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FLAVONOID PIGMENTATION IN THE SEDGES (CYPERACEAE)*

H. T. CLIFFORD

Botany Department, University of Queensland, Brisbane

and

J. B. HARBORNE

Phytochemical Unit, Botany Department, University of Liverpool

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Abstract—A survey of pigmented leaf, inflorescence or seed of eighteen species representing fifteen genera of the Cyperaceae failed to reveal the presence of any of the common anthocyanins, earlier reported as being widespread in the supposedly related grasses (Poaceae). Instead, a new pigment, carexidin (probably a 3-desoxyanthocyanidin), was found in three species and the yellow aurone aureusidin, not previously known in the monocotyledons, was found in eight species. In addition, the chalcone okanin has been detected in the seed of *Kyllinga*. The taxonomic and phyletic significance of these results are discussed in relation to the existing classification of the grasses and sedges.

INTRODUCTION

A PREVIOUS survey[1] has shown that anthocyanin pigmentation, based mainly on cyanidin, is very common in leaf, inflorescence and seed of the grasses (Poaceae) and that the systematic distribution is not correlated, as it is in several dicotyledonous families,[2–4] with the major taxonomic groupings of species into tribes or subfamilies. This, it was argued, was to be expected for a wind-pollinated family where there is no selection for colour by pollen vectors, as there is in families such as the bird- and bee-pollinated Gesneriaceae,[2] Plumbaginaceae[3] and Primulaceae.[4]

In order to determine whether this conclusion based upon data solely for the grasses could be extended into a generalization for other wind-pollinated plant groups, it was decided to investigate the nature and distribution of flavonoid pigments amongst the Cyperaceae. The sedges are generally accepted as being allied to the grasses and an added incentive for studying their pigmentation, which has hardly been examined at all in the past,[5] was to compare the flavonoid pattern with that of the grasses.

RESULTS

Direct and acid-hydrolysed extracts of coloured leaf, inflorescence or seed of eighteen species of the Cyperaceae (Table 1) were examined for flavonoids by standard procedures.[6] No anthocyanins were detected in any of the tissues; nor were any common flavonoids found.

* Part IX in the series "Comparative Biochemistry of the Flavonoids".

[1] H. T. CLIFFORD and J. B. HARBORNE, *Proc. Linn. Soc. Lond.* **178**, 125 (1967).
[2] J. B. HARBORNE, *Phytochem.* **6**, 1643 (1967).
[3] J. B. HARBORNE, *Phytochem.* **6**, 1415 (1967).
[4] J. B. HARBORNE, *Phytochem.* **7**, 1215 (1968).
[5] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Vol. 2, pp. 124–133, Birkhäuser-Verlag, Berlin (1963).
[6] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, New York (1967).

Anthocyanidins were found in hydrolysed tissue of three species, but since no anthocyanin was present in the respective direct extracts, these were almost certainly derived from leucoanthocyanidins. Indeed, Bate-Smith[7] has recorded leucocyanidin or leucodelphinidin in leaf of seven of fourteen Cyperaceae species he examined.

TABLE 1. DISTRIBUTION OF FLAVONOID PIGMENTS AMONGST A SAMPLE OF SEDGE GENERA

Sub-family, tribe, genus and species*	Presence of		Other constituents
	Aureusidin	Carexidin	
CYPEROIDEAE			
Hypolytreae			
Lepironia articulata (Retz) Domin	+	+	—
Scirpeae			
Fimbristylis dichotoma (L.) Vahl	—	—	Leucodelphinidin
Eleocharis acuta R. Br.	+	—	—
Scirpus nodosus Rottb.	+	—	—
S. mucronatus L.	—	—	—
Rhynchosporeae			
Schoenus apogon Roem. et Schult.	+	—	—
S. brevifolius R. Br.	—	+	—
Gahnia clarkei G. Benl	+	—	—
G. aspera Spreng.	—	—	—
Caustis recurvata Spreng.	—	—	Leucocyanidin
Cladium junceum R. Br.	—	—	Unidentified yellow pigment
Ptilanthelium deustum (R. Br.) Kukenth	+	—	—
Lepidosperma laterale R. Br.	—	—	Leucocyanidin
Remirea maritima Aubl.	+	—	Second aurone
Cypereae			
Cyperus rotundus L.	+	—	—
Kyllinga brevifolia R. Br.	—	—	Okanin, leucodesoxyanthocyanidin
CARICOIDEAE			
Sclerieae			
Scleria hebecarpa Nees	—	+	—
Cariceae			
Carex riparia Curt.	—	+	—
C. acutiformis Ehrh.	—	+	—

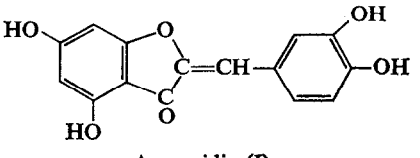
* Arranged according to Schultze-Motel.[12] Tissue examined was inflorescence or seed, except in the case of *Remirea* (leaf base).

The only anthocyanin-like pigment detected in the survey was what appears to be a new 3-desoxyanthocyanidin, called carexidin. This pigment occurs in the inflorescence of *Carex riparia* and the seed of *Lepironia articulata* and *Schoenus brevifolius*. It appears from R_f values to be present in these tissues without any glycosidic attachment. In its spectral properties, it resembles luteolinidin, but clearly differs from it or any other known 3-des-

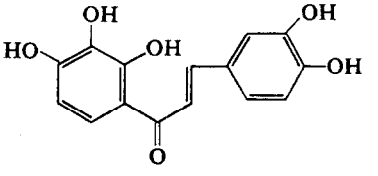
[7] E. C. BATE-SMITH, *J. Linn. Soc. (Bot.)* **60**, 383 (1968).

oxyanthocyanidin in chromatographic mobility (see Experimental). Insufficient material has been available for its further characterization.

A deep-yellow pigment was, however, extracted by hot alcohol from tissues of eight species (Table 1) and this was readily identified, by direct comparison with authentic material, as the aurone aureusidin (I). Aureusidin was accompanied in *Remirea* by a second aurone with a higher R_f value, but insufficient material was available for its characterization. It was different in colour and R_f from sulphuretin and is possibly a monomethyl ether of aureusidin. Aureusidin, previously known to occur in glycosidic form in petals of the Compositae and several other sympetalous families,[6] is present, quite remarkably, in these sedges in the free state, i.e. without any sugar attachment.



Aureusidin (I)



Okanin (II)

At least two other types of yellow phenolic pigment were detected in sedges during the present survey. One present in *Cladium* remains unidentified but another occurring in seed of *Kyllinga brevifolia* was determined as being the chalcone, okanin (II), on the basis of comparing its colour, spectra and R_f properties with authentic material. This result is of some general systematic interest, since chalcones have only been recorded once before in the Monocotyledoneae, in *Xanthorrhoea* (Liliaceae).[8,9]

From Table 1, it is apparent that both aureusidin and carexidin are widely distributed in the family. The distribution of these pigments within genera are certainly not very consistent, judging from the variations observed in *Gahnia*, *Scirpus* and *Schoenus*. Likewise, there is no constancy within the tribes; the lack of aureusidin from the subfamily Caricoideae is interesting but the data are too few to be accepted as being statistically significant. The pigments thus appear to be randomly distributed, which fits in with the conclusion, already drawn from the earlier grass survey,[1] that in predominantly wind-pollinated families, no correlation between pigment systems and taxonomic groups can be expected.

Whilst the grasses and sedges are alike in respect to their pollination mechanisms, they differ markedly in the pattern of their flavonoid pigments. The results of the present survey, together with those obtained by Bate-Smith[7] in studying the leaf constituents, are summarized in Table 2. They seem to differ in almost every character. Sedges have long been regarded as closely related to grasses and, in many taxonomic works, the two families constitute a single order. In more recent years, they have been accepted as differing at ordinal level and doubt has been expressed as to their being closely related.[10–12] The chemistry to date (Table 2) certainly indicates that they should be separated. While it would be tempting to relate the flavonoid characters to phyletic advancement of these families in the monocotyledons,

[8] H. DUEWELL, *J. Chem. Soc.* 2562 (1954).
[9] A. J. BIRCH and P. HEXTALL, *Australian J. Chem.* **8**, 263 (1955).
[10] J. HUTCHINSON, *The Genera of Flowering Plants*, Vol. 1, Oxford University Press (1964).
[11] A. TAKHTAJAN, *Die Evolution der Angiospermen*, Jena (1959).
[12] W. SCHULTZE-MOTEL, in *Syllabus der Pflanzenfamilien* (edited by H. MELCHIOR), Vol. 2, pp. 602–607, Springer-Verlag, Berlin (1964).

wider surveys are clearly needed before much weight can be given to the presence of the "advanced" aurone character in sedges and its concomitant absence from the grasses.

TABLE 2. DISTRIBUTION OF FLAVONOID CLASSES IN GRASSES AND SEDGES

Flavonoid character	Distribution in Grasses*	Distribution in Sedges
Anthocyanin	+++	–
3-Desoxyanthocyanidin†	+	++
Aurone	–	+++
Glycoflavone	+++	–
Leucoanthocyanidin	+	+++
Common flavonol	+	+
Flavone tricin	++	–
Chalcone	–	+

* Key: +++ = common, ++ = uncommon, + = rare, – = not detected. Data from Refs. 1, 5–7.

† The only occurrence in grasses of this class is in *Sorghum vulgare* (see Ref. 1).

EXPERIMENTAL

Plant Material

Plants were collected in the field in Australia and identified by H. T. C. and sent by airmail to J. B. H. or collected in England (*Carex*).

Pigment Analysis

Direct and acid hydrolysed extracts of leaf, inflorescence and seed tissues were examined for flavonoids by standard procedures. Aureusidin was isolated in quantity from leaf of *Ptilanthelium deustum* and identified by comparison with authentic material from *Antirrhinum majus* by u.v. spectra (of the pigment and its acetate) and by co-chromatography on paper (six solvents) and silica gel layers (two solvents). It was similarly identified in other tissues by co-chromatography and spectral analysis. *Carexidin* was obtained in quantity by extracting deep-crimson fruits of *Lepironia articulata* with hot EtOH. It was orange-red in solution ($\lambda_{max}^{MeOH-HCl}$ 491 nm, λ_{max}^{NaOMe} 550 nm) and appeared as a fluorescent yellow spot on paper in u.v. light. Its R_f values were 0·53 (Forestal), 0·41 (BAW), 0·01 (1% aq. HCl) and 0·37 (in BuHCl); it separated when chromatographed with known 3-desoxyanthocyanidins.[6] It was unaffected by heating with 2 N HCl for 0·5 hr. The unidentified yellow pigment extracted by EtOH from *Cladium* fruits had λ_{max} 281 and 324 nm, λ_{max}^{alk} 378 nm with R_f 0·01 in H_2O, 0·35 in BAW. It was decolorized on heating with acid. The second aurone in *Remirea* had R_f 0·59 in BAW (aureusidin 0·46) and 0·52 in Forestal (aureusidin 0·30), was similar in colour properties on paper, and separated when chromatographed with sulphuretin (R_f 0·75 in BAW). The yellow chalcone in *Kyllinga* fruits was identified as okanin by co-chromatography in six solvents and spectral comparison with authentic pigment.

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