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DISTRIBUTION AND TAXONOMIC SIGNIFICANCE OF FLAVONOIDS IN THE LEAVES OF THE CYPERACEAE*

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Abstract-Flavonoids were surveyed in leaves of 62 species from 11 genera of the Cyperaceae. Over half the sample contained glycoflavones, one half tricin, a third luteolin, while a sixth contained quercetin and/or kaempferol. Tricin was found as the 5-glucoside in Carex *riparia* and C. *acutiformis,* the same two species also yielding iso-orientin. Free tricin and a luteolin 7-arabinosylglucoside were obtained from leaves of *Cyperus longus.* Quercetin 3-rutinoside was detected in leaves of five Carex species. The presence of the characteristic leaf flavonoids (glycoflavones, tricin) of the grasses in this family shows that the Cyperaceae and the Gramineae are more closely linked chemically than a previous study of their inflorescence pigments suggested.

INTRODUCTION

PREVIOUS studies' of flavonoids in the sedges (Cyperaceae) established the presence of the aurone aureusidin and a novel desoxyanthocyanidin carexidin in the family. Anthocyanins-common pigments in the grasses-were found to be absent from pigmented tissue of 18 species from 15 sedge genera, but leucoanthocyanidins were occasionally detected.[1] These latter compounds were also detected in a leaf survey of 14 species from 7 genera by Bate-Smith,[2] who also found quercetin in four species, but did not record any flavones in the group. Although based on a small sample, these results indicated that there were differences in flavonoid pattern (in the inflorescence) between the sedges and the grasses, the family most closely allied to sedges in most taxonomic treatments.[1]

In order to gain further insight into the flavonoid pattern of this interesting family, a wider survey has now been undertaken, particularly with regard to the leaf constituents. These studies are part of an extensive survey among monocotyledonous families at one time or another associated from the morphological or evolutionary point of view with the grasses (Gramineae).[3-6] The opportunity was also taken to examine the flavonoids of *Carex,* the largest genus in the family, and of which over 60 species are native to the British Isles.' The results have shown for the first time that luteolin, tricin and glycoflavones are characteristic flavonoids in the leaves of the sedges.

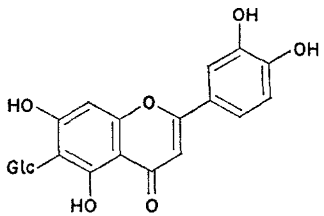
* Part XVI in the series "Comparative Biochemistry of the Flavonoids"; for Part XV, see C. W GLENNIE and J. B. HARBORNE, Phytochem. **10, 1379** (1971).

[1] H. T. CLIFFORD and J. B. HARBORNE, *Phytochem. 8, 123* (1969).
[2] E. C. BATE-SMITH, *J. Linn. Soc. Botan. 60,383* (1968).
[3] J. B. HARBORNE and H. T. CLIFFORD, *Phytochem.* **8, 2071** (1969).
[4] E. C. BATE-SMITH and J. B. HARBORNE, *Phytochem.* 8, 1035 (1969).
[5] H. T. CLIFFORD and J. B. HARBORNE, *Proc. Linn. Soc. Lond. 178, 125* (1967).
[6] C. A. WILLIAMS, J. B. HARBORNE and H. T. CLIFFORD, *Phytochem.* 10, 1059 (1971).
[7] A. C. JERMY and T. G. TUTIN, *British Sedges,* B.S.B.I. (1968).

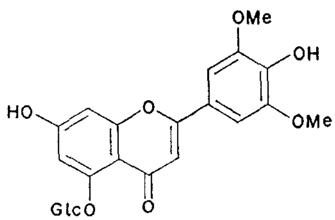
RESULTS

Direct and acid hydrolysed extracts of leaf material from 62 sedge species were examined for flavonoids by standard procedures, with the results shown in Table 1. Of the four flavonoid characters recorded in this Table, three have not been reported in the family before, namely presence of tricin, luteolin and glycoflavones. The fourth, quercetin, was detected by Bate-Smith,* but the present survey shows that flavonols are relatively rare, occurring in only nine taxa (15 % of the sample). Quercetin is apparently completely absent from the Cypereae and Rhynchosporeae, but it does occur in the Scirpeae (2 records) and in Curex (7 records). In all the ***Curex*** species, quercetin was accompanied by kaempferol and it occurred combined as the commonest of all glycosides, as the 3-rutinoside (rutin).

One of the most significant discoveries of the present survey is that glycoflavone occurs in over half (55 per cent) of the sedge species examined, since this is a character new to the family. The actual glycoflavone present was not identified in most instances, but from R_fs and colour reactions, the compounds present seem to be of the usual type, i.e. based on luteolin or apigenin. One such compound, iso-orientin or 6-*C*-glucosylluteolin (I), was positively identified in ***Carex*** *acutiformis* and C. *riparia* and a survey of direct extracts of ***Carex*** leaves suggested that the same (or similar) substance was present in most of the taxa of this genus recorded in Table 1 as containing glycoflavone.



(I) Iso-orientin



(II) Tricin 5-glucoside

By contrast with the rarity of flavonols in the leaves of sedges, flavones are very common (Table 1) and two such compounds, luteolin and tricin, were detected as regular constituents (37 and 47% respectively) in the group. Since tricin, unlike luteolin, is generally very rare in plants,' its identification was confirmed by exact mass spectral measurement on material isolated from ***Carex*** *riparia*. More detailed study of the presence of tricin in C. *riparia* and the related C. *acutiformis* indicated it was present mainly as the 5-glucoside (II), a compound which has previously been found in ***Triticum*** (Gramineae).[9,10] However, similar

[8] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, London (1967).
[9] J. B. HARBORNE and E. HALL, *Phytochem.* 3,421 (1964).
[10] C. W. GLENNIE and J. B. HARBORNE, *Phytochem.* 10, 1379 (1971).

TABLE 1. FLAVONOIDS IN THE HYDROLYSED EXTRACTS OF LEAVES OF MEMBERS OF THE CYPERACEAE

Sub-family, tribe, genus and species	Presence/absence of			
	Kaempferol and/or Quercetin	**Glycoflavone**	**Luteolin**	Tricin
CYPEROIDEAE				
Scirpeae				
Eriophorum latifolium Hoppe	+	+	−	−
E. gracile Roth.	−	+	+	+
Eleocharis palustris (L.) Roem. **& Schult.**	−	−	−	−
Blysmus compressus **(L.) Panz.** ex Link	−	−	+	+
Scirpus holoschoenus **L.**	−	−	−	−
S. sylvaticus L.	−	+	−	+
S. *radicans* **Schkuhr**	−	+	−	−
S. maritimus **L.**	−	−	+	+
Fuirena pubescens Kth.	+	−	−	−
Rhynchosporeae				
Schoenus nigricans L.			−	−
Rhynchospora alba (L.) Vahl	−	+	−	+
Bolboschoenus maritimus (L.) **Palla**	−	−	+	+
Cladium mariscus **(L.)** Pohl	−	+	+	−
Cypereae				
Cyperus fuscus L.		−	+	−
C. ***glomeratus*** L.	−	−	+	−
C. ***difformis*** L.	−	−	+	−
C. ***vegetus*** Willd.	−	−		−
C. ***longus*** L.	−	+	+	+
CARICOIDEAE				
Cariceae*				
Carex subgenus Primocarex				
C. ***capitata*** L.	−	+	−	
C. ***pulicaris*** L.*	−	+	−	−
C. ***microglochin*** Wahl.	−	+	−	+
C. ***rupestris*** All.*	−	+	−	−
C. ***davalliana*** Sm.*	−	+	−	−
Carex subgenus Vignea				
C. ***arenaria*** L.	−	−	−	+
C. ***echinata*** **Murr.**	−	−	−	−
C. ***muricata*** **L.** ssp. ***leersii*** **Aschers. &** Graeb.	−	+	−	−
C. ***spicata*** Huds.	−		−	+
C. ***divulsa*** L.	+	−	+	
C. ***otrubae*** Podp.	−	+	−	+
C. ***remota*** L.	−	+	−	−
Carex subgenus **Eucarex**				
C. ***aquatilis*** Wahl.	−	+	+	−
C. ***nigra*** (L.) Reichardt	−	−	−	+
C. ***flacca*** Schreb.	−	+	+	+
C. ***pendula*** Huds.	−	+	+	−
C. ***umbrosa*** Host	−	+	−	+
C. ***digitata*** L.	−	+	+	+
C. ***magellanica*** Lam.	−	+	−	+
C. ***retrocurva*** Dewey	−	+	+	−
C. ***sempervirens*** Vill.	−	−	+	+
C. ***strigosa*** Huds.	−	−	−	+
C. ***sylvatica*** **Huds.***	−	+	−	+
C. ***scabrata*** Schwein.	−		+	−

TABLE I—*continued*

Sub-family, tribe, genus and species	Presence/absence of: Kaempferol and/or Quercetin	Glycoflavone	Luteolin	Tricin
C. brevicollis DC.	—	+	+	—
C. *pilosa* Scop.	—	+	—	+
C. depauperata Good.		+	—	+
C. *extensa* Good.*		—	+	+
C. *distans* L.		+	+	
C. hostiana DC.*	+	+	—	—
C. *flava* L.*	+	—	—	—
C. *lepidocarpa* Tsch.	+		—	—
C. *demissa* Hornem.*	+	—	—	—
C. *serotina* Merat. ssp. *serotina**	+	—	—	—
C. serotina ssp. *pulchella**	+	—	—	—
C. pseudocyperus L.		+	—	—
C. *squarrosa* L.	—	—	+	—
C. *rostrata* Stokes		+	4	+
C. *riparia* Curt.*			—	+
C. *acutiformis* Ehrh.	—	+	—	+
C. *trifida* Cav.			—	+
C. nutans Host			—	+
C. *hirta* L.	—	+	—	+
C. *aristata* R. Br.	—	+	—	+

* Material from Leicester Herbarium; remainder from Reading herbarium, except for those examined as fresh material (see Experimental). *Carex* species are arranged according to Kukenthal.[11] The majority of *Carex* spp. examined showed the presence of chlorogenic acid on two-dimensional chromatography of direct extracts.

study of fresh alcoholic extracts of *Carex trifida* and *Cyperus longus* yielded free tricin and it thus appears that this compound occurs in these species either in the free state or in some labile combined form which is broken down by alcoholic extraction.

Finally, two tricin-containing species, *Carex nigra* and C. *sylvatica*, were examined to see if any chemical differences occurred at the population level. C. *sylvatica* is a stable species, morphologically invariant and hybridizing with difficulty with neighbouring species (only one hybrid, with C. *hirta,* is known). By contrast, C. *nigra,* a member of a difficult group of closely related species has been variously defined and even when interpreted in a strict sense, as here, shows very great morphological variation. Neither species in fact shows any significant variation in chemical pattern in the populations examined (11 and 7 specimens respectively).

DISCUSSION

While a previous survey[1] of flavonoids of pigmented tissue (mainly inflorescence) in sedges established that there were significant differences from the pattern found in the grasses (Gramineae), the present study of leaf flavonoids shows that the two families are relatively similar in their leaf constituents. This is reassuring, since the two families are generally regarded by taxonomists as being closely associated on morphological grounds.

[11] G. KUKENTHAL, *Des Pflanzenreich* IV, 20, Heft 38 (1909).

The general leaf pattern now observed in the Cyperaceae (regular occurrence of tricin, luteolin and glycosylflavones, rarity of flavonols), is very similar to that found not only in the grasses but also in the palms (Palmae).[6] Other neighbouring monocotyledonous families have quite different flavonoid patterns in the leaves, which indicates that these three families are especially closely related, a suggestion which has recently emerged quite independently by means of numerical analysis of the distribution of morphological characters in these families.[12]

With regard to the systematic arrangement of the taxa within the Cyperaceae, the present survey has not revealed any striking new correlation between chemistry and classification. However, the presence of flavonols in seven of the 44 *Carex* species is of phylogenetic interest, suggesting as it does that these are the more primitive taxa within the genus. It is interesting that six of these seven flavonol-containing species are particularly closely related morphologically (see Table 1 and Ref. 7). Since there are at least two conflicting views of evolutionary trends within the genus,[11,13] further studies of flavonoids in a wider sample of *Carex* would undoubtedly be worthwhile.

The discovery of tricin in the Cyperaceae in no less than 29 taxa from seven genera is of special interest because of the relative rarity or elusiveness of this flavonoid in nature. One might expect it to occur regularly in plants because of its structural similarity to two widespread phenolics, the anthocyanidin malvidin and the cinnamic acid, sinapic acid. However, it has only been reported twice in the dicotyledons (*Orobanche*, Orobanchaceae and *Medicago*, Leguminosae) and twice in the monocotyledons (in several genera of the Gramineae and in Crocus, Iridaceae). In the Gramineae, it is as expected accompanied by water soluble sinapic acid esters, but the same does not seem to be true in the Cyperaceae, where the major cinnamic ester is chlorogenic acid. Tricin has subsequently been found once in the palms, in *Chamaerops*,[6] and wider surveys of these and other families may well reveal further sources. Its occurrence in fresh leaves of some *Carex* species, apparently in the free state, is also exceptional and this phenomenon is under further investigation.

EXPERIMENTAL

Plant Material

Carex riparia, *C. acutiformis*, *C. pseudocyperus* and *Cyperus longus* were collected locally and voucher specimens are deposited in the University of Reading herbarium. Fresh material of *Carex trifida* was kindly provided by Dr. D. M. Moore from his South American collection. Dried herbarium tissue was obtained either from the University of Reading or from the University of Leicester herbaria and the flavonoid results obtained have been annotated on the sheets from which material was taken.

Flavonoid Analyses

Acid hydrolysed and fresh extracts of leaf material were examined for flavonoids by standard procedures.[8] Rutin and chlorogenic acid were identified in direct extracts by comparison with markers on two dimensional chromatograms run in BAW and 5% HOAc. Glycoflavones were recorded in acid-hydrolysed leaf extracts if the ethyl acetate of more usually the amyl alcohol fractions showed the presence of flavones mobile in water, with high R_f in Forestal and medium to low R_f in BAW. Distinction between glycoflavones and difficultly hydrolysable flavone *O*-glycosides is not completely possible on this basis and the records of glycoflavones in the Table may be overestimated. More detailed studies of direct extracts of three species (see below) showed, however, that all three contained glycoflavones. By contrast, the presence of tricin was very readily determined, particularly on account of its characteristic yellow fluorescence in UV light $+NH_3$ and its high mobility in the phenol solvent.

[12] H. T. Clifford, *J. Linn. Soc. Botan.* Suppl. 1, 63, 25 (1970).
[13] V. I. Kreezetowiez, *J. Bot. U.S.S.R.* 21, 395 (1936).

Flavonoids of Carex

A two-dimensional chromatogram run in BAW and 15% HOAc of fresh leaf extract of ***Carex riparia*** showed the presence of four phenolic constituents: Cl, R_fs 0·76/0·05, dark brown → fluorescent yellow (colours in UV light without and with NH_3); C2, R_fs 0·57/0·66, blue-+ green; C3, R_fs 0·43/0·33, dark brown → light brown; and C4, R_fs 0·23/0·07, blue white fluorescence → yellow fluorescence. A similar chromatogram of C. ***acutiformis*** showed the presence of C2, C3 and C4.

Cl was identified as tricin by UV spectral analysis and by co-chromatography with authentic material in six solvents. It was further identified by mass spectral measurement (mol. wt. found 330.0742, $C_{17}H_{14}O_7$ required 3300739). C2 was identified as chlorogenic acid by co-chromatography in six solvents and by hydrolysis to caffeic acid. C3 was identified as iso-orientin (I) by UV and mass spectral analysis, by co-chromatography in six solvents with authentic material, by interconversion to orientin on acid heating and by degradation to luteolin on treatment with HBr in PhOH (1: 1) at the b.p. for 16 hr. C4 was identified as tricin 5-glucoside (II) (for details, see Ref. 10).

Free tricin was also detected in fresh leaves of ***Carex trifida***, together with a glycoside, provisionally identified as tricin 7-glucuronide on the basis of spectral and R_f data and the detection of tricin and glucuronic acid after acid hydrolysis.

Flavonoids of Cyperus

A two-dimensional chromatogram of fresh leaf extracts of ***Cyperus longus*** showed the presence of three constituents: L1, 0·76/0·05, dark brown → fluorescent yellow; L2, 0·29/0·12, brown → bright yellow; and L3, 0·38/0·12, brown → bright yellow. L1 was identified as tricin (see above), L2 was identified as a glycosylluteolin (λ_{max} 258, 271, 351 nm, positive shifts with all reagents) containing glucose and arabinose as 0-glycosidic sugars. L3 was identified as a luteolin 7-0-arabinosylglucoside from spectral and R_f data and from the identification of the hydrolytic products.

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