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Review

Isoflavonoids in non-leguminous taxa: A rarity or a rule?

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Abstract

Isoflavonoids are characteristic metabolites in legumes and an overwhelming number of reports concerning them come from the Leguminosae. Nevertheless, the spectrum of isoflavonoid producing taxa includes the representatives of four classes of multicellular plants, namely the Bryopsida, the Pinopsida, the Magnoliopsida and the Liliopsida. At least 59 non-leguminous families have been reported to produce isoflavones *sensu lato*; coumestans have been reported in 3 families, coumaronochromones in 3, pterocarpans in 9 and rotenoids in 8 families. Prenylated isoflavones have been found in 15 non-leguminous families and isoflavone dimers, heterodimers or oligomers in three families. More than two hundred different isoflavonoid aglycones have been reported in non-legumes altogether. The number of individual structures is even greater if the variety of glycosides are considered.

Enzymology and genetics of isoflavonoid biosynthesis have been studied almost exclusively in legumes, with the exception of a few model plants (i.e. *Beta vulgaris*, *Arabidopsis thaliana*, *Nicotiana tabacum* and *Zea mays*). The key step at the very beginning of the isoflavonoid metabolic pathway is the oxidation of flavanone connected with the migration of aryl moiety from C2 to C3 mediated by a CYP450 enzyme isoflavone synthase (IFS), which has been identified and cloned in multiple legumes and in sugar beet (*Beta vulgaris*, Chenopodiaceae). No information is available about the enzyme(s) responsible for the biosynthesis of isoflavonoid core in other taxa. Experimental data demonstrates the capability of numerous enzymes of non-legume origin to metabolize isoflavones as alternative substrates to other phenolics.

Keywords: Isoflavonoid; Pterocarpan; Rotenoid; 3-Arylcoumarin; Coumestan; Coumaronochromone; Non-leguminous; Chemotaxonomy

Contents

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1. Introduction

Isoflavonoids constitute a specific branch of flavonoid metabolism, differing from the other flavonoids by the position of the phenolic ring B. Up to now about 1600 isoflavonoids have been described including glycosides (Veitch, 2007). They are involved in the interactions between plant and their environmental partners – from bacteria and fungi to herbivorous insect, molluscs and vertebrates (Dakora and Phillips, 1996). Several dozens of isoflavonoids have interesting pharmacological activities – endocrinological, antibacterial, antiviral, anti-inflammatory, etc. (Cornwell et al., 2004). Estrogenically active isoflavones – genistein, daidzein and few others – belong to the most studied phenolics (8051 records on WOS as of 27th July 2007). The overwhelming majority of isoflavonoids which have been described have been from legumes. Common underlining of this fact sometimes leads to overlooking that the presence of isoflavonoids has been reported in numerous taxonomically distant species. The aim of this study is to summarize the taxonomical distribution of individual isoflavonoid subgroups and to discuss the possibility of their occurrence in additional, still undiscovered taxa.

2. Categorization of isoflavonoids

Isoflavonoids are biosynthetically derived from the same precursors as the majority of complex flavonoids (Schijlen et al., 2004). These are the simple flavanones – naringenin (5,7,4′-trihydroxyflavanone), liquiritigenin (7,4′-dihydroxyflavanone) and perhaps even also some other 7-hydroxyflavanones. It seems that 7-hydroxyflavanones without the hydroxy group at position 4′ may also be potential precursors (Kim et al., 2003). In isoflavonoid-producing plants these flavanones are converted to the most basic isoflavones by the action of isoflavone synthase. Further sequences of enzymic reactions then give rise to more complex structures including glycosides, rotenoids, pterocarpans, 3-arylcoumarins, coumestans, coumaronochromones, prenylated isoflavones, isoflavonoid dimers and conjugates (Fig. 1) (Crombie and Whiting, 1998; López-Meyer and Paiva, 2002; Tahara and Ibrahim, 1995; Veitch, 2007).

3. Distribution of isoflavonoids in non-legumes

One of the first isoflavones obtained from a natural source was iridin from *Iris florentina* (Iridaceae) – a

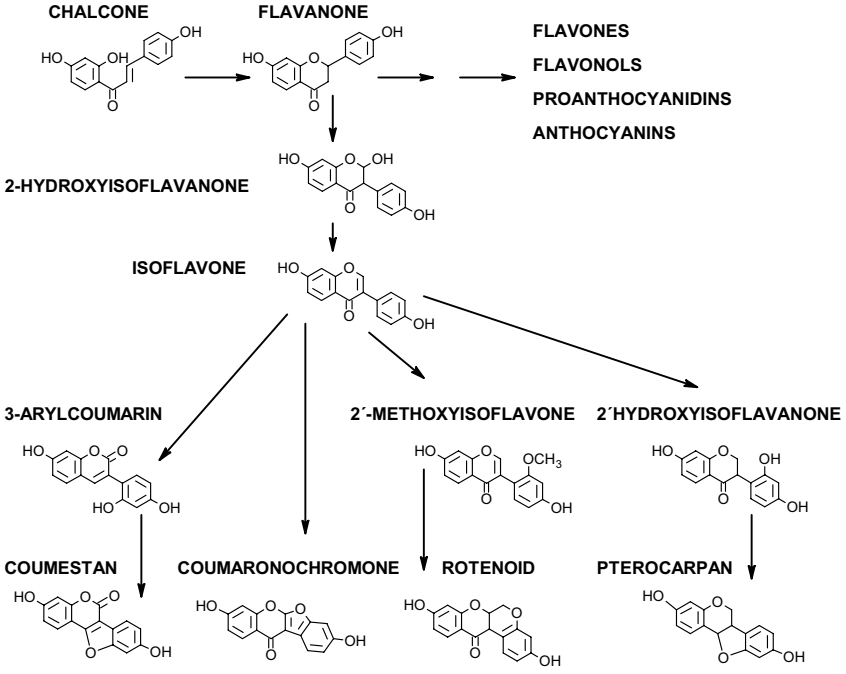

Fig. 1. A simplified scheme of biosynthetic relationships between structural types of isoflavonoids. (The arrows indicate the direction of metabolism, not individual reaction steps). For detailed description of biosynthesis of individual classes of compounds, see, e.g. López-Meyer and Paiva (2002), Schijlen et al. (2004), and Veitch (2007).

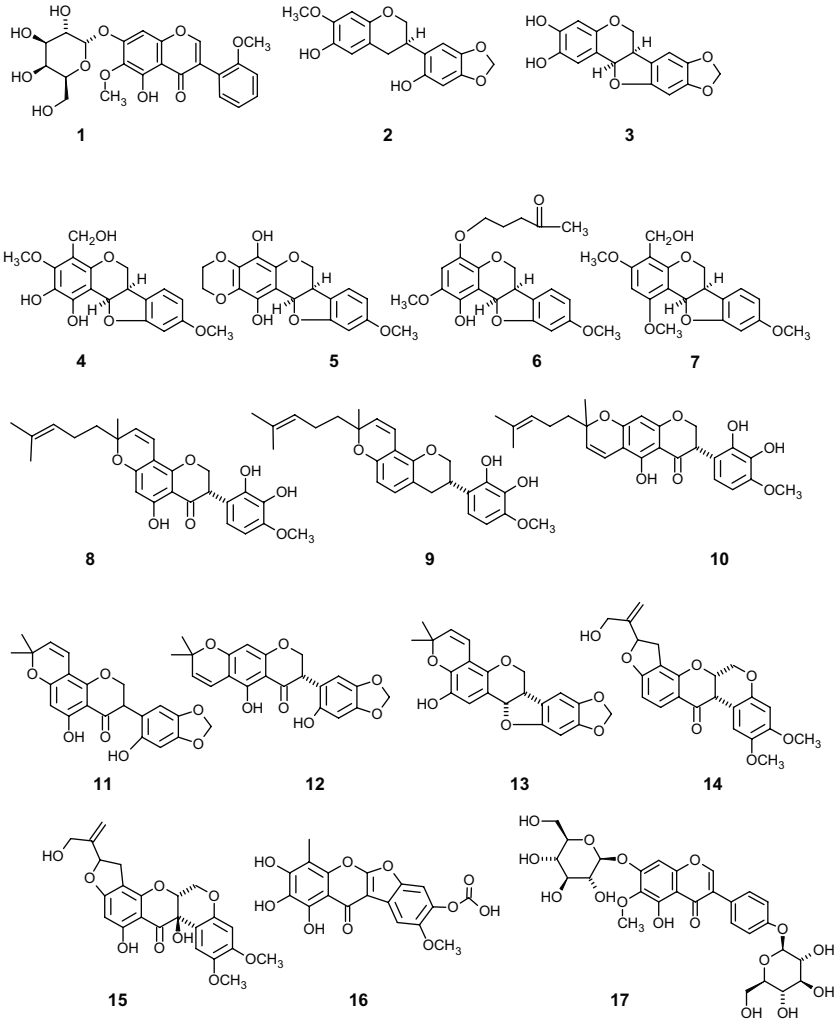

Fig. 2. Examples of isoflavonoids reported in non-leguminous taxa since the last review: 5-hydroxy 6,2′-dimethoxy isoflavone 7-*O*-beta-D-galactopyranoside (**1**) from *Liriodendron tulipifera* (Magnoliaceae); hildegardiol (**2**) and 2-hydroxymaackiain (**3**) from *Hildegardia barteri* (Sterculiaceae); atricarpans A–D (**4**–**7**) from *Zygophyllum eurypterum* (Zygophyllaceae), discoloranone B (**8**), nitidulin (**9**), isodiscoloranone B (**10**), discoloranone A (**11**), isodiscoloranone A (**12**), (6a*S*,11a*S*)-2-hydroxyleiocarpin (**13**), amorphigenin (**14**) and dabinol (**15**) from *Berchemia discolor* (Rhamnaceae); urophyllumol (**16**) from *Urophyllum chinensis* (Rubiaceae); tectorigenin 4′,7-di-*O*-glucoside (**17**) from *Viola hondonensis* (Violaceae).

non-legume and moreover a monocot (de Laire and Tiemann, 1893). In 1910, Finnemore reported the occurrence of prunetin in the bark of *Prunus* species (Rosaceae) (Whiting, 2001). Five non-leguminous families (i.e. Amaranthaceae, Moraceae, Podocarpaceae, Rosaceae and Iridaceae) were mentioned as sources of isoflavonoids in Harborne's Comparative Biochemistry of Flavonoids from 1967 (Harborne, 1967). Dewick's review from 1993 found literary references to 33 genera in 20 families (Dewick, 1993). Twelve years later, Reynaud et al. (2005) enumerated altogether 164 isoflavonoids reported in 31 non-leguminous angiosperm families, three gymnosperm families possessing 15 structures and 3 isoflavonoids found in one Bryophyte. A year later, Macková et al. (2006) drew attention to 49 further examples from additional 17 families not cited in the former review. Most recently, isoflavonoids of different structural types have been for the first time reported in Violaceae (Moon et al., 2005a,b), Sterculiaceae (Meragelman et al., 2005), Magnoliaceae (Kuanar, 2006), Rhamnaceae (Chin et al., 2006), Rubiaceae (Guo et al., 2007) and Zygophyllaceae (Ahmad et al., 2006) (Fig. 2). Preliminary data on the detection of simple isoflavones in two Cannabaceae species was published as a conference abstract by Koblovská (2006). Moreover, additional evidence was given for the presence of isoflavones in Rutaceae (Wang et al., 2006) and Poaceae (Benavides et al., 2007). It appears that at least 225 isoflavonoids from 59 non-leguminous plant families have been described to date. The relationships between isoflavonoid-producing taxa are unclear and this ambiguity is further emphasized by the fact that complex structural types of isoflavonoids (namely coumestans, pterocarpans and rotenoids) were found in representatives of both classes and almost of all subclasses of flowering plants (Table 1).

Table 1
Taxonomical occurrence of isoflavonoid structural types

Division	Class	Subclass	Order	Family	Isoflavones	3-arylcoumarins	Coumaronochromones	Coumestans	Pterocarpans	Rotenoids	Prenylation	Bisisoflavones
Bryophyta	Bryopsida	Bryideae	Bryales	Bryaceae	+							+
Coniferophyta	Pinopsida		Pinales	Araucariaceae	+							
				Cupressaceae	+							
				Podocarpaceae	+							
Magnoliophyta	Liliopsida (Monocots)	Commelinidae	Cyperales	Poaceae (Gramineae)	+			+				
				Cyperaceae	+						+	
			Eriocaulales	Eriocaulaceae	+							
			Juncales	Juncaceae	+							
		Liliidae	Liliales	Asphodelaceae	+	+						
				Iridaceae	+				+	+	+	
				Liliaceae	+				+			
				Stemonaceae	+					+		
				Smilaceae	+							
		Zingiberidae	Zingiberales	Zingiberaceae	+				+		+	
	Magnoliopsida (Dicots)	Asteridae	Asterales	Asteraceae (Compositae)	+			+			+	
			Gentianales	Apocynaceae	+		+					
				Asclepiadaceae	+					+		
			Lamiales	Verbenaceae	+							
			Rubiales	Rubiaceae	+		+					
			Scrophulariales	Scrophulariaceae	+					+	+	
			Solanales	Solanaceae	+							
				Convolvulaceae	+				+		+	
		Caryophyllidae	Caryophyllales	Amaranthaceae	+							
				Chenopodiaceae	+			+				
				Nyctaginaceae	+		+			+	+	
			Polygonales	Polygonaceae	+							
		Dilleniidae	Capparales	Brassicaceae	+							
			Ebenales	Sapotaceae	+							+
			Malvales	Bombacaceae	+							
				Malvaceae	+							
				Sterculiaceae	+				+			
			Theales	Clusiaceae	+						+	
				Ochnaceae	+						+	+
			Violales	Cucurbitaceae	+							
				Violaceae	+							
		Hamamelidae	Myricales	Myricaceae	+				+	+	+	
			Urticales	Cannabaceae	+							
				Moraceae	+						+	
				Urticaceae	+						+	
		Magnoliidae	Laurales	Lauraceae	+							
			Magnoliales	Magnoliaceae	+							
				Myristicaceae	+				+			

	Nymphaeales	Nymphaeaceae	+							
	Papaverales	Papaveraceae	+							
	Ranunculales	Menispermaceae	+							
Rosidae	Apiales	Apiaceae	+							
	Celastrales	Celastraceae	+						+	
	Euphorbiales	Euphorbiaceae	+					+	+	
	Fabales	Leguminosae	+	+	+	+	+	+	+	+
	Linales	Erythroxylaceae	+							
	Myrtales	Melastomataceae	+							
		Myrtaceae	+							
	Polygalales	Polygalaceae	+							
	Rhamnales	Vitaceae	+							
		Rhamnaceae	+				+	+	+	
	Rosales	Connaraceae	+							
		Crassulaceae	+							
		Rosaceae	+							
	Sapindales	Rutaceae	+							
		Zygophyllaceae					+			
Families altogether			59	2	4	4	10	9	16	4

For references on individual taxa, see Reynaud et al. (2005), Macková et al. (2006) and Section 3 of this article.

4. Isoflavonoid enzymology

Isoflavonoid metabolism may be divided into two essential stages. The aryl-migrating step resulting in synthesis of the most basic isoflavones is the primary stage of biosynthesis. All subsequent enzymic reactions may be regarded as secondary.

4.1. Synthesis of the isoflavonoid skeleton

Oxygenation of the flavanone precursor accompanied with migration of the phenolic moiety is an unusual albeit not unique reaction (Holscher and Schneiuder, 2005). The enzyme responsible for this step is 2-hydroxyisoflavanone synthase (2-HIS), most often referred to in literature as isoflavone synthase (IFS). The genes which encode IFS have been identified in numerous legumes (e.g. in soy, alfalfa, licorice, chickpea, lentils, mung bean, and clover) and in sugar beet (*Beta vulgaris*, Chenopodiaceae) (Akashi et al., 2000; Overkamp et al., 1999; Steele et al., 1999; Kim et al., 2006a). In phylogenetically distant taxa, knowledge is limited to the level of metabolites; the genetic data is lacking. All known IFS belong to the CYP93C subfamily of cytochrome P450. Soybean IFS has been expressed in several non-legumes – i.e. in *Arabidopsis thaliana*, tobacco, maize and rice. The transgenes produced not only genistein, but also its glycosylated forms – genistin and/or 6″ malonylgenistin (Jung et al., 2000, 2003; Sreevidya et al., 2006). Sawada et al. (2002) demonstrated that a single amino acid mutation is able to switch the catalytic activity of IFS from the aryl migration to the 3β-hydroxylation of flavanone skeleton. Moreover, certain mutations enabled IFS to produce both types of metabolites, i.e. isoflavones and 3-hydroxyflavanones. The latter type of reaction has been assigned to non-P450 enzymes, namely to 2-oxoglutarate-dependent dioxygenases, in several recent plants (Britsch et al., 1992; Pelt et al., 2003). Sawada hypothesized that flavanone 3β-hydroxylase of the P450 type was present in past plant species, and could be the evolutionary predecessor of IFS.

4.2. Functionalization of the isoflavonoid skeleton

In contrary to unusual character of the aryl-migrating step, subsequent functionalization of isoflavone skeleton may be executed by enzymes which may not be recognized as the ones metabolizing isoflavones. Enzymes show three levels of specificity: for the substrate, for the reaction and for the product. The broad substrate specificity of certain enzymes enables them to modify multiple compounds thus giving rise to a substantially higher number of secondary metabolites than would be anticipated from the number of known genes. The general aspects of this phenomenon have been discussed, e.g. by Schwab (2003). A few examples of isoflavonoid metabolism in non-legumes will be given here.

4.2.1. Glycosyltransferases

Glycosyltransferases are strictly specific with respect to the sugar moiety, however, some of them have relatively broad spectrum of possible acceptors of the glycosyl group (Hefner et al., 2002). UDP-glucosyl transferase from strawberries (*Fragaria ananassa*) was shown to glycosylate flavanones, flavones and also isoflavones with comparable efficacy (Cheng et al., 1994); glucosyl transferase from tobacco was able to modify substrates as different as coumarins, phenylpropanoic acids, flavones and isoflavones (Taguchi et al., 2000). Broad substrate specificities to flavonoids and isoflavonoids were observed in glucosyltransferases from *Allium cepa* and *Maclura pomifera* assigned UGT73G1 and UGT75L4, respectively (Kramer et al., 2003; Tian et al., 2006). UDP-glucosyltransferase from *Bacillus cereus*, BcGT-1 is able to use apigenin, genistein, kaempferol, luteolin, naringenin and quercetin as substrates. The enzyme preferentially glycosylated at the 3-hydroxyl group, but it could transfer a glucose group onto the 7-hydroxyl group when the 3-hydroxyl group was not available (Hyung Ko et al., 2006). Vice versa, broad substrate specificities were observed also in "isoflavonoid-specific" leguminous glycosyltransferases. Formononetin 7-*O*-glucosyltransferase from *Glycyrrhiza echinata*, designated UGT73F1, displayed remarkable activity to different non-isoflavonoid phenolics, e.g. naringenin, baicalein, ferulic acid and scopoletin (Nagashima et al., 2004). Glycosyltransferase UGT71G1 from *Medicago truncatula* glycosylates flavonoids, isoflavonoids, and triterpenes. It can transfer glucose to each of the five hydroxyl groups of the flavonol quercetin and to the 7-hydroxyl of the isoflavone genistein (He et al., 2006).

4.2.2. Methyltranferases

S-Adenosyl-L-methionine utilizing methyltransferases are widely abundant in plants. Class B *O*-methyltransferases (OMT) catalyze methylation of the hydroxyl groups of flavonoids, chalcones and isoflavones (Roje, 2006). The OMT genes are present in multiple copies in plant genomes, e.g. in *Arabidopsis thaliana* at least 17 homologues to leguminous isoflavone 7-OMT have been recorded (Lapčík et al., 2006). Some OMT display relatively wide spectrum of possible acceptors of methyl group (Vogt, 2004). Methyltransferases able to methylate isoflavonoids together with other phenolics have been described, e.g. in carnation (*Dianthus caryophyllus* L., Caryophylaceae) and poplar (*Populus deltoides* Marsh., Salicaceae), both species belonging to families so far not reported to produce isoflavones (Curir et al., 2003; Kim et al., 2006b).

4.2.3. Oxidases/reductases

Regioselective oxidations and/or reductions belong to substantial biosynthetic steps which are executed by numerous oxidoreductases, namely by the members of CYP450 family of enzymes. The CYP450 genes are present in multiple homologues in plant genomes, metabolic function of substantial number of them remains still unclear. It is worthy to mention the wide taxonomical distribution of "isoflavone-reductase like" and "isoflavone-reductase related" genes in the spermatophytes. Expression of these genes has been recorded, e.g. in *Cryptomeria japonica* (Cupressaceae), *Oryza sativa* and *Zea mays* (Poaceae), *Betula verrucosa* (Betulaceae), *Citrus paradisi* (Rutaceae), *Arabidopsis thaliana* (Brassicaceae), *Nicotiana tabacum* and *Solanum tuberosum* (Solanaceae) (Karamloo et al., 2001; Kim et al., 2005; Lers et al., 1998).

4.2.4. Isoprenylating enzymes

Prenyltransferases are regarded as key enzymes in the formation of many phytoalexins. While they are strictly specific with respect to the isoprenyl donor, some of them are able to prenylate several acceptors (Laflamme et al., 1993; Yamamoto et al., 1997). Prenyltransferase derived from the microsomal fractions of cell cultures of *Morus nigra* was shown to be able to prenylate both chalcones with a 2′,4′-dihydroxy substitution and the isoflavone genistein (Vitali et al., 2004). Microsomal fraction from *Sophora flavescens* was able to prenylate several flavanones, flavones and the isoflavone genistein (Yamamoto et al., 2000). These studies were, however, performed with microsomal fractions from elicited plant cell cultures which could have contained several enzymes in one preparation. Pure enzymes are necessary for better understanding of the specificity of prenylation.

5. Screening for new isoflavonoid producers

Most likely, the number of isoflavonoid-producing families is higher than currently known. Despite the possibility that characteristic compounds may be structurally complex in particular taxa, their simple precursors could be present at transient levels. Coincidentally, owing to the fact that the simplest isoflavones have been attracting the attention of biomedical science for several decades due to their possible impact on human health, numerous sensitive and specific methods are available for their analysis (Wu et al., 2004; Umphress et al., 2005).

Systematic screening for intermediates of the isoflavonoid metabolic pathway could reveal a higher number of isoflavonoids in each species where at least one isoflavonoid has been reliably detected, and moreover, the availability of sophisticated methodologies for the detection of simple isoflavones gives the opportunity to systematically seek out the presence of isoflavonoid pathway in taxa not studied from this point of view so far. Recently, we have applied specific immunoassays for screening of isoflavonoids in several non-leguminous families, using HPLC-MS as the confirmatory method. This approach revealed for the first time the occurrence of isoflavonoids, e.g. in the Rutaceae (Lapčík et al., 2004; Koblovská et al., in press), the Myrtaceae (Lapčík et al., 2005) and the Cannabaceae (Koblovská et al., 2006) families. The presence of isoflavonoid metabolism in the Rutaceae family

was subsequently verified by Wang et al. (2006), who have isolated six new methoxylated isoflavonoid glycosides from *Glycosmis pentaphylla*.

6. Conclusions

Isoflavonoids are synthesized by at least 60 families that belong to substantially distant higher taxonomical units, i.e. the Bryophyta, the Coniferophyta and the Magnoliophyta. Relations between isoflavonoid-producing families are unclear. Biosynthetically advanced types of isoflavonoids (i.e. coumestans, pterocarpans and rotenoids) occur in several unrelated families that belong to different subclasses of both the monocots and the dicots.

Virtually no information is available about the enzymes responsible for the aryl-migrating step in the biosynthesis of isoflavones in non-legumes, however, the capability to metabolize isoflavones has been demonstrated in numerous non-legumes including species not known to synthesize them.

The number of isoflavonoid-producing families may be higher than currently known. Early products of the isoflavonoid pathway may be used as screening markers of its presence in new taxa.

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