

LEAF FLAVONOID DIVERSITY IN THE AUSTRALIAN GENUS *PATERSONIA*

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Key Word Index—*Patersonia*; Iridaceae; flavonol and flavone glycosides; scutellarein 7-methyl ether; quercetagenin 3,6,3'-trimethyl ether; glycoflavones; biflavonoids; isoflavonoids.

Abstract—In a leaf survey of 10 *Patersonia* species, six different flavonoid classes were detected: flavonol and flavone glycosides, 6-hydroxyflavonoids, flavone C-glycosides, biflavonoids and isoflavones. Twenty-nine flavonol glycosides, including seven with aliphatic acyl substitution, were found variously in these plants. *Patersonia glabrata* differed from the other nine taxa in producing amentoflavone and glycoflavones. Amentoflavone was found also in *P. pygmaea* but here and in the remaining species flavonol glycosides were the major constituents. However, all the taxa could be distinguished by their flavonoid profiles. For example, the unique presence of four unusual constituents scutellarein 7-methyl ether, quercetagenin 3,6,3'-trimethyl ether and the isoflavones tectorigenin and iristectorigenin A in the narrow-leaved *P. occidentalis* var. *angustifolia* clearly support its separation from the broad-leaved typical var. *occidentalis* as an independent species. Similarly, the seven differences in flavonoid pattern between *P. sericea* and *P. longifolia* support their recognition as separate species. The occurrence of biflavonoids in *P. glabrata* and *P. pygmaea* supports the placement of *Patersonia* in the Nivenioideae and the inclusion of *Isophysis* in the Iridaceae.

INTRODUCTION

The taxonomic position of the Iridaceous genus *Patersonia*, with some 19 species, 17 of which are endemic to Australia and the other two to Malesia, is uncertain. It has variously been considered a member of the subfamily Sisyrinchioideae, placed in tribe Aristeae of subfamily Iridoideae [1, 2] together with *Aristea*, *Klattia*, *Nivenia* and *Witsenia*, and most recently positioned in the subfamily Nivenioideae with the same four genera plus the Madagascan saprophyte *Geosiris* [3]. Morphologically, the genus is highly unusual with, for example, secondary thickening in its rhizome.

We have previously screened six *Patersonia* species for their flavonoid aglycones as part of a wider survey of the family Iridaceae [4, 5]. However, flavonoid glycosides were identified in only four taxa: *P. fragilis*, *P. glabrata*, *P. longifolia* and *P. sericea*. These results gave some support to the suggestion [4, 5] that *Patersonia* and other members of the Nivenioideae as constituted by Goldblatt [1–3] may be 'primitive' within the Iridaceae. But it was the discovery of the biflavonoid, amentoflavone in both *P. glabrata* [4] and *Isophysis tasmanica* (Isophysidoideae) [6], the species, which is postulated to most closely resemble an ancestral Iridaceae, that led us to extend our detailed analysis to another six *Patersonia* taxa in the hope of finding further biflavonoids. We report here our new results and compare them with our previous findings.

RESULTS

The results of the leaf flavonoid analysis of 10 *Patersonia* species are presented in Table 1. Dried leaf material was used throughout. The data refer to both flavonoid

glycosides and aglycones identified by standard procedures from methanolic leaf extracts after separation and purification by PC, TLC and in some cases HPLC.

A number of new or unusual flavonol glycosides were found during the course of the survey but as most occurred as complex mixtures the amounts that could be isolated of most were usually too small to allow full identification. However, two acylated flavonol glycosides were partially characterized from *P. occidentalis* var. *angustifolia* after purification by HPLC: quercetin 3-(acetylglucuronoside) and isorhamnetin 3-(3-methylbutyrylglucuronoside). The latter is a new compound and this is the first report of 3-methylbutyric acid as an acylating acid, although 2-methylbutyric acid has been recorded previously in *Valeriana wallichii* (Valerianaceae) [7]. A compound with the same R_f values was also found in *P. fragilis*, together with another unidentified acylated isorhamnetin 3-rutinoside. From their UV spectral data the five other acylated flavonol glycosides also appear to have aliphatic acyl groups. Four of these were found in *P. umbrosa*, where quercetin and isorhamnetin 3-xylosylglucosides and their acylated derivatives, an acylated kaempferol 3-xylosylglucoside and an acylated quercetin 3-glucosylxylosylrhamnoside were isolated but the sequence of the sugars and the identity of the acyl groups were not determined.

It is also of note that although the quercetin 3-arabinoside found in *P. maxwellii* and *P. fragilis* co-chromatographed with quercetin 3- α -L-arabinopyranoside (guajaverin) in four solvents on TLC, it was clearly separated from this compound and the 3- α -L-arabinofuranoside (avicularin) by HPLC (see Experimental). As it tended to decompose on keeping, it is most probably a furanoside and so could be the so far undescribed quercetin 3- β -L-arabinofuranoside.

Three accessions of *P. glabrata* were distinguished by the presence of flavone C-glycosides and amentoflavone. In the remaining nine *Patersonia* species and in a fourth accession of *P. glabrata* flavonol glycosides were the major leaf constituents and glycoflavones were absent.

The deviant accession of *P. glabrata* was also distinguished by the presence of free tricetin. This is the first record of tricetin in Nivenioideae and it is rare in the Iridaceae, being reported before only once in the Iridoideae–Sisyrinchieae, in eight members of the Iridaceae, in one member of the Tigrideae and in nine taxa of the Ixioideae. As the identity of the aberrant *P. glabrata* sample, among the four examined, has been thoroughly verified by the staff of the New South Wales Herbarium, it seems there must be chemical variation in this species and that further sampling of plant populations is needed to see how much variation occurs.

Amentoflavone was isolated from only one completely new source, *P. pygmaea*, where it occurs together with another unknown biflavonoid. However, some unidentified compounds were present in *P. sericea*, *P. maxwellii* and *P. occidentalis*, which might possibly, from their colour reactions and chromatographic mobilities, be biflavonoids.

The nine flavonol-producing species may all be distinguished from each other by their glycosidic patterns. The sugars: arabinose, rhamnose, glucose and galactose are all common but xylose is rare; myricetin and quercetin 3-xylosides were characterized only in *P. maxwellii*. Myricetin monoglycosides have otherwise been identified only in *P. occidentalis*, *P. sericea* and *P. longifolia*. Myricetin was detected also in *P. babilanoides* but there was insufficient plant material to allow the glycosides to be identified. Quercetin glycosides, on the other hand, were universally present and isorhamnetin glycosides occurred in all taxa except *P. longifolia*. In *P. umbrosa* some unusual quercetin and isorhamnetin di- and triglycosides and their acylated derivatives were partially characterized. A quercetin triglycoside and isorhamnetin 3-rutinoside were also isolated from *P. maxwellii* and both rutin and isorhamnetin 3-rutinoside and their acylated derivatives were present in *P. occidentalis* var. *angustifolia*. Kaempferol was found only twice: as the 3-glucoside in *P. occidentalis* var. *occidentalis* and as an acylated diglycoside in *P. umbrosa*. Typical *P. occidentalis* may also be distinguished from both its narrow-leaved variety *angustifolia* and all the other species examined by the presence of the 6-hydroxyflavonoids: scutellarein 7-methyl ether and quercetagenin 3,6,3'-trimethyl ether and the isoflavonoids, tectorigenin and isotectorigenin A. In the Iridaceae, isoflavonoids have been identified previously only from 10 *Iris* species and the closely related *Belamcanda chinensis* [8], both genera being in the Iridoideae–Irideae. The discovery of isoflavonoids in *Patersonia* is therefore of note. However, its significance in relation to Goldblatt's more recent classification remains to be resolved.

DISCUSSION

The present survey of *Patersonia* has revealed an unusually wide variety of flavonoid constituents in the genus. The finding of 6-hydroxyflavonoids and isoflavones is especially interesting, since these two classes are relatively rare in the family as a whole. Due to the structural richness encountered in the genus, it is not surprising that all 10 species examined may be easily

distinguished by their leaf flavonoid patterns. The most similar profiles were those of *P. maxwellii* and *P. occidentalis* var. *occidentalis*, which are thought to be closely related species [9]. However, the flavonoid pattern of the broad-leaved typical *P. occidentalis* was so different from the narrow-leaved variety, var. *angustifolia*, that one wonders whether they should not be treated as two separate species. As *P. occidentalis* is known to be morphologically variable, further study of both its chemistry and morphology would be worthwhile.

Taxonomically, *Patersonia longifolia* is treated as a variety of *P. sericea* in the latest "Flora of Australia" [9] but their very different flavonoid patterns suggest that they should continue to be recognised as two separate species. Thus, while both taxa produce myricetin 3-rhamnoside, 3-glucoside and 3-galactoside they may be distinguished by the presence of four isorhamnetin glycosides, myricetin 3-arabinoside and quercetin 3-rhamnoside in *P. sericea* and an acylated rutin derivative in *P. longifolia*.

Patersonia fragilis shows chemical similarity with *P. occidentalis* var. *angustifolia* in producing both quercetin 3-(acetylrutinoside) and the new isorhamnetin 3-(3-methylbutyrylrutinoside). However, it differs from all other *Patersonia* species by having another unique acylated isorhamnetin 3-rutinoside in its tissues. As it is a morphologically variable species [9], it is unfortunate that as only one accession was available it was not possible to test for chemical variation.

Patersonia glabrata differs from all the other taxa so far studied in producing flavone C-glycosides and amentoflavone. The only other species in which amentoflavone was detected was *P. pygmaea*, where it co-occurs with flavonol glycosides. These do not appear to be closely related species since they are geographically separate. Thus, *P. glabrata* is widespread on the coast and tablelands of South Queensland (NSW) and coastal Victoria, while *P. pygmaea* occurs in south western Western Australia. However, both taxa occur in similar habitats, i.e. in woodlands and on sandy heathland.

The flavonol glycoside data described here and elsewhere [5] supports a close relationship between *Patersonia* and the woody South African genera, *Klattia*, *Nivenia* and *Witsenia*, which has been established from anatomical studies of the leaves [10].

The occurrence of biflavonoids in *P. glabrata* and *P. pygmaea* also supports the placement of *Patersonia* in the Nivenioideae amongst the least specialised members of the Iridaceae and suggests a closer relationship with *Isophysis tasmanica* (Isophysidoideae) than was earlier envisaged. It also indirectly supports the inclusion of the latter taxon in the Iridaceae which is of some systematic importance as there have been suggestions that it should be considered a separate family.

EXPERIMENTAL

Plant material. Verified plant material was received from Dr T. D. Macfarlane [Western Australian Herbarium (Perth)], where voucher specimens have been retained.

Identification of leaf flavonoids. Leaf flavonoids were extracted in hot 80% MeOH and 2D-PCs of the extracts run in BAW and 15% HOAc to compare the flavonoid patterns of the 10 *Patersonia* species. Initial isolation was carried out on PC on 3 MM paper in (i) BAW and (ii) 15% HOAc. In complex mixtures further purification of glycosides was carried out on PC in CAW

(1:1) and by HPLC. Known glycosides were characterized on the basis of UV spectral analysis, R_f , acid hydrolysis to aglycone and sugar and where possible by direct comparison with authentic markers. Flavone C-glycosides were identified by standard procedures: R_f and UV spectral data, resistance to 4 hr acid hydrolysis and comparison with authentic markers. Flavone C-glycoside O-glycosides were determined from R_f , UV spectral data and 40 min acid hydrolysis to give sugar and parent glycoflavone and isomer.

Identification of quercetin 3-(acetylrutinoside) and isorhamnetin 3-(3-methylbutyrylrutinoside) from *P. occidentalis* var. angustifolia. Quercetin 3-(acetyl RG) and isorhamnetin 3-(3-methylbutyryl RG) were isolated from a direct 80% MeOH leaf extract by PC in BAW, 15% HOAc and CAW (1:1) followed by HPLC (Waters 600 system) on a Spherisorb S5 C₈ column (25 cm × 4.9 mm i.d.) using solvents: A: H₂O–HCOOH (19:1) and B: MeCN with a flow rate of 1.2 ml/min and detector at 365 nm. Initial conc: A 80 B 20 changing by 2% min. to A 40 B 60 after 20 min. and then immediately back to initial conditions and held for 10 min. R_f and R_i data are given in Tables 2 and 3, respectively.

Isorhamnetin 3-(3-methylbutyrylrutinoside). UV $\lambda_{\max}^{\text{MeOH}}$ 255, 267', 357; + NaOAc 273, 370; + H₃BO₃ 255, 267', 358 nm. FAB-MS gave a molecular ion at m/z 708 (C₃₃H₄₀O₁₇, requires 708) and a fragment at m/z 316 for isorhamnetin. The 3-methylbutyryl acyl group was determined from the 200 MHz ¹H NMR spectrum in pyridine-*d*₅ in the aliphatic region, which showed two 3-proton signals at δ 1.90 and 1.75 indicative of a *gem*-dimethyl group. Alkaline hydrolysis gave isorhamnetin 3-rutinoside.

Quercetin 3-(acetylrutinoside). FAB-MS (+ve and –ve modes) gave a molecular ion at m/z 652 (C₂₉H₃₂O₁₇, requires 652). Alkaline hydrolysis gave rutin.

Other flavonol glycosides. A number of unusual flavonol di- and tri-glycosides, some acylated, were partially characterized from R_f data, acid hydrolysis to aglycone and sugar and HPLC R_i s (see Table 3). However, the sequence of the sugars and the nature of the acyl group were not determined and not all the

compounds could be separated from each other to obtain good UV spectra.

Flavones. Luteolin and chrysoeriol were identified by standard procedures.

The characterization of scutellarein 7-methyl ether and quercetagetin 3,6,3'-trimethyl ether from *P. occidentalis*. Both compounds were isolated from an 80% MeOH leaf extract by PC on 3 MM paper in BAW followed by an overnight run in 15% HOAc before purification by HPLC using the conditions described above. Quercetagetin 3,6,3'-trimethyl ether was further purified by TLC on silica gel in toluene–HOAc (4:1).

Scutellarein 7-methyl ether. UV $\lambda_{\max}^{\text{MeOH}}$ 275, 335; + NaOAc 275, 367; + H₃BO₃ 277, 339; + NaOH 276, 328, 395. It co-chromatographed with an authentic marker in 7 solvents: on TLC cellulose in BAW, 50% HOAc, Forestal and CAW (2:1) (R_f s: 93, 78, 83 and 91) and on silica gel in toluene–HOAc (4:1), CHCl₃–*n*-hexane–HOAc (40:40:3) and toluene–MeOH–HOAc (45:3:2) (R_f s: 17.07 and 13). MS m/z 300 [M⁺] (C₁₆H₁₂O₆ required 300) 285 [M–Me], 257 [M–CO–Me], 207, 167 [tetraoxygenated A-ring fragment], 128, 119.

Quercetagetin 3,6,3'-trimethyl ether. UV $\lambda_{\max}^{\text{MeOH}}$ 255, 269, 346; + NaOAc 275, 312, 380; + H₃BO₃ 255, 269, 350. R_f s: TLC on cellulose BAW 93, Forestal 90, 50% HOAc 74, CAW (2:1) 94 and on silica gel toluene–HOAc (4:1) 30, CHCl₃–*n*-hexane–MeOH (40:40:3) 14 and toluene–MeOH–HOAc (45:3:2) 21. An authentic marker was not available. Demethylation gave quercetagetin (co-chromat. in the 7 solvents above). MS m/z 360 [M⁺] (C₁₈H₁₆O₈ requires 360), 345 [M–15], 330 [M–30] and 151 (B-ring with one hydroxyl and one methoxyl). These data and the increase in intensity of the long wave band in the UV spectrum with NaOH indicates its structure as quercetagetin 3,6,3'-trimethyl ether.

Biflavonoids. Biflavonoids were isolated from 80% MeOH leaf extracts by PC on 3 MM paper in BAW, where they ran near the solvent front. They were purified on TLC silica gel in toluene–HCOOEt–HCOOH (5:4:1) (TEF) followed by PC on

Table 2. R_f data for some unusual flavonol glycosides from *Patersonia* species

Flavonol† glycoside	Solvents			
	BAW	15% HOAc	H ₂ O	CAW(1:1)
Quercetin				
3-RG	31	58	24	50
3-Xyl RG	26	70	30	43
3-(Acetyl RG)	61	74	34	59
3-Xylglc acylated*	63	75	40	85
3-GalGlcRha	51	82	50	32
3-GlcXylRha	17	81	51	37
3-GlcXylRha acylated	41	87	67	72
Isorhamnetin				
3-RG	41	70	40	71
3-XylGlc*	63	75	40	85
3-GlcXylRha	41	87	67	72
3-XylGlcacylated*	63	75	40	85
3-RGacylated	65	78	53	71
3-(3-methylbutyryl RG)	88	81	52	92
Kaempferol				
3-XylGlcacylated*	63	75	40	85

*These compounds were present as a mixture, which was resolved only by HPLC.

†The order of sugars is not known in the di- and triglycosides apart from the rutinosides.

Table 3. HPLC R_f data* for some flavonol glycosides from *Patersonia* species

Flavonol glycoside†		Retention time (R_f)	Plant source‡
Myricetin	3-Ara	5.22	Max
	3-Gal	3.13	Max
	3-Glc	3.67	Max
	3-Xyl	4.49	Max
Quercetin	3-Ara	6.82	Max
	3- α -L-Arabinopyranoside (Gyaijaverin)	6.57	Marker
	3- α -L-Arafuranoside (Avicularin)	6.44	Marker
	3-Gal	4.05	Max
	3-Glc	4.49	Max
	3-Xyl	5.30	Max
	3-RG	5.00	Marker
	3-(acetyl RG)	5.22	Occ-an
	3-XylGlc	4.42	Um
	3-XylGlcacylated	6.33	Um
	3-GalGlcRha	4.68	Max
	3-GlcXylRha	3.97	Um
	3-GlcXylRhaacylated	5.53	Um
Isorhamnetin	3-RG	6.18	Um
	3-(3-methylbutyryl RG)	12.01	Occ-an
	3-XylGlc	5.86	Um
	3-XylGlcacylated	8.53	Um
	3-GlcXylRha	5.21	Um
Kaempferol	3-XylGlcacylated	7.86	Um

*For HPLC conditions see Experimental.

†The sugar sequence in di- and tri- glycosides other than the rutinosides is not known.

‡Max = *Patersonia maxwellii*; Um = *P. umbrosa* and Occ-an = *P. occidentalis* var. *angustifolius*.

3 MM paper in *n*-BuOH–1 M (NH₄OH (1:1) (BN) or TLC on silica gel in toluene–pyridine–HCOOH (100:20:7) (TPF). Amentoflavone, previously characterized in *P. glabrata* [3] was identified in *P. pygmaea* by co-chromatography on TLC in 5 solvents: TEF, TPF on silica gel and BN cellulose (see above) and toluene–MeCOEt–MeOH (4:3:3) on Merck polyamide 11 and nitromethane–MeOH (4:3) on microcrystalline polyamide (Schleicher and Schull).

Isoflavonoids from P. occidentalis. Isoflavonoids were isolated from an 80% MeOH leaf extract by PC in BAW, where they ran near the solvent front. They were separated from methylated flavonol aglycones by running on PC overnight in 15% HOAc. Further purification on TLC silica gel in 1% MeOH in CHCl₃ gave two compounds: tectorigenin and iristectorigenin A. R_f s respectively: on silica gel 1% MeOH in CHCl₃ 18, 38; 8% MeOH in CHCl₃ 86, 96; EtOAc–petrol (1:1) 100, 100 and on cellulose BAW 92, 92 and 15% HOAc 33, 33.

Tectorigenin. UV spectral data $\lambda_{\max}^{\text{MeOH}}$ 267, 335'; +NaOAc 273, 340; +H₃BO₃ 267, 335'; +NaOH 277, 332; +AlCl₃ 275, 315, 365 and AlCl₃/HCl 277, 315, 365 nm. MS: m/z 300 [M⁺] (C₁₆H₁₂O₆ requires 300) 285 [M–15], 282, 257, 139, 118 [B-ring]. It co-chromatographed in 4 solvents with an authentic sample from our collection.

Iristectorigenin A. UV spectral data $\lambda_{\max}^{\text{MeOH}}$ 268, 335'; +NaOAc 273, 339; +H₃BO₃ 268, 335; +NaOH 280, 325; +AlCl₃ 276, 310, 380 and +AlCl₃/HCl 278, 310, 383. MS m/z 330 [M⁺] (C₁₇H₁₄O₇ requires 330) 315 [M–15], 312, 287, 149. The spectral data are identical with lit. values but we have not

been able to rule out completely the less likely isomeric structure, iristectorigenin B, for our substance.

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