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FLAVONOID EVIDENCE AND THE CLASSIFICATION OF THE ANARTHRIACEAE WITHIN THE POALES

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Key Word Index—*Anarthria*; Anarthriaceae; *Georgiella*; *Ecdeiocolea*; Ecdeiocoleaceae; *Lyginia*; *Hopkinsia*; Restionaceae; Poales; flavonols; flavonol 5-methyl ethers; chemotaxonomy.

Abstract—A survey of the flavonoids of all seven known species of *Anarthria* showed the regular presence of myricetin, quercetin and isorhamnetin and traces of kaempferol. Quercetin 5,4′-dimethyl ether was uniquely present in *Anarthria laevis*, while quercetin 3-methyl ether occurred in *A. gracilis*. Proanthocyanidins were detected in two species and tricin in *A. polyphylla*. The flavonols largely occurred as 3-glycosides, but some unknown conjugates were also found to be characteristic of the genus. Two related taxa in the Ecdeiocoleaceae could be separated by the fact that they only produced quercetin and isorhamnetin. These results were compared with the flavonoid patterns of the Restionaceae *sensu latu* and support the view derived from chloroplast DNA data that Anarthriaceae diverged early from the Restionaceae and that Ecdeocoleaceae is a sister group.

INTRODUCTION

Anarthria are rhizomatous herbs which range from about 10 cm to 1 m tall. This small genus of seven species native to South Western Australia, has been variously treated taxonomically as a genus within the Restionaceae or as a monotypic family, the Anarthriaceae. Its position within the order Poales has therefore still been a matter of controversy. Both *Anarthria* and *Ecdeiocolea* were included on morphological grounds within the Restionaceae in most of the older treatments of this group. However, recent chloroplast DNA data support their separation as individual families and indicate that the Ecdeiocoleaceae is a sister taxon to the Restionaceae and that the Anarthriaceae diverged at an earlier date [1]. Until recently, the related *Ecdeiocolea* (or Ecdeiocoleaceae) was considered to be monotypic. However, two of us (LASJ and BGB) recently discovered another undescribed species, which we have decided to place in the new genus, '*Georgiella*' and have named '*G. hexandra*' [2]. Both these genera, like *Anarthria*, are endemic to South Western Australia.

Two further genera, *Hopkinsia* and *Lyginia*, need to be mentioned here, since they have been included formerly in the Restionaceae, but have some affinities with *Anarthria*. They are now considered separate monotypic families, Hopkinsiaceae and Lyginiaceae. Nevertheless, DNA sequence data and embryology indicate they have a closer affinity with Anarthriaceae than previously thought [3–5].

In a previous paper we have reported some flavonoid data for the Anarthriaceae [6] and have exhaustively surveyed the Restionaceae [7 and Williams *et al.*, unpublished results], although most of the data obtained for Australian members have not been published yet. Most Australian Restionaceae have characteristic flavonoid profiles in which the 8-hydroxyflavonoids, hypolaetin (8-hydroxyluteolin) and gossypetin (8-hydroxyquercetin) together with tricin are frequent constituents. By contrast the South African genera show a simpler flavonoid pattern in which common flavonols, flavones and glycoflavones and occasionally myricetin methyl ethers occur [7]. However, one of the more 'primitive' Australian genera of the Restionaceae, *Sporadanthus*, together with *Hopkinsia* also have this simple flavonol pattern.

Previously we examined two *Anarthria* species, *A. scabra* and *A. prolifera*, and found quercetin glycosides in the leaf tissue of both and in the inflorescence of the latter and both quercetin and kaempferol in the inflorescence of *A. scabra*. In view of the conflicting evidence regarding the position of

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Table 1. A stem/leaf flavonoid survey of the Anarthriaceae and Ecdeiocoleaceae

Family† genus species	Flavonols My	Qu	Km	Isorh	Qu 5,4′-DIME (**5a**)	Other aglycones	Collector's name and number
Anarthriaceae							
Anarthria							
A. laevis R.Br.♀	−	−	−	−	+	B/Y unknown*	B. G. Briggs 7452
	−	−	−	−	+	B/Y unknown*	B. G. Briggs 8511
A. scabra R.Br.♀	+	+	(+)	−	−	Pro Cy	B. G. Briggs 7596
♂	+	+	(+)	−	−	Pro Cy, Free My di ME	T. E. H. Aplin 3307
A. gracilis R.Br.♀	+	+	−	+	−	Free Qu 3 ME	B. G. Briggs 7653
♂	+	+	−	+	−	Free aglycones nd	B. G. Briggs 7626
A. humilis Nees	−	+	−	(+)	−	−	B. G. Briggs 8511
A. polyphylla Nees♂	(+)	+	(+)	+	−	Tricin, (Pro Cy), (Pro Dp)	B. G. Briggs 7890
♀	−	+	(+)	+	−	Tricin, (Pro Cy), (Pro Dp)	B. G. Briggs 7891
A. 'egrallata' L. A. S. Johnson & B. G. Briggs unpubl.	+	+	−	+	−		B. G. Briggs 9369
A. prolifera R.Br.	+	+	−	+	−	−	B. G. Briggs 7536
♀	+	+	−	+	−	−	B. G. Briggs 7536
Ecdeiocoleaceae							
Georgiella							
'*G. hexandra*' B. G. Briggs	−	+	−	+	−	−	B. G. Briggs 6395
& L. A. S. Johnson unpubl.	−	+	−	+	−	−	B. G. Briggs 2684
Ecdeiocolea							
E. monostachya F.	−	−	−	+	−	−	R. Coveny 7875
Muell.	−	−	−	+	−	−	B. G. Briggs 7495
	−	−	−	+	−	−	B. G. Briggs 7456
	−	−	−	+	−	−	K. L. Wilson 2582
	−	−	−	+	−	−	S. Krauss 131
	−	−	−	+	−	−	S. Krauss 135

Key: My = myricetin, Qu = quercetin, Km = kaempferol, Isorh = isorhamnetin and DIME = dimethyl ether.
* R_f s BAW 67, Forestal 41, 50% acetic acid 17 and CAW (2:1) 12; possibly a My dimethyl ether.
† Classification according to Linder *et al.* [3,4].

the Anarthriaceae in the Poales, we have now carried out a comprehensive survey of this family. We have also screened the related Ecdeiocoleaceae, which has not yet been previously investigated for its flavonoid constitutents.

RESULTS

The results of a leaf flavonoid survey of all seven known *Anarthria* species (two accessions of six) and '*Georgiella hexandra*' (two accessions) and six accessions of *Ecdeiocolea monostachya* from the Ecdeiocoleaceae are presented in Table 1. The results refer to aglycones identified in leaf tissue after acid hydrolysis by direct comparison with authentic markers and were confirmed by 2 DPC of direct leaf extracts and detailed analysis of the flavonoid glycosides present in each species (see Table 2).

Five flavonoid aglycone patterns can be distinguished within the Anarthriaceae with *A. scabra*, *A. humilis* and *A. laevis* all having their own distinctive constituents. By contrast the three species, *A. gracilis*, *A. prolifera* and *A. 'egrallata'* are similar in having myricetin, quercetin and isorhamnetin. Finally, *A. polyphylla* is probably closest to the latter three species but differs from all other taxa in producing a small amount of tricin. *Anarthria laevis* has the most unusual profile, in which tamarixetin 5-methyl ether (quercetin 5,4′-dimethyl ether) is the major aglycone constituent, which appears as a distinctive fluorescent yellow colour in UV light. Another minor component, a possibly related myricetin derivative with the same colour in UV light, was also present. This is the first report of tamarixetin 5-methyl ether in nature, although the 3′-isomer, isorhamnetin 5-methyl ether has been characterised from the leaf resin of *Cistus laurifolius* [8]. Both compounds occur as 3-glycosides in the plant and have a characteristic blue colour in UV light changing to yellow in the presence of ammonia vapour. HPLC analysis of the myricetin-based glycoside suggested there may be both a myricetin dimethyl ether and a trimethyl ether present in the minor blue to yellow glycoside spot on the 2 DPC.

The flavonoid results are especially interesting for the Ecdeiocoleaceae, where the new monotypic '*Georgiella*' is clearly distinguished from the other monotypic member of the family, *Ecdeiocolea*, by the presence of quercetin in the former and its absence in the

Table 2. The flavonoid glycosides identified in *Anarthria*, *Ecdeiocolea* and *Georgiella* species and two representative taxa from the Lyginiaceae and Restionaceae

Species	Accession number	Flavonoid glycosides identified
Anarthriaceae		
Anarthria egrallata	Briggs 9369	A mixture of Qu and Isorh conjugated diglucosides
A. gracilis	Briggs 7653	Qu 3-glucoside, a mixture of Qu, My and Isorh conjugates, free Qu 3ME
A. humilis	Briggs 8511	Qu 3-glucosylarabinoside* and 3-rutinoside
A. laevis	Briggs 7452	Qu 5,4′-DIME (tamarixetin) 3-glycoside (**5**), My DIME and triME glycosides
A. polyphylla	Briggs 7891	QU and Isorh 3-glucosides, Tricin 7-glucoside (trace), a mixture of Qu and Isorh conjugated diglucosides
A. prolifera	Briggs 7563	Qu 3-glucoside, 3-rhamnoside and 3-diglucoside*, My 3-rhamnoside and a My conjugate (**1**), Isorh 3-diglucoside*
A. scabra	Briggs 3307	My and Qu conjugates (**2** and **3**), free My 3,3′DIME and 7 unidentified free aglycones with λ max at 320 nm
Ecdeiocoleaceae		
Ecdeiocolea monostachya	Briggs 7456	Isorh 3-glucoside, Isorh 3-diglucoside*
	Briggs 2582	Isorh 3-ferulyldiglucoside* (**4**)
'*Georgiella hexandra*'	Wilson 2684	Qu 3-glucoside and 3-galactoside, Isorh 3-glucoside and 3-galactoside, Qu and Isorh 3-diglucoside*? and 3-digalactoside*?, acylated Qu and Isorh 3-glucoside and 3-galactoside
Lyginiaceae		
Lyginia imberbis R.Br.	Briggs 8273	Qu 3-glucoside, 3-rutinoside, 3-dialloside*, Isorh 3-acylglucoside, 3-diglucoside* and dialloside
Restionaceae		
Lepyrodia anarthria F. Muell.	Krauss 98	Or, Iso Or, two Lu di-*C*-glycosides, Qu 3-glucuronide, My 3-acyl-glucoside

* The order of the sugars was not determined.

Key: Qu = quercetin, My = myricetin, Isorh = isorhamnetin, Lu = luteolin, Tr = tricin, Or = orientin, Iso Or = Iso-orientin, DIME = dimethyl ether, triME = trimethyl ether.

latter. Both taxa produce isorhamnetin as a major culm/or stem constituent but *E. monostachya* is unique in the family Ecdeiocoleaceae and differs from all the *Anarthria* species in producing only isorhamnetin glycosides. However, '*G. hexandra*' cannot be distinguished by its flavonoid aglycone pattern from *A. humilis* in the Anarthriaceae although it does have a very different flavonoid glycoside pattern, in which both galactosides and glucosides were identified (see Table 2). Flavonoid galactosides were not found in any other taxon from the Anarthriaceae or from *E. monostachya* but both flavone and flavonol galactosides have been found frequently in members of the Restionaceae (Williams *et al.*, unpublished results).

A more detailed analysis of the flavonoid glycosides was carried out on a representative specimen of each species of the Anarthriaceae and Ecdeiocoleaceae and for comparative purposes this was extended to include *Lyginia imberbis* (Lyginiaceae) and *Lepyrodia anarthria* (Restionaceae), as representatives of two other possibly closely related genera. The flavonoid glycosides identified are listed in Table 2. A number of simple flavonol glycosides, e.g. quercetin 3-glucoside and rhamnoside and myricetin 3-rhamnoside were easily identified by standard procedures. However, characterisation of some of the other major flavonol glycosides in three of the *Anarthria* species: *A. gracilis*, *A. prolifera* and *A. scabra*, proved unexpectedly difficult, since although the pure compounds were easily acid hydrolysed to the aglycone (myricetin, quercetin and isorhamnetin), no sugar was detected in equivalent amount. All these conjugates were mobile at pH 4.4 and had very high R_f values on cellulose TLC in water, suggesting they might be acid-resistant glucuronides or have acyl substituents. However, no trace of glucuronic acid was detected after acid treatment and all these (**1**–**3**) compounds remained unchanged by alkaline hydrolysis. *Anarthria laevis*, as already described above, is distinguished by tamarixetin 5-methyl ether, which is present as a 3-glycoside and the other three species, *A. 'egrallata'*, *A. humilis* and *A. polyphylla* are distinguished by the presence of flavonol diglycosides. The tamarixetin 5-methyl ether 3-glycoside from *A. laevis* was mobile on electrophoresis at pH 4.4 and very mobile in water but only glucose was produced on acid hydrolysis and it was not possible to determine its conjugation further because of lack of material.

In the Ecdeiocoleaceae acylated flavonol glycosides are characteristic constituents of both genera. In *E. monostachya* an isorhamnetin 3-feruloyldiglucoside was partially identified and in '*G. hexandra*' acylated derivatives of quercetin and isorhamnetin 3- mono

and diglucoside and galactoside were found. In both cases the corresponding simple non-acylated mono and diglycosides co-occurred. Acylated flavonol glycosides were found also in the representatives of the Lyginiaceae and Australian Restionaceae, i.e. an isorhamnetin 3-acylglucoside in *Lyginia imberbis* and a myricetin 3-acylglucoside in *Lepyrodia anarthria*, respectively. However, *Lyginia imberbis* was easily distinguished from all the Anarthriaceae and Ecdeiocoleaceae taxa and *Lepyrodia anarthria* by the production of the unusual sugar, allose, in combination with both quercetin and isorhamnetin as their 3-diallosides. *Lepyrodia anarthria*, on the other hand, differed from all the other taxa in the present study by producing flavone *C*-glycosides (orientin, iso-orientin and two luteolin di-*C*-glycosides) and quercetin 3-glucuronide. Flavone *C*-glycosides have been found in 46% of the *Lepyrodia* species surveyed (Williams *et al.*, unpublished results), where they could prove useful in indicating relationships within the genus.

DISCUSSION

The present flavonoid data appear to be useful at both generic and family levels. Thus, within the Anarthriaceae, three species, *A. 'egrallata'*, *A. gracilis* and *A. prolifera*, have identical flavonoid aglycone patterns suggesting they are closely related. *Anarthria scabra* shares many of the characteristics of these two species, notably myricetin and the unusual unidentified flavonol conjugates. The presence of myricetin in all three taxa suggests that they may be chemically the most primitive members of the family, whereas the presence of a dimethylated flavonol in *A. laevis*, tamarixetin 5-methyl ether, indicates this may be the most chemically advanced taxon of the group.

Anarthria 'egrallata' is a newly described species, thought to be closely related to *A. polyphylla*. The present results generally support its separation based upon the absence of tricin and kaempferol, which are trace constituents of *A. polyphylla*. However, the presence of the same flavonol diglycoside conjugates in both species provides a strong link between the two.

In the Ecdeiocoleaceae both the aglycone and glycoside data clearly separate the new genus '*Georgiella*' from *Ecdeiocolea* by the presence of quercetin and galactose (in combination with both quercetin and isorhamnetin) in the former and their absence from the latter. '*Georgiella hexandra*' has an aglycone pattern very similar to *A. humilis* but can be distinguished from all *Anarthria* species by the presence of flavonol galactosides, while *E. monostachya* is unique in producing only isorhamnetin glycosides. Also neither taxon produces the distinctive, unidentified *Anarthria* conjugates.

The flavonoid glycoside profiles of representative taxa of the Restionaceae and Lyginiaceae analysed are again very different from each other and from those in the Anarthriaceae, Ecdeiocoleaceae and Hopkinsiaceae in the presence of allosides in *Lyginia imberbis* and flavone *C*-glycosides in *Lepyrodia anarthria*. However, *L. imberbis* does produce some simple flavonol glycosides e.g. quercetin 3-glucoside, 3-rutinoside and a 3-diglucoside, which also occur in African Restionaceae, some *Anarthria* species and in the Ecdeiocoleaceae which indicates the ancestral condition of this group of plants.

A comparison of the flavonoid aglycone data for the two families under study with that for all other relevant taxa (see Introduction) are presented in Table 3. Most of the characteristic Restionaceae characters, such as simple flavones, 8-hydroxyflavones and flavonols, are absent from both the Anarthriaceae and Ecdeiocoleaceae. However, among the more 'primitive' members of the Restionaceae, e.g. *Sporadanthus*, and in the Hopkinsiaceae, simple flavonoid aglycone profiles similar to those found in the Anarthriaceae and Ecdeiocoleaceae do occur. The high frequency of myricetin in *Sporadanthus* appears to be especially significant. In *Lyginia* the more chemically advanced 8-hydroxyflavonol, gossypetin and/or its 7-methyl ether is present in at least one species, and in *Lepyrodia* flavone *C*-glycosides occur in nearly half of the species surveyed, suggesting that these genera are more distantly related to the Anarthriaceae and Ecdeiocoleaceae than *Sporadanthus* and *Hopkinsia*. Thus, at the family level, the flavonoid data are only in part agreement with the new classification now adopted and with recent morphological and cpDNA inversion data [3–5], which place *Hopkinsia* with *Lyginia* as basal within the Restionaceae or exclude them from this family completely.

In conclusion, the flavonoid evidence suggests that the Anarthriaceae is distinct from, and chemically more primitive than the Ecdeiocoleaceae, Lyginiaceae and most Restionaceae. However, there are sufficient similarities between the Anarthriaceae, Ecdeiocoleaceae, Hopkinsiaceae, Lyginiaceae and some genera of the Restionaceae to indicate that they are closely related families. Thus, the results are congruent with the chloroplast DNA data, which suggest that the Anarthriaceae diverged early from the Restionaceae and that the Ecdeiocoleaceae is a sister group of the Restionaceae [1].

EXPERIMENTAL

Plant material. Dried stem/leaf material was used throughout the study, most of which was collected and verified by two of us (BGB and LASJ), and voucher specimens have been deposited in the National Herbarium of New South Wales, Royal Botanic Gardens, Sydney, Australia. The remaining specimens supplied by other collectors were also verified by BGB and LASJ and voucher specimens lodged in NSW.

Identification of stem/culm flavonoids. The flavonoid constituents were extracted from stem/culm material in hot 80% MeOH and run two dimensionally in BAW and 15% HOAc on Whatman no. 1 paper to obtain a flavonoid profile for each taxon. The fla-

Table 3. The % occurrence of flavonoid aglycones in taxa of the Ecdeiocoleaceae and Anarthriaceae compared with the Lyginiaceae, Hopkinsoniaceae and the Restionaceae and two of their chemically most closely related genera

Family genus	No. of taxa	My	Qu	Km	Flavonols* Isorh	Tam 5 ME (5)	Goss	Goss 7 ME	Lu	Ap	Flavones* Chrys	Tr	Hyp	Hyp 7ME	Lu	Free aglycones Tr	Chrys	Pro Cy	Flavone C-glycs	Flavonoid sulphates
Ecdeiocoleaceae	2	0	50	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anarthriaceae	7	71	85	28	71	14	0	0	0	0	0	14	0	0	0	0	0	14	0	0
Lyginiaceae	3	0	100	0	66	0	0	33	0	0	0	0	0	0	0	0	0	0	0	0
Hopkinsiaceae	2	0	100	0	100	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0
Australian Restionaceae	116	9	34	7	14	0	12	4	56	17	9	22	51	7	15	6	17	17	15	28
Lepyrodia	13	38	92	31	31	0	15	0	0	0	0	0	0	0	0	0	0	0	46	15
Sporadanthus	6	83	100	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Detected after acid hydrolysis with 2 M HCl.

Key: My = myricetin, Qu = quercetin, Km = kaempferol, Isorh = isorhamnetin, Tam = tamarixetin, Goss = gossypetin, Lu = luteolin, Ap = apigenin, Chrys = chrysoeriol, Tr = tricin, Hyp = hypolaetin, ProCy = procyanidin and ME = methyl ether.

vonoid constituents in the MeOH leaf extracts were sepd from interferring cinnamic acid and other phenolic constituents by either multiple 2DPPC in BAW and 15% HOAc or 1DPPC in 15% HOAc or BAW as appropriate. The flavonoid spots/bands were cut out, combined and eluted with 80% MeOH giving a partially purified flavonoid for each taxon. The concd eluents were acid hydrolysed and the flavonoid aglycones identified by comparison with authentic markers using standard procedures.

Flavonoid glycosides. Known glycosides, isolated and purified by standard procedures, were characterised on the basis of UV spectral analysis, R_f and R_t data, acid hydrolysis to aglycone and sugar and where possible by direct comparison with authentic markers. Flavone *C*-glycosides were confirmed by 4 hr acid hydrolysis with 2 M HCl, extraction into iso-amyl alcohol and 2DPC in BAW and 15% HOAc and 1DTLC on cellulose in BAW and H_2O compared with vitexin and isovitexin. Flavonoid sulphates were detected by paper electrophoresis of direct MeOH leaf extracts at pH 2.2 (HOAc–HCOOH buffer) for 2 hr at 400 V. HPLC analysis was carried out on a Waters 600 multisolvent delivery system, fitted with a reverse phase Bondapak phenyl column, dimensions 4 mm ID + 300 mm. HPLC conditions: solvent A = 2% HOAc, solvent B = MeOH–HOAc–H_2O (18:1:1) using either the glycoside programme 75% A:25% B → 35% A:65% B or the aglycone programme 40% A:60% B → 100% B in 23 min in linear mode at 25°, flow rate 1 ml min^{-1} with diode array detection at 260 and 350 nm.

Other flavonol glycosides. A number of other flavonol glycosides isolated by standard procedures were partially identified in the process of confirming the aglycone results.

Unusual flavonol derivatives from A. gracilis *and* A. scabra **(1–3)**. These compounds, which were mobile on electrophoresis at pH 4.4 and had high mobility on TLC in H_2O, on acid hydrolysis gave known aglycones (myricetin, quercetin or isorhamnetin) but no recognisable sugar and were unchanged by alkaline hydrolysis. Therefore, they are not glucuronides or acylated compounds. FAB-MS failed to give meaningful results in all cases. R_f and R_t data and electrophoretic mobility at pH 4.4 is given for three of these compounds **(1–3)** in Table 4 and UV spectral data in Table 5.

Flavonol diglycosides. Twelve flavonol diglycosides were identified from R_f, R_t and UV spectral data (Tables 4, 5) and acid hydrolysis to aglycone and sugar, but there was insufficient material to determine the sugar linkages. The diglucosides and digalactosides of quercetin and isorhamnetin from '*G. hexandra*' could only be sepd by HPLC using the glycoside programme (see above and Table 4). An isorhamnetin 3-diglucoside from *E. monostachya* gave a FAB-MS of +ve 641 m.u. and −ve 693 m.u. confirming its $[M]^+$ as 640.

Acylated flavonol glycosides. Seven acylated fla-

Table 4. The R_f and R_t data for some new and unusual partially characterised flavonoid glycosides from the Anarthriaceae, Ecdeiocoleaceae, Lyginiaceae and Restionaceae

Flavonoid glycoside	Colour in UV + NH_3	BAW	$R_f \times 100$ in 15% HOAC	$R_f \times 100$ in H_2O	CAW (1:1)	R_t†	Electrophoresis mobility at pH 4.4 in mm
Myricetin							
3-Glyc (**1**)^{Pro}	DK/Y	29	17	54	01	nd	11
3-Glyc (**2**)^{s}	DK/Y	38	23	75	02	12.62	7.5
3-acyl Glc^{Lep}	DK/Y	12	22	49	01	nd	14
Quercetin							
Rutin Marker	DK/Y	42	47	33	07	16.07	0
3-Glyc (**3**)^{s}	DK/Y	51	31	75	02	16.76	11
3-Glc Ara^{h}	DK/Y	37	44	42	03	14.73	0
3-Di Glc^{Pro}	DK/Y	48	48	16	13	nd	0
3-Di Glc?^{G*}	DK/Y	48	58	22	50	15.41	5
3-Di Gal?^{G*}							
3-Acyl Glc^{G*}	DK/Y	76	32	03	76	23.84	5
3-Acyl Gal^{G*}							
3-Di All^{Ly}	DY/Y	20	41	15	13	10.62	nd
Isorhamnetin							
3-Di Glc^{Pro}	DK/Y	52	55	24	37	nd	0
3-Di Glc^{E}	DK/Y	23	59	17	19	19.53	0
3-ferulyl di Glc (**4**)^{E}	DK/Y	23	26	03	33	22.01	0
3-Di Glc?^{G*}	DK/Y	48	58	22	50	18.44	5
3-Di Gal?^{G*}							
3-Acyl Glc^{G*}	DK/Y	76	32	03	76	25.77	5
3-Acyl Gal^{G*}							
3-Acyl Gly^{Ly}	DK/Y	50	48	18	56	17.58	nd
3-Di Glc^{Ly}	DK/Y	32	41	15	38	14.37	nd
3-Di All^{Ly}	DK/Y	25	41	15	38	14.61	nd
Tamarixetin							
3-Gly (**5**)^{Lae}	B/Y	21	28	66	71	23.41	10
Isorhamnetin							
3-Glc marker	B/Y	19	28	04	73	22.86	0

* 3-Glucosides and 3-galactosides can only be separated from each other by HPLC. The R_f data is given for two mixtures: (1) quercetin and isorhamnetin 3-diglucosides and digalactosides and (2) their equivalent acylated derivatives.

† HPLC glycoside programme (see Experimental).

Key: Lae = *Anarthria laevis*, Pro = *A. prolifera*, h = *A. humilis*, s = *A. scabra*, E = *Ecdeiocolea monostachya*, G = '*Georgiella hexandra*', Lep = *Lepyrodia anarthria*, Ly = *Lyginia imberbis*, Glc = glucoside, Glyc = glycoside, Gal = galactoside, All = alloside, Dk = dark, Y = yellow, B = blue, BAW = *n*-butanol–acetic acid–water, 4:1:5, top layer and CAW- = chloroform–acetic acid–water.

vonol glycosides were partially characterised from their R_f, R_t and UV spectral data (Tables 4 and 5), acid hydrolysis to aglycone and sugar and alkaline hydrolysis to the deacylated glycosides. An isorhamnetin 3-acylglucoside from *Lyginia imberbis* gave FAB-MS +ve 625 and −ve 623 indicating a $[M]^+$ of 624, which suggested it was isorhamnetin 3-glucoside with a *p*-coumaric acid acyl group. However, no *p*-coumaric acid was detected after alkaline hydrolysis. The acylated digalactosides and diglucosides of quercetin and isorhamnetin present in '*G. hexandra*' could only be separated by HPLC using the glycoside programme (see above and Table 4). A cinnamic acid-acyl group was detected by HPLC after alkaline hydrolysis of the mixture at R_t 11.24 (ferulic acid 12.89, isoferulic 14.75) and λ_{max} 290′, 320 nm using the glycoside programme but was not further identified.

Identification of an isorhamnetin 3-feruloyldiglucoside from E. monostachya (**4**). Compound **4** gave isorhamnetin, glucose and ferulic acid. Alkaline hydrolysis gave isorhamnetin 3-diglucoside and ferulic acid, which was identified by HPLC comparison with an authentic marker: R_t 12.83 (**4**), 12.89 (ferulic acid) and 14.75 (isoferulic acid) using the glycoside programme (see above). A band II of 332 nm in the neutral spectrum of **4** (Table 5) also indicated acylation with a cinnamic acid and this was confirmed as ferulic by the FAB-MS of +ve 817, −ve 815 giving a $[M]^+$ of 816 for **4**. The R_f and R_t data (Tables 4, 5) also indicated acylation. The sugar linkage and position of attachment of the ferulic acid was not determined. However, the dark to yellow colour of **4** in UV + NH_3 and the positive NaOAc shift suggested that all three moieties were attached at the 3-position.

Table 5. UV spectral data for some partially characterised flavonoid glycosides and a new aglycone from the Anarthriaceae, Ecdeiocoleaceae, Lyginiaceae and Restionaceae

Flavonoid	λ_{max}^{MeOH}	+NaOAc	$+H_3BO_3$	+NaOH	$+AlCl_3$	$+AlCl_3$–HCl	HPLC λ_{max}
Myricetin							
3-Glyc (**1**)^{Pro}	266,342	274,403	268,376	nd	nd	nd	nd
3-Glyc (**2**)^{s}	263,360	268,375	263,379	274,404 Dec.	268,423	271,358,406	260,355
3-Acyl Glc^{Lep}	263,363	273,389	261,384	279,325,418	262,403	269,364	nd
Quercetin							
3-Glyc (**3**)^{s}	259,357	273,385	264,378	279,410 Dec.	270,402	268,363,400′	255,265′,355
3-Glc Ara^{h}	258,357	274,391	266,378	282,419	247,274,427	262,359	256,267′,355
3-Di Glc^{Pro}	265,361	271,406	264,379	281,419	nd	nd	nd
3-Di Glc?^{G}*	256,356	274,385	266,363	276,416	267,364,404	267,360,399	nd
3-Di Gal?^{G}*							
3-Acyl Glc^{G}*	257,315,370′	274,315,370′	266,315,370′	275,369	274,310,400′	274,308,400′	nd
3-Acyl Gal^{G}*							
3-Di All^{Ly}†	258,264,355	273,384	272,363	274,412	280,354,414	280,354,400	255,267′,355
Isorhamnetin							
3-Di Glc^{Pro}	265,356	272,371	265,360	274,418	nd	nd	nd
3-Di Glc^{E}	256,355	257,360	257,360	257,263,275,329,413	255,359,400′	256,357,400′	256,267′,354
3-ferulyl di Glc (**4**)^{E}	250,269′,332	272,360	250,269,332	270,394	nd	nd	248,270′,340
3-Di Glc^{G}*	256,356	274,385	266,363	276,416	267,364,404	267,360,399	nd
3-Di Gal?^{G}*							
3-Acyl Glc^{G}*	257,315,370′	274,315,370′	266,315,370′	275,369	274,310,400′	274,308,400′	nd
3-Acyl Gal^{G}*							
3-Acyl Glc^{Ly}	nd	nd	nd	nd	nd	nd	254,267′,355
3-Di Glc^{Ly} + some Qu	nd	nd	nd	nd	nd	nd	255,267′,355
3-Glc	256,264′,355	274,387	269,362	273,413	268,358,400	268,358,400	254,267′,355′
3-Di All^{Ly}†	258,254,355	273,384	272,363	274,412	280,354,414	280,354,400	255,267′,355
Tamarixetin 5ME (**6**)	nd	nd	nd	nd	nd	nd	251,368
3-Glyc (**5**)	nd	nd	nd	nd	nd	nd	250,262′,350
Isorhamnetin 5 ME	nd	nd	nd	nd	nd	nd	252,365
3-Glc	nd	nd	nd	nd	nd	nd	255,265′,346

* The diglucosides and digalactosides of quercetin and isorhamnetin were not separated except by HPLC—the data given is for a mixture.

† Quercetin and isorhamnetin 3-diallosides could only be separated on HPLC and the spectral data given is for the mixture except for the HPLC λ_{max}.

Key: Pro = *Anarthria prolifera*, s = *A. scabra*, E = *Ecdeiocolea monostachya*, G = '*Georgiella hexandra*', Lep = *Lepyrodia anarthria*, Ly = *Lyginia imberbis*, Glc = glucoside, Glyc = glycoside, Gal = galactoside, All = alloside, Qu = Quercetin and ME = methyl ether.

Identification of tamarixetin 5-methyl ether 3-glycoside (**5**) *from* A. laevis. Compound **5** was isolated from an 80% MeOH leaf extract of *A. laevis* by multiple 2D PPC in BAW and 15% HOAc followed by 1D PC in H_2O. It appeared blue to yellow in UV + NH_3. R_f, R_t and UV spectral data are given in Tables 4, 5. It was mobile on electrophoresis at pH 4.4 and very mobile in H_2O on TLC suggesting acylation but there was insufficient material to confirm this by alkaline hydrolysis. However, acid hydrolysis with 2 M HCl for 30 min, gave glucose (but no glucuronic acid or obvious acyl group) and a fluorescent yellow aglycone (**5a**) with similar R_fs to isorhamnetin 5-methyl ether: BAW 78 (77), Forestal 51 (51), 50% HOAc 24 (24) and CAW (2:1) 72 (69). However, the HPLC R_t and UV spectrum λ_{max} for **5a** were slightly different from the isorhamnetin 5-methyl ether marker; R_f 11.30 (10.78) and λ $HPLC_{max}$ 251, 268 (252, 365) so it was suspected that **5a** may be the closely related tamarixetin 5-methyl ether, a new aglycone, which would be stable in NaOH, whereas isorhamnetin 5-methyl ether is not. UV spectral analysis proved that **5a** was stable with 2M NaOH after 20 mins and the neutral spectrum was restored by addition of an equivalent amount of 2 M HCl λ_{max}^{MeOH} 256, 267′, 364, +NaOH 273, 328, 375 and NaOH + HCl 256, 267′, 364, indicating that methylation is at the 4′- rather than the 3′-position. The position of another methyl at the 5-position is indicated by the blue to yellow colour (in UV + NH_3) of the glycoside (**5**) and the fluorescent yellow of the aglycone **5a**. Demethylation of **5a** gave quercetin and a quercetin monomethyl ether, which co-chromatographed with isorhamnetin in 50% HOAc, BAW, Forestal and CAW 2:1 but was probably tamarixetin for which no marker was available but does not separate from isorhamnetin in these conditions. There was insufficient demethylated mixture to compare with isorhamnetin on HPLC. The position of the glucose on **5a** is thought to be at the 3-position because of the ready acid hydrolysis of **5** but confirmation with a positive NaOAc shift in the UV spectrum was not obtained due to lack of material.

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