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FLAVONOID PATTERNS IN THE MONOCOTYLEDONS. FLAVONOLS AND FLAVONES IN SOME FAMILIES ASSOCIATED WITH THE POACEAE*

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Abstract—Flavonoid pigments have been identified in leaves of representative members of the Anarthriaceae, Araceae, Flagellariaceae, Sparganiaceae, Typhaceae and Palmae. While the first five families have flavonol glycosides as their major leaf constituents, the Palmae contain luteolin glycosides, glycosylflavones and in one case tricin (*Chamaerops humilis*) and thus show many similarities with patterns found in the Poaceae and Cyperaceae. The results support a recent numerical analysis based on morphological characters linking the palms and the grasses. New glycosides found during the survey are quercetin 3-neohesperidoside in *Typha latifolia* and quercetin and kaempferol 3-rutinoside-7-galactosides in *Oreodoxa regia*. The discovery of myricetin (as the 3-glucoside and 3-rutinoside) uniquely in *Sparganium* suggests that the Sparganiaceae is one of the more primitive families of this group.

INTRODUCTION

IN CONTINUATION of chemosystematic studies of monocotyledonous families related to the grasses (Poaceae),[1-5] we have now examined flavonoids in representative species from six further families and the results are presented in this paper. Many of the taxa studied have been surveyed by Bate-Smith,[6] but, according to Hegnauer,[7] in none of these families have the flavonoids been the subject of detailed studies.

RESULTS AND DISCUSSION

The results of identifying flavonoids in leaves of representative members of the six families, of interest because of their systematic association with the grasses, are presented in Table 1. These results will be discussed in turn by family.

Both the Sparganiaceae and Typhaceae are similar to the grasses in that they are wind-pollinated and show a preference for swampy habitats. Both are monogeneric families and British representatives of each family have been available for examination. According to

* Part XIV in the series "Comparative Biochemistry of the Flavonoids"; for Part XIII, see J. B. HARBORNE and C. A. WILLIAMS, *Phytochem.* **10**, (1971).

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[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**, 1025 (1969).
[5] J. B. HARBORNE and H. T. CLIFFORD, *Phytochem.* **8**, 2071 (1969).
[6] E. C. BATE-SMITH, *J. Linn. Soc.* **60**, 383 (1968).
[7] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Vol. II, Birkhauser Verlag, Switzerland (1963).

TABLE 1. FLAVONOIDS IDENTIFIED IN THE LEAVES OF SOME MONOCOTYLEDONOUS PLANTS

Order, family, genus and species	Leaf flavonoids identified
TYPHALES	
Sparganiaceae	
Sparganium erectum L.	Quercetin and myricetin 3-rutinosides, myricetin 3-glucoside
Typhaceae	
Typha latifolia L.	Quercetin and kaempferol 3-glucosides, quercetin and kaempferol 3-galactosides, quercetin 3-neohesperidoside
RESTIONALES	
Flagellariaceae	
Flagellaria indica L.	Four kaempferol 3-glycosides†
Anarthriaceae	
Anarthria scabra R.Br.	Quercetin (as 4'-glucoside) (quercetin and kaempferol in inflorescence)
A. prolifera R.Br.	Quercetin 3-glucoside (quercetin in inflorescence)
ARALES	
Araceae	
Gymnostachys anceps R.Br.	Kaempferol 3-sophoroside-7-rhamnoside
ARECALES	
Palmae*	
Chamaedorea sp.	Glycosylapigenin
Chamaerops humilis L.	Tricin, glycosylflavone and leucocyanidin
Howea forsteriana (F. Muell.) Becc.	Luteolin 7-glucoside, luteolin 7-diglucoside and glycosylapigenin
Oreodoxa regia H. B. & K.	Quercetin and kaempferol 3-rutinoside-7-galactosides, kaempferol 3-glucoside and glycosylflavone
Phoenix dactylifera L.	Luteolin 7-glucoside, luteolin 7-rutinoside and glycosylapigenin

* A survey of some 35 taxa showed glycosylflavones in 23, leucoanthocyanidins in 22 and flavonols (usually quercetin) in eight.

† Sugars occurring in these glycosides are glucose, galactose, arabinose and rhamnose in various combinations.

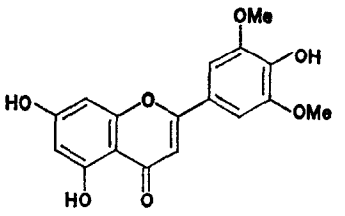
Bate-Smith,[6] the branched burr-reed *Sparganium erectum* contains quercetin and leucocyanidin, but we have found, in addition, myricetin, present as the 3-glucoside and 3-rutinoside (Table 1). This is interesting since myricetin is rare in the monocotyledons, the only other sources being the Zingiberaceae and the Iridaceae (*Crocus* and *Iris*).[6] Since myricetin represents a primitive 'woody' character in the dicotyledons, it is possible that its presence in *Sparganium* has a similar indication here. Leaf of the reedmace, *Typha latifolia*, contains five flavonol glycosides (Table 1), four being well known compounds but the fifth, quercetin 3-neohesperidoside, representing the first report in Nature of a flavonol neohesperidoside. This disaccharide (rhamnosyl-$\alpha(1 \rightarrow 2)$-glucose), an isomer of the very common rutinose, has been found up to the present amongst the flavonoids only as a sugar component of flavanones.[8]

The only representative of the Flagellariaceae, a small family of three genera, available for study was *Flagellaria indica*. Kaempferol was the only aglycone detected in acid hydrolysed extracts, and this agrees with the parallel finding of Bate-Smith[6] of kaempferol alone in *F. guineensis*. There was, however, considerable complexity in the glycosides present in

[8] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, London (1967).

F. indica and, because of shortage of material, it proved impossible to identify fully any of the components. Four glycosides were present, each with two or three different sugars attached at the 3-position (Table 1). The Flagellariaceae has often been taxonomically associated with the grasses but the fact that the only flavonoid detected in two species was a flavonol, present in complex glycosidic combination, rules out any obvious similarity in flavonoid pattern. However, further species need to be examined in order to confirm the point.

In spite of the size and economic importance of the Palmae, hardly anything is known of the flavonoid pattern in the family. Bate-Smith[6] simply records leucoanthocyanidins in 13 of 17 taxa surveyed, and flavonols in two of the same 17 taxa. We are in the process of carrying out a detailed study of the flavonoids of the palms; at present we report analyses of four species (Table 1) which seem from our surveys to be representative. *Howea* and *Phoenix* are very similar in containing the flavone luteolin and glycosylapigenin derivatives. The most significant taxonomic finding, however, is of the rare flavone tricin (I) in



Tricin (I)

Chamaerops humilis. Tricin has been previously reported in the monocotyledons only in the grasses (where it is widespread) and in the more distant Iridaceae (present in three species of *Crocus*). The importance of finding tricin in the palms is underlined by its recent discovery, too, in a member (*Carex*) of the sedge family, the Cyperaceae.[9] Just as they are in the grasses, flavonols seem to be uncommon in the palms; a survey showed them to be present in only eight of 35 taxa, whereas glycosylflavones, characteristic grass flavonoids, were present in 23 of 35 taxa examined. One flavonol-containing palm is *Oreodoxa regia* and a detailed study of its leaf flavonoids revealed the presence of two new flavonol glycosides, namely kaempferol and quercetin 3-rutinoside-7-galactoside. It is perhaps not without significance that the structurally related flavonol glycoside, kaempferol 3-rutinoside-7-glucoside has been found in *Crocus*[8] (compare the parallel occurrence of tricin in these sources, see above).

Gymnostachys (Araceae) differs from most other members of the family in having long linear leaves and is regarded by Deyl[10] as being intermediate between Araceae and *Sparganium*. Chemical examination showed the presence of a single flavonoid, which was identified as kaempferol 3-sophoroside-7-rhamnoside, a relatively rare flavonol glycoside type,[8] by standard procedures. Thus, the presence of a flavonol in *Gymnostachys* does show a link with *Sparganium*, although the latter has two different flavonols (quercetin and myricetin) in different glycosidic combination. The absence of glycoflavone from it may be equally taxonomically significant, since it shows it is different from the Lemnaceae, a family usually classified with the Araceae, and one which is rich in glycosylflavones.

[9] J. B. HARBORNE, unpublished results.
[10] M. DEYL, *Acta Musei Nationalis, Prague* **116**, 3 (1955).

A study of flavonoid patterns in *Anarthria* is of interest since it is a small genus until recently included in the Restionaceae but now split off as the Anarthriaceae.[11] Examination of two species showed it to be another flavonol-rich group, but there was no trace of gossypetin or related compounds which appear from our earlier work to characterize the Restionaceae proper.[5] Chemistry thus supports the newer classification of *Anarthria.*

The results of flavonoid analysis in the above families, taken with earlier results on other families associated with grasses, show that several patterns are emerging and that families can be grouped together on the basis of their flavonoid constituents (Table 2). In many instances, sampling has been very limited and more work is needed to confirm, or otherwise, these family groupings. Nevertheless, the results do, generally, agree with modern taxonomic thinking on these family relationships. For example, Cronquist[12] considers that, apart from the historical precedent, *Sparganium* and *Typha* could be united into a single family and the chemical data (Table 2) are in agreement with this. Perhaps the most striking aspect of

TABLE 2. GROUPINGS OF MONOCOTYLEDONOUS FAMILIES BASED ON FLAVONOID PATTERNS

Flavonols alone	6- or 8-Hydroxy Flavonols	Flavones (luteolin, tricin) and glycosylflavones
	Eriocaulaceae Restionaceae*	
Sparganiaceae Typhaceae* Flagellariaceae Anarthraceae	Flavonols and Glycosylflavones	Palmae* Cyperaceae* Poaceae (= Gramineae)
	Commelinaceae† Araceae* Lemnaceae	

* These families have a majority of members which are leucoanthocyanidin positive. As Bate-Smith has pointed out, the phyletic significance of this character in the monocotyledons differs from its role as a 'woody' indicator in the dicotyledons.

† Bate-Smith[6] records kaempferol or quercetin in five of 13 taxa and our own survey (unpublished) suggests quercetin in four and glycosylflavone in another four of some 43 taxa examined. The family as a whole is generally very poor in recognizable flavonoid constituents.

these data are the suggested association of the grasses with the Cyperaceae and the Palmae, particularly the latter association. Morphological data, on the whole, have not been used in the past for connecting the grasses and the palms, but it is especially interesting that a recent numerical analysis[13] of morphological characters in these and other families, indicated a close association between these two very large and economically important plant groups.

[11] D. F. CUTLER and H. K. AIRY-SHAW, *Kew Bull.* **19**, 489 (1961).
[12] A. CRONQUIST, *The Evolution and Classification of Flowering Plants*, Nelson, London (1968).
[13] H. T. CLIFFORD *Bot. J. Linn. Soc.* **63**, Suppl. 1 (1970).

EXPERIMENTAL

Plant Material

Fresh leaves of *Typha*, *Sparganium* and *Howea* were obtained from plants growing at the University of Reading and voucher specimens have been deposited in the Botany Department herbarium. The Palmae specimens were obtained, otherwise, from the Royal Botanic Gardens, Kew. The *Anarthria* species were collected and identified by Eleanor M. Bennett 17 miles west of Walpole, South Coast, Western Australia. The remaining taxa were grown by or collected by one of us (H.T.C.) in Queensland, Australia.

Flavonoid Identifications

Standard procedures were used for the extraction, isolation, purification and identification of the flavonoids. Known pigments were identified by hydrolytic studies and by detailed u.v. spectral and chromatographic comparison with authentic samples. Flavonol 3-glycosides were further characterized by identifying the sugars released on H_2O_2 oxidation. Many monocotyledonous tissues are rich in interfering impurities and the purification of flavonoids, even after removal of lipids by petroleum extraction, is difficult and requires repeated paper chromatographic separations.

Quercetin 3-neohesperidoside. Isolated from *Typha latifolia* leaf and separated by paper chromatography from co-occurring quercetin and kaempferol 3-monoglucosides and 3-monogalactosides. It gave glucose and rhamnose on acid hydrolysis, was identical to quercetin 3-rutinoside (rutin) in colour reactions and u.v. spectrum (with shifts) and was also very similar in R_f in BAW (0·40, rutin 0·34) and in PhOH (0·40, rutin 0·43). However, it was well separated from rutin in BEW (0·64, rutin 0·58) and H_2O (0·43, rutin 0·24). H_2O_2 oxidation gave a disaccharide identified as neohesperidose by co-chromatography with authentic material (prepared from natural naringenin 7-neohesperidoside) in 4 solvents and by co-electrophoresis on paper in borate buffer pH 10 at 15 V/cm for 6 hr (M_G values: *Typha* Sugar and neohesperidose 0·16, rutinose 0·51).

Kaempferol and quercetin 3-rutinoside 7-galactosides. Isolated from *Oreodoxa regia* leaf, along with kaempferol 3-glucoside and glycosylapigenins. Acid hydrolysis gave the respective aglycones and approximately equal amounts of glucose, rhamnose and galactose. The colour reactions on paper and the u.v. spectral data indicated they were 3,7-diglycosides and R_f values also gave this indication. R_fs for kaempferol 3-rutinoside 7-galactoside were 0·33 in BAW, 0·47 in BEW, 0·43 in PhOH, 0·76 in H_2O and 0·77 in 15% HOAc; for the quercetin derivative 0·36 in BAW, 0·48 in BEW, 0·43 in PhOH, 0·31 in H_2O and 0·47 in 15% HOAc. The structures as 3-rutinosides 7-galactosides were established by H_2O_2 oxidation, which gave rutinose, identified by co-chromatography in 4 solvents and by co-electrophoresis (conditions as above), in which it clearly separated from robinobiose (galactosyl-α,1→6-rhamnose), which had M_G value 0·31. Finally, β-glucosidase hydrolysis removed the galactose at the 7-position, yielding the corresponding 3-rutinoside.