# THE PHYTOCHEMICAL RICHNESS OF THE *IRIDACEAE* AND ITS SYSTEMATIC SIGNIFICANCE

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ABSTRACT - Just as the family *Iridaceae* is abundant in its morphological and anatomical diversity, so is it rich in its secondary metabolites. Many varied phytochemicals have been described from the family. Isoflavones, first recorded in *Iris florentina*, have recently been detected in *Iris pseudopumila*. Almost all common classes of flavonoid are present, while the rarer biflavonoids characterise *Isophysis* and *Patersonia*. The anthocyanins in flowers are generally distinctive at the generic level. Quinonoid and xanthone pigments have systematically interesting distribution patterns. Distinctive chemicals in *Iris* rhizome oils and in *Crocus* styles are useful economically. Yet other phytochemicals such as non-protein amino acids and special storage carbohydrates have restricted distribution patterns.

KEY WORDS - Iridaceae; flavonoids; xanthones; naphthoquinones; proanthocyanidins

### INTRODUCTION

The *Iridaceae* is an ornamental plant family of about 1500 species and 85 genera, which has an almost worldwide distribution. For its size, it is particularly rich in its phytochemistry, having a wealth of chemical structures, especially of the phenolic type (Hegnauer 1963; 1986). Classes of phenolic present include anthocyanins, flavonols, flavones, isoflavones, xanthones, biflavones, naphthoquinones and proanthocyanidins. Other groups of secondary metabolite recorded in the family are saponins, fructans, non-protein amino acids, sterols, and bufadienolides. Some individual metabolites are of economic importance. The sesquiterpene  $\alpha$ -irone from *Iris florentina* L. is valued for its characteristic scent of violets and is used in perfumery. The yellow carotenoid-like pigment crocein from styles of *Crocus sativus* L. is employed as a food colourant. Again, the isoflavone iridin is a major component of orris root, obtained from the rhizome of *Iris florentina*, and still used in perfumery.

The main purpose of the present review is to consider those phytochemical studies which have included significant surveys within the family and to assess the systematic value of chemical markers. However, it is also of importance to consider the *raison d'être* of this proliferation of secondary chemistry and this topic will be considered before the systematic implications.

## FUNCTION OF IRIDACEAE SECONDARY METABOLITES

A major hypothesis propounded over recent years to explain the widespread occurrence of secondary constituents in plants points to a variety of ecological functions. Thus some compounds, such as the coloured anthocyanins, are important in pollination biology. Other constituents provide the plant with defences against herbivory and microbial infection (Harborne, 1993). The reason why there is a considerable variety of anthocyanins present in the flowers of *Iridaceae* is that many different colours need to be produced to attract a wide variety of pollinators from bees and beetles to butterflies and sunbirds (Goldblatt, this volume). Selection for blue flower colour is apparent in some genera of the family, particularly *Iris*, a colour favoured by bee pollinators. In such a case, it is likely that the anthocyanin present, a delphinidin derivative, is copigmented by flavones to produce the necessary shift in colour from purple to blue. Copigmentation of this type has been established by Asen *et al.* (1970) in the case of cultivated *Iris* flowers.

The purpose of secondary metabolites in leaves of *Iridaceae* is less obvious, because of lack of experimentation. Although alkaloids are apparently absent from the family, some very toxic constituents have been characterised in two South African genera, *Homeria* Vent. and *Moraea* Miller. These are bufadienolides, a class of plant toxin also present in toad venoms. They are poisonous to mammals and could be assumed to provide defence against animal grazing. Another class of mammalian feeding deterrents present in *Iridaceae* are the proanthocyanidins. These polymeric tannins have been variously detected in 45 of 255 species surveyed (Williams *et al.*, 1986) and occur in 18 genera, including *Dietes* Salisb., *Iris* L., *Nivenia* Vent. and *Patersonia* R. Br. The effectiveness of tannins as feeding deterrents depends very much on the concentrations present in the leaves, and quantitative analyses are still needed to confirm that they are important protectants in this family.

Another ecological role for secondary metabolites in leaves is protection against microbial invasion. Phenolic constituents are abundant in leaves of *Iridaceae* and they have often been implicated as antimicrobial agents. The isoflavones irigenin and tectorigenin of *Iris* rhizomes are known to be effective antifungal agents, as are a number of flavanones recently reported in *Iris* tissues. The only experimental evidence that the isoflavonoids of *Iris* are antifungal has been obtained from studies in *Iris pseudacorus* L. Treatment of leaves with cupric chloride solution, a method commonly used to mimic phytoalexin induction, led to the production in the leaves of 10 isoflavones and six coumaronochromones. Described as stress metabolites, several of these were antifungal. In particular, the compound 5,7,2'-trihydroxyisoflavone, newly reported from this *Iris* species, was fungitoxic against *Cladosporium herbarum* (Pers.: Fr.) Link at a concentration of 25 mg cm<sup>-2</sup> in a special bioassay test system (Hanawa *et al.*, 1991).

## CHEMOSYSTEMATICS OF IRIDACEAE

The chemosystematics of this family is not as well advanced as that in many other families of the monocotyledons (Williams and Harborne, 1988). Most phytochemical reports on *Iridaceae* cover only single species and there have not been many surveys. Several early attempts were made to relate chemistry to taxonomy e.g. in the case of storage carbohydrates in rhizomes or corms, but these are now mainly of historical interest (Hegnauer, 1986). In recent years, there have been two comprehensive surveys

on non-protein amino acids (Larsen *et al.*, 1981) and on polyphenols (Williams *et al.*, 1986). The study of meta-carboxy-substituted aromatic amino acids and glutamyl peptides was based on storage organs of 116 species. It showed that these unusual amino acids were characteristic of the subfamily *Iridoideae*, but were absent from the *Ixioideae* and *Sisyrinchoideae*. Thus they were useful markers for taxa within the *Iridoideae*, but of little value outside this subfamily (Larsen *et al.*, 1981).

Here, attention will be mainly focussed on the more recent survey of phenolic patterns in the family, which covered 255 species from 57 genera (Williams *et al.*, 1986). Some general conclusions relating these phenolic profiles with tribal classification will be mentioned later. In the meantime, the systematic implications of the distribution of individual subclasses of phenolic will be considered. The subclasses considered here are the anthocyanins, isoflavones, xanthones, biflavonoids and quinones.

### ANTHOCYANINS

The anthocyanins reported in floral tissues of *Iridaceae* are listed in Table 1. Some 95 pigments have been characterised from about 30 plant species, so that there is considerable variation with possible taxonomic interest. It is the different glycosidic patterns that appear to vary from one genus to another.

Plant species	Pigments present	Ref.
IRIDOIDEAE		
<i>Iris</i> (15 spp.)	Dp or Mv 3-(p-coumarylrutinoside)-5- glucoside	1, 2
Sisyrinchium sp.	Pt 3-diglucosylrhamnoside, Pt 3-rutinoside	3
IXIOIDEAE		
Anomatheca laxa	Cy 3-rutinoside	3
A. verrucosa	Cy 3-rutinoside	2
Babiana stricta	Mv 3-glucoside-5-(6"-malonylglucoside)	4
	Mv 3-glucoside-5-(3"-sulfatoglucoside)	
	Mv 3-glucoside-5-(2"-sulfato-6"-malonylglucoside)	
Chasmanthe ethiopica	Cy and Pn 3-rutinosides	2
Crocosmia masonii	Cy 3-rutinoside-5-glucoside	2
	Cy 3,5-diglucoside	
Crocus antalyensis	Pt and Mv 3,7-di-(6"-malonylglucosides)	5
	Dp and Pt 3-rutinosides	
	Dp and Pt 3,5-diglucosides	
	Dp 3-glucoside-5-(6"-malonylglucoside)	
	Dp and Pt 3,7-diglucosides	
Freesia cv.	Mv 3-glucoside	2
Gladiolus gandavensis	Pg, Cy, Dp, Pn, Pt and Mv 3-rutinosides 3-rutinoside-5-glucosides	6
	and 3,5-diglucosides	
Lapeyrousia corymbosa	Dp 3-diglucoside	3
Schizostylis coccinea	Cy 3-glucoside	3
Tritonia cv. Prince of Orange	Pg 3-gentiobioside	2
Watsonia meriana, W.	Pg 3-glucoside, Pg 3-sophoroside and Pg 3-sophoroside-7-glucoside	2
rosea, W. tabularis		

TABLE 1 - ANTHOCYANIN PIGMENTS IN FLOWERS OF THE IRIDACEAE

Key: Pg = pelargonidin, Cy = cyanidin, Pn = peonidin, Dp = delphinidin, Pt = petunidin, Mv = malvidin. Ref.: 1, Williams *et al.*, 1997; 2, Harborne, 1997; 3, Williams *et al.*, 1986; 4, Toki *et al.*, 1984; 5, Norback and Kondo, 1998; 6, Akaria *et al.*, 1981. A unique feature of these anthocyanins is the presence of sulfated pigment glycosides in *Babiana stricta* (Ait.) Ker (Toki *et al.*, 1994). Many sulfated flavones and flavonols have been described in nature but this is the first time that sulfated anthocyanins have been reported. Another unusual feature is the 3,7-diglycoside substitution pattern that occurs separately in *Crocus* L. and *Watsonia* Miller (Table 1). Malonylation is another structural feature which is present rarely in the family, being reported only so far in *Crocus antalyensis* B. Mathew. A survey of the family for malonylated pigments, which can be done quite simply by paper electrophoresis at pH 4.4, showed that they are very uncommon in the family.

Clearly, it is too early to comment extensively on the taxonomic value of these glycosidic patterns, since so relatively few species have been analysed. It is already apparent that the pattern in *Iris* is relatively consistent with delphanin, delphinidin 3-*p*-coumarylrutinoside-5-glucoside, occurring widely. The malvidin analogue has been reported from *Iris ensata* Thunb.. One may also note the presence of isomeric 3-diglucosides in *Tritonia* Ker-Gawler and *Watsonia*. The former genus has the 3-gentiobioside, in which the two glucoses are linked  $\beta_1 \rightarrow 6$ , while in *Watsonia* occurs the 3-sophoroside, where the two glucoses are linked  $\beta_1 \rightarrow 2$ .

### **ISOFLAVONES**

The presence of isoflavones in rhizomes or aerial parts is one of the most distinctive chemical features of the genus *Iris*. One of the earliest reports on this genus was of the isoflavone irigenin and its 7-glucoside iridin in orris root, *Iris florentina*. Their chemical structures were determined by Baker (1928). Orris root is a preparation of the powdered rhizome. Iridin is a major component and must contribute to the use that is made of orris root in perfumery. Since then, irigenin and/or iridin have been isolated from at least nine other species. These are *I. germanica* L., *I. kamaonensis* Wall. Ex G. Don, *I. marsica* I.Ricci and Colas., *I. lutescens* Lam., *I. nepalensis* Wall., *I. pallida* Lam., *I. setina* Colas., *I. tingitana* Boiss. and Reut. and *I. unguicularis* Poir.

The isoflavone irigenin has a unique substitution pattern, which is rarely observed outside *Iris*. Within *Iris*, irigenin is frequently accompanied by one or more related structures but particularly by tectorigenin, first obtained from *I. tectorum* Maxim.. Some thirty other isoflavones have been described variously from *Iris* species. A recent review described several new structures from *Iris japonica* Thunb. (aerial parts), *I. nigricans* Dinsm. (rhizomes) and *I. kashmiriana* Bak. (rhizomes) (Boland and Donnelly, 1998).

Isoflavones have been reported from two other genera in *Iridaceae*, *Belamcanda chinensis* and *Patersonia*, but otherwise appear to be absent from most species in the family (Williams *et al.*, 1986). According to Ingham's review (1983), the rhizome of *Belamcanda* Adans. contains tectorigenin 7-glucoside and iridin. This occurrence is very expectable, since this plant has always been regarded as being closely related to *Iris*; the report of tectorigenin and iristectorigenin A in *Patersonia occidentalis* R. Br. var. *angustifolia* is more surprising. Isoflavones appear to be rare in *Patersonia*, however, and they could not be detected in the closely related subspecies with broad leaves, *P. occidentalis* var. *occidentalis* (Williams et al., 1989).

Finally, it should be emphasised that isoflavones, while occurring in the *Leguminosae* and at least a dozen other dicotyledonous families, are relatively rare in

## XANTHONES

Mangiferin is a unique C-glucosylxanthone which, unlike other xanthones, has a widespread if scattered distribution in nature. It was first identified in *Mangifera indica* L. (*Anacardiaceae*), and hence the name, but has subsequently been reported in some ferns and in a variety of dicotyledons (in 28 genera from 19 families) (Hostettmann and Hostettmann, 1989). In the monocots, it was first recognised as a component of *Iris germanica* flowers (Bate-Smith and Harborne, 1963), but it does occur in other *Iridaceae* (see below) as well as in the *Liliaceae*. It is a biologically active chemical, with anti-inflammatory, antihepatotoxic and antiviral properties. It is particularly easily detected in surveys by its distinctive colour reactions on paper or TLC and its characteristic ultraviolet spectrum.

Within the genus *Iris*, surveys have indicated that mangiferin has a discrete distribution, largely following subgeneric, sectional or series divisions. Thus, it is found in all species surveyed of subgenus *Iris*, including 15 taxa from section *Iris*. It is present in *I. humilis* Georgi from section *Psammiris* and in *I. hoogiana* Dykes from section *Regelia*. In subgenus *Limniris* section *Limniris*, mangiferin is present in members of four of the ten series surveyed: *Tripetalae*, *Laevigata*, *Ensatae* and *Unguiculares*. Finally, it was detected in both taxa of the subgenus *Hermodactyloides* examined: *I. histriodes* (G.F. Wilson) S. Arnot and *I. reticulata* M. Bieb. (Williams *et al.*, 1986).

Besides occurring regularly in the genus *Iris*, mangiferin has been detected in the Irideae in *Belamcanda punctata* and in two *Gynandriris* Parl. species. The other major source of mangiferin in *Iridaceae* is in the tribe *Tigrideae*. It is found in species of *Eleutherine* Herb., *Ennealophus* N.E. Br., *Fosteria, Gelasine* Herb., *Rigidella* Lindl., *Sessilanthera* Molseed & Cruden and *Tigridia* Juss., but is absent from four other genera surveyed. In *Tigridia*, it is generally present but is noticeably absent from *T. pavonia* (L.f.) DC., a morphologically distinctive taxon within that genus (Williams *et al.*, 1986).

Mangiferin can occur in *Iridaceae* in association with its structural isomer, isomangiferin. In *Iris*, a number of other mangiferin derivatives may be present, including 0-methyl ethers, like irisxanthone the 5-methyl ether, *O*-glycosides and acylated *O*-glycosides. Two such derivatives are useful taxonomic markers for diploid species within the bearded *Iris* group, also occurring in derived allopolyploids. Thus, a mangiferin methyl ether is present in the diploid *Iris pseudopumila* Tineo and in the allopolyploids *I. lutescens, I. marsica* and *I. bicapitata* Colas. A second mangiferin derivative, an acylglycoside, is present in the diploid *I. reichenbachii* Heuff. and in the tetraploids, *I. lutescens, I. marsica* and *I. albicans* Lange. The phyletic significance of these results is discussed elsewhere (Williams *et al.*, 1997).

## BIFLAVONOIDS

Biflavonoids are apparently primitive chemical markers in higher plants, since they occur primarily in bryophytes, tracheophytes and gymnosperms. They are, by contrast, far less common in dicotyledonous angiosperms. A typical and common member of this subclass of flavonoids is amentoflavone. This compound is a dimer of the common flavone apigenin (5,7,4'-trihydroxyflavone), the two flavone units being linked together by a carbon-carbon bond between the 3'-position of one with the 8-position of the other.

Amentoflavone occurs in *Ginkgo biloba* L. (*Ginkgoaceae*), *Podocarpus montana* Lodd. (*Podocarpaceae*), *Cycas revoluta* Thunb.(*Cycadaceae*) and other gymnosperms (Geiger, 1994). Its discovery in *Iridaceae* in *Patersonia glabrata* R.Br. and *P. pygmaea* Lindl. was therefore surprising (Williams *et al.*, 1989). Interestingly, it occurs here in association with myricetin, a flavonol which in the dicots is associated with the primitive woody habit.

A comprehensive survey of 255 *Iridaceae* species showed one further definitive source of amentoflavone, namely in the atypical taxon *Isophysis tasmanica* (Hook.) T. Moore, endemic to Tasmania, the only known species of the *Isophysidoideae*. In *Isophysis* T. Moore ex Seemann, amentoflavone is accompanied by dihydroamentoflavone and one partly identified amentoflavone methyl ether (Williams *et al.*, 1986). Whether other plants of the *Iridaceae* have biflavones is not yet clear. If so, it is likely to be in closely related species of *Patersonia*, in *Klattia partita* Bak. and in *Witsenia maura* (L.) Thunb. (Williams and Harborne, 1985). Further work, however, is needed to confirm these preliminary records.

The presence of amentoflavone in *Patersonia glabrata* and *Isophysis tasmanica* is of phytogeographical significance since both these taxa are confined in their natural distribution to Australasia. They appear to be primitive members of the family, so that the occurrence in them of amentoflavone could be regarded as the retention of a primitive relict in an otherwise highly specialised plant family. The floral structure and leaf form of *Isophysis*, in particular, has led Goldblatt (1985) to postulate that the ancestral *Iridaceae* resemble *Isophysis*.

#### QUINONES

The discovery of the hydroxynaphthoquinone, plumbagin, in the *Iridaceae* was unexpected (Williams and Harborne, 1985) since it is a characteristic constituent of three quite unrelated families in the dicotyledons: *Droseraceae*, *Ebenaceae* and *Plumbaginaceae* (Thomson, 1997). This quinone was characterised from leaves of *Aristea alata* Bak. and was present in all five other species of *Aristea* Sol. surveyed. Other unidentified quinone pigments were also present.

A survey of 47 genera and one 200 taxa of the *Iridaceae* revealed only two other generic sources of plumbagin: *Sparaxis* Ker-Gawler, in the *Gladoleae*; and *Sisyrinchium* L. in tribe *Sisyrincheae*. Neither of these genera are closely related to each other or to *Aristea*. Plumbagin is present in *Sparaxis tricolor* (Schneev.) Ker-Gawler, but is not found in *S. grandiflora* Poepp. It was identified in leaves of only two of 22 *Sisyrinchium* species, *S. brachypus* (Bickn.) J. Henry and *S. californicum* (Ker) Dryand. It also occurs in the flowers of *S. brachypus* and in flowers of a third species *S. tinctorium* HBK (Williams and Harborne, 1985). Finally there is an isolated report of plumbagin in *Sisyrinchium acaule* Klatt. (Thomson, 1997). Our discovery of this quinone in *Iridaceae* has been confirmed by Kumar *et al.* (1985) who reported it in leaves and rhizomes of *Aristea ecklonii* Bak.

Other quinone pigments have been described from the family, but it is unlikely that any of them are widespread. Thus anthraquinones have been reported in *Libertia coerulescens* Kunth, but our survey of 255 species failed to yield any further sources and they were not present in the related *Libertia chilensis* Klotz ex Bak. Again several benzoquinones, including irisquinone [6-methoxy-2-(1 O-heptadecenyl)-1,4-benzoquinone] have been reported in seed oils or seed coats of some six *Iris* species. However, they have not been found in any other *Iridaceae*, apart from *Belamcanda chinensis* which is close to *Iris* (Thomson, 1997).

## PHENOLICS AND TRIBAL CLASSIFICATION

A survey has been carried out in *Iridaceae*, both for the special classes of phenolic described above, and for the common flavonoid constituents. These latter include simple flavones (occurring as O-glycosides), the C-glycosylflavones and the various common flavonols. As a result, it is possible to distinguish the different tribes from each other to some degree based on phenolic patterns (Table 2).

The *Irideae* and *Tigrideae* can be distinguished by the regular presence of the xanthone mangiferin. The *Trimezieae* and *Sisyrincheae*, by contrast, can be separated by the absence of flavonols combined with the regular presence of glycoflavones. The *Aristeae* is distinguished by the occurrence of the quinone plumbagin, although this quinone does occur in isolated instances in two other tribes. The *Aristeae* is also notable for the absence of glycoflavones in the leaves.

Members of the *Watsonieae* are separated by the fact that flavonols are dominant constituents. The *lxieae* also have mainly flavonols, but they also contain a number of distinctive flavones, such as tricin, acacetin and scutellarein derivatives.

Nothing has been said here about the various glycosides of the above flavones and flavonols recorded in the family. However, a rich array of such glycosides have been described from 16 plant species and many other await detection in these plants (Williams *et al.*, 1986). In summary, then, there is no doubt that the *Iridaceae* contain a heterogenous collection of flavonoid and related phenolic constituents. Many of these are potentially useful as taxonomic markers, but further detailed surveys are still needed to establish whether or not they are important for the classification of this diverse family.

Tribe	Dominant Phenolics
Irideae	Glycoflavones common, mangiferin typical
Tigrideae	Glycoflavones common, mangiferin typical
Trimezieae	Glycoflavones, but no flavonols
Sisyrincheae	Glycoflavones, but no flavonols
Aristeae (including Nivenieae)	Plumbagin occasional, but glycoflavones rare
Watsonieae	Flavonols dominant, e.g. isorhamnetin, myricetin
lxieae	Flavonols dominant, e.g. isorhamnetin, myricetin

TABLE 2 - PHENOLICS AND TRIBAL CLASSIFICATION OF THE *IRIDACEAE*, BASED ON A SURVEY BY WILLIAMS *ET AL*. (1986)

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