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# FLAVONOID PATTERNS OF THE RESTIONACEAE. GOSSYPETIN IN *RESTIO* AND A NEW FLAVONE IN *HYPOLAENA**

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**Abstract**—A survey of nine species of six genera of the Restionaceae revealed the presence of the yellow flavonol gossypetin in stem or inflorescence of three *Restio* species and of *Calorophus lateriflorus* and of a new flavone of related structure in *Hypolaena fastigiata*. These represent the first records in the monocotyledons of flavonols and flavones with extra 8-hydroxyl substituents. The new flavone, hypolaetin, was identified as 5,7,8,3′,4′-pentahydroxyflavone and, on acid treatment, it isomerized to the known 5,6,7,3′,4′-isomer, 6-hydroxyluteolin. Cyanidin 3-glucoside was detected in stems and shoots of two *Restio* species and leucocyanidin in all three *Restio* species examined. The results of the survey are discussed in relation to the known flavonoid patterns in the Cyperaceae, Poaceae, and Eriocaulaceae.

## INTRODUCTION

THE CLASSIFICATION of the grasses (Poaceae = Gramineae) and families related to it has caused considerable difficulty and most taxonomic treatments of these vary at the ordinal level. This is the kind of situation where chemistry may be of help and, indeed, surveys that have been carried out to date in the monocotyledons have shown that flavonoid pigments, in particular, are useful characters at the family level.

Thus, an earlier survey[1] of flavonoids in the grasses indicated the presence of the flavone tricin and of *C*-glycosylflavones as distinctive leaf constituents and of cyanidin 3-glycosides as characteristic pigments of leaf, inflorescence and fruit.[2] A study of the supposedly related sedges (Cyperaceae) showed that anthocyanins were absent, but that the aurone aureusidin and a new 3-desoxyanthocyanidin, carexidin, were typical pigments in the family.[3] More recently,[4] a study of *Eriocaulon* (Eriocaulaceae) showed the presence, in nearly all species examined, of the yellow flavonol, quercetagetin, a substance not previously recorded in the monocotyledons.

This survey has now been extended to the Restionaceae, the flavonoids of which have not yet been examined,[5] and has led to the discovery, for the first time in monocotyledons, of

* Part XII in the series "Comparative Biochemistry of the Flavonoids"; for Part XI, see Ref. 4.

[1] J. B. HARBORNE and E. HALL, *Phytochem.* **3**, 421 (1964).
[2] H. T. CLIFFORD and J. B. HARBORNE, *Proc. Linn. Soc. Lond.* **178**, 125 (1967).
[3] H. T. CLIFFORD and J. B. HARBORNE, *Phytochem.* **8**, 123 (1969).
[4] E. C. BATE-SMITH and J. B. HARBORNE, *Phytochem.* **8**, 1035 (1969).
[5] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Vol. II, Birkhauser verlag, Switzerland (1963).

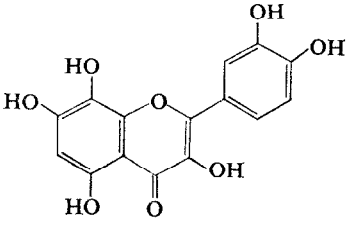
gossypetin, a yellow flavonol isomeric with quercetagetin, and of a new flavone, the structure of which is described in this paper. The Restionaceae, with about 400 species grouped into twenty to thirty genera, has been variously classified near the Eriocaulaceae,[6] the Poaceae[7] and both the Poaceae and Cyperaceae.[8]

## RESULTS

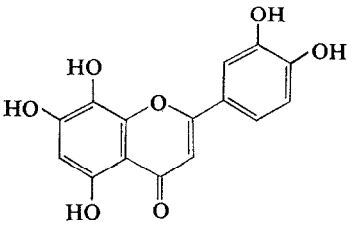
The results of limited survey of nine species of living plants of the Restionaceae native to Eastern Australia are presented in Table 1. The study of the flavonoids was hindered by the large quantities of hydroxycinnamic acid esters present. Acid hydrolysis yielded all four of the common acids, namely *p*-coumaric, caffeic, ferulic and sinapic acids. This result agrees with earlier observations of Bate-Smith[9] on two species.

Visible brown or red colours were present in some part of all species examined; that in shoot and stem of two of the *Restio* species was provisionally identified as cyanidin 3-glucoside, a pigment very common in the grasses. Colour in the fruits of most species appeared to be insoluble and presumably associated with the cell wall. Leucoanthocyanidins based on cyanidin were found specifically in the three *Restio* species.

Quercetin was found in one species, *Restio tetraphyllus*, but none of the other common flavonols and flavones of higher plants was detected in acid-hydrolysed extracts of these plants. Instead, the relatively rare yellow flavonol gossypetin (I) was discovered in four species. It was identified by comparison with authentic material and the sodium acetate test was used to distinguish it from the closely similar quercetagetin.[10] More detailed examination of direct extracts of one of the four species *R. tetraphyllus* showed that it was present as the 7-glucoside, gossypitrin. In addition, a new yellow flavone was found (in glycosidic form) in stems of *Hypolaena fastigiata*, a monotypic species. The aglycone was named hypolaetin, and identified as 5,7,8,3′,4′-pentahydroxyflavone (II), i.e. the flavone analogue of gossypetin, from the following data.




Gossypetin (I)




Hypolaetin (II)

The molecular weight of hypolaetin, determined by mass spectral analysis, indicated that it was a pentahydroxyflavone. U.v. spectral analysis in neutral solution ($\lambda_{max}$ 280, 342 nm) indicated that it was a flavone rather than a flavonol; a positive borate shift showed that it contained a 3′,4′-dihydroxyl grouping and the magnitude of the $AlCl_3$ shift showed it contained a 5-hydroxyl. Its dark-absorbing colour in u.v. light indicated the presence of

[6] U. HAMANN, in *Syllabus der Pflanzenfamilien*, Vol. II, p. 555, Borntraeger, Berlin (1964).
[7] A. TAKHTAJAN, *Die Evolution der Angiospermen*, p. 288, Fischer, Jena (1959).
[8] J. HUTCHINSON, *The Families of Flowering Plants*, Vol. 2, p. 517, Clarendon Press, Oxford (1959).
[9] E. C. BATE-SMITH, *J. Linn. Soc. (Botany)* **60**, 325 (1968).
[10] J. B. HARBORNE, *Phytochem.* **8**, 177 (1969).

TABLE 1. FLAVONOIDS OF THE RESTIONACEAE

| Genus/Species | Yellow flavonol | Anthocyanin | Leucoanthocyanidin | Unidentified aurone | Unidentified mauve compd. |
|---|---|---|---|---|---|
| *Calorophus lateriflorus* (R. Br.) F. Muell. | Gossypetin | – | – | – | + |
| *Coleocarya gracilis* S. T. Blake | – | – | – | + | + |
| *Hypolaena fastigiata* R. Br. | Hypolaetin | – | – | + | + |
| *Leptocarpus brownii** Hook. f. | – | – | – | – | – |
| *Lepyrodia interrupta* F. Muell. | – | – | – | + | + |
| *L. muelleri** Benth. | – | – | – | – | – |
| *Restio pallens* R.Br. | Gossypetin (stem) | Cyanidin 3-glucoside | Leucocyanidin | + | + |
| *R. tenuiculmis* S. T. Blake | Gossypetin (stem) | Cyanidin 3-glucoside | Leucocyanidin | + | + |
| *R. tetraphyllus* Labill. | Gossypetin‡ (inflorescence) | – | Leucocyanidin | + | + |

* Fruits only examined for these plants; with other species, shoots, stems, inflorescence and fruits were examined separately.

† Unidentified aurone (?) showed a very low $R_f$ in Forestal and BAW and gave a yellow fluorescence changing to orange in u.v. + $NH_3$. The unidentified mauve compound had $R_f$ 0·25 in Forestal. All species examined as stem showed the presence of *p*-coumaric, caffeic, ferulic, sinapic and gentisic acids.

‡ As the 7-glucoside, gossypitrin. Quercetin 3-glucoside is also present in the inflorescence.

6- or 8-hydroxylation, and that it was thus either 5,6,7,3′,4′- or 5,7,8,3′,4′-pentahydroxyflavone. It was spectrally different from the known 6-isomer, 6-hydroxyluteolin, and also had a slightly higher $R_f$ in most solvents. It could thus be formulated as the 8-isomer (II), a new flavone, and this structure was confirmed when, on acid treatment, it underwent ring opening and isomerization to 6-hydroxyluteolin, identified by direct comparison with an authentic sample. The 6-hydroxy isomer is known to be the stable structure[11] and attempts to convert this compound back to hypolaetin expectedly failed. While flavones with extra hydroxylation (or methoxylation) in the 6-position or the 6- and 8-positions are well known,[12] this appears to be the first report of a flavone with the 5,7,8,3′,4′-hydroxylation pattern. The only comparable substance is wogonin (5,7-dihydroxy-8-methoxyflavone), which occurs in the roots of *Scutellaria baicalensis*,[13] but which lacks B-ring hydroxyls.

## DISCUSSION

The discovery of the relatively rare flavonol gossypetin (I) and the related flavone (II) in the Restionaceae is of general systematic interest, since this class of compound has only previously been known in the dicotyledons. It confirms the already expressed view[9,12] that the two major angiosperm groups are broadly similar in flavonoid pattern. Apparent differences in pattern noted earlier[14] are, as this present paper shows, certainly due to the fact that the monocotyledons have not been as intensively surveyed as the dicotyledons.

It is interesting to contrast the presence of gossypetin in the Restionaceae with the occurrence of the isomeric 5,6,7,3′,4′-pentahydroxyflavonol, quercetagetin, in the Eriocaulaceae.[4] In the Sympetalae, gossypetin occurs in the relatively less advanced families Ericaceae, particularly the very "ancient" genus *Rhododendron*, and the Plumbaginaceae,[10] whereas quercetagetin (or the related flavone, 6-hydroxyluteolin) is found in the more advanced Compositae, Bignoniaceae, Globulariaceae and Scrophulariaceae.[15] Within this group of families quercetagetin might be accepted as a more "advanced" character than gossypetin. Accepting the same relationships to apply in the monocotyledons the Eriocaulaceae are to be regarded as chemically more advanced than the Restionaceae. Such an opinion is in accord with the phylogenetic speculations of Hutchinson[8] and Takhtajan[7] who regard the Restionaceae as a relatively unspecialized family ancestral to the Poaceae and the Eriocaulaceae as a specialized family terminating a separate line of evolution.

From the systematic point of view, the occurrences of these yellow pigments apparently distinguishes the restiads not only from the Eriocaulaceae but also from the sedges and grasses. Further surveys among a wider range of material are however necessary to test the consistency of these differences in flavonoid patterns.

## EXPERIMENTAL

*Plant Material*

The plants were collected and identified in the field by H. T. C. and airmailed to England for examination by J. B. H.

[11] T. R. SESHADRI, in *Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN), p. 184, Pergamon Press, Oxford (1962).
[12] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, London (1967).
[13] S. HATTORI, *Acta Phytochim. (Japan)* **5**, 99 (1930).
[14] J. B. HARBORNE, in *Comparative Phytochemistry* (edited by T. SWAIN), p. 271, Academic Press, London (1966).
[15] J. B. HARBORNE, unpublished results.

*Identification of Known Phenolics*

Plant organs were examined separately for flavonoid aglycones using standard procedures. Gossypetin was identified in hydrolysed extracts by co-chromatography in four solvents, by use of colour tests and by u.v. spectral analysis. Hydroxycinnamic acids in hydrolysed stem extracts were identified by two-dimensional TLC on Cellulose MN300 using benzene–acetic acid–water (6:7:3) and 15 per cent acetic acid.

Cyanidin 3-glucoside was identified in stems and shoots of *Restio pallens* and *R. tenuiculmis*, by co-chromatography, colour reactions and spectral measurements. Under these conditions, it is not distinguished from the 3-galactoside and its identification as the 3-glucoside is thus provisional.

A concentrated 70 per cent EtOH extract of *R. tetraphyllus* inflorescences, on chromatographic separation in butanol–acetic acid–water and 5 per cent HOAc on No. 3 paper, yielded two major flavonol bands. The faster-moving band ($R_f$ 0·50; 0·08) was identified as quercetin 3-glucoside and the slower-moving band ($R_f$ 0·25; 0·02) as gossypetin 7-glucoside (gossypitrin). Identification was based in both cases on co-chromatography with authentic samples in six solvents, spectral analysis and hydrolysis to give glucose and the appropriate aglycone.

*Isolation and Identification of Hypolaetin*

Whole plants of *Hypolaena fastigiata* were hydrolysed in 2 N HCl and the ethyl acetate extract was chromatographed on Whatman No. 3 paper in 50 per cent HOAc and BAW. Hypolaetin was obtained as deep yellow microneedles, m.p. 296°, which gave a red-brown colour in the gossypetone test. Mass spectral analysis showed a single peak at 302 ($C_{15}H_{10}O_6$ requires m.w. 302) and it was unaffected by heating with pyridinium chloride (conditions for demethylation). It had EtOH $\lambda_{max}$ 257, 280 and 342 nm; it gave a bathochromic shift with alkali (broad band at 380 nm), with NaOAc–$H_3BO_3$ (359 nm), with NaOAc (284 nm) and with $AlCl_3$ (346, 386 nm). On reductive cleavage, it gave 3,4-dihydroxyphenylpropionic acid and the corresponding alcohol and traces of pyrogallol but no phloroglucinol. On paper chromatograms, it gave a dull black colour in u.v. light, changing to dull green with ammonia and it had the following $R_f$s ($\times$ 100), 6-hydroxyluteolin values in parentheses: 50 (45) in Forestal; 54 (45) in butanol–acetic acid–water (4:1:5); 36 (29) in 50 per cent HOAc; 45 (38) in butanol–ethanol–water (4:1:2·2); and 38 (38) in PhOH. After heating with N HCl–EtOH (1:1) for 7 hr at the b.p., 70% was converted to 6-hydroxyluteolin, identified by spectral and $R_f$ comparison with authentic material, isolated from *Catalpa bignonioides* leaf or by demethylation of sinensetin (5,6,7,3′,4′-pentamethoxyflavone).

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