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GOSSYPETIN AND HERBACETIN AS TAXONOMIC MARKERS IN HIGHER PLANTS*

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Abstract—Four yellow flavonol pigments earlier reported in the Leguminosae, Ericaceae and Papaveraceae as quercetagetin glycosides have now been found to be the isomeric gossypetin derivatives. Reliable methods are outlined for distinguishing between these isomeric 6- and 8-hydroxyquercetin derivatives on a microscale, including a simple colour test which can be carried out on chromatograms. The yellow flavonol monomethyl ether reported in *Lotus corniculatus* flower is now shown to be gossypetin 7-methyl ether. Gossypetin, besides occurring in *Rhododendron* petals, is also widespread in the leaves of this (in 76 of 103 species examined) and nine related genera of the Ericaceae. It also occurs as a leaf constituent in the related *Empetrum* (Empetraceae). In a survey of legume leaves, gossypetin was only detected once, in *Acacia constricta*. Surveys indicate that herbacetin, the kaempferol analogue of gossypetin, is much less common, but it has been detected as principal flower pigment in *Meconopsis paniculata* (Papaveraceae). Consideration of the known natural distribution of the two yellow flavonols indicates that they are of most interest as taxonomic markers at the generic and subfamilial levels.

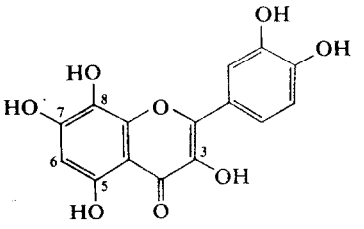
INTRODUCTION

YELLOW flavonol pigments, as distinct from the three common near-colourless flavonols, are relatively rare in higher plants[1,2] and the study of their natural distribution is interesting both taxonomically and from the point of view of their function as flower pigments.

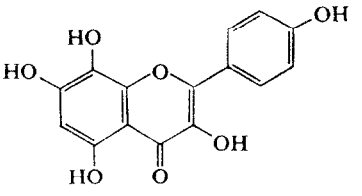
Earlier studies[3] revealed such pigments in several unrelated angiosperm families and the compounds then appeared to be based on quercetagetin (6-hydroxyquercetin), a pigment first isolated from *Tagetes* in 1877.[4] The apparent distinction from the closely similar 8-hydroxy isomer gossypetin (I), a pigment of *Gossypium* flowers,[5] was based mainly on R_f values, colour reactions and conversion to the corresponding anthocyanidin. Further studies[6] carried out with more reliably identified marker compounds, then showed that the two isomers are identical in R_f and can only be distinguished by detailed u.v. and i.r. spectral analysis. Re-examination of a pigment in the Primulaceae, earlier reported as quercetagetin 3-gentiotrioside,[3] showed it to be the gossypetin derivative.[6] Corrected structures are presented here for the other pigments misidentified in the earlier studies. Further studies of the distribution of gossypetin (I) and the related herbacetin (II) are also reported and the taxonomic value of these pigments is discussed.

* Part VIII in the series "Comparative Biochemistry of the Flavonoids"; for Part VII, see J. B. HARBORNE, *Phytochem.* **7**, 1215 (1968).

[1] J. B. HARBORNE, in *Chemistry and Biochemistry of Plant Pigments* (edited by T. W. GOODWIN), pp. 247–278. Academic Press, New York (1965).
[2] J. B. HARBORNE, *Bull. Nat. Inst. Sci. India* 52 (1965).
[3] J. B. HARBORNE, *Phytochem.* **4**, 647 (1965).
[4] LATOUR and MAGNIER DE LA SOURCE, *Bull. Soc. Chim. Paris.* **228**, 337 (1877).
[5] A. G. PERKIN, *J. Chem. Soc.* **75**, 825 (1899).
[6] J. B. HARBORNE, *Phytochem.* **7**, 1215 (1968).



Gossypetin (I)



Herbacetin (II)

RESULTS

Identification of Gossypetin and Distinction from Quercetagetin

When these yellow flavonols are isolated in quantity from a plant source, the distinction between them can be made on the basis of physical properties, preparation of derivatives,[7] degradative studies[8] and NMR spectral analysis.[9] In phytochemical surveys, where such compounds are usually only available in milligram amounts or less, other methods have to be adopted. In surveys of hydrolysed leaf or petal plant extracts, the presence of either flavonol is readily apparent during paper chromatography in strongly-acid solvents, such as Forestal, when they both appear as visible yellow spots, dull black in u.v. light with the colour unchanged by ammonia. Although there are slight differences in R_f and also in the colour on

TABLE 1. CHROMATOGRAPHIC, SPECTRAL AND COLOUR PROPERTIES OF GOSSYPETIN, QUERCETAGETIN AND HERBACETIN

Property	Gossypetin*	Quercetagetin*	Herbacetin†
	λ_{max} (long wave bands in nm)		
Spectral:			
In 95% EtOH	338, 386	365	330, 380
+$NaBH_4$	435	405	435
+NaOAc	370, 625	381	370, 625
	l.r. max in the 9–10·5 μ region		
KBr disc	9·5, 10·1, 10·3 (medium intensity)	9·25, 9·75, 10·45 (strong intensity)	9·5, 10·1, 10·5 (medium or weak intensity)
Colour:			
With EtOH–NaOAc‡	Blue	Yellow	Blue
With *p*-benzoquinone in EtOH (satd. soln.)	Purple–red	Yellow	Wine–red

* Chromatography in a range of solvents on paper and on TLC (cellulose, polyamide and silica gel) failed to separate gossypetin and quercetagetin. E.g., on paper: Forestal R_fs both 0·26; BAW R_fs 0·31. Both give dull black colours in u.v. light.

† The spectral max. for herbacetin is given in Ref. 13 as 370 nm; this was measured on an impure sample, and is now known to be incorrect.

‡ Colour test can be carried out in solution, when blue colour fades within 5–10 min. The colour on paper chromatograms is more permanent (lasts 48 hr) but takes *ca.* 30 min to develop. The "Gossypetone test" is relatively insensitive and does not lend itself to use as a colour reaction on paper.

[7] T. A. GEISSMAN (editor), *Chemistry of Flavonoid Compounds*, Pergamon Press, Oxford (1962).
[8] T. A. GEISSMAN and C. STEELINK, *J. Org. Chem.* **22**, 946 (1957).
[9] M. B. THOMAS and T. J. MABRY, *Phytochem.* **7**, 787 (1968).

paper or TLC, these are not sufficient for distinguishing between the isomers and, on co-chromatography, they show no separation. A simple colour test, based on dipping chromatograms in alcoholic sodium acetate when gossypetin gives a blue-grey colour and quercetagetin is unchanged, has now found to be an effective means of distinction. Confirmation can then be based on differences in spectral and other colour properties, as summarized in Table 1.

Differences that are apparent in these tests (Table 1) are clearly sufficient to distinguish between the two isomers and these have been carefully checked for reliability. Other micro-scale tests have been sought, so far without success. An earlier attempt[10] to use differences in the products obtained on reductive cleavage has subsequently been found to be unreliable in that it is not always possible to detect the pyrogallol formed from gossypetin, but not from quercetagetin, probably because of its considerable instability in alkaline media.

Identification of 8-hydroxykaempferol (herbacetin, II) on a micro-scale follows from its similarity in properties (Table 1) to gossypetin and analogous differences from quercetagetin.[6] 6-Hydroxykaempferol is not known as a natural product and a synthetic sample was not available.

Correction of Earlier Structures for Yellow Flavonols

Re-investigation of structures earlier thought[3] to be quercetagetin-based, as has already been reported briefly,[6] showed them all to be in fact gossypetin derivatives. Thus, the "quercetagetin 3-galactoside" reported in flowers of *Lotus corniculatus* and *Rhododendron* species is gossypetin 3-galactoside. Again, the "quercetagetin 7-glucoside" in *Papaver nudicaule* petals is gossypetin 7-glucoside (gossypitrin), a fact confirmed by direct comparison with authentic material from *Chrysanthemum segetum*.[8] Finally, the "quercetagetin 7-methyl ether 3-galactoside" of *L. corniculatus* must be the corresponding glycoside of gossypetin 7-methyl ether since it has now been found to give gossypetin, and not quercetagetin, on demethylation. Assignment of the methyl group to the 7-position is based on spectral analysis (e.g. lack of acetate shift, positive borate shift) and its behaviour on reductive cleavage, when it yields some 3,4-dihydroxyphenylpropionic acid, but also large amounts of diphenylpropane derivatives, a characteristic of 7-*O*-methylated flavonoids.[10] Attempts to confirm this structure by synthesis from gossypetin, by complete methylation followed by selective demethylation, have so far failed.

There have been several earlier reports of the occurrence of quercetagetin in leaves of plants by Bate-Smith and others (cf. Ref. 11), which were based solely on R_f and colour reactions. Clearly these reports could equally well refer to the presence of gossypetin and confirmatory colour and spectral tests are necessary to settle the structure of these pigments. Re-examination of the leaf pigment in *Empetrum nigrum*[11] has shown that the compound is, in fact, gossypetin and not the 6-hydroxyisomer, quercetagetin, a result expectable on taxonomic grounds (see below).

Natural Distribution of Gossypetin and Herbacetin

Continued surveys have been carried out of the occurrence of these yellow flavonols, particularly in the families already known to contain them. A detailed survey of the flavonoids of the Primulaceae has recently been published[6] and similar surveys in the Ericaceae and the Compositae will be reported separately. It is, however, worth summarizing (Table 2) the

[10] H. M. HURST and J. B. HARBORNE, *Phytochem.* 6, 1111 (1967).
[11] E. C. BATE-SMITH, *Mem. Bull. Soc. Bot. France* 16 (1965).

TABLE 2. NATURAL DISTRIBUTION OF GOSSYPETIN AND HERBACETIN

Plant source*		Organ	Pigment present†	References
ARCHICHLAMYDEAE				
Papaveraceae	*Meconopsis paniculata* (Don) Prain	P	Herbacetin 3-glucoside	This paper
	Papaver nudicaule L.	P	Gossypetin 7-glucoside†	This paper
Leguminosae	*Acacia constricta* Benth.	L	Gossypetin (as glycoside)	This paper
	A. catechu Willd.	Wood	Gossypetin (?)	15
	Lotus corniculatus L.	P	Gossypetin 3-galactoside† 7-*O*-Methylgossypetin 3-galactoside†	This paper
Crassulaceae	*Sedum album*	S	Gossypetin, herbacetin	17
	Sedum acre L. var. *sexangulare* (L.) Koch	S	8-*O*-Methylherbacetin	17
Malvaceae	*Gossypium arboreum* L. *G. herbaceum* L., *G. neglectum* Tor.	P	Gossypetin and herbacetin 7-glucosides	7
	Hibiscus esculentus L. *H. sabdariffa* L., *H. vitifolius* L.	P	Gossypetin and hibiscetin (as glucosides)	7
SYMPETALAE				
Ericaceae‡	*Rhododendron* spp.	76 spp L. 10 spp P.	Gossypetin 3-galactoside†	This paper
	Erica arborea L. var. *alpina*	L		
	E. ciliaris L. var. *aurea*	L		
	Kalmia latifolia L.	L		
	K. angustifolia L.	L		
	Ledum columbianum Piper	L		
	L. groenlandicum Gunn	L		
	L. palustre L.	L		
	Phyllodoce empetriformis D. Don	L		
	P. caerulea (L.) Bab.	L		
	P. nipponica Mak.			
	× *Phyllothamnus erectus* Schncid.	L		
	Rhodothamnus chamaecistus (L.) Rchb.	L		
Empetraceae	*Empetrum nigrum* L.	L	Gossypetin (as glycoside)	
	E. hermaphroditum Hagerup	L		
Primulaceae	*Primula* 7 spp.	P	Gossypetin 3-gentiotrioside†	6
	Dionysia aretioides (Lehm) Boiss.	L	Gossypetin (as glycoside)	6
	Douglasia vitaliana B & H	P		
Scrophulariaceae	*Mimulus luteus* L.	P	Herbacetin 7-glucoside†	6
Compositae	*Chrysanthemum segetum* L.	P (ray)	Gossypetin 7-glucoside	8

Key: P = petal, L = leaf, S = shoot.

* Species which may have either quercetagetin or gossypetin are *Vauquelinia californica* (Rosaceae)[11] and *Coronilla glauca* (Leguminosae).[3] A yellow flavonol of the same R_f as gossypetin has also been detected in *Eriocaulon decangulare* leaf but not in sufficient quantity for further characterization (E. C. Bate-Smith and J. B. Harborne, unpublished observations).

† Pigments marked thus are "corrected" structures.

‡ Full details of the survey of the Ericaceae will be published separately. Gossypetin has been tentatively identified also in leaf of *Bruckenthallia speciliforum*, *Loiseluria procumbens*, *Leiophyllum buxifolium* and *Menziesia lasiophylla*. Significantly, it could not be detected in leaf of thirty-six species from other families in the order Ericales (i.e. it was absent from twenty-five spp. from fourteen genera of the Epacridaceae, four spp. of *Clethra*, Clethraceae and seven spp. from two genera of the Pyrolaceae).

present known distribution patterns of these compounds, including reports from other laboratories.

It is probable that quercetagetin, far from being fairly widespread as was originally envisaged, is in fact rare and confined to one or two tribes of the Compositae[7,12] and to part of the Leguminosae (see below); quercetagetin methyl ethers are, however, known in a few other families (see e.g. Ref. 13).

Herbacetin, by contrast to gossypetin, seems to be very uncommon and it occurs mainly in conjunction with gossypetin. It was earlier found on its own in *Mimulus luteus*[6] and with gossypetin in *Primula alpicola* and *P. vulgaris*.[6] During the present work, following on the discovery of gossypitrin in *Papaver nudicaule*, another good source of herbacetin has been found in petals of *Meconopsis paniculata* where it occurs as the principal pigment. Yellow flavonols, however, compete as yellow pigments in poppies with the nitrogen-based nudicaulin[3] (in *P. nudicaule* and *M. cambrica*) and with carotenoids (*M. integrifolia*) and are not common.

The distribution of gossypetin in the Leguminosae has also been studied, following the recognition that the pigments in *L. corniculatus* flowers are the 3-galactosides of gossypetin and its 7-methyl ether and not the related quercetagetin derivatives. It has, however, not been possible to recheck the possibly mistaken report[3] of quercetagetin in *Coronilla glauca* flowers. The Leguminosae is, however, one of the few families with both isomers, since quercetagetin has been reliably reported in petals of *Leucaena glauca* Benth.[14] and, less definitely, in wood of *Acacia catechu*.[15] This latter report may in fact refer to gossypetin, since a survey of *Acacia* leaf tissue revealed the presence of gossypetin in another species *A. constricta*. It was discovered during a survey of herbarium material for isoflavonoids and was successfully identified in a few leaf fragments from a sheet dated 1885. Gossypetin, however, was not found in forty-one other *Acacia* species or in some 200 other legumes examined, and is clearly rare as a leaf character.

DISCUSSION

The yellow flavonols gossypetin and herbacetin have now been found in nine angiosperm families and are probably present in at least two or three other families as well (Table 2). These are, however, in any classification, widely scattered plant groups and their occurrence therefore lacks any broad taxonomic significance. Their primary function, together with quercetagetin, is as yellow petal pigments and coloration is presumably achieved by simple introduction of extra hydroxyl groups into the widely occurring common flavonols, kaempferol and quercetin, though this has yet to be proved by biosynthetic studies. Contrary to an earlier belief,[1–3] 8-hydroxylation is much more common than 6-hydroxylation, a fact which may reflect the greater reactivity of the 8-position in flavonols to anionic attack.

Gossypetin and its relatives make a significant contribution to flower colour in several families and in at least two their occurrence is correlated with systematics. In the Primulaceae, gossypetin appears to specifically replace carotenoid as the major yellow flower pigment in at least two sections, Sikkimenses and Vernales, of the genus *Primula*; it also occurs significantly in two related genera but not in more distant taxa.[6] In the Ericaceae, gossypetin is also limited as a petal pigment to species of a few sections of *Rhododendron*,[3] and again, it appears

[12] T. ANYOS and C. STEELINK, *Arch. Biochem. Biophys.* **90**, 63 (1960).
[13] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, New York (1967).
[14] A. R. G. NAIR and S. S. SUBRAMANIAN, *Current Sci. (India)* **31**, 504 (1962).
[15] D. E. HATHWAY and J. W. T. SEAKINS, *Biochem. J.* **65**, 32 (1957).

to replace carotenoid as major flower pigment when it occurs. Curiously, gossypetin is widespread as a leaf constituent in *Rhododendron*, being present in three-quarters of the 100 or so species surveyed; its function here is at present obscure. It is of taxonomic interest as a leaf constituent since it also occurs in eight other genera of the Ericaceae (see Table 2) more or less related to *Rhododendron*. Thus gossypetin is present in two genera (*Ledum, Menziesia*) of the same tribe Rhododendreae and in five genera of the related tribe Phyllodoceae; it also occurs in the more distant tribe Ericeae (*Erica, Bruckenthallia*) of the subfamily Ericodeae. Systematically, it is interesting to find it also in *Empetrum* (Empetraceae) since this genus and family have long been recognized as being very closely related to the Ericaceae.

Evidence has been obtained that gossypetin and quercetagetin are of taxonomic interest in the Compositae, since one occurs in *Chrysanthemum* and the other in *Chrysanthemum, Coleostephus, Hymenoxys* and *Tagetes*. Surveys are now being extended in the tribes Heleniae and Anthemideae. It is satisfying to find that at present they are not known to co-occur with other yellow flavonoid pigments of the Compositae, the chalcones and aurones, which are confined almost entirely to the Coreopsidineae (Heliantheae).

Finally, the occurrence of yellow flavonols in the Papaveraceae may well be of chemotaxonomic interest, particularly if they are not detected, as they have not yet been, in the Cruciferae and its relatives. Gossypetin might thus provide yet another chemical character (like presence of alkaloid, absence of sulphur compounds) distinguishing the Papaveraceae from other families in the order Rhoedales, *sensu* Engler, and providing support[16] for the removal of this family from the order, as proposed by Takhtajan.

EXPERIMENTAL

Plant material was obtained from University of Liverpool Botanic Gardens at Ness. Dried leaf material was obtained from University of Liverpool herbarium. Flowers of *Lotus corniculatus* L. were collected locally.

Methods of isolation and identification of pigments. Methods described in earlier publications (cf. Ref. 13) were used. The identification of gossypetin and herbacetin in various plant species was carried out as for *Primula*.[6] In survey work, identification of gossypetin was based at least on (1) co-chromatography in four to six solvent systems, (2) a positive blue colour on paper with NaOAc and (3) correct spectral maxima in the visible region.

Gossypetin 7-methyl ether. The properties of this compound are as described for Lotus FlA in an earlier paper.[3] On demethylation, it gave gossypetin, and not quercetagetin as stated earlier.[3] Assignment of the methyl group to the 7-position was based partly on its spectral properties and partly on its behaviour on reductive cleavage. Its spectral maxima in 95% EtOH (260, 270*, 335*, 381 nm) were very similar in position and intensity to those for gossypitrin (gossypetin 7-glucoside) (261, 275*, 345, 387 nm). It failed to give a NaOAc shift (7-position blocked), but gave a normal H_3BO_3 shift (3′,4′-dihydroxyl free) and a large $AlCl_3$ shift (3-hydroxyl free). It gave a very weak positive reaction in the gossypetone test but failed to respond to the NaOAc colour test. On reductive cleavage with Na–Hg, it gave 3,4-dihydroxyphenylpropionic acid and the corresponding alcohol, and also three unidentified catechols, which from their R_f values on two-dimensional silica gel plates,[10] were diphenylpropane derivatives.

Herbacetin 3-glucoside was isolated from petals of *Meconopsis paniculata* by chromatography in butanol–acetic acid–water (BAW), when it was obtained as deep-yellow needles. Its spectral properties were as follows: $\lambda_{max}^{95\%\ EtOH}$ 279, 308, 331, 372* nm, $\lambda_{max}^{EtOH-NaOH}$ 384 nm changing in 2–3 min to 356 nm, $\lambda_{max}^{AlCl_3}$ 358 nm. On acid hydrolysis, it gave glucose and herbacetin, identified by direct u.v., i.r. and R_f comparison with material from *Primula alpicola*.[6] On oxidation with H_2O_2, it gave only glucose. It is formulated as the 3-glucoside from this evidence, its spectral properties (e.g. the large hypsochromic shift when compared with herbacetin) and its R_fs, which were 0·53 (BAW), 0·17 (H_2O) and 0·50 (PhOH). The glucoside of herbacetin isolated earlier from *Mimulus luteus*,[3] and called mimulin, can now be formulated as the known 7-glucoside, herbacitrin,

* Inflection.

[16] A. KJAER, in *Comparative Phytochemistry* (edited by T. SWAIN), pp. 187–194, Academic Press, New York (1966).

[17] M. COMBIER, K. MARKHAM, H. AUDIER, P. LEBRETON, T. MABRY and M. JAY, *Compt. Rend.*, in press (1968).

first isolated from *Gossypium herbaceum* by Neelankantam and Seshadri.[18] Thus, in its spectral properties, it resembles herbacetin as gossypitrin does gossypetin, but fails to give a NaOAc shift (7-hydroxyl blocked). Again, its R_fs are similar to the 3-glucoside in BAW (0·51) and PhOH (0·46) but differ expectedly in H_2O (0·02).

Acknowledgements—The author is grateful to the Science Research Council for financial support and to Miss C. A. Williams for experimental assistance.

[18] K. Neelankantam and T. R. Seshadri, *Proc. Indian Acad. Sci.* **4A**. 54 (1936).