Biflavonoids, Quinones and Xanthones as Rare Chemical Markers in the Family Iridaceae

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Amentoflavone has been characterised from leaves of *Patersonia glabrata.* This is the first report of a biflavone in the Monocotyledoneae. The quinone plumbagin, a characteristic constituent of three dicotyledonous families, is now found to be a useful marker for the genus *Aristea;* it also occurs in two *Sisyrinchium* species and in *Sparaxis tricolor.* Mangiferin, a C-glucosylxanthone known previously in the Iridaceae only from *Crocus, Iris* and *Gynandriris* has now been found in *Eleutherine, Rigidella, Gelasine* and *Tigridia.* The chemotaxonomic significance of these results is discussed.

Introduction

As part of a continuing chemotaxonomic survey of flavonoids and related phenolics in families of the Monocotyledoneae (see *e.g.* [1, 2]), we have been investigating the ornamentally important family, the Iridaceae. This is a family of petaloid plants of the Liliales, of some 1500 species in 85 genera, which are distributed throughout the world. In an earlier survey of the phenolics of the leaves, Bate-Smith [3] found a particularly wide range of flavonoid patterns to be present. In particular, the distributions of the flavonol myricetin and of proanthocyanidins were useful for indicating phylogenetic trends within members of the Iridoideae and Crocoideae. Other phenolic constituents known in the family include a series of isoflavones in *Iris* [4], hydroxyquinones in *Eleutherine* and *Libertia* [5] and the C-glucosylxanthone mangiferin in *Iris* and *Crocus* spp. [3, 6].

In our own studies of the Iridaceae, we have already reported on the first identification of 6-hydroxyflavones in the family, in three species of *Crocus;* a range of other *Crocus* flavonoids were also identified [7]. We now report the discovery of a new class of flavonoid, represented by the biflavone amentoflavone, in the family in *Patersonia.* We also describe the results of surveying these plants for quinones and xanthones.

Results

Biflavonoids are apparently primitive chemical markers in higher plants, since they occur primarily (and widely) throughout the gymnosperms but have only been reported relatively rarely in a few dicotyledonous angiosperm groups [8]. Our discovery of amentoflavone in leaves of *Patersonia glabrata,* an Australian member of the tribe Aristeae, was therefore quite unexpected. It was, however, unambiguously identified by direct comparison with an authentic specimen (see Experimental). Amentoflavone was also found in the inflorescence of this plant but was not detected in any of the other ca. 200 species of the Iridaceae surveyed. However, other biflavonoids may be present in *Klattia partita, Witsenia maura* and in two more *Patersonia* species (Table I): *P. occidentalis* and *P. sericea,* but there was insufficient plant material to allow their identification. It is of note that *P. glabrata* also differs from the other *Patersonia* species and the other members of the Aristeae examined in accumulating flavone C-glycosides instead of flavonols (Table I). *Patersonia longifolia, P. occidentalis* and *P. sericea* are also unusual in producing myricetin glycosides as major leaf constituents.

This discovery of amentoflavone in the Iridaceae represents the first report of a biflavone in the monocotyledons. Indeed, there is only one other mention of a biflavonoid occurring in these plants, namely of a 3,8-linked biflavanone from the stems of *Lophiola americana* Wood [9]. This plant is of uncertain taxonomic affinities, having been placed both in the Haemodoraceae and in the Amaryllidaceae. If the latter placement is correct, then there is some relationship with our discovery of amentoflavone in *Patersonia,* since Amaryllidaceae and Iridaceae are usually regarded as being closely related [10].

Our discovery of the hydroxynaphthoquinone, plumbagin, in the Iridaceae was also unexpected, since it is a characteristic constituent of three quite

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Table I. Phenolic constituents of the tribe Aristeae.

Species	Flavone C-glycosides	Flavonols	Others	Source*	Accession number
Aristea alata Baker	–	Qu, Km	Plumbagin	K	084-81.01253
A. compressa Klaus	–	Qu, Km	Plumbagin	K	080-61-08002
A. ensifolia Muir	–	Qu, Km	Plumbagin	K	039-64.03902
A. lugens Hort. ex Steud.	–	Qu, (Km) (Isorh)	Plumbagin	M	
A. platycaulis Baker	–	–	Plumbagin	K	000-69.52032
A. singularis H. Weim	–	Qu, Km	Plumbagin	MO	P. Goldblatt 7253
Klattia partita Baker var. *flava* Lewis	–	Qu, Km, My Isorh	DK/DK (biflavonoid?)	MO	P. Goldblatt 6920
Nivenia fruticosa (L. f.) Baker	–	Qu, Km, Isorh	–	MO	Esterhuysen 36149
Nivenia sp. nov.	–	Qu, Km	–	MO	PG 7178
Patersonia fragilis (Labill.) Asch. & Graebn.	–	Qu, Km Isorh	ProCy, ProDp	NSW	P. Hind 3582
P. glabrata R. Br.	+	–	Amentoflavone ProCy	NSW	R. Coveny 11831 & C. Miller
P. glabrata R. Br.	+	–	Amentoflavone	NSW	R. Coveny 11834 & C. Miller
P. longifolia R. Br.	–	My, (Qu) (Isorh)	(ProCy)	NSW	R. Coveny 11835 & C. Miller
P. longiscapa Sweet	–	Qu, Isorh	–	M	
P. occidentalis R. Br.	–	Qu, Km My, Isorh	ProDp Biflavonoid?	K	149-83.01737
Patersonia sericea R. Br.	–	Qu, Isorh My	(ProCy)	NSW	R. Coveny 11837 & C. Miller
P. sericea R. Br.	–	Qu, Isorh My	2 Biflavonoids?	NSW	P. Hind 3544
Witsenia maura Thunb.	–	Qu, My Isorh	ProDp (biflavonoid?)	MO	Orchard 35

* K, Plants from the living collection; The Royal Botanic Gardens, Kew; M, material supplied by Brian Mathew of The Royal Botanic Gardens, Kew from his own private garden; MO, material received from Dr. Peter Goldblatt, Missouri Botanic Garden and NSW, National Herbarium of New South Wales.

Key: Qu, quercetin; Km, kaempferol; Isorh, isorhamnetin; My, myricetin; ProCy, procyanidin; ProDp, prodelphinidin; ( ), trace constituent.

unrelated families in the Dicotyledoneae, in Droseraceae, Ebenaceae and Plumbaginaceae [5]. This quinone was characterised from leaves of *Aristea alata* and confirmed in the other five species of *Aristea* (Table I) that were available for screening. In all but one species it co-occurred with simple flavonols and other unidentified quinone pigments were present in most taxa. However, quinones were not detected in the four other genera (see Table I) examined in the tribe Aristeae. Indeed, during a survey of 47 genera and over 200 taxa of the Iridaceae, plumbagin was found in only two other genera: *Sparaxis* (in the Gladeoleae) and *Sisyrinchium* (tribe Sisyrincheae), which are not closely related to each other or to the Aristeae. Thus, it co-occurred with flavonols in *Sparaxis tricolor* (Schneev.) Ker-Gawler (K, 312-77.06928) but was not detected in *S. grandiflora* (de la Roche) Ker-Gawler, the only other *Sparaxis* species surveyed. Similarly, plumbagin was identified in the leaves of only two of 22 *Sisyrinchium* species examined, in *S. brachypus* (Bickn.) J. Henry (K, 473-57.47301) and *S. californicum* (Ker-Gawler) Dryander (K, 424-83.05371). It was also detected in the flowers of *S. brachypus* and in the flowers (but not leaves) of *S. tinctorium* H, B & K (K, 473-69.09279). The flowers of *S. tenuifolium* Homboldt & Bonpland (K, 312-83.03722) contained another unidentified quinone pigment.

Plumbagin sometimes occurs naturally in bound colourless form as a quinol glycoside and this is true of its presence in roots and leaves of the Plumbaginaceae [11]. In the Iridaceae, it appears to be free, both in the leaf and flower, since it is readily and rapidly extracted from fresh tissue by methanol or ether. Its contribution to yellow flower colour in two *Sisyrinchium* species is unusual, since quinones

Table II. Distribution of mangiferin in the Iridaceae.

Previous reports [4]	New results	Source	Accession number
Iridoideae			
Irideae			
Belamcanda punctata[**] Moench.			
Gynandriris	*Gynandriris australis* Goldblatt	K	306-83.03483
Iris sisyrinchium L.	*G. setifolia* (L.f.) Foster	K	312-77.02410
(= *Gynandriris*			
sisyrinchium (L.) Parl.			
Iris[*] subgenus *Iris*			
Section *Iris*			
I. aphylla L.	*I. cengialtii* Ambr. (= *I. pallida*	R	Mathew 403
I. chamaeiris Bertol	subsp. *cengialtii* (Ambr.) Foster)		
(= *I.*[a] *lutescens* Lam.)	*I. mellita* Janka	S	
I. flavescens DC.	(= *I. suaveolens* Boissier & Reuter)		
I. germanica CV.			
I. imbricata Lindl.	*I. pumila* L.	R	20002 Jugoslavia
Section *Onocyclus*			
I. sari Schott ex Baker			
Subgenus *Limniris*			
Section *Limniris*			
Series Laevigatae			
I. kaempferi Sieb.			
(= *I. ensata* Thunb.)			
I. pseudoacorus L.			
I. versicolor L.			
Series *Unguiculares*			
I. unguicularis Poir.	*I. unguicularis* Poir. cv.	R	760539
	'Cecilia Christy-Miller'		
	I. unguicularis Poir. var.	R	780957
	lazica Alboff		
Pardanthopsis			
Iris dichotoma Pallas			
(= *Pardanthopsis dichotoma*			
(Pallas) Lenz)			
Tigrideae			
	Eleutherine bulbosa Urb.[c]	K	312-83.03664
	E. bulbosa Urb.[c]	K	312-83.03666
	Gelasine azurea Herb.[b]	K	306-83.03471
	Rigidella orthantha Lem.[c]	K	312-83.03569
	R. orthantha Lem.[c]	K	312-83.03528
	Rigidella sp.[c]	K	312-83.03568
	Tigridia alpestris Molseed[b]	K	312-83.03880
	T. alpestris[b]	K	312-83.03883
	Tigridia sp.[b]	K	312-83.03875
	Tigridia sp.[b]	K	312-83.03891
	Tigridia sp.[b]	K	312-83.03881
	Tigridia sp.	K	312-83.03822
	Tigridia sp.	K	441-75.04555
	Tigridia sp.	K	312-83.0384
	Tigridia sp.[c]	K	312-83.03840
Croceae			
Crocus aureus Sibrh. & Sm.[a]			
C. stellaris Haw.			

[*] Classification of *Iris* species according to B. Mathew [10].
[**] Reference [18].
[a] Report confirmed in present survey.
[b] A mangiferin O-glucoside also present.
[c] Isomangiferin also present.

are not often recognized as floral pigments. In *S. tinctorium,* plumbagin is not the only yellow petal pigment, since co-occurring carotenoids were also detected.

Our discovery of plumbagin in the Iridaceae has been confirmed independently by Kumar *et al.* [12] who have just reported it in the leaves and rhizomes of a sixth *Aristea* species, *A. ecklonii* Baker, where it occurs with two plumbagin dimers. Another reported source of quinones in the Iridaceae is the South American *Libertia coerulescens,* the leaves of which reputedly contain several anthraquinone pigments [13]. We have not found any evidence of anthraquinones during our surveys, and could not find any quinones present in the leaves of the related *Libertia chilensis.*

Another systematically interesting phenolic we encountered during our survey is the C-glucosylxanthone mangiferin, which is very easily detected in plant extracts because of its striking fluorescence in UV light. Previous reports of its occurrence in Iridaceae are mainly from *Iris,* including several *Iris* species which have subsequently been transferred to the genera *Gynandriris* and *Pardanthopsis* [3, 6]. Otherwise it has only been found in two *Crocus* species [3, 7]. The present findings of mangiferin in the family are listed in Table II together with the previous reports for comparison. Thus, in the present survey mangiferin has been found in a further five *Iris* taxa: three in subgenus *Iris,* section *Iris* and two in subgenus *Limniris,* section *Limniris,* series *Unguiculares.* Mangiferin together with isomangiferin has also been detected in another two *Gynandriris* species. However, the most interesting findings of the current leaf survey of the Iridaceae are in the tribe Tigrideae, where mangiferin was characterised for the first time in the four genera: *Eleutherine, Gelasine, Rigidella* and *Tigridia.* In the two *Eleutherine,* the two *Rigidella* and one of the *Tigridia* species examined isomangiferin also occurred, while in *Gelasine azurea, Tigridia alpestris* and three unidentified *Tigridia* species a mangiferin O-glucoside was additionally present, but in species of another four genera of the Tigrideae: *Calydorea, Cipura, Sphenostigma* and *Herbertia* mangiferin and its derivatives were not detected and were also absent from *Tigridia meleagris* and *T. pavonia.*

Discussion

In most systems of plant classification, the Iridaceae are placed near the Liliaceae and the Amaryllidaceae, although the precise relationships that exist between the three families are still disputed. From the present and earlier results on the phenolic constituents [14] of the three families (Table III), it is clear that there are some chemical links, particularly between the Iridaceae and Liliaceae. Nevertheless, it is also apparent that the Iridaceae is the most chemically distinctive, with a wider range of characters than the other two.

The discovery of the naphthoquinone plumbagin in three genera, for example, extends the number of known quinone pigments, which now range from the simple 2,6-dimethoxybenzoquinone in *Iris milesii* rhizomes, the modified naphthoquinone eleutherin in *Eleutherine bulbosa* tubers to chrysophanol and emodin, anthraquinones in the roots and leaves of *Libertia coerulescens.* By contrast, in the Liliaceae the main type of quinone are the anthraquinones of the Aloineae, although derivatives of the naphthoquinone 7-methyljuglone have been reported from

Table III. Distribution of Primitive Phenolic Markers in the Iridaceae and related families.

Chemical character	Occurrences in Iridaceae	Liliaceae	Amaryllidaceae
Biflavonoid	*Patersonia*	–	*Lophiola*
Glycosylxanthone	8 genera (see Table II)	*Anemarrhena* and *Smilax*	–
Quinones	*Aristea, Eleutherine, Sisyrinchium, Sparaxis*	widespread in Aloineae; *Dianella* and *Stypandra*	–
Myricetin	at least 10 genera (inc. *Patersonia*)	–	–
Proanthocyanidins	at least 15% of species	10% of species	rare, in *Doryanthes*
Flavone C-glycosides	in 64% of species	in 1% of species	–

roots of *Stypandra grandis* and two *Dianella* species [5].

These new chemical results are probably of most significance in relation to distribution patterns within the Iridaceae. Most of the phenolic characters recorded in Table III, and especially the biflavones, could be regarded as "primitive" markers, so that their main interest is in their correlative occurrence in morphologically unspecialized members of the family. For example, the concentration of three primitive features, biflavones, myricetin and C-glycosylflavones, in the Australasian genus *Patersonia* would suggest that it is a primitive group within the Iridaceae. This ties in well with the fact that the genus shows woody characteristics and has aberrant secondary thickening typical of monocots [10]. Again, the South African genus *Aristea* is regarded as unspecialized in terms of its floral parts and of its unmodified storage organs [15]. The presence of the quinone pigment, plumbagin, throughout all members surveyed would support this view. In particular, *Aristea singularis,* which is probably the most primitive species of the genus is positive for plumbagin. On the other hand, quinone pigments appear to be completely lacking in all the more highly specialized genera of the family.

The distribution of mangiferin in the Iridaceae is perhaps more difficult to interpret in terms of its phyletic significance but it does have systematic interest. Thus, it is a characteristic constituent of some groups of *Iris;* namely subgenus *Iris,* section *Iris* (the bearded or pogon irises) and subgenus *Limniris,* section *Limniris,* series *Laevigatae* and *Unguiculares*. It is also present in all three *Gynandriris* species, which have been surveyed [16]. Within the Tigrideae mangiferin was detected in *Eleutherine, Gelasine, Rigidella* and in some of the *Tigridia* species but was absent from the *Calydorea, Cipura, Sphenostigma* and *Herbertia* taxa. This pattern of distribution does not agree entirely with Goldblatt's recent division of the Tigrideae on the basis of chromosome number [17]. However, there are some correlations and further taxa need to be surveyed before definite conclusions can be drawn.

Experimental

Sources of plant material are given in Tables I and II and the text.

Identification of phenolic constituents

Amentoflavone. Amentoflavone was isolated from an 80% methanolic leaf extract of *Patersonia glabrata* by (1) PC on Whatman 3MM paper in BAW (*n*-butanol:acetic acid:water, 4:1:5 top layer) (2) TLC on silica gel in TEF (toluene:ethyl formate:formic acid, 5:4:1) and (3) PC on 3MM paper in 30% acetic acid. R_f and UV spectral data were identical with those of an authentic marker. UVλ_{max} MeOH 271, 338; +NaOAc 276, 370; +H_3BO_3 272, 335; +NaOH 277 sh, 400; +$AlCl_3$ 280, 301 sh, 350, 388 and $AlCl_3$/HCl 280, 301 sh, 345, 388. R_fs on TLC cellulose were: BAW 100, Forestal 91, CAW (chloroform: acetic acid:water, 2:1 sat. with water) 72 and *n*-butanol:2M ammonia (1:1, top layer) 31; on TLC silica gel: TEF 42, TPF (toluene:pyridine:formic acid, 100:20:7) 09 and on polyamide TLC in toluene:methyl ethyl ketone: methanol, 4:3:3, 11. The compound remained unchanged on demethylation with pyridinium chloride for 4 hours.

Molec. wt. by FAB-MS in glycerol: $[M + H]^+$ 539, $C_{30}H_{19}O_{10}$ requires 539.

Mangiferin. Mangiferin was isolated from 80% methanolic leaf extracts of representative species of *Tigridia, Eleutherine, Rigidella* and *Gelasine* by cutting out and combining the relevant spots from a number of 2D PCs on 3MM paper of each taxon. In all cases the R_f and spectral data of the isolated compound was identical with an authentic marker of mangiferin. UVλ_{max} MeOH 235, 258, 317, 366; +NaOH 235, 247 sh, 273, 304, 393. R_f data on PC Whatman No. 1 paper for mangiferin and isomangiferin, respectively: BAW 39, 22; H_2O 11, 07; 15% acetic acid 35, 14 and CAW (1:1 sat. with water) 22, 12. Isomangiferin was identified by comparison with R_f data and a mangiferin O-glucoside, isolated from *Tigridia alpestris* (R_f, BAW 58 and 15% acetic acid 53) gave mangiferin and glucose (PC in 4 solvents) on acid hydrolysis with 2N HCl for 40 minutes.

Plumbagin. Plumbagin was isolated from an 80% methanolic leaf extract of *Aristea alata* by PC on 3MM paper in BAW. It was identified by direct comparison with an authentic specimen of plumbagin, by means of spectral properties (and shifts with NaOH and $AlCl_3$), of MS fragmentation (M^+188, $C_{11}H_8O_3$ requires 188) and of co-chromatography by TLC using 6 solvents and 3 adsorbents. For screening purposes, identification was based on spectral measurements, TLC on silica gel in petrol-ether (7:3) and toluene-petrol (2:1) and TLC on polyamide in MeOH-toluene (4:1).

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