Phytochemistry, 1979, Vol. 18, pp. 1323–1327. Pergamon Press Ltd. Printed in England.

CORRELATIONS BETWEEN FLAVONOID CHEMISTRY, ANATOMY AND GEOGRAPHY IN THE RESTIONACEAE

JEFFREY B. HARBORNE

Phytochemical Unit, Plant Science Laboratories, The University, Reading, U.K.

(*Received* 29 *January* 1979)

Key Word Index—*Restio*; *Elegia*; *Chondropetalum*; Restionaceae; flavonoids; gossypetin; gossypetin 7-methyl ether; syringetin 3-galactoside; larycitrin 3-galactoside; chemistry and phytogeography; chemistry and anatomy.

Abstract—Comparisons of the flavonoid patterns in stems and inflorescences between Australasian and South African members of the Restionaceae indicate significant differences with geography. Nine of 14 Australasian species contain gossypetin or a related 8-hydroxyflavonoid and proanthocyanidins are uncommon. By contrast, the 33 South African taxa studied contain common flavonols, flavones and glycoflavones, while proanthocyanidins are present in 29. Two anatomically related South African genera, *Chondropetalum* and *Elegia*, contain, in addition, myricetin 3-galactoside, together with the 3-galactosides of the myricetin methyl ethers, larycitrin and syringetin. These results confirm the conclusions derived from anatomy that members of *Hypolaena*, *Leptocarpus* and *Restio*, genera represented in both Australia and South Africa, have the distinctive flavonoids characteristic of their geographic origin rather than of their systematic position. The family as a whole is different in flavonoid pattern from other monocotyledonous families with which it is sometimes associated.

INTRODUCTION

The Restionaceae are a rush-like group of xeromorphic plants, of some 30 genera and 300 species, which grow almost exclusively either in South Africa or in Australasia. The classification within the family has given considerable trouble, partly because of their almost leafless habit and the fact that many species are dioecious. In spite of the geographical separation, some of the generic names are used for plants from both continents [1, 2]. Following a detailed anatomical survey of the family, Cutler [3, 4] found significant discontinuities among the different groups of plants and concluded that all South African species and genera are distinct from those in Australia and vice versa. These conclusions have not yet been accepted fully by taxonomists in the present generic treatment of the family and it was clearly of interest to see if the chemical data would support, or otherwise, the division of the restiads according to anatomy and geography.

That chemical study of the family might be rewarding was indicated by the earlier discovery of some new and rare flavonoid structures in several Australian members. Of particular note was the report of the uncommon yellow flavonol, gossypetin, in four species and the first record of a new flavone 8-hydroxyluteolin in *Hypolaena fastigiata*, the compound being named hypolaetin from the generic source [5]. The opportunity was afforded the author of studying a representative selection of South African Restionaceae for flavonoids and the present paper reports these results, together with a further exploration of Australasian members of the family.

RESULTS

South African Restionaceae

The results of surveying 33 South African members of the Restionaceae for flavonoids are shown in Table 1. These data refer to analyses of stem tissue. Some of the same compounds were also present in the inflorescences, but the stems gave the more consistent results. Although the inflorescences were usually highly coloured (various shades of brown), separate extraction of these tissues failed to yield recognizable water-soluble pigments, except in the case of *Chondropetalum hookerianum*, which gave some cyanidin 3-glycoside.

The flavonoids present in the stems are mainly well known flavonol glycosides, flavone glycosides or *C*-glycosylflavones. More unusual flavonol glycosides were found specifically in species of *Chondropetalum* and *Elegia*, which were marked off from all the other taxa by the presence of myricetin derivatives. In addition to myricetin 3-galactoside, these plants contain the 3-galactoside of myricetin 3′-methyl ether (larycitrin) and of myricetin 3′,5′-dimethyl ether (syringetin). Until recently these two methyl ethers appeared to be rare in plants, but there is increasing evidence that they have a significant distribution. Thus, one or other or both have been reported variously in a number of dicotyledons: in *Lathyrus odoratus* [6] and other legumes [7], in *Limnanthes* (Limnanthaceae) [8] and in *Soymida febrifuga* (Meliaceae) [9]. They are also present in gymnosperms, in needles of *Larix* [10].

The presence of syringetin in these two restiad genera represents the third report of its occurrence in monocotyledons; it has previously been detected in *Philydrum lanuginosum* (Philydraceae) [11] and in *Hedychium stenopetalum* (Zingiberaceae) [12]. By contrast, larycitrin is reported for the first time from the monocotyledons; its 3-galactoside, here described, is a new compound. A new bioside, probably the 3-rhamnosylgalactoside, is also present in these plants.

While other genera among the South African plants can be distinguished by different flavonoids (Table 1), *Chondropetalum* and *Elegia* are identical in pattern in

Table 1. Flavonoids in South African Restionaceae

Genus and species	Presence/absence procyanidin	Flavonol glycosides	Flavones and other constituents
Cannamois virgata (Rottb.) Steud	+	Qu 3-glucoside, Qu 3-galactoside and Qu 3-rutinoside	Lu 7-glucoside
Chondropetalum hookerianum (Mast.) Pillans	+	My 3-galactoside and Lar 3-galactoside	Cy 3-glycoside
C. mucronatum (Nees) Pillans	+	My 3-galactoside	—
C. tectorum (L.f.) Pillans	+	Syr 3-galactoside	—
Elegia capensis (Burm.f.) Schelpe	+	My 3-galactoside, My 3-arabinoside, My 3-rhamnoside and Qu 3-galactoside	—
E. cuspidata Mast.	+	Syr 3-galactoside	—
E. galpinii N.E.Br.	+	My 3-galactoside, Lar 3-galactoside, Syr 3-galactoside and Lar 3-diglycoside	—
E. parviflora Kunth	+	Lar and Syr 3-galactosides	Sulphates present
E. persistens Mast.	+	My 3-galactoside, Lar 3-galactoside and 3-diglycoside	—
E. spathacea Mast.	+	Syr 3-galactoside	—
Hypodiscus albo-aristatus (Nees) Mast.	+	—	n.d.
H. aristatus (Thunb.) Nees	+	—	Ap and Chry glycosides
H. argenteus (Thunb.) Mast.	+	Qu 3-glycoside	Lu glycoside
Leptocarpus asper Pillans	—	—	Flavone glyc.
L. hyalinus Pillans	+	—	Glycoflavones
L. paniculatus Mast.	+	Qu 3-galactoside	Lu 7-glucoside, Lu diglycoside and Chry 7-diglycoside
Mastersiella diffusa (Mast.) Gilg & Benedict	+	—	Orientin and iso-orientin
M. digitata (Thunb.) G. & B.	+	Qu 3-glucoside and Qu 3-rutinoside	—
Restio bifarius Mast.	—	Qu 3-galactoside	Orientin, lucenin
R. bifidus Thunb.	+	—	Glycoflavones
R. callistachyus Kunth.	+	Qu 3-glycoside	Lu glycoside
R. egregius Hochst.	+	—	Glycoflavones (?)
R. festuciformis Nees ex Mast.	—	—	Glycoflavones
R. obtussisimus Steud.	+	Qu 3-glycoside	—
R. purpurascens Nees ex Mast.	+	Qu 3-glycoside	Orientin, iso-orientin
R. similis Pillans	+	—	Orientin, iso-orientin
R. subverticillatus Mast.	+	—	Lu 5-glucoside
R. triflorus Rottb.	+	Unidentified flavonol	—
R. triticeus Rottb.	+	—	Glycoflavones
Staberoha cernua (L.f.) Dur. & Schinz.	—	Gossypetin 7-methyl ether	—
Thamnochortus dichotoma (Rottb.) R. Br.	+	Flavonol glycoside	—
T. gracilis Mast.	+	—	Glycoflavones
T. insignis Mast.	—	—	Orientin, lucenin
Willdenowia argentea (Nees) Hieron.	+	—	Lu 7-glucoside and Lu 7-diglucoside

Key: My = myricetin; Syr = syringetin; Lar = larycitrin; Qu = quercetin; Lu = luteolin; Ap = apigenin; Chry = chrysoeriol; n.d. = not determined.

having the above four myricetin derivatives in common. These two genera are extremely similar anatomically and Cutler [3] remarks that, with regard to the three *Chondropetalum* species studied here, they are practically indistinguishable from *Elegia*. Furthermore, the genera are linked by an unusual anatomical feature: the presence of gaps between some epidermal cells of the outer layer, through which the outer wall of a cell from the inner layer can be seen. The chemical results mirror this close anatomical inter-relationship.

There is chemical variation within these two genera in the degree of methylation of myricetin at the species level. In fact, species can be placed in a progressive series of increasing B-ring methylation. In *Chondropetalum*, there is *mucronatum* (myricetin alone), followed by *hookerianum* (myricetin + larycitrin) and *tectorum* (syringetin alone). Similarly in *Elegia*, there is the series: *capensis* and *spathacea* (My alone); *persistens* (My + Lar); *galpinii* (My + Lar + Syr); *parviflora* (Lar + Syr); and *cuspidata* (Syr alone).

Only one flavonoid character separates *Elegia* from *Chondropetalum*, the presence in *E. parviflora* of sulphated derivatives. This is the only occurrence of sulphated flavonoids in the South African members. There is, however, a single report of sulphation in one of the Australian species, in *Hypolaena fastigiata*. Sulphation is thus rare in the family.

The only plant in Table 1 which shows any clear relationship in flavonoid constituents with those of the Australasian group is *Staberoha cernua*. This plant contains gossypetin 7-methyl ether, a type of compound otherwise only known in the Australian taxa (see below). The exceptional chemistry in *Staberoha* is not entirely unexpected, since anatomically, the genus fails to show a particular affinity with other taxa in the family [3]. Its chemical alliance with the Australasian plants hints that it could represent a link between the two geographically separated parts of the family.

Australasian Restionaceae

The results of re-examining the stem flavonoids in nine Australian Restionaceae previously surveyed for inflorescence pigments [5] and of looking at a further six taxa in this group are shown in Table 2. No less than nine of the 14 species contain 8-hydroxyflavonoids so this chemical feature characterizes the group. The infrequency of proanthocyanidins is also noteworthy, since these derivatives are almost universally present (Table 1) in South African plants. It may also be noted that glycoflavones and common flavone glycosides are apparently absent from Australian taxa.

Hypolaetin (8-hydroxyluteolin), previously reported only in *Hypolaena fastigiata* [5], has now been found in two further sources: in *Restio complanatus*, where it occurs in association with gossypetin, and in *Leptocarpus tenax*. Thus, hypolaetin is almost as good a taxonomic marker for the group as gossypetin, which is now reported in 4 of 5 *Restio* species, and from both *Calorophus* species studied. A derivative of gossypetin—the rare 7-methyl ether [13]—has also been found for the first time in these plants; it occurs once in *Leptocarpus similis*. Interestingly, this is the only New Zealand plant examined in this survey, all the other plants being of Australian origin.

Table 2. Flavonoids identified in Australasian Restionaceae

Genus and species	Presence/absence procyanidin	Flavonoids present
Calorophus lateriflorus (R. Br.) F. Muell	—	Gossypetin (as 7-glucoside)
C. minor Hk. f.	—	Gossypetin (as 7-glucoside)
Coleocarya gracilis S.T. Blake	—	—
Hypolaena fastigiata R. Br.	—	Hypolaetin (as glucoside)
Leptocarpus brownii Hook.f.	—	—
L. similis Edgar	—	Gossypetin 7-methyl ether
L. tenax (Labill.) R. Br.	—	Hypolaetin
Lepyrodia interrupta F.V. Muell	—	—
L. scariosa R. Br.	—	My, Qu
Restio complanatus R. Br.	—	Gossypetin (as 7-glucoside) and hypolaetin
R. pallens R. Br.	+	Gossypetin, cyanidin 3-glucoside
R. tenuiculmis S.T. Blake	+	Gossypetin, cyanidin 3-glucoside
R. tetraphyllus Labill.	+	Gossypetin (as 7-glucoside)
subsp. *meiostachyus*	+	—

DISCUSSION

The present results confirm the earlier suggestion [5] that plants of the Restionaceae have an interesting flavonoid chemistry. Thus, they contain a number of relatively rare pigments: gossypetin and its 7-methyl ether, hypolaetin, syringetin and larycitrin. The purpose behind the synthesis of these particular constituents in the family is not clear, since they do not appear to contribute to visible colour. Their presence is, however, of biological interest since their distribution is correlated with anatomical and geographical discontinuities in the family.

The major geographical separation of the Restionaceae between two continents is clearly reflected in the flavonoid chemistry and there is little overlap between the two patterns. Thus, the Australian plants are characterized by the presence of 8-hydroxyflavonoids, infrequency of proanthocyanidin and probable absence of glycosylflavone. By contrast, the South African species are distinguished by the regular presence of myricetin and quercetin, of glycosylflavones and of proanthocyanidins, with the concomitant absence of 8-hydroxyflavonoids (except in *Staberoha*).Such large variations in flavonoid pattern with geography within families have not often been recorded before. Similar differences are, however, present in the Cyperaceae, a cosmopolitan family represented in four continents, where the African and Australian members also seem to be different in their flavonoids [14]. The flavonoid differences between the two main groups of restiads suggest that further exploration of the family and of *Leptocarpus*, a genus which is unique in the group in occurring also in Asia and South America [4], would be rewarding.

The correlation between flavonoid chemistry and plant anatomy in the restiads is also striking. As already mentioned, Cutler [3, 4] has criticized on anatomical grounds earlier taxonomic treatments of the family which allowed that species of *Hypolaena*, *Leptocarpus* and *Restio* be represented in both South Africa and Australasia. He found considerable discontinuities in these genera and clear divisions on a geographical basis. Such differences are also apparent in the flavonoids (Table 3) and reinforce the need for a revision of the generic names used for these plants.

There are problems in comparing the flavonoid data obtained here on restiads with similar data from other families in that most of the data relates to stem rather than leaf tissue. However, since the culms or stems of Restionaceae have taken over the functions of photosynthesis and transpiration in these plants, one may presume that the flavonoid chemistry of the stem may have undergone some modification as a result of this adaptation. Certainly, on the basis of comparison with leaf analyses in other monocot families, it does appear that the Restionaceae are different in a number of features

Table 3. Flavonoid comparisons of *Hypolaena*, *Leptocarpus* and *Restio* from different continents

Genus	Flavonoids of Australian taxa	Flavonoids of South African taxa
*Hypolaena**	Hypolaetin (1/1 sp.) as sulphate	3-Glucoside and 3-rutinoside of quercetin in 1/2 spp.
Leptocarpus†	Hypolaetin in 1/3 spp; gossypetin 7-methyl ether in 1/3 spp.	3-galactoside of quercetin and flavone glycoside in 1/3 spp.; glycoflavones in 2/3 spp.
Restio‡	Gossypetin in 4/5 spp. Cyanidin 3-glucoside in inflorescence of 2/5 spp.	Quercetin in 2/11 spp.; glycosylflavones in 8/11 spp.

* South African taxa are now usually referred to as *Mastersiella* (as in Table 1) but were earlier included in *Hypolaena*.

† Cutler [3] indicates that South African taxa should be referred to as *Calopsis*.

‡ Cutler [3] points out that Australian taxa should be assigned to new genera, but there are nomenclatural difficulties in dealing with these plants.

from any other group associated with them. For example, Juncaceae are anatomically close [4] but the flavonoid markers of the rushes [15] are notably absent from restiads. Similarly, the Gramineae are often taxonomically related to restiads (e.g. [16]), but here again tricin, which is characteristic of grasses [17], is not found at all in restiads.

EXPERIMENTAL

Plant material. South African plants were collected in the Kogelberg Nature Reserve, nr. Betty's Bay, except for *Willdenowia argentea* which was collected at Elim, Bredasdorp and *Elegia galpinii* which was from Swellendam. All the plants were in flower and were identified by H. R. Tolken, and vouchers are deposited in the U.R. herbarium. Sources of many Australian species have been given earlier [5]. Further species were collected near Sydney by C. J. Quinn and vouchers are deposited in the U.N.S.W. herbarium. *Leptocarpus similis* was collected near Milton Otago, South Island, New Zealand (UNSW voucher No. 5500).

Flavonoid identifications. Plant 70% EtOH extracts were routinely studied by 2D PC and acid-hydrolysed extracts by 1D PC and TLC. PC in $CHCl_3$–HOAc–H_2O (3:1:1) was particularly useful for distinguishing larycitrin and syringetin from quercetin and kaempferol respectively. Large quantities of blue fluorescent materials were present in most species and their presence sometimes obscured flavonoid spots. There was particular difficulty with the detection of glycosylflavones and their distribution reported here may require some modification. Glycoflavones were securely identified, following purification from these impurities, in at least 5 spp. (see Table 1). In general, known compounds were identified by standard procedures and structures were confirmed by direct comparison with authentic markers.

Syringetin 3-galactoside was obtained from *E. cuspidata* as yellow needles; λ_{max}^{EtOH} nm: 253, 264, 305, 360; +NaOH, 428; +NaOAc, 256, 267; +$AlCl_3$, 366, 396; +H_3BO_3 364. R_f (×100) 51 in BAW, 08 in H_2O, 38 in 15% HOAc, 86 in PhOH. It gave galactose on acid or β-glucosidase hydrolysis and syringetin, identified by co-chromatography and spectral comparison with authentic material from *Lathyrus odoratus* [6]. Identity of syringetin was confirmed by MS (parent ion 346.0684; $C_{17}H_{14}O_8$ requires 346.0689) and by demethylation to myricetin.

Larycitrin 3-galactoside was isolated from *E. galpinii* and had a similar spectrum to syringetin 3-galactoside, except that it gave a 20 nm bathochromic shift in the presence of borate. R_f (×100) 43 in BAW, 09 in H_2O, 32 in 15% HOAc, 60 in PhOH (cf. myricetin 3-galactoside values: 37, 09, 26 and 33 respectively). It gave galactose on hydrolysis and larycitrin, identified by MS and by R_f and spectral comparison with lit. data [10, 11]. A larycitrin 3-diglycoside was obtained from the same plant; since it gave rhamnose and galactose in approximately 1:1 ratio on acid hydrolysis and had R_fs (×100) 25 in BAW, 24 in H_2O, 42 in 15% HOAc, 57 in PhOH, it was provisionally identified as the 3-rhamnosylgalactoside.

The gossypetin derivative from *Staberoha* was isolated as a glycoside; λ_{max}^{EtOH} nm: 262, 278, 347, 384, R_f (×100) 45 in BAW, 11 in H_2O, 35 in 15% HOAc, 69 in PhOH, visible yellow on paper, dark black in UV. It was not hydrolysed by β-glucosidase, but acid hydrolysis gave glucose and galactose. Spectral data indicated sugar linkage in the 3-position, so the glycoside is provisionally identified as a 3-glucosylgalactoside. The aglycone, formed on acid hydrolysis, was identified as gossypetin 7-methyl ether, by direct comparison with a synthetic sample (cf. [13]). Identification was aided by the fact that the 7-methyl ether has a particularly characteristic UV spectrum [13] and it absorbs in the visible at a higher wavelength (392 nm) than gossypetin itself (386 nm) or any other related flavonol. Gossypetin was produced from it on demethylation with pyridinium chloride. Identity was confirmed by MS ($C_{16}H_{12}O_8$ requires MW 332, found MW 332). The same gossypetin derivative was also obtained from *Leptocarpus similis* and was identical in every way with the *Staberoha* compound.

Acknowledgements—The author is especially grateful to H. R. Tolken, South African Liaison Officer at Kew Herbarium 1975–1977, for the provision and identification of African plant material. He is also grateful to Dr. C. J. Quinn, Univ. of New South Wales, Kensington and Prof. G. Bayliss, Univ. of Dunedin, New Zealand, for the provision and identification of Australasian plants. He is also indebted to Dr. D. F. Cutler for valuable discussions on the anatomy and taxonomy of these plants.

REFERENCES

1. Gilg-Benedict, C. (1930) in *Die Natürlichen Pflanzenfamilien* (Engler, A. and Prant, K., eds.) 2nd edn, Vol. 15a, pp. 8–27.
2. Pillans, N. S. (1950) in *Flora of the Cape Peninsula* (Adamson, R. S. and Salters, T. M., eds.). Cape Town.
3. Cutler, D. F. (1969) in *Anatomy of the Monocotyledons* (Metcalfe, C. R., ed.) Vol. IV, Juncales. Clarendon Press, Oxford.
4. Cutler, D. F. (1972) in *Taxonomy, Phytogeography and Evolution* (Valentine, D. H., ed.) pp. 73–83. Academic Press, London.
5. Harborne, J. B. and Clifford, H. T. (1969) *Phytochemistry* **8**, 2071.
6. Harborne, J. B. (1969) *Phytochemistry* **8**, 177.
7. Jay, M., Hasan, A., Voirin, B., Favre-Bonvin, J. and Viricel, M. (1978) *Phytochemistry* **17**, 827.
8. Parker, W. H. and Bohm, B. A. (1975) *Phytochemistry* **14**, 553.
9. Pardhasaradhi, M. and Sidhu, G. S. (1972) *Phytochemistry* **11**, 1520.
10. Niemann, J. G. (1973) *Phytochemistry* **12**, 2056.
11. Bohm, B. A. and Collins, F. W. (1975) *Phytochemistry* **14**, 315.
12. Williams, C. A. and Harborne, J. B. (1977) *Biochem. Syst. Ecol.* **5**, 221.

13. Harborne, J. B., Saleh, N. A. M. and Smith, D. M. (1978) *Phytochemistry* **17**, 589.
14. Williams, C. A. and Harborne, J. B. (1977) *Biochem. Syst. Ecol.* **5**, 45.
15. Williams, C. A. and Harborne, J. B. (1975) *Biochem. Syst. Ecol.* **3**, 181.
16. Dahlgren, R. (1975) *Bot. Not. (Lund)* **128**, 119.
17. Harborne, J. B. and Williams, C. A. (1976) *Biochem. Syst. Ecol.* **4**, 267.