
	Naringenin 7-rhamnosylglucoside†	P–m–	4
Cinnamic acid	*p*-Coumarylglucose	All, including albinos	17
	Caffeylglucose		17
	Ferulylglucose		17

* All other genotypes referred to in this column are dominant for this gene, i.e. Pal.

† Nature of the disaccharide present has not been unequivocally established. Since this pigment is chromatopographically identical with naringin[4] (naringenin 7-neohesperidoside, the sugar appears to be neohesperidose (rhamnosyl $\alpha 1 \rightarrow 2$ glucose) rather than rutinose (rhamnosyl $\alpha 1 \rightarrow 6$ glucose).

EXPERIMENTAL

Plant Material

Stocks of all the available *Antirrhinum majus* genotypes and a collection of *Antirrhinum* species were grown at this Institute in the glasshouse.

Separation and Identification of Flavonoids

The general procedures described in earlier papers in this series were used. Special precautions are necessary with *Antirrhinum* extracts to avoid contamination of the flavonoids with the aucubins[18] that are present. In most instances, fresh petal material was used and the flavonoid bands on chromatograms of concentrated extracts, developed in butanol–acetic acid–water, were eluted and immediately purified by chromatography in 15% acetic acid.

[18] A. R. TRIM and R. HILL, *Biochem. J.* **50**, 310 (1951).

Chromatograms left exposed to air for more than 24 hr often became stained brown at R_Fs between 0·2 and 0·4 as a result of the breakdown of the aucubin materials.

Identification of Chrysoeriol

The aglycone formed on hydrolysis of F2, did not separate from authentic chrysoeriol (supplied by Dr. R. M. Horowitz) when chromatographed in BAW, PhOH, Forestal solvent, 50% aqueous acetic acid and n-propanol–acetic acid–water (1:1:1). It also had the same distinctive colour (bright green) as the authentic specimen when exposed to NH_3 vapour in ultraviolet light (by comparison, apigenin is dull yellow-green and luteolin dull yellow). It also had identical spectral properties, i.e. λ_{max} (mμ) 95% EtOH 252, 269, 295 (inflection), 350; EtOH/NaOAc 264, 272 (inflection); EtOH/$AlCl_3$ 261, 272, 293, 351; EtOH/NaOH 408; and EtOH/H_3BO_3 252, 269, 295 (inflection), 350 mμ. On demethylation with pyridinium chloride, it gave luteolin.

Methylation and Hydrolysis of Flavone Glycosides

F10 was heated with excess dimethyl sulphate in acetone in the presence of potassium carbonate at 100° for 4 hr. The methylated product was purified by chromatography in 15% acetic acid, and then hydrolysed with β-glucuronidase in acetate buffer at pH 4·9. The hydrolysed product was identified as apigenin 5-methyl ether, from its blue florescence in ultraviolet light and its spectral properties: λ_{max} (mμ) 95% EtOH 234, 253 (inflection), 292, 326; EtOH/NaOAc 240 (i.e. 7-hydroxyl free); EtOH/$AlCl_3$ 292, 326 (i.e. 5-hydroxyl blocked); NaOEt 386 mμ (i.e. 4'hydroxyl free). Its R_F values were as follows (values for apigenin 5,4'-di-*O*-methyl ether in parentheses): 0·79 (0·84) in BAW, 0·78 (0·85) in n-propanol–acetic acid–water (1:1:1) and 0·08 (0·14) in 15% acetic acid.

Methylation and hydrolysis of F2 and F3 gave products chromatographically and spectrally identical with luteolin 5,3',4'-tri-*O*-methyl ether.

Delphin from Antirrhinum cornutum

The anthocyanin, isolated by the usual methods,[19] gave delphinidin and glucose on hydrolysis. Its spectral properties were λ_{max} 276, 536 mμ (O.D. ratios 440/536 15 per cent, 310/536 19 per cent and 276/536 50 per cent). Its R_F values were 0·13 in BAW, 0·03 in BuHCl 0·07 in 1% HCl and 0·23 in HOAc–HCl–H_2O (15:3:82), and it did not separate from authentic delphinidin 3,5-diglucoside (delphin).

Acknowledgements—The author is grateful to Dr. R. M. Horowitz, Pasadena, for a sample of chrysoeriol; to Dr. K. Egger, Heidelberg, for a sample of kampferol 3,7-diglucoside; to Prof. R. Hänsel, West Berlin, for a sample of bractein; and to Dr. J. R. S. Fincham and Mr. B. J. Harrison, of this Institute, for provision of plant material and for valuable discussions.

[19] J. B. HARBORNE, *Biochem. J.* **74**, 262 (1960).