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PLANT POLYPHENOLS—XV

FLAVONOLS AS YELLOW FLOWER PIGMENTS*

J. B. HARBORNE

John Innes Institute, Bayfordbury, Hertford, Herts.

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Abstract—The flavonol quercetagetin has been identified as a yellow flower pigment in species of *Coronilla*, *Lotus*, *Papaver*, *Primula* and *Rhododendron*; it has been isolated as the 3-galactoside, 3-gentiotrioside or 7-glucoside. It is accompanied in *Lotus corniculatus* by its 7-methyl ether. A flavonol tentatively identified as the related 6-hydroxykaempferol has been found in *Mimulus luteus*. Quercetagetin is accompanied in *Papaver nudicaule* by nudicaulin, a water-soluble yellow pigment earlier considered to be a flavylium salt; spectral studies now indicate that this is unlikely. Syringetin and isorhamnetin have been found as yellow flower pigments in *Lathyrus pratensis*. While the above compounds, unlike the common flavonols, undoubtedly contribute to yellow flower colour, they accompany yellow carotenoids in many of the plants that have been examined. During the present survey, a water-soluble carotenoid has been found in the petals of only one plant, *Nemesia strumosa*. This pigment was readily distinguished by spectral means from the yellow flavonols and was identified as crocein.

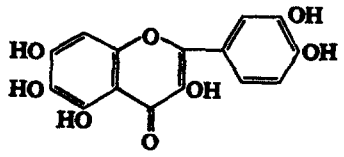
INTRODUCTION

LITTLE is known about the contribution of flavonoids to yellow colour in higher plants, although Seybold[1] has shown that the majority of yellow-petalled plants have flavonols as well as carotenoids. The widely-occurring flavonols (i.e. kaempferol, quercetin and myricetin) do not contribute to yellow colour, since they are more or less colourless at the pH values normally found in cells and are often abundant in white, ivory or cream flower petals. Again, chalcones and aurones, two groups of flavonoid which are distinctly yellow, are relatively rare.[2] Flavonols were therefore isolated from a range of yellow-petalled plants in order to assess their contribution, if any, to flower colour.

Attention was first drawn to this problem when an unusual flavonol was found in *Rhododendron campylocarpum* (Ericaceae) as the principal yellow pigment.[3] It had a characteristic dark brown colour on paper in u.v. light, and appeared yellow in daylight but was not identified. Similar substances had been noted earlier in the flowers of several other plants, especially in the primrose, *Primula vulgaris*, and related species. These pigments have now been identified as derivatives of quercetagetin (I), previously known in *Tagetes* (Compositae)[4,5] but hitherto thought to be a rare petal constituent. The present paper describes the identification of quercetagetin derivatives and related flavonols and discusses their importance as flower pigments.

* Part XIV. J. B. HARBORNE, *Phytochem.* 4, 107 (1965).

[1] A. SEYBOLD, *Sitzber. heidelberg Akad. Wiss. Math-naturw. Kl.* 2, (1953–4).
[2] M. SHIMOKORIYAMA, In *Chemistry of the Flavonoid Compounds* (Edited by T. A. GEISSMAN), pp. 286–316, Pergamon Press, Oxford (1962).
[3] J. B. HARBORNE, *Arch. Biochem. Biophys.* 96, 171 (1962).
[4] LATOUR and MAGNIER DE LA SOURCE, *Bull. Soc. Chim. Paris* 228, 337 (1877).
[5] P. S. RAO and T. R. SESHADRI, *Proc. Indian Acad. Sci.* 14A, 289 (1941).



(I) Quercetagetin

RESULTS

Identification of Quercetagetin

Quercetagetin was identified in acid-hydrolysed extracts of the flowers of *Coronilla*, *Lotus*, *Papaver*, *Primula* and *Rhododendron* species by direct comparison with authentic

TABLE 1. CHROMATOGRAPHIC AND SPECTRAL PROPERTIES OF QUERCETAGETIN AND RELATED FLAVONOLS

Flavonols	R_f value in* BAW	Forestal	PhOH	PAW	Colour in u.v. light
Kaempferol	0·85	0·55	0·39	0·47	Bright yellow
Quercetin	0·66	0·40	0·21	0·32	
Myricetin	0·40	0·25	0·07	0·20	
Quercetagetin	0·31	0·26	0·12	0·19	Dull black
Patuletin	0·68	0·48	0·56	—	Bright green-yellow
Lotus F1A	0·64	0·45	0·46	0·64	Dull yellow
Primula F3A	0·72	0·46	0·85	0·65	Bright yellow
Gossypetin	0·21	0·41	0·03	0·34	Golden yellow
Mimuletin	0·59	0·42	0·45	—	Dull black
Lathyrus aglycone†	0·68	0·55	0·80	0·70	Bright yellow
			0·87		
Isorhamnetin	0·68	0·55	0·80	0·69	

	λ_{max} in EtOH (mμ) Alone Band I	Band II	+NaOAc Band I	+H_3BO_3 Band II	+NaOH Band II	+$AlCl_3$ Band II
Quercetagetin	259	364	248	384	decomp.	425
	272		285			
Gossypetin	261	383	280	398	450 (unstable)	442
Patuletin	258	373	264	394	decomp.	443
Lotus F1A	260	381	260	395	decomp.	440
	270 (inf.)		270 (inf.)			
Primula F3A	255	380	265	388	435	360
	272		280			436
Mimuletin	263	338	—	—	decomp.	—
	278	386				
Lathyrus aglycone†	254	374	255	376	408 (unstable)	435

* R_f values were also recorded in 50% HOAc and in BEW.
† A mixture (see text); acetate had λ_{max} 255 and 307 mμ.

material isolated from *Tagetes*. Identification was based on spectral measurements and on paper chromatography and co-chromatography in six solvent systems (Table 1). Quercetagetin differs in R_f value from any of the other known flavonols and is unusual in appearing on paper chromatograms as a dull black spot in u.v. light unaffected by ammonia vapour. The isomeric gossypetin (3,5,7,8,3′,4′-hexahydroxyflavone) had quite different R_f values (Table 1) and appeared in u.v. light as a bright golden yellow. In its spectral properties, quercetagetin is also quite distinctive, exhibiting as it does a double peak in the short u.v. and a broad band (λ_{max} 364 mμ) at longer wavelengths. Identification of quercetagetin was confirmed in *Papaver*, *Primula* and *Rhododendron* by reductive acetylation followed by hydrolysis to 6-hydroxycyanidin.

TABLE 2. CHROMATOGRAPHIC AND SPECTRAL PROPERTIES OF FLAVONOL GLYCOSIDES

Flavonol glycoside	Sugar and position of subst.	R_f value in BAW	R_f value in H_2O	R_f value in PhOH
Quercetagetin glycosides				
Rhododendron F6*	3-Galactoside	0·42	0·10	0·40
Primula F4	3-Gentiotrioside	0·15	0·40	0·30
from *Papaver nudicaule*	7-Glucoside	0·31	0·02	0·30
Lotus F1	3-Galactoside	0·58	0·19	0·67
from *Coronilla*	3-Glycoside	0·42	0·34	0·53
Other glycosides				
Primula F3	3-Gentiotrioside (?)	0·20	0·50	0·49
Mimulin	Glucoside	0·51	0·02	0·46
Lathyrus glycoside†	Not known	0·27	0·58	0·70

Glycoside	λ_{max} in EtOH (mμ): Alone Band I	Alone Band II	+NaOAc Band I	+H_3BO_3 Band II	+NaOH Band II	+$AlCl_3$ Band II
Rhododendron F6	264 278	352	295	363	403	375
Primula F4	264 280	350	305	360	392	375
Papaver	261 278 (inf.)	347 388	260 278 (inf.)	400	decomp.	452
Lotus F1	263	352	262	390	419	—
Coronilla	262 280	347	310	—	decomp.	365
PrimulaF3	260 278	360 370	285	360 370	425	365
Mimulin	247 288	335 388	247 288	— 386	decomp.	—
Lathyrus glycoside†	253	364	257	364	412	370 (inf.) 405

* Lotus F2 had identical properties to Rhododendron F6 and did not separate from it when they were co-chromatographed in six solvent systems.

† A mixture (see text).

Quercetagetin 3-Galactoside in Rhododendron

The occurrence in *Rhododendron campylocarpum* of a flavonol galactoside (designated F6) which on hydrolysis gave an unusual aglycone (F6A) has already been described.[3] F6A has now been identified as quercetagetin (see above) and F6 has been identified as the 3-galactoside (Table 2). Thus, the R_f and spectral data show that it is a 3-glycoside and micro-analysis and oxidation with H_2O_2, which gave galactose alone, confirmed that only one sugar unit was present. The only glycosides of quercetagetin previously reported are the 3-glucoside (tagetiin)[6] and the 7-glucoside (quercetagitrin),[5] both of which occur in Tagetes *erecta*.[7]

TABLE 3. DISTRIBUTION OF FLAVONOLS IN FLOWERS OF *Rhododendron* SPECIES

Species	Flower colour	Flavonols present: Quercetagetin	Myricetin	Quercetin	Kaempferol	Azaleatin
campylocarpum	Primrose yellow	+	−	+	−	−
*chryseum**		+	−	+	−	−
herpesticum		+	−	−	−	+
telopium		+	−	+	−	−
trichocladum		+	−	+	+	−
wardii		+	−	−	−	−
ambiguum	Yellow or orange	−	+	+	−	−
cv. "Lady Chamberlain"		−	−	+	−	+
wightii		−	−	+	−	−
aberconwayi	Cream or white	−	+	+	+	−
campanulatum		−	+	+	−	−
fictolacteum		−	−	+	−	−
hyperythrum		−	−	−	+	−
irroratum†		−	+	+	−	−
racemosum var. album		−	−	−	−	+
roxieanum		−	−	−	+	−

* Two forms, Y.U. 14641 and F. 20432, were examined and found to be identical.
† Two forms, type form and R. 59620, were examined and found to be identical.

Unlike the common flavonol glycosides such as rutin, F6 has a strong yellow colour when present on chromatograms and this suggests that it is the main yellow pigment in *Rhododendron* flowers. Its distribution in a range of yellow- and white-flowered *Rhododendron* species are given in Table 3. It will be seen that quercetagetin occurs (as the 3-galactoside) in six out of the nine yellow-flowered species, but in none of the seven white-flowered species examined. Thus its occurrence is correlated with yellow flower colour, irrespective of whether carotenoid also occurs in the genus or not. There are, in fact, no reports of carotenoids in *Rhododendron* (cf. Ref. 8) and none could be detected in two of the quercetagetin-containing species, i.e. *R. wardii* and *R. campylocarpum*. However, carotenoids are presumably present in the three yellow-flowered species which lack quercetagetin (see Table 3). It is significant that ordinary

[6] N. MORITA, *J. Pharm. Soc. Japan* 77, 31 (1957).
[7] S. S. SUBRAMANIAN and M. N. SWAMY, *Current Sci. India* 32, 308 (1963).
[8] T. W. GOODWIN, *Comparative Biochemistry of the Carotenoids*, pp. 45–54, Chapman and Hall, London (1952); and also *Chem. Abstr.* 1951–1961.

flavonols (i.e. rhamnosides of kaempferol, quercetin, axaleatin and myricetin) occur randomly in all sixteen species examined, so that their distribution is not related to flower colour.

Quercetagetin 3-Gentiotrioside in Primula

During earlier surveys of the flavonoids of the Primulaceae,[9] two unidentified flavonols were noted in acid hydrolysates of several yellow-flowered *Primula* species, namely: the primrose, *P. vulgaris*; the oxlip, *P. elatior*; the cowslip, *P. veris*; *P. polyanthus*; and several less common yellow-flowered species, e.g. *P. sino-purpurea* and *P. silva-taroucana*. These two flavonols appeared to be important yellow pigments, particularly as carotenoids had not been identified in these plants. One of these flavonols (Primula F4A) has now been identified as quercetagetin (see above). The other (Primula F3A) has not been fully identified; it gives quercetagetin on demethylation and thus appears to be a new methyl ether of this flavonol.

Two pieces of evidence indicate that quercetagetin derivatives contribute to yellow flower colour in *Primula*. First, both flavonols were found in *P. polyanthus* in yellow, brown and purple colour forms but were absent from white, purple-blue and blue varieties. By contrast, kaempferol and quercetin were uniformly present (compare the situation in *Rhododendron*, mentioned above). Second, an aqueous-alcoholic extract of primrose corollas, from which the deeper yellow eyes had been excised, contained no yellow pigment soluble in petroleum-ether. On chromatography of the aqueous extract, quercetagetin derivatives were the only substances which were yellow in visible light. There was no evidence for the presence of the water-soluble crocein of *Crocus* pollen, a sample of which was available for comparison. Carotenoids were present in the eye of the primrose and of *P. sinensis* and throughout the corollas of *P. veris* and *P. polyanthus*, but they were not studied further.

The major flavonol glycoside of primrose flowers was then isolated by chromatography (Primula F4 in Table 2); it required careful purification, because it was not easily separated from several closely related glycosides. The purified material was identified as quercetagetin 3-gentiotrioside. On acid hydrolysis it gives gentiobiose, glucose and quercetagetin and the spectral data indicate that it has a sugar only in the 3-position. The high R_f value in water indicates that it contains a trisaccharide. On oxidation with H_2O_2, it gives a sugar identical with that combined in the flavonol and anthocyanidin triglucosides previously isolated from *Primula sinensis*.[9] That this trisaccharide is probably gentiotriose (*O*-β-D-glucopyranosyl-(6→1)-*O*-β-D-glucopyranosyl-(6→1)-β-D-glucopyranose) follows from the facts that (1) it gives only gentiobiose and glucose on acid hydrolysis, and (2) it is not appreciably hydrolysed by β-glucosidase but neither it is attacked by maltase. ΔR_m values, calculated from the R_f values reported[9] for the flavonoid monoglucosides, gentiobiosides and triglucosides are also consistent with this structure; e.g. in the peonidin series in 1% HCl ΔR_m (3-glucoside to 3-gentiobioside) is 0·37 and ΔR_m (gentiobioside to triglucoside) is 0·38.

Quercetagetin 7-Glucoside and Nudicaulin in Papaver nudicaule

A water-soluble yellow pigment was isolated by Price, Robinson and Scott-Moncrieff[10] as the principal colouring matter of petals of *Papaver nudicaule* and *Meconopsis cambrica* (both Papaveraceae). The pigment, nudicaulin, was not fully characterized but was suggested to be a diglucoside of a flavylium salt containing nitrogen. Price *et al.* had difficulty in purifying nudicaulin and similar difficulties were encountered in the present work. When the crude pigment was chromatographed on thick paper in water, a minor yellow pigment (R_f 0·02) was

[9] J. B. Harborne and H. S. A. Sherratt, *Nature* **181**, 25 (1958); *Biochem. J.* **78**, 298 (1961).
[10] J. R. Price, R. Robinson and R. Scott-Moncrieff, *J. Chem. Soc.* 1465 (1939).

separated from the main substance (R_f 0·90). The minor pigment gave quercetagetin on hydrolysis and other studies (Table 2 and Experimental) indicate that it is the 7-glucoside of quercetagetin.

The main pigment, after further purification, agreed in all its properties with those described for nudicaulin. On acid hydrolysis, it gave glucose and an aglycone which did not correspond with any known flavonoid. On treatment with anthocyanase (it was not attacked by β-glucosidase) it gave two intermediate glycosides, indicating that it is present as a triglucoside. The pure nudicaulin and its aglycone have identical spectral properties; there are two main absorption maxima at 258 and 467 mμ and a minor band at 330 mμ. The absorption spectrum in ethanol is unaltered by changes in pH or by the addition of $AlCl_3$, indicating that there are no free phenolic groups present in the pigment. Thus, the spectral data and solubility properties indicate that nudicaulin is neither a flavonoid nor a carotenoid. The possibility that it is a betaxanthin is ruled out by the facts that its spectral properties are different (betaxanthins have λ_{max} at 476 to 485 mμ and show no absorption between 300 and 400 mμ) and that it forms no amino acid when degraded with 2 N acid.

Flavonols of the Leguminosae

Following the discovery of 2',4',4-trihydroxychalcone in the yellow flowers of gorse, *Ulex europeaus*,[11] other yellow-flowered legumes were briefly examined. Although no other species had chalcones, several contained flavonols which appeared to be yellow *in vivo*. These species were further tested by shaking aqueous-alcoholic petal extracts with petroleum ether; in most cases, an appreciable portion (up to 30 per cent) of the colouring matter remained in the aqueous phase after exhaustive extraction. The flavonols in three species (*Coronilla glauca*, *Lotus corniculatus* and *Lathyrus pratensis*) were chosen for further examination.

As expected, quercetagetin was identified in acid-hydrolysates of fresh petals of *Coronilla glauca*. The glycoside present was not studied in detail but the R_f values and spectrum suggest that it has a di- or trisaccharide attached to the 3-hydroxyl group. An extract from *Lotus* flowers also gave quercetagetin on hydrolysis, but it was accompanied by a second flavonol (Lotus F1A). The quercetagetin glycoside was also isolated and was readily identified as the 3-galactoside by direct comparison with the pigment in *Rhododendron* (see above). The other new glycoside (Lotus F1) also gave galactose on hydrolysis and the new aglycone (Lotus F1A) was identified as quercetagetin 7-methyl ether on the following basis. It gives quercetagetin on demethylation and the R_f values, spectrum and microanalysis show that it must be a monomethyl ether. It cannot be the known 6-methyl ether, patuletin, which has different R_f values and colour reactions (Table 1) and the spectral measurements show that the methyl group can be on neither the 3'- or 4'-hydroxyl (positive boric acid shift) nor on the 3- or 5-hydroxyl (compare spectrum in neutral solution with that of quercetagetin). Hence, it must be the 7-methyl ether, a conclusion supported by the lack of a sodium acetate shift in the spectrum (Table 1). This is the first record of the occurrence of quercetagetin 7-methyl ether in nature.

The third legume studied, *Lathyrus pratensis*, contained no derivatives of quercetagetin in its flowers; traces of kaempferol or quercetin were present, as reported by Pecket,[12] but the major flavonol aglycones of both flower and leaf appears to be a mixture of methyl ethers of quercetin and myricetin. All attempts to separate the components of the aglycone failed and the glycosides in the unhydrolysed extracts were also difficult to separate. That two components are present is clear from the following experiments. Chromatography in phenol (but

[11] J. B. HARBORNE, *Phytochem.* **1**, 203 (1963).
[12] R. C. PECKET, *New Phytologist* **59**, 138 (1960).

in no other solvent) showed the presence of two flavonols, one corresponding to isorhamnetin, the other (with a higher R_f) to a new dimethyl ether of myricetin. Reductive acetylation yielded two anthocyanidins, readily identifiable as peonidin (from isorhamnetin) and malvidin (from myricetin 3',5'-dimethyl ether). On demethylation the aglycone mixture gave myricetin, quercetin and an intermediate, which is presumably myricetin 3'-methyl ether.

Myricetin 3',5'-dimethyl ether was synthesized by Heap and Robinson in 1929[13] and named syringetin, but it has never been found in plants, although it has a close biogenetic relationship with the widely-occurring phenolics, malvidin and sinapic acid. A comparison of the *Lathyrus* aglycone with synthetic syringetin is needed to confirm the identification of the former and this is in progress.

Mimulin and Crocein in the Scrophulariaceae

During a study of the distribution of aurones in the Scrophulariaceae (cf. Ref. 14), water-soluble yellow pigments were noted in the petals of two species, *Mimulus luteus* and *Nemesia strumosa*. Neither were aurones but that in *Mimulus luteus*, called mimulin (Table 2), has similar properties to the quercetagetin glycosides. Mimulin appears to be a simple glucoside, and is accompanied by carotenoids which are presumably similar to those present in other *Mimulus* species.[15]

The aglycone, mimuletin, has uniformly higher R_f values than quercetagetin in all solvents (Table 1), which suggests that it is the related 6-hydroxykaempferol. However, lack of material and the pigment's instability have prevented further work on this substance. While at least two methyl ethers of 6-hydroxykaempferol are known in plants,[16] the substance itself has only been reported once before in nature, in petals of *Galega officinalis*.[17] Its identification in this plant is not unequivocal and studies are in progress to confirm the natural occurrence of this substance.

The water-soluble pigment in flowers of *Nemesia strumosa* had similar R_f values on paper chromatograms (0·27 in butanol–acetic acid–water and 0·36 in water) to flavonol glycosides. However, its spectral properties showed at once that it was a carotenoid, not a flavonoid, and it was therefore compared directly with the only known water-soluble carotenoid, crocein (the digentiobiose ester of crocetin) from *Crocus* pollen. It proved to be identical (Table 4), as did the *Nemesia* aglycone with crocetin. This carotenoid, because of its carboxyl groups, has very different chromatographic properties from the common carotenoids such as β-carotene and is not mobile on silica gel plates in the solvent systems commonly employed for carotenoids. However, it proved to be mobile in solvents used for separating aliphatic dicarboxylic acids[18] and these were employed in the present identification (Table 4). Crocetin, first isolated from petal and pollen of *Crocus* spp., is a rare pigment;[19] it is interesting that one of the few other records of its occurrence is *Verbascum*,[20] which is in the same family as *Nemesia*.

[13] T. Heap and R. Robinson, *J. Chem. Soc.* 67 (1929).
[14] J. B. Harborne, *Phytochem.* 2, 327 (1963).
[15] T. W. Goodwin and D. M. Thomas, *Phytochem.* 3, 47 (1964).
[16] J. Gripenberg, In *Chemistry of the Flavonoid Compounds* (Edited by T. A. Geissman), p. 422, Pergamon Press, Oxford (1962).
[17] N. P. Maxyutin and V. I. Litvinenko, *Dokl. Akad. Nauk. SSSR.* 154, 1123 (1964).
[18] E. V. Truter, *Thin Film Chromatography*, p. 175, Cleaver Hume Press, London (1963).
[19] W. Karrer, *Konstitution und Vorkommen der Organischen Pflanzenstoffe*, p. 743, Birkhäuser Verlag, Basel (1958).
[20] L. Schmid and E. Kotter, *Monatsh.* 59, 341 (1932).

TABLE 4. CHROMATOGRAPHIC AND SPECTRAL COMPARISON OF *Nemesia* GLYCOSIDE AND AGLYCONE WITH CROCEIN AND CROCETIN

Solvent system	Support	R_f value of *Nemesia* glycoside and crocein	R_f value of *Nemesia* aglycone and crocetin	R_f value of β-carotene
45% EtOAc in C_6H_6	Silica gel-G	0·00	0·00	0·83
Toluene: HCO_2Et: HCO_2H (5:4:1)	Silica gel-G	0·00	0·54	0·85
20% HOAc in $CHCl_3$	Silica gel-G	0·00	0·90	1·00
EtOH: 2 N.NH_3 (25:7)	Silica gel-G	0·15	0·70	0·90
BAW (4:1:5)	Whatman No.1 paper	0·27	1·00	1·00
Water	Whatman No.1 paper	0·36	0·00	0·00

Spectral maxima (in mμ)		
Nemesia glycoside	Crocein	Solvent
410, 433, 455	411, 437, 458	95% EtOH
Nemesia aglycone	Crocetin	
403, 427, 452	403, 427, 452	95% EtOH
403, 434, 462	404, 432, 464	Pyridine
415, 435, 463 (inf.)	415, 433, 462 (inf.)	$CHCl_3$
406, 427, 454	405, 422, 450	EtOAc

DISCUSSION

Although our knowledge of the yellow flavonols is still scanty, two striking facts emerge. First, a significant proportion of the yellow-petalled plants that have been examined contain both carotenoid and flavonoid, pigments produced by quite unrelated biosynthetic pathways. Second, it is clear that for a flavonol to contribute to yellow flower colour it must possess some structural feature absent from the commonly-occurring flavonols such as kaempferol and quercetin.

The first point is evident from a consideration of the three legumes studied. All three contain carotenoids in their petals and yet they also have appreciable amounts of flavonols, which are yellow in colour. Similarly, the chalcone and aurone pigments are nearly always associated with carotenoids. These and other examples have been discussed in more detail elsewhere.[21] That flavonols alone can produce visible pigmentation is shown by such plants as the primrose and yellow-flowered rhododendrons in which, significantly, related white species lack the flavonol or flavonols that colour the yellow forms.

The second point—that only structurally unusual flavonols contribute to yellow colour—is evident in every example so far studied. The commonest pigment found in the present survey is quercetagetin, so that the introduction of a hydroxyl group into the 6-position in

[21] J. B. HARBORNE, In *Biochemistry of Plant Pigments* (Edited by T. W. GOODWIN), pp. 201–232, Academic Press, London (1965).

quercetin has a profound effect on its colour properties. A similar introduction of a hydroxyl group into the 8-position gives gossypetin, well known as the yellow pigment of cotton flowers.[22] It is also clear that methylation of quercetin and myricetin can give substances which are yellow pigments, as in *Lathyrus pratensis*. Although it seems at present that ordinary flavones and flavonols do not actively contribute to yellow petal colour, they might conceivably do so if present in very high concentrations or as metal chelates (analogous to the anthocyanin-iron and -aluminium complexes in the cornflower.)[23]

Although carotenoids and flavonoids are the most important yellow colouring matters, other structures contribute to yellow colour in restricted groups of plants. Chief among these are the yellow betaxanthins of the Centrospermae.[24] Other yellow pigments are the alkaloids berberine, present in *Berberis* flowers and roots, and flavocarpine of the stem bark of *Pleiocarpa mutica* (Apocynaceae).[25] To this group must now be added the poppy pigment nudicaulin, since spectral studies have disposed of the earlier suggestion that it is a flavylium salt.

EXPERIMENTAL

Plant Material

Flowers of the *Rhododendron* species were collected from plants grown in the Royal Park, Windsor. Flowers of *Primula elatior* were kindly collected by Miss R. Brett in Cambridgeshire. Other plants were grown from seed in the glasshouses or collected locally.

Authentic Pigments

Quercetagetin was isolated from petals of *Tagetes erecta*, the African marigold, and patuletin from petals of *Tagetes patula*, the French marigold. A sample of gossypetin, kindly provided by Professor T. R. Seshadri, was purified by chromatography in 50% HOAc and BAW. Crocein was isolated from the pollen of *Crocus laevigatus* and several other *Crocus* spp.

Paper Chromatography and Spectroscopy

Solvents used for paper chromatography were BAW, *n*-butanol: acetic acid: water (4:1:5); PhOH, water-saturated phenol; Forestal, acetic acid: conc. HCl: water (30:3:10); PAW, *n*-propanol: acetic acid: water (1:1:1); BBzPW, *n*-butanol: benzene: pyridine: water (5:1:3:3); BEW, *n*-butanol: ethanol: water (4:1:2–2); and various proportions of acetic acid–water. Spectra were measured on a Unicam S.P. 500.

Reductive Acetylation of Quercetagetin

Authentic material and the aglycones from *Papaver nudicaule*, *Primula vulgaris* and *Rhododendron campylocarpum* were heated with acetic anhydride, sodium acetate and zinc dust, using the method of King and White.[26] The products, after heating with acid and extracting into pentyl alcohol, were chromatographed in Forestal. The eluted bands containing the 6-hydroxycyanidin all had $\lambda_{max}^{MeOH-HCl}$ 518 mμ, E_{440}/E_{max} 25% and R_f values of 0·30 in Forestal (cyanidin 0·48) and 0·38 in BAW (cyanidin 0·58). The pigment had a brighter red colour on paper than cyanidin, which is a dull magenta.

[22] A. G. Perkin, *J. Chem. Soc.* **109**, 145 (1916).
[23] E. Bayer, *Chem. Ber.*, **91**, 1115 (1958); **92**, 1062 (1959).
[24] M. Piattelli, L. Minale and G. Prota, *Phytochem.* **4**, 121 (1965).
[25] G. Buchi, R. E. Manning and F. A. Hochstein, *J. Am. Chem. Soc.* **84**, 3393 (1962).
[26] H. G. C. King and T. H. White, *J. Chem. Soc.* 3901 (1957).

Isolation and Identification of the Flavonol Glycosides

Methods described in earlier papers in this series were used. Quercetagetin 3-galactoside isolated from *Rhododendron campylocarpum* was obtained as deep yellow prisms, m.p. 200° (Found: C, 48·12; H, 4·78. $C_{21}H_{20}O_{13}$. $2\frac{1}{2}$ H_2O required: C, 48·0; H, 4·8%). Both this pigment and the quercetagetin 7-glucoside from *Papaver nudicaule* were rapidly hydrolysed by β-glucosidase, gave only galactose or glucose on oxidation with H_2O_2 and were hydrolysed by acid directly to aglycone and sugar.

The trisaccharide gentiotriose was isolated from Primula F4 by H_2O_2 oxidation. It had the following R_G values (values for gentiobiose in parentheses): 0·28 (0·45) in BAW, 0·30 (0·45) in BEW, 0·20 (0·35) in BBzPW and 0·53 (0·61) in PhOH. On acid hydrolysis, it gave gentiobiose and glucose and on β-glucosidase hydrolysis, it gave traces of glucose; it was not attacked by maltase.

Lotus F1A (Quercetagetin 7-Methyl Ether)

This substance was isolated by chromatography from acid hydrolysates of *Lotus corniculatus* flowers and on crystallization from aqueous ethanol gave 4·3 mg of pale yellow needles, m.p. 252–4° (Found: OMe, 9·1. $C_{16}H_{12}O_8$ required: OMe, 9·4%). On demethylation with pyridinium chloride at 140° for 3 hr, it gave quercetagetin, identified by co-chromatography with authentic material.

Lathyrus aglycone (isorhamnetin and myricetin 3,'5'-dimethyl ether)

This mixture was obtained as a pale yellow solid, m.p. 250°, after paper chromatography of the solid aglycone obtained by hydrolysing alcoholic extracts of *Lathyrus pratensis* flowers. Repeated paper chromatography failed to separate the two components present. The spectral data (Table 1) are consistent with the suggestion that the mixture contains isorhamnetin and myricetin 3',5'-dimethyl ether, except that the spectrum unexpectedly failed to show a shift in the short u.v. band in the presence of NaOAc. However, the mixed glycoside (Table 2) gave a small shift. A pure sample of isorhamnetin also failed to give the expected shift (λ_{max}^{EtOH} 255 mμ, $\Delta\lambda^{NaOAc}$ 0 mμ), but isorhamnetin 3,4'-diglucoside (dactylin) did (λ_{max}^{EtOH} 255 and 268 mμ, giving a single peak at 270 mμ in the presence of NaOAc).*

On demethylation with pyridinium chloride at 140° for 6 hr, *Lathyrus* aglycone gave myricetin and quercetin. An intermediate, isolated after demethylation for 2 hr, had R_f values of 0·55 in BAW and 0·36 in Forestal and λ_{max}^{EtOH} 380 mμ, $\lambda_{max}^{EtOH-H_3BO_3}$ 425 and 455 mμ. On reductive acetylation followed by hydrolysis the mixture gave peonidin ($\lambda_{max}^{MeOH-HCl}$ 533 mμ) and malvidin ($\lambda_{max}^{MeOH-HCl}$ 543 mμ). These two anthocyanidins were further identified by co-chromatography on paper with authentic pigments in Forestal, Formic and BAW, and by co-chromatography on layers of silica gel-G in ethyl-acetate–formic acid–2 N HCl (85:9:6).

Nudicaulin

This was obtained as an orange powder, after chromatography of fresh petal extracts of *Papaver nudicaule* in BAW, H_2O, BAW and BAW. It had $\lambda_{max}^{EtOH-HCl}$ 258, 330 and 467 mμ and $\lambda_{max}^{aq.\ HCl}$ 340, 445 and 458 mμ; in water and aqueous alkali it only showed weak absorption in the visible with max at 465–470 mμ. The aglycone, obtained by acid hydrolysis, had identical absorption in EtOH—HCl to the glycoside. On alkaline fusion, the aglycone

* An anomalous lack of sodium acetate shift has also been reported for 5,7–dihydroxy–6,8–dimethoxy–3',4'–methylenedioxyflavone, lucidin, by H. H. LEE and C. H. TAN, *J. Chem. Soc.* 2743 (1965).

failed to yield any recognizable phenolic fragments. On hydrolysis with anthocyanase for 1½ hr at pH 4·0 and 37°, nudicaulin gave two intermediates with similar absorption maxima and the aglycone. R_f values of nudicaulin, the two intermediates and the aglycone were: 0·25, 0·37, 0·46 and 0·98 in BAW, and 0·70, 0·54, 0·24 and 0·00 in H_2O.

Crocein and Crocetin from Nemesia

Fresh corollas were extracted with hot 95% EtOH and the yellow pigment glycoside present, which was insoluble in petroleum ether, was purified by paper chromatography in BAW, H_2O and BEW. The algycone, produced on hydrolysis with 2 N HCl at 100° for 30 min or 2 N NaOH at 15° for 10 min, was obtained as orange-red prisms, m.p. *ca.* 300° (lit. m.p. for crocetin 295°). The spectral and chromatographic properties of glycoside and aglycone, together with those of authentic crocein and crocetin from *Crocus*, are recorded in Table 4. Maxima for crocetin are reported[27] in CS_2 and petroleum ether, but values could not be obtained in these solvents in this laboratory because of its insolubility. Values are given in four other solvents in the table; there was also good agreement between authentic and isolated pigments in the relative intensities of the three maxima in the 400–500 mμ region.

[27] B. H. Davies, In *Biochemistry of Plant Pigments* (Edited by T. W. Goodwin), pp. 489–532, Academic Press, London (1965).