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PRENYLATED ISOFLAVONOIDS—AN UPDATE

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Key Word Index—Isoflavonoids; prenyl; isopentenyl; geranyl; structural diversity; prenyltransferase; fungal metabolism; biological activities.

Abstract—This review deals with some bioorganic chemical aspects of isoflavonoids. Its highlights are rather restricted to the structural modifications and the diverse oxygenated side attachments originating from the isoprenoid substituents, 3,3-dimethylallyl (prenyl), 1,1-dimethylallyl and geranyl groups. Some aspects of naturally occurring isoflavonoids, their new classes and biological functions are also described.

INTRODUCTION

One of the representative classes of plant secondary metabolites, the flavonoids (C_6–C_3–C_6) consist of two main groups, namely flavonoids (in the narrow sense) and isoflavonoids. The isoflavonoids are structurally distinct from other flavonoid classes in that they contain a C_{15} skeleton based on 1,2-diphenylpropane (**I**). There are some 870 isoflavonoid aglycones including *ca* 65 microbial metabolites of natural origin described in the latest review that covers the literature until the end of 1990 [1]. Since then, more than 140 new isoflavonoids have been reported in *Phytochemistry*, *Chem. Pharm. Bull.*, *J. Nat. Prod.* and *Heterocycles* (1991–1993). Other flavonoids possessing a basic C_{15} skeleton of 1,3-diphenylpropane (**II**) are ubiquitous in all terrestrial plants except the Anthocerotopsida, and the number of known compounds, both as aglycones and glycosides, is estimated to be more than 4000. The biosynthetic pathways for isoflavonoids as well as their relationships among other isoflavonoid groups are shown in Fig. 1. The isoflavonoid skeleton is established by an aryl migrating enzyme known as isoflavone synthase [2–5]. All isoflavonoids are believed to be derived from a restricted number of simple isoflavones, e.g. daidzein (**1**, 5-deoxyisoflavonoids), genistein (**2**, 5-hydroxyisoflavonoids) and so on. Therefore, isoflavone synthase is a key enzyme in isoflavonoid biosynthesis, with a pronounced specificity to some simple flavanones. The 870 naturally occurring isoflavonoid aglycones are divided into 14 classes and 23 subclasses based on their skeletal modifications (see Table 1 and Fig. 1). In contrast with other groups of flavonoids, the distribution of isoflavonoids among plants is relatively sparse, probably owing to the sporadic occurrence of isoflavone synthase. According to Dewick's review [1], 759 isoflavonoids have been isolated from leguminous plants, while another 80 have been reported from non-leguminous plants, belonging to 33 genera in 20 families. To date, an additional 20 or more compounds have been described from non-leguminous plants, some of which belong to plant families previously not known to contain isoflavonoids, such as the Apocynaceae [6], Meliaceae [7], Pinaceae [8] and Polygalaceae [9], in addition to new genera of the Compositae (*Gaillardia* sp.) [10] and Myristicaceae (*Pycnanthus* sp.) [11].

The authors describe mainly the isoprenoid substituted isoflavonoids (complex isoflavonoids) and their relatives on the basis of chemical and biological aspects. Details of the structure and distribution of the most common isoflavonoids and their prenylated derivatives, may be found in the following reviews (author, covered period and number of isoflavonoids): Ollis (–1961), 51 [12]; Wong (–1969), 135 [13]; Wong (–1973), 180 [14]; Dewick (–1980), 456 [15]; Ingham (–1981), 510 [16]; Dewick (–1985), 629 [17]; and Dewick (–1990), 870 [1]. Biosynthetic studies of isoflavonoids have been remarkably promoted by the increasing interest in their role as defence metabolites of leguminous plants, and these topics have been reviewed in detail [17–21, and refs cited therein].

COMPLEX ISOFLAVONOIDS

These are isoflavonoids with additional carbons involving a cyclic or acyclic side structure that originated from

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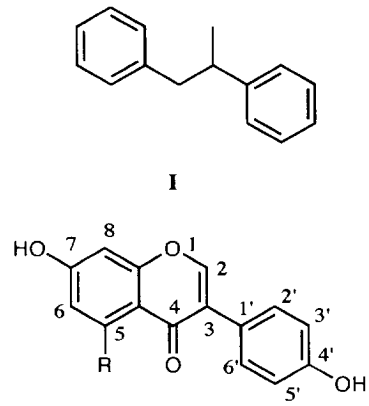
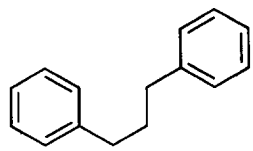
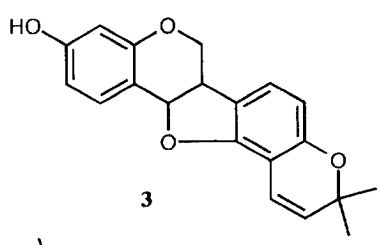


I



II

1 R=H daidzein
2 R=OH genistein



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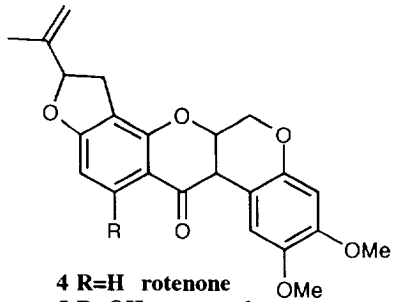
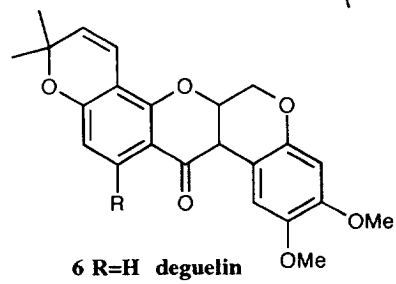
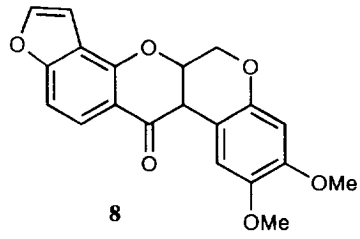


4 R=H rotenone
5 R=OH sumatrol



6 R=H deguelin
7 R=OH toxicarol



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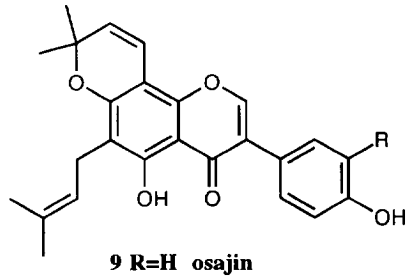


9 R=H osajin
10 R=OH pomiferin

dimethylallyl (prenyl) or geranyl groups. As with isoflavone synthase, the restricted distribution of prenyl- or geranyltransferase has resulted in a smaller number of plants that express this type of complex isoflavonoid. As shown in Table 1, respectively 56 and 17% of leguminous and non-leguminous isoflavonoids are of the complex type.

In addition to leguminous plants, some belonging to nine genera in seven families and known to produce complex isoflavonoids (family, complex/total isoflavonoids, genus, isoflavonoid class) are as follows: Celastraceae, 1/1, *Eunomyces*, furanoisoflavone; Compositae, 3/14, *Wyethia*, prenylisoflavones; Euphorbiaceae, 1/2, *Macaranga*, dihydrofuranorotenoid; Moraceae, 4/5, *Cudrania* and *Maclura*, 1 dihydrofuranoisoflavone and 3 prenylpyranoisoflavones; Scrophulariaceae, 3/3, *Sopubia* and *Verbascum*, prenylisoflavone aglycone, glycoside and dihydrofuranorotenoid; and Zingiberaceae, 2/2, *Costus*, dihydrofurano- and pyrano-pterocarpans. In 1993, cultured cells of *Pinus thunbergii* (Gymnosperma, Pinaceae) were reported to yield phaseollin (**3**), a pyranopterocarpan under stress conditions [8]. Although species from the Iridaceae, Chenopodiaceae, and Nyctaginaceae yield a total of 75 isoflavonoids (-1993), they are all simple isoflavonoids [22–24].

Plants belonging to the Moraceae are rather notable for the production of complex flavonoids (flavones and flavanones), some of which have complicated structures, that are probably derived via intra-molecular and inter-molecular Diels–Alder reactions [25].

The first complex isoflavonoids to have their structures elucidated were the piscicidal and insecticidal rotenoids, such as rotenone (**4**) [26–28] and deguelin (**6**) [29, 30], as well as in 1932, toxicarol (**7**) [31, 32], sumatrol (**5**) [33] and elliptone (**8**) [34] later in that decade. The structures of the complex isoflavones osajin (**9**), pomiferin (**10**) [35, 36] and toxicarol isoflavone (**11**) [37] were reported in the early 1940s.

Structural diversity of isoflavonoids

The structural diversity of naturally occurring isoflavonoids is dependent on: (a) the modification of the skeletal cyclic systems (14 classes), (b) oxygenation of the

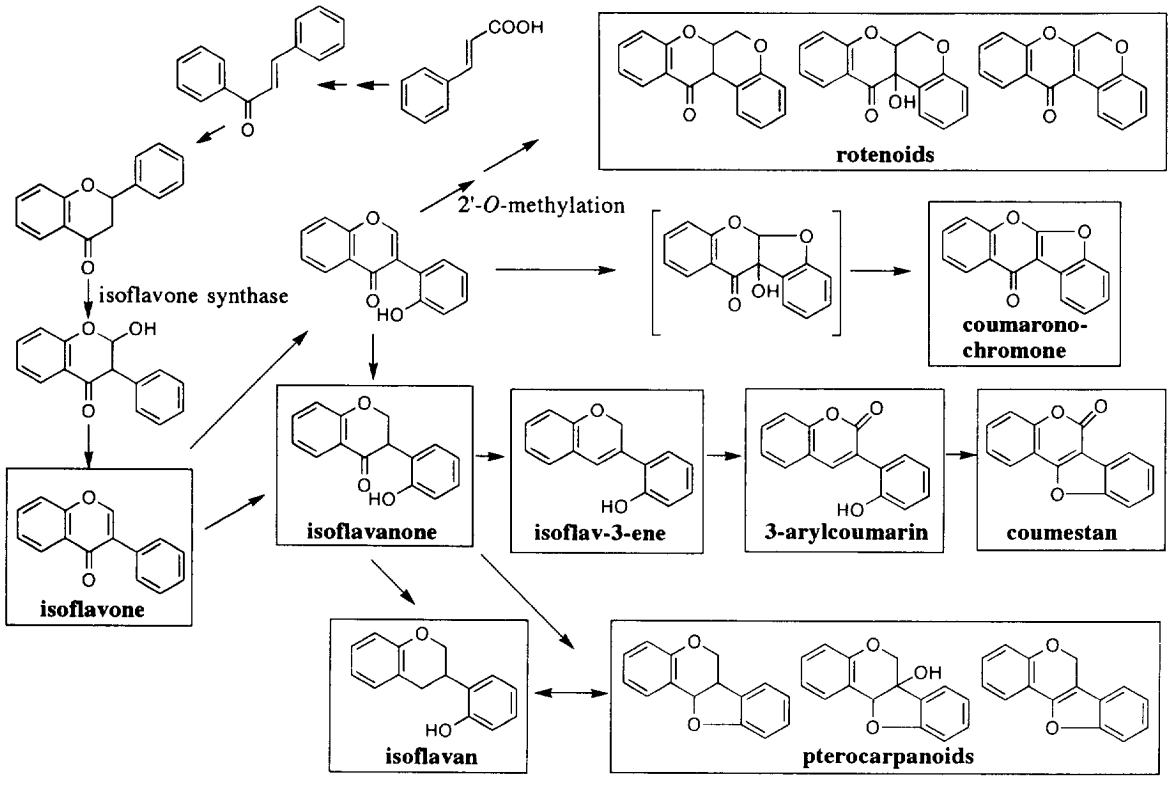


Fig. 1. Major isoflavonoid classes and their biosynthetic relationships (modified from Dewick [1]).

Table 1. Isoflavonoids found in terrestrial plants (-1990) [1]

Isoflavonoid class	Total compounds	Leguminous plants					Non-leguminous plants				
		Number	Complex	Simple	5-OH	5-H	Number	Complex	Simple	5-OH	5-H
Isoflavones	334*	293	170	123	190	103	[58]	10	48	52	6
Pterocarpanoids	152†	152	78	74	16	136	4	2	2	0	4
Rotenoids	72‡	62	54	8	18	44	13	2	11	12	1
Isoflavanones	62	62	36	26	37	25	0	0	0	0	0
Isoflavans	55	55	24	31	5	50	0	0	0	0	0
Coumestans	45§	41	22	19	8	33	5	0	5	4	1
3-Arylcoumarins	22	22	19	3	17	5	0	0	0	0	0
Isoflavonoid oligomers	22	22	0	22	2	20	0	0	0	0	0
Coumaronochromones	14	14	13	1	14	0	0‖	0	0	0	0
Isoflav-3-enes	11	11	2	9	1	10	0	0	0	0	0
Miscellaneous	26	25	7	18	3	22	1	0	1	1	0
Total	815¶	759	425	334	311	448	81	14	67	69	12

*Seventeen isoflavones are found both in leguminous and non-leguminous plants.
†Four pterocarpanoids are found both in leguminous and non-leguminous plants.
‡Three rotenoids are found both in leguminous and non-leguminous plants.
§One coumestan is found both in leguminous and non-leguminous plants.
‖Non-leguminous plants for example, Nyctaginaceae and Iridaceae have been known to yield simple coumaronochromones [38, 39].
¶Twenty-five isoflavonoids in total are found both in leguminous and non-leguminous plants.

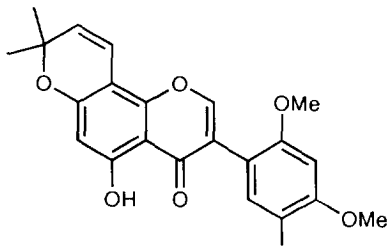
aromatic carbons, (c) *C*- or *O*-alk(en)ylation: *C*- or *O*-methylation, and *C*- or *O*-isopentenylation (prenylation or geranylation), and (d) the modification of substituents: oxygenation of side attachments, and the cyclization with a phenolic hydroxyl group to yield a methylenedioxy ring, as well as variously oxidized five- and six-membered cyclic ethers. The biogenetically related skeletal structures (nine major classes) are depicted in Fig. 1, and the

number of compounds in each isoflavonoid class is shown in Table 1. Complex isoflavonoids and 5-oxygenated isoflavonoids are found both in leguminous and non-leguminous plants.

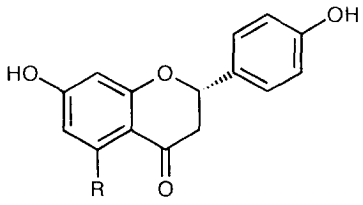
An examination of the oxygenation pattern of aromatic carbons in the isoflavone skeleton, reveals that the 293 isoflavones in the Leguminosae are divided into 40 patterns based on the oxygenated carbons (number of compounds): 5,7,4′ (56), 5,7,2′,4′ (50), 5,7,3′,4′ (33), 5,7,2′,4′,5′ (13), 7,4′ (13), 7,3′,4′ (12), 7,2′,4′,5′ (11), as well as 33 other oxygenation patterns, each of which consists of less than 10 compounds, and 11 patterns each consisting of only one compound. Forty-one non-leguminous isoflavones consist of 18 oxygenation patterns, 11 of which are not known among leguminous isoflavones. The major groups of non-leguminous isoflavones possess the following oxygenation patterns (number of compounds): 5,6,7,3′,4′ (10), 5,6,7,2′ (5), 5,7,3′,4′ (5), 5,6,7,3′,4′,5′ (4), among others.

Biosynthetic considerations

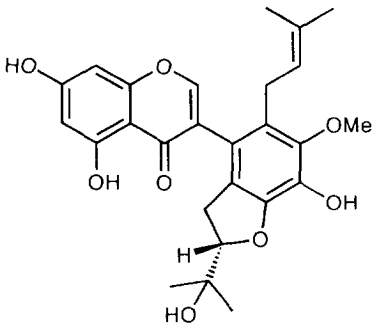
Based on the scheme of isoflavonoid biogenesis [17, 18], the first isoflavonoids are believed to be synthesized by aryl migration of the 5-hydroxy- and 5-deoxyflavanones, naringenin (**12**) and liquiritigenin (**13**) which is catalysed by the cytochrome P_{450}-, O_2- and NADPH-dependent isoflavone synthase, yielding initially the respective 2-hydroxyisoflavanones, followed by dehydration resulting in genistein (**2**) and daidzein (**1**), respectively [2–5]. According to the reaction mechanism, the enzymatic aryl migration seems to require a 4′- or a 2′-hydroxyl group [5, 40]. Therefore, almost all isoflavonoids are oxygenated at the C-4′ or C-2′ position of the corresponding flavone or isoflavone B-ring, 10 out of the 334 isoflavones studied lack both the 2′- and 4′-oxygenation. Nine of the 293 leguminous isoflavones, and 15 of the 41 non-leguminous isoflavones lack the 4′-oxygenation.



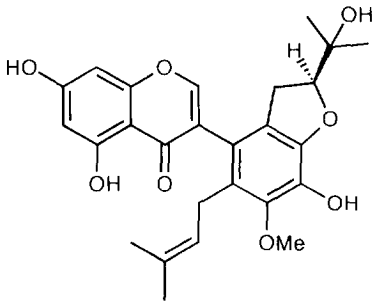
11 toxicarol isoflavone



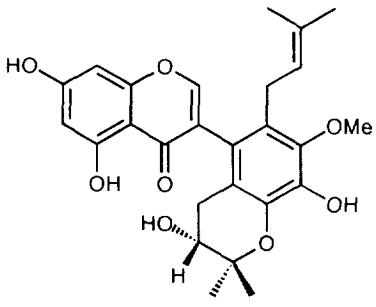
12 R=OH naringenin
13 R=H liquiritigenin



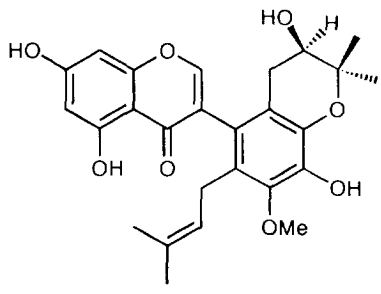
14 erythbigenol A



15 erythbigenol B



16 erythbigenone A



17 erythbigenone B

About 60% of leguminous isoflavones belong to the 5-deoxy type, however the latter are of less common occurrence (*ca* 15%) in non-leguminous plants. Only two 7-deoxyisoflavones have, thus far, been reported as plant constituents.

The enzymatic hydroxylation of aromatic carbons in the isoflavonoids [41–43] and methylenedioxy-ring formation [44] are catalysed by cytochrome P_{450}-dependent monooxygenases. The enzymatic *O*-methylation is catalysed by position-specific *O*-methyltransferases [45, 46] with *S*-adenosyl-L-methionine serving as the methyl donor. The oxygenation and *O*-methylation patterns found in naturally occurring isoflavones are summarized in Table 2.

It has generally been recognized that isoprenoid substituents are added to the different classes of isoflavonoids after the basic skeletons have been partly or completely constructed. A prenyltransferase specific to isoflavonoids has been reported in elicitor-treated soybean cotyledons [47, 48] and cell suspension cultures [49]. The enzyme catalyses the transfer of the dimethylallyl group from dimethylallyl pyrophosphate (DMAPP) to position 2 or 4 of 3,6a,9-trihydroxypterocarpan. The membrane bound DMAPP:trihydroxypterocarpan dimethylallyl transferase

Table 2. *O*-Alk(en)ylation of phenolic hydroxyl groups in 334 plant isoflavones

Oxygenated carbon	Oxygenated compound no.*	Free OH	*O*-Methyl	*O*-Alkenyl *O*-cyclic	Methylene-dioxy	OH/OH + OR (%)
C-5	226	188	36	2		83
C-6	76	9	46	0	21†	12
C-7	332	177	49	82	3	53
C-8	33	7	23	0		21
C-2′	120	71	46	3		59
C-3′	119	42	45	1	31	35
C-4′	312	134	92	38	17	43
C-5′	57	4	36	0		7
C-6′	2	0	2	0		0

*Compound numbers possessing the oxygenated C-*n* carbon in 334 plant isoflavones.
†Twenty-one compounds possess a 6,7-methylenedioxy part structure.

Table 3. Complex isoflavonoids: Substitution, position and structure of substituent

Isoflavonoid class	Complex isoflavonoid (no.)	Substitution number			Position of substitution*						Substituent structure† Cyclic ether		
		Mono	Di	Tri	C-6	C-8	C-2′	C-3′	C-5′	C-6′	Chain	5-membered	6-membered
Isoflavones	180‡	95§	70	3	88	65	2	53	11	5	120	27	79
Pterocarpan-oids	78	62‖	16	0	44	13	—	26	10	0	48	15	30
Rotenoids	54	54	0	0	10	44	—	0	0	0	2	40	12
Isoflavanones	36	28¶	8**	0	17	10	0	8	9	0	28	6	10
Isoflavans	24	20††	4	0	7	11	0	8	2	0	11	2	15
Coumestans	22	22‡‡	0	0	10	1	0	2	6	3	12	2	8
3-Arylcoum-arins	19	16	3	0	15	6	0	0	0	1	6	3	13
Coumarono-chromones	13	7	4	2	7	6	0	8	0	0	13	2	1
Others	9	9	0	0	6	1	0	2	0	0	4	2	3

Carbon numbering is from the isoflavone skeleton. Total numbers of side chains and other attachments having C–C bond on each skeletal carbon are counted.
*A compound duplicately or triplicately substituted is counted twice or three times, respectively.
†A compound possessing two different substituents is counted twice.
‡Including 12 *O*-prenyl or *O*-geranyl substituted.
§Including 2 1,1-dimethylallyl substituted.
‖Including 3 geranyl substituted.
††Including 5 1,1-dimethylallyl and 3 prenylpyrano substituted.
¶Including 1 prenylpyrano substituted.
**Including 2 prenyl and geranyl substituted.
‡‡Including 1 geranyl substituted.

(prenyltransferase) has recently been solubilized and partially characterized [50]. In addition, the prenyltransferases from *Lupinus albus* roots and cell suspension cultures catalysed the introduction of a dimethylallyl group into positions 6, 8 and 3′ of the isoflavones genistein and 2′-hydroxygenistein [51]. The variations in product ratios obtained from various microsomal preparations that were solubilized with different detergents, suggested that prenylation at positions 6, 8 and 3′ of the isoflavone ring system is catalysed by distinct prenyltransferases [51]. Further studies on the biosynthesis of prenylated isoflavones in white lupin cell suspension cultures indicated their high turnover and their secretion into the nutrient culture medium [52].

More than half of the leguminous isoflavonoids are substituted with 3,3-dimethylallyl (prenyl) group(s) or side attachment(s) that are variously modified from the prenyl substituent (Table 3). Isoprenoid substituents found in naturally occurring isoflavonoids are depicted in Fig. 2. These and other differently modified isoprenoid side structures are also found in flavonoids [1, 25], coumarins [53–55], xanthones [56], chromones [57] and alkaloids [58].

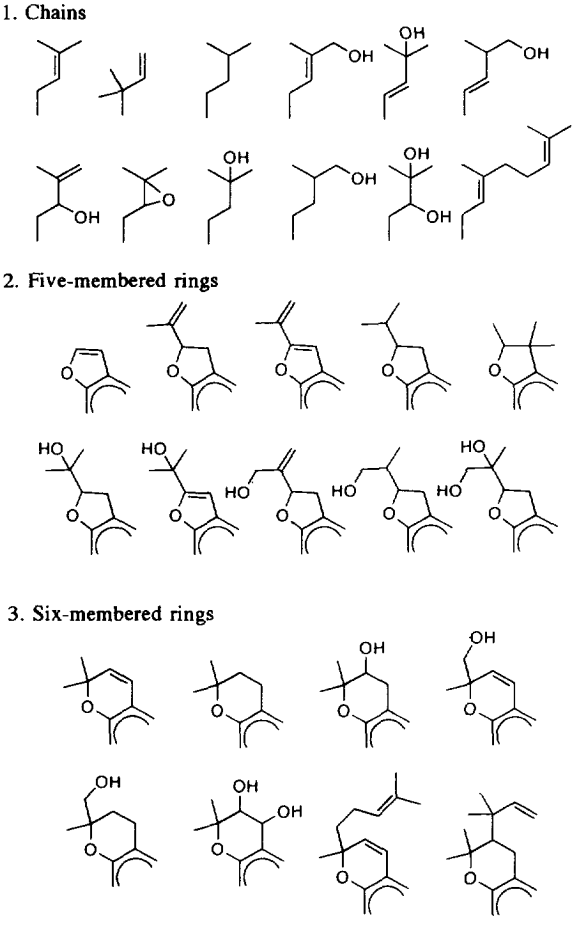


Fig. 2. Isoprenoid forms found in naturally occurring isoflavonoids [1, 72, 73].

More than half of the isoflavonoids (*ca* 57%) belong to those compounds that lack an asymmetric centre in the skeleton, e.g. isoflavones, isoflav-3-enes, 3-arylcoumarins, coumestans and coumaronochromones. Even though an asymmetric centre does not exist in the isoflavonoid skeleton, prenylation followed by structural modification sometimes introduces both an asymmetric centre and axis chirality in isoflavonoid molecules. The stereochemistry of skeletons and side attachments has been fully analysed except for some compounds [1, 14, 15, 17, 59–61]. The first atropisomers (rotational isomers) have been found in the derivatives of *Piscidia* 2′,6′-diprenylated isoflavones, erythbigenols A (**14**) and B (**15**), and erythbigenones A (**16**) and B (**17**), which have been isolated and characterized on the basis of chemical and spectroscopic properties [62].

STRUCTURAL MODIFICATION OF PRENYLATED ISOFLAVONES

A detailed survey of the isoflavonoid constituents in white lupin roots (*Lupinus albus*, Leguminosae) revealed the presence of various isoflavones with part structures (**d–i**) in Fig. 3 [63–65]. These metabolites were believed to be easily biosynthesized via a putative intermediate (**c**), although no reliable biogenetic studies of lupin isoflavonoids have, as yet, been carried out.

Some enzymatic prenyl cyclizations have been studied on rotenoids, pterocarpans and coumarins. Although an epoxy-intermediate has long been postulated in the biosynthetic pathway of dimethylpyrano and isopropenyldihydrofurano derivatives of isoflavonoids [53, 66], further studies are required to support this hypothesis.

According to a recent paper by Crombie *et al.* [67], deguelin cyclase, a non-heme iron protein from *Tephrosia vogelii*, catalyses the oxidative cyclization of rot-2-enoic acid (**18**) to deguelin (**6**) as shown in Fig. 4. The dimethylhydroxydihydropyrano derivative (**19**), which had been formerly postulated as a possible intermediate in the pathway from rotenoic acid to deguelin, via an epoxy-intermediate (**j**), was not transformed into deguelin in a *T. vogelii* cell-free system.

Although prenyl cyclization into rotenone has been observed in the germinating seeds of *Amorpha fruticosa* (Fig. 5), the reaction has not been demonstrated in the cell-free system [68]. Recently, however, a cell suspension culture of *T. vogelii* has been shown to produce rotenone together with the dihydrofurano-side attachment [69]. This culture may provide an excellent system to establish the enzymatic steps involved in the dehydrogenation and cyclization of rotenone biosynthesis [68].

In contrast with rotenoids, prenylated pterocarpans (glyceollidins for example, **20**) are directly transformed into a mixture of isopropenyldihydrofurano (**22**: rotenone-type side attachment) and dimethylpyrano (**21**: deguelin-type side attachment) pterocarpans as shown in Fig. 6 [70]. The reaction is reported to be catalysed by a cytochrome P_{450}-dependent monooxygenase-type enzyme, requiring O_2 and NADPH, from a microsomal

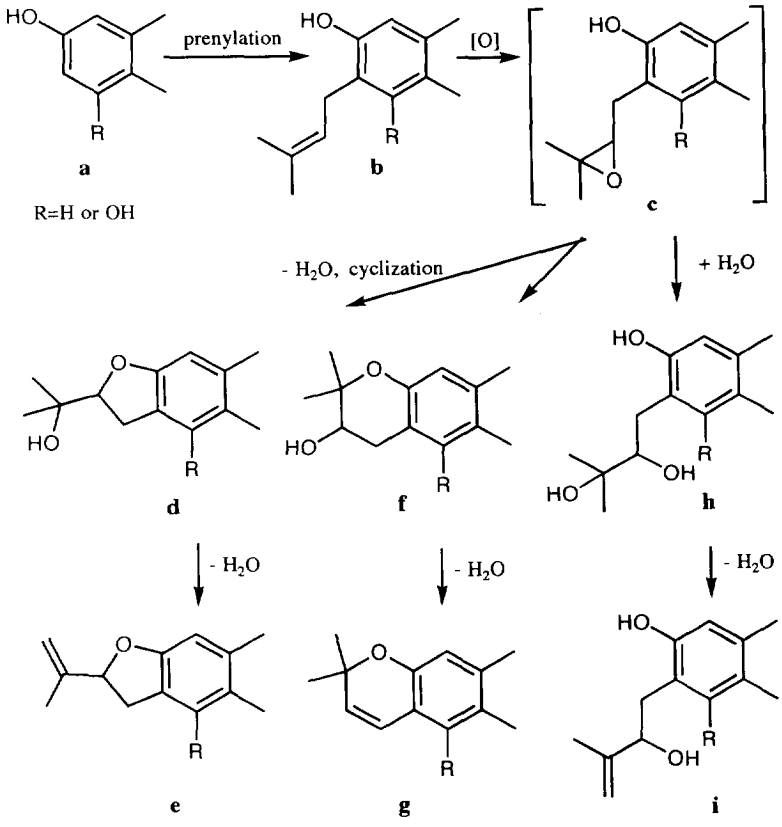


Fig. 3. Side-attachments found in the complex isoflavones from white lupin (*Lupinus albus* L. cv. Kievskij Mutant). (a) Simple isoflavones (three forms), (b) prenylation at C-6 and/or C-3′ (21 compounds*), (c) a proposed metabolic intermediate, (d) 15 compounds, (e) three compounds, (f) two compounds, (g) one compound, (h) 11 compounds, and (i) three compounds. *Duplicately substituted compounds are counted twice.

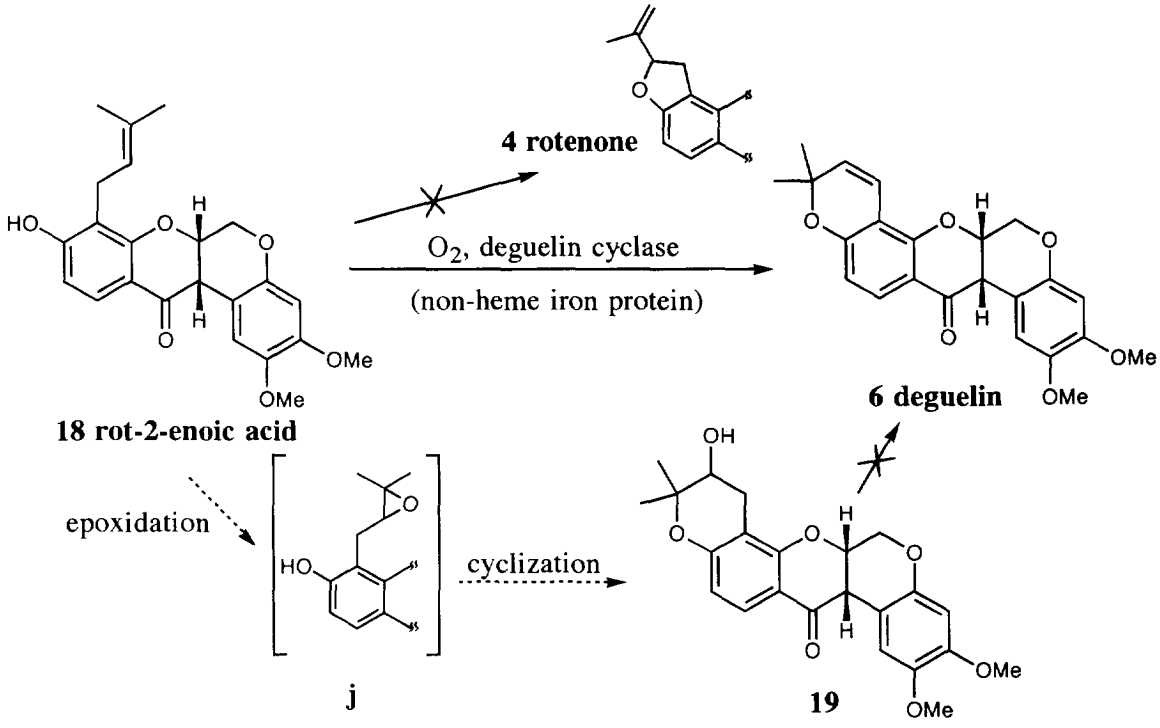


Fig. 4. Cyclization of rot-2-enoic acid to deguelin by deguelin cyclase from *Tephrosia vogelii* [66]. (j) Formerly proposed epoxy-intermediate.

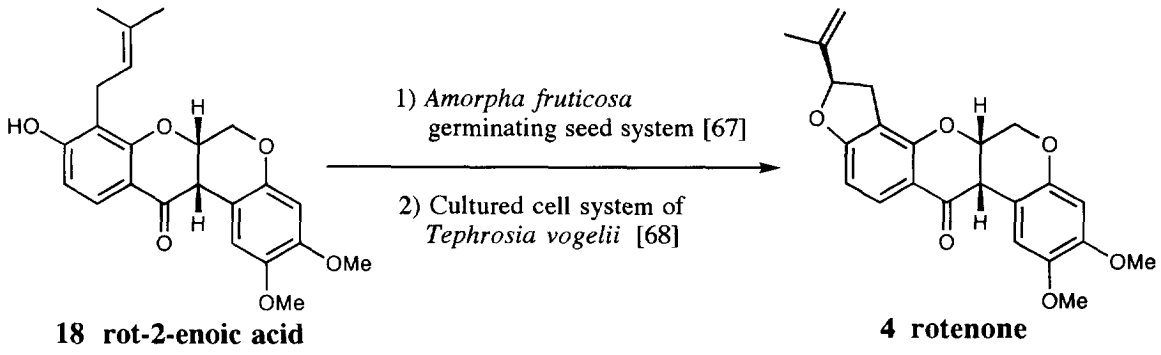


Fig. 5. Cyclization of rot-2-enoic acid to rotenone in germinating seed and cultured cell systems. The presence of dehydrating and cyclizing enzyme(s) is presumed [67].

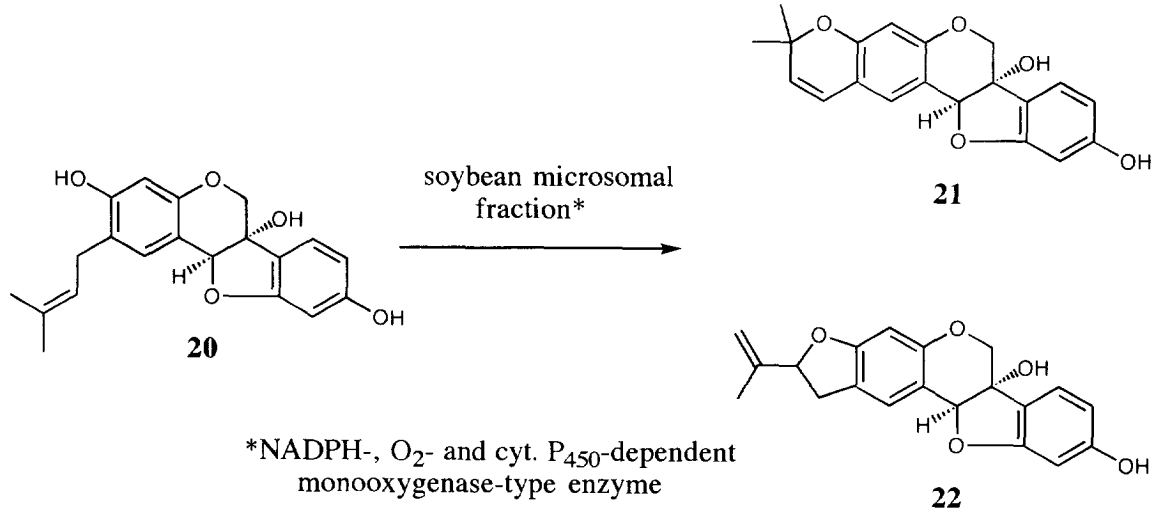


Fig. 6. Enzymatic cyclization of a prenylated pterocarpan to glyceollins [69]. The scheme is depicted for cyclization of 2-prenylglycinol (**20**) to yield glyceollins II (**21**) and III (**22**).

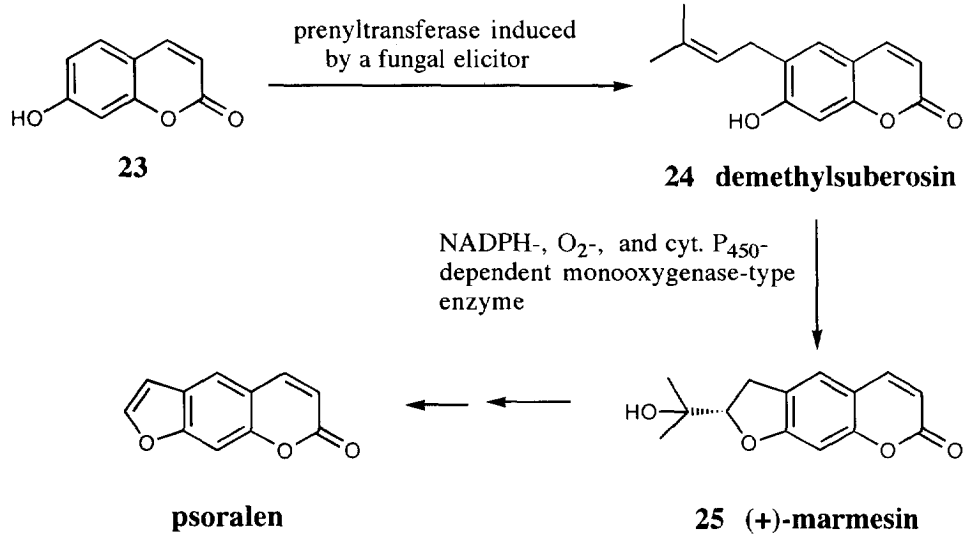


Fig. 7. Conversion of 7-hydroxycoumarin (**23**) to demethylsuberosin (**24**) and (+)-marmesin (**25**) by microsomal enzymes from elicitor-treated *Ammi majus* cell culture [70, 71].

fraction of an elicitor-challenged soybean culture. However, no reaction intermediate was postulated, and the detailed reaction mechanism has not yet been elucidated.

A situation similar to pterocarpans, but without the equivalent prenyl cyclization has been reported in the elicitor-treated cell suspension cultures of *Ammi majus* (Apiaceae) [70, 71]. 7-Hydroxycoumarin (**23**) is first

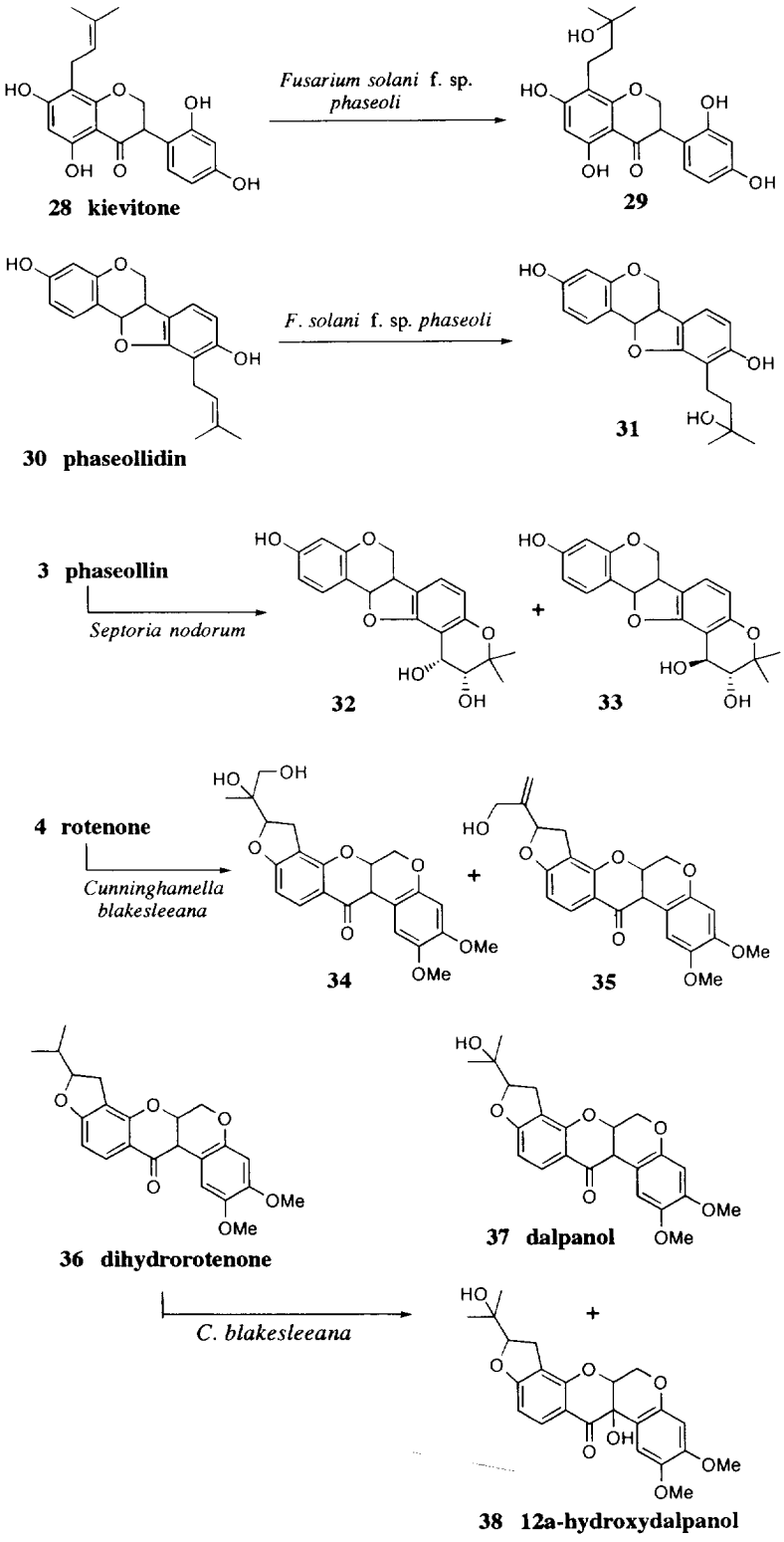


Fig. 8. Fungal metabolism of complex isoflavonoids [See refs in the text].

prenylated by a prenyltransferase induced by a biotic elicitor [71], and the side chain is then cyclized to yield (+) − marmesin (*S*-configuration) (**25**) (Fig. 7). The latter step is catalysed by a microsomal cytochrome P_{450}-dependent enzyme requiring O_2 and NADPH [72]. Subsequent oxidative cleavage of the side chain in the product gives rise to a furocoumarin, psoralen [72]. In the latter reaction, however, the authors doubt the existence of an epoxy-intermediate between demethylsuberosin (**24**) and (+)-marmesin (**25**) [72].

MICROBIAL METABOLISM OF THE PRENYL SIDE CHAIN

It has been suggested that prenylation of isoflavonoids, especially isoflavones, increases their antifungal [47, 63, 75] and antifeedant activity [76]. Prenylated isoflavones, such as wighteone (**26**) and luteone (**27**) in leguminous plants are known both as pre-infectional and post-infectional fungitoxins [77–79].

Fungal metabolism of isoflavonoids has received much interest especially in terms of pathogenicity and detoxification of such plant fungitoxins [80], and the topic has been thoroughly reviewed [17, see also Biological Activities section]. When isoflavonoids contain prenyl or cyclized isoprenoid substituents, the side attachments are frequently modified. A prenylated isoflavanone, kievitone (**28**) and a pterocarpan, phaseollidin (**30**) have been shown to be detoxified by hydration at the double bond of the prenyl side chain to yield **29** and **31**, respectively [81, 82]. An enzyme catalysing the hydration of kievitone (**28**), and the phytoalexin phaseollidin (**30**) has been purified from *Fusarium solani* [83–85]. A dimethylpyranopterocarpan, phaseollin (**3**) was oxidized to the glycol derivatives (**32**, **33**, Fig. 8) by *Septoria nodurum* [86]. Hydroxylation of the terminal methyl group of rotenoids has also been reported in the *Cunninghamella blakesleeana* system which resulted in the formation of **34** and **35** [87], as well as for dihydrorotenone (**36**) by *Streptomyces griseus* yielding **37** and **38** [88] (Fig. 8).

In 1984, a new metabolic pathway characteristic of the prenylated isoflavone luteone (**27**) was reported [89]. The fungitoxic luteone (**27**) was metabolized by *Aspergillus flavus* to a major product, luteone hydrate (**39**), which was not the case when the former (**27**) was administered to *Botrytis cinerea* cultures. In addition to luteone hydrate (**39**), both *A. flavus* and *B. cinerea* transformed luteone (**27**) into the cyclic ether derivatives **40** and **41**, as well as luteone glycol (**42**, Fig. 9). These latter metabolites were shown to be far less fungitoxic than the substrate luteone (**27**) [89].

By using the prenylated isoflavones wighteone (**26**) [90], licoisoflavone A (**43**) [91], 2′-hydroxylupalbigenin (angustone A, **44**) [92], 2,3-dehydrokievitone (**45**) [93], piscerythrone (**46**) and piscidone (**47**) [94], as well as the prenylated pterocarpan edunol (**48**) [95], and the flavone topazolin (**49**) as substrates [94], the general features for the fungal metabolism were established, as shown in Fig. 10 [94]. The initial fungal modification involves epoxidation of the double bond in the prenyl substituent. The resulting epoxide becomes liable to nucleophilic attack by water, giving rise to a glycol, or by an *ortho*-phenol group to yield either dihydrofurano (neutral condition) and dihydropyrano (acidic condition) rings, as shown in a model organo-synthetic system [96]. However, the postulated epoxide-intermediate, corresponding to the administered substrate, could not be detected in the metabolizing cultures.

When cyclization was prevented by using the improved substrate, 2,3-dehydrokievitone 7-*O*-methyl ether (**50**), lacking a free hydroxyl group *ortho* to the prenyl side

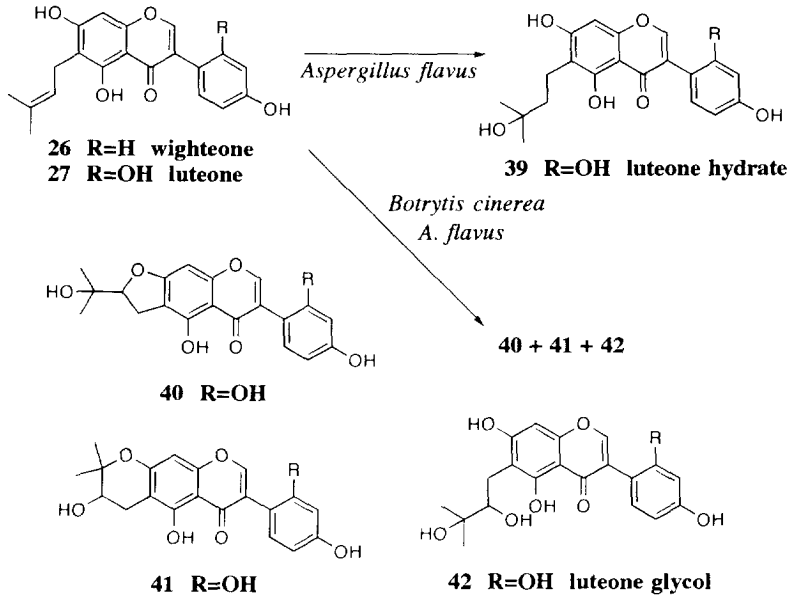


Fig. 9. Fungal metabolites of the prenylated isoflavone luteone (R=OH).

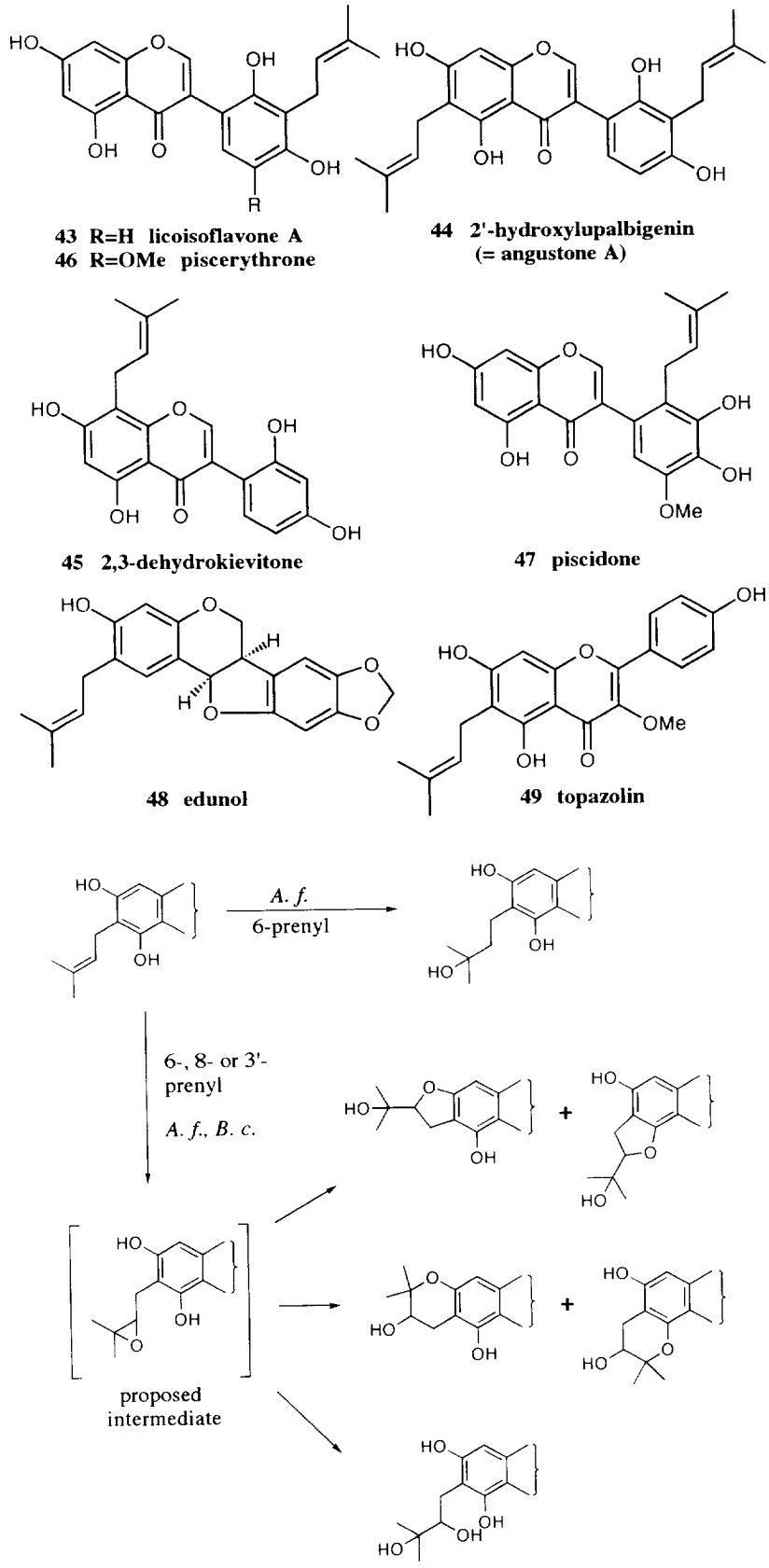


Fig. 10. Prenylated flavonoid metabolism in *Aspergillus flavus* (*A. f.*) and *Botrytis cinerea* (*B. c.*). The prenyl substituted carbons are those of the isoflavone skeleton.

chain, the epoxide (**51**) was successfully isolated and characterized from the metabolic culture of *B. cinerea* [97], The *S*-configuration at the side chain C-2 of the epoxide was established by chemical correlation with that of the corresponding (2*S*)-glycol metabolite (**52**) associated with the epoxide (Fig. 11). The absolute stereochemistry of the glycol metabolite was determined by CD spectroscopy after complexation with osmium tetroxide/pyridine complex [98]. The glycol (**52**) possessing the same chirality as the metabolite was also produced by stereo-conservative acid hydrolysis of the epoxide (**51**) [97]. In a similar manner, the stereochemistry of the 1-

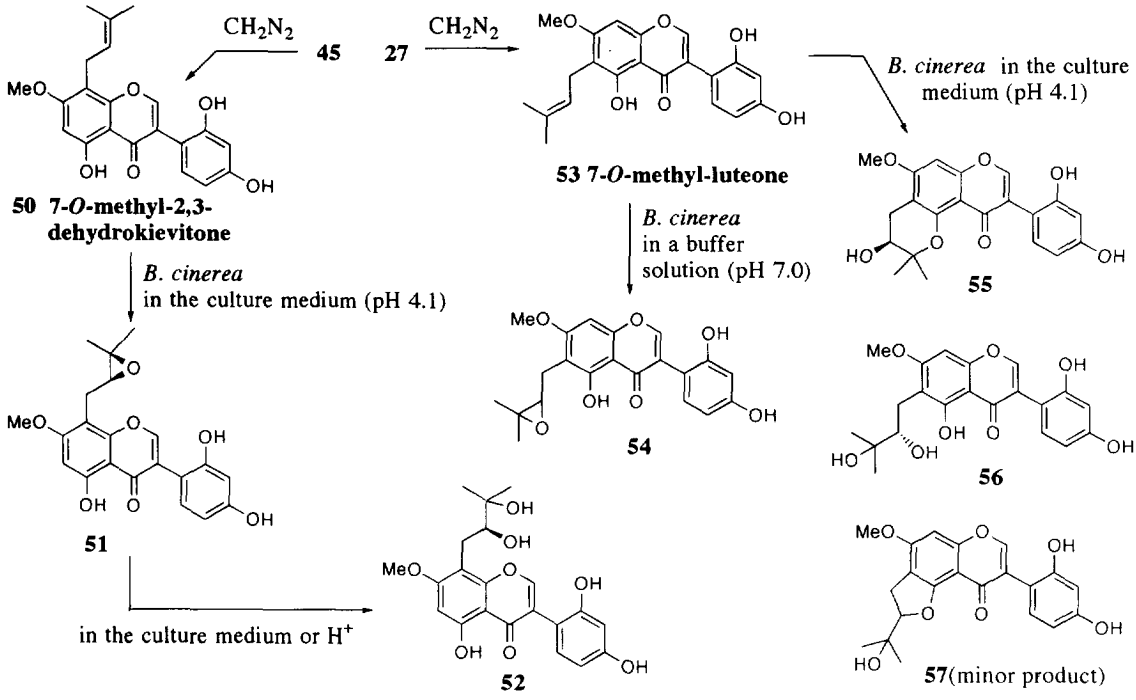


Fig. 11. Fungal metabolism of 6- or 8-prenylated isoflavone 7-*O*-methyl ethers by *Botrytis cinerea* and identification of the epoxy-intermediates.

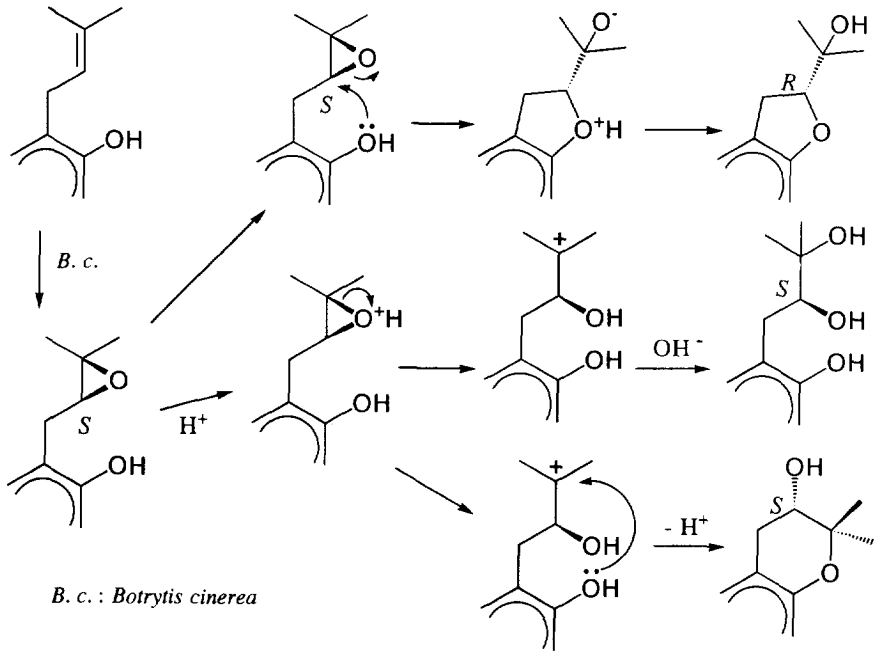


Fig. 12. Metabolic pathways and stereochemistry for fungal metabolism of prenylated flavonoids.

hydroxy-1-methylethyldihydrofurano-isoflavone metabolite (**40**) was elucidated by the CD method, when applied to the osmate ester/pyridine complex, after dehydration of the latter compound [59, 99].

Finally, the presence of the epoxide intermediate of a prenylisoflavone possessing an *ortho* hydroxyl group (**54**) was confirmed using a *B. cinerea* culture, in neutral buffer, metabolizing 7-*O*-methylluteone (**53**) followed by HPLC analysis (Fig. 11) [100]. Absolute configuration of the optically active dihydropyrano metabolite (**55**) was determined [101] by the improved Mosher's method which determines the ^{1}H NMR shielding effect of MTPA derivatives [100, 102].

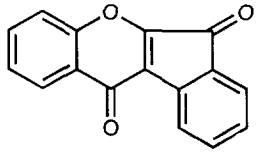
Thus, the reactions in the pathway and the stereochemistry for fungal metabolism of prenylated isoflavones have been established as shown in Fig. 12 [100]. The pathway consists of a stereospecific epoxidation, catalysed by the fungal enzyme (possibly a cytochrome P_{450}-dependent oxygenase), followed by non-enzymatic cyclization and hydrolysis of the resulting epoxide. The *S*-configuration in the epoxy-intermediate was conserved in both the glycol and dihydropyrano metabolites, and was inverted in the dihydrofurano metabolite.

It is interesting to note that metabolic experiments on luteone (**27**) in a rat liver homogenate revealed that the substrate was predominantly oxidized at the terminal methyl groups to yield metabolites with a variously oxygenated side chain [103].

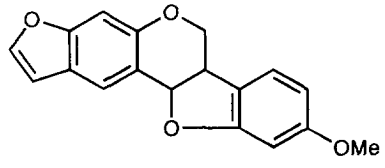
SOME UNUSUAL ISOFLAVONOIDS

Isoflavonoids with a new skeleton

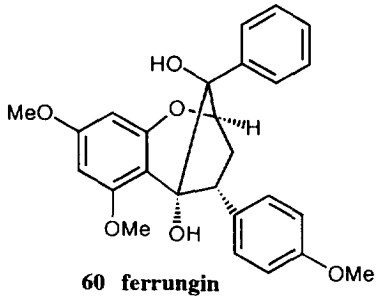
A novel isoflavonoid, wrightiadione (**58**) has been isolated from *Wrightia tomentosa* (Apocynaceae), which is a new family as an isoflavonoid source, and whose structure was identified by X-ray crystallography [6]. However, neither this compound (**58**) nor 9-*O*-methylneodunol (**59**), a pterocarpan found in a marine organism [104], has been shown to be synthesized through



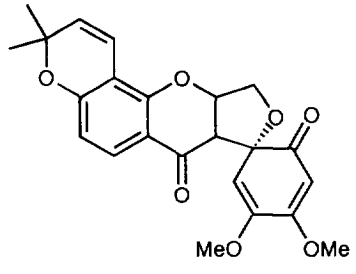
58 wrightiadione



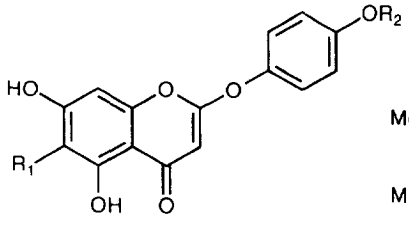
59 9-*O*-methylneodunol



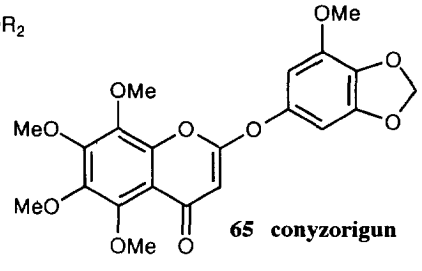
60 ferrungin



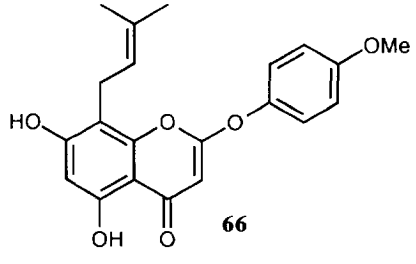
61 amorphispirone



62 R_1=OMe, R_2=H capillarisin
63 R_1=R_2=H
64 R_1=H, R_2=Me



65 conyzorigun



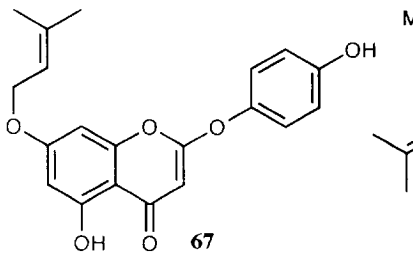
66

pathways established for ordinary isoflavonoids. A far more exotic isoflavonoid with a new skeletal structure, ferrungin (**60**), has been found in *Agraria ferruginaea* (Meliaceae) [7]. Amorphispirone (**61**), a cytotoxic rotenoid with a spiro part structure, was also isolated from *Amorpha fructicosa* [105].

Capillarisin (**62**) and its relatives, 6-demethoxycapillarisin (**63**), 6-demethoxy-4'-methylcapillarisin (**64**), 4'-*O*-methylcapillarisin and 7-*O*-methylcapillarisin have long been known as a group of 2-phenoxychromones from *Artemisia capillaris* (Compositae) [106]. Interestingly, **63** and **64** were also found in *Rosa rugosa* [107]. 6-Demethoxycapillarisin (**63**) has also been reported as a fungal-induced phytoalexin of *Cassia obtusifolia* (Leguminosae) [108]. This compound was associated with apigenin in *R. rugosa* [107], and its formation in *C. obtusifolia* was stimulated after elicitation with *Alternaria cassiae*. [^{14}C]Phenylalanine was incorporated into the phytoalexin (**63**) [108], although it is not clear whether these phenoxychromones are biosynthesized via the corresponding flavonoids. Recently, however, a highly oxygenated simple 2-phenoxychromone, conyzorigun (**65**) was isolated from *Ageratum conyzoides* (Compositae) [109]. In addition to 6-demethoxy-7-*O*-methylcapillarisin, two other prenylated 2-phenoxychromones (**66** and **67**) have been reported from *Epimedium sagittatum* (Berberidaceae) [110], and glyasperin E (**68**), the first known 3-phenoxychromone has been isolated from *Glycyrrhiza* (Leguminosae) [111].

Coumaranochroman-4-ones

Four complex isoflavonoids belonging to a new class, coumaranochroman-4-one, have been isolated and their structures unambiguously elucidated by dehydration to yield the corresponding coumaronochromones, e.g. piscerythrol (**69**) to lisetin (**70**, Fig. 13) [112]. These are piscerythrol (**69**) from Jamaican dogwood (*Piscidia erythrina*), lupinols A (**71**) and B (**72**) from white lupin, and lupinol C (**73**) from yellow lupin [112].



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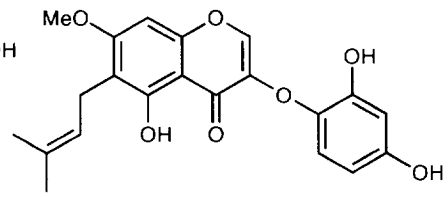
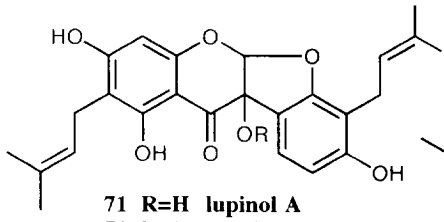
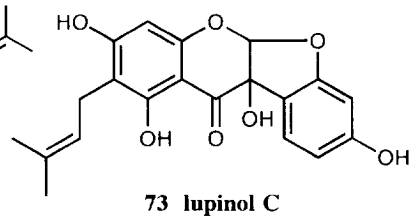


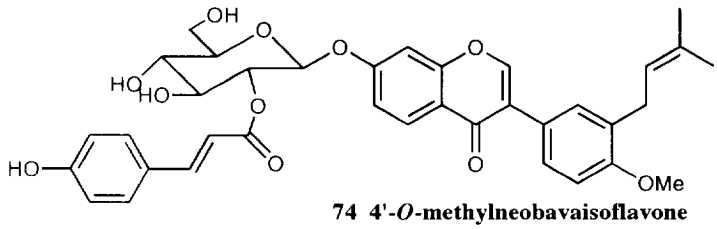
68 glyasperin E



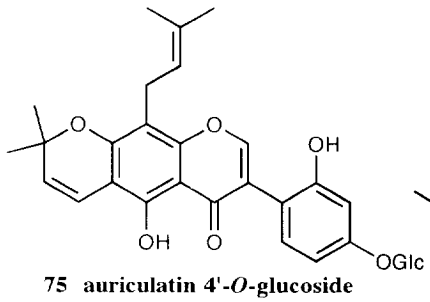
71 R=H lupinol A
72 R=Me lupinol B



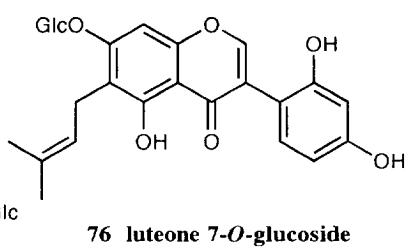
73 lupinol C



74 4'-*O*-methylneobavaisoflavone 7-*O*-(2"-*O*-*p*-coumaroyl)glucoside



75 auriculatin 4'-*O*-glucoside



76 luteone 7-*O*-glucoside

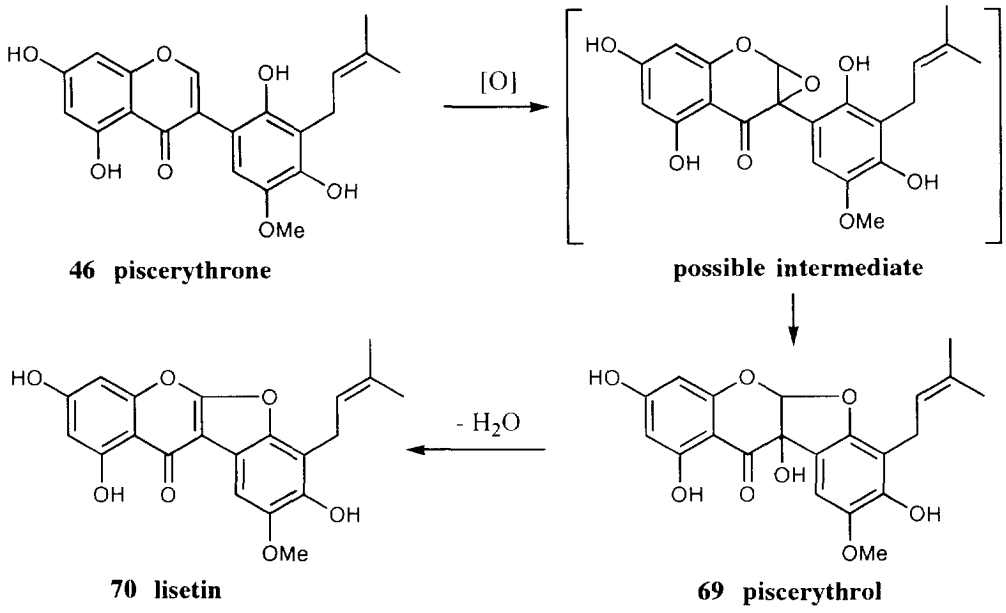


Fig. 13. Hypothetical pathway from a 2′-hydroxyisoflavone piscerythrone (**46**) to lisetin (**70**).

Lisetin (**70**), from *P. erythrina*, the first compound in a rare naturally occurring class of isoflavonoid coumaronochromones [113], is believed to be formed by the direct oxidation of 2′-hydroxyisoflavone, piscerythrone (**46**). A second coumaronochromone, millettin was isolated in 1981 from *Millettia auriculata* [114]. Some time later, the presence of five new coumaronochromones (lupinalbins A–E) was reported in 1985 [115] and three more relatives were isolated, all from white and yellow lupins [64, 65, 116]. It is notable that all these coumaronochromones are accompanied by the structurally related 2′-hydroxyisoflavones in each plant. The number of known coumaronochromones, including the triprenylated derivatives from *Euchresta japonica* [117], is now 30 (–1993), and not only are they found in leguminous plants, but also in species from the Iridaceae and Nyctaginaceae [23, 38]. The presence of 3-hydroxycoumaranochroman-4-ones in lupins and Jamaican dogwood, strongly suggests that the latter compounds are derived from the corresponding 2′-hydroxyisoflavones as shown in Fig. 13.

Complex isoflavone glycosides

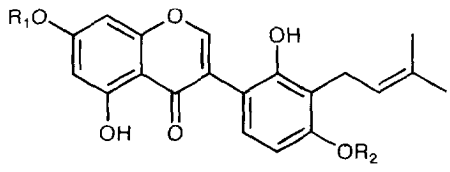
Whereas glycosides of isoflavonoids are not particularly rare, their numbers are extremely small when compared with the vast range of flavonoid glycosides. In addition to the 150 isoflavonoid glycosides which have been identified [1, 16], the following complex isoflavone glycosides have been recently reported: 4′-*O*-methylneobavaisoflavone 7-*O*-(2″-*O*-*p*-coumaroyl)glucoside (**74**) from *Sopubia delphinifolia* (Scrophulariaceae) [118], and auriculatin 4′-*O*-glucoside (**75**) from the stem bark of *Erythrina eriotricha* (Leguminosae) [119].

White lupin yields not only malonylated glycosides of simple isoflavones, but also the prenylated isoflavone glucosides, luteone 7-*O*-glucoside (**76**), as well as the 7-*O*- and 4′-*O*-glucosides of licoisoflavone A (**77** and **78**) [120]. Rotenoid glycosides, the majority of which are complex isoflavonoids [16, 121] are formed, not via a phenolic hydroxyl, but via an alcoholic hydroxyl group, such as dalpanol 3′-*O*-glucoside (**79**) [121]. In fact, dalpanin (**80**) is the only known example of complex isoflavonoid *C*-glucoside [122].

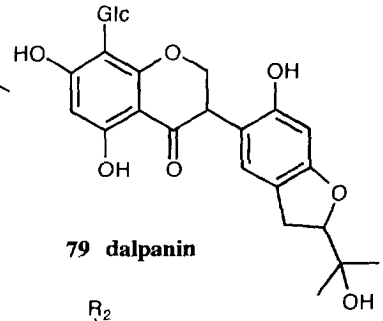
Heteroatom-containing isoflavonoids

While almost all isoflavonoids consist solely of C, H, and O atoms, there exist a small number of naturally occurring isoflavonoids that contain chlorine or nitrogen atoms. For example, 6-chlorogenistein (**81**) [123], 8-chloroorobol (**82**) [124], 6,3′-dichlorogenistein (**83**) [123], and 6,8-dichlorogenistein (**84**) [125] have all been detected in the culture media of *Streptomyces* spp. However, it has recently been revealed that these halogenated compounds (**83** and **84**) are not actually products of *Streptomyces* metabolism, but are derived from the corresponding isoflavone genistein (**2**) in the fermentation media [125].

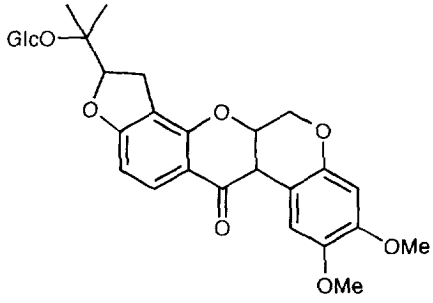
Nitrogen-containing isoflavonoids have recently been found in the root bark of *Piscidia erythrina* (Leguminosae). These include two 4′-aminoisoflavones, piscerythramine (**85**) [126] and isopiscerythramine (**86**) [127], and a third which possesses an oxazole ring, piscerythoxazole (**87**) [127]. However, owing to the small amounts isolated, the biological activity of these peculiar isoflavone derivatives could not be investigated. To date, eight *N*-containing flavonoids have been reported, all of which consist of a flavonoid skeleton with a *N*-containing cyclic side attachment that is connected via a C–C bond. These are ficine (**88**) and isoficine (**89**) from *Ficus pantoniana* (Moraceae) [128], phyllospadine (**90**) from *Phyllosphadix*



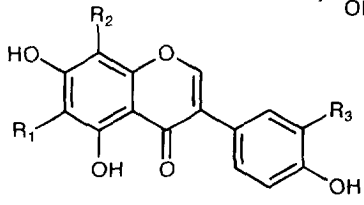
77 R_1=Glc, R_2=H licoisoflavone A 7-*O*-glucoside
78 R_1=H, R_2=Glc licoisoflavone A 4'-*O*-glucoside



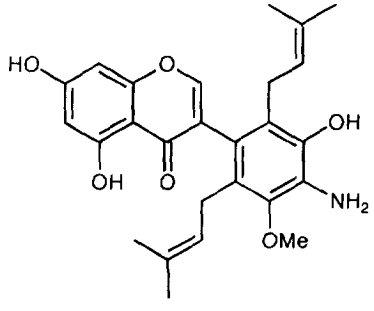
79 dalpanin



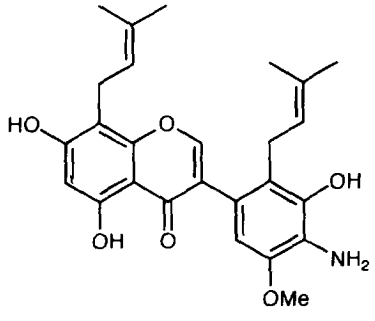
80 dalpanol 3'-*O*-glucoside



81 R_1=Cl, R_2=R_3=H 6-chlorogenistein
82 R_1=H, R_2=Cl, R_3=OH 8-chloro-orobol
83 R_1=R_3=Cl, R_2=H 6,3'-dichlorogenistein
84 R_1=R_2=Cl, R_3=H 6,8-dichlorogenistein



85 piscerythramine



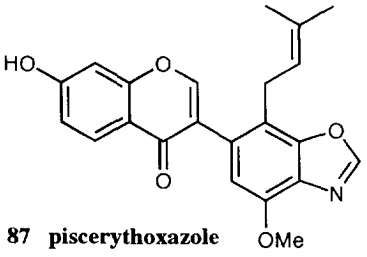
86 isopiscerythramine

iwatensis) (Potamogetonaceae) [129], vochysine (**91**) from *Vochysia guianensis* (Vochysiaceae) [130], buchenavianine (**92**) and its relatives (**93** and **94**) from *Buchenavia* sp. (Combretaceae) [131], and lilaline (**95**) from *Lilium candidum* (Liliaceae) [132]. *Buchenavia* flavonoid alkaloids have been rediscovered in *B. capitata* as moderate cytoprotective agents against HIV in cultured human lymphoblastoid cells [133].

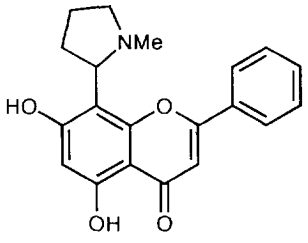
From a biogenetic viewpoint, these nitrogen-containing isoflavones and flavonoids are very interesting, especially concerning the source of the nitrogen atom, and the step at which it is incorporated into the molecule. Although phenylalanine is the obligate precursor of the isoflavone B-ring, it is not known whether *p*-aminophenylalanine found in *Vigna* [134] may act as the precursor of 4'-aminoisoflavones.

IN VITRO PRODUCTION OF ISOFLAVONOIDS

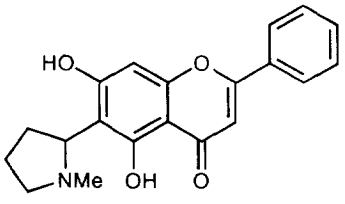
While the capability of plant tissue and cell cultures to produce a variety of secondary metabolites has been well documented [for recent reviews, see 135–137 and refs. therein], there is, nevertheless, a noticeable paucity of reports relating to the *in vitro* production of isoflavonoid compounds. The ability of legume species to produce isoflavonoid phytoalexins, in response to microbial attack, or elicitation by abiotic/biotic elicitors, is a trait that is also expressed in their cell cultures. This trait has been demonstrated with cell cultures of French bean (*Phaseolus vulgaris*) which produce phaseollin (**3**), and smaller amounts of phaseollidin (**30**), phaseollinisoflavan and kievitone (**28**) [138, 139]; soybean (*Glycine max*) cultures which accumulate mainly glyceollins **21** and **22**, as well as small amounts of 2-prenylglycinol (**20**) and its 4-prenylated isomer [48]; and alfalfa (*Medicago sativa*) cell cultures which accumulate medicarpin [140]. The accumulation in *Vigna angularis* of the simple 5-deoxyisoflavone, daidzein (**1**), 2'-hydroxydaidzein and their 7,4'-diglucosides was reported to increase dramatically upon treatment of the culture with the transcription inhibitor actinomycin D [141]. On the other hand, lupin (*Lupinus albus*) root cell cultures constitutively synthesize the entire



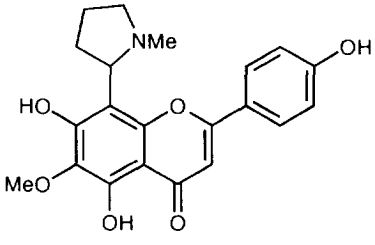
87 piscerythoxazole



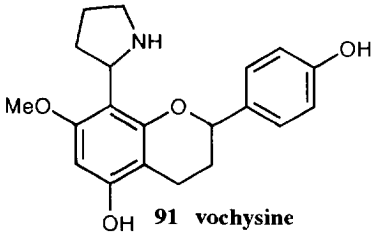
88 ficine



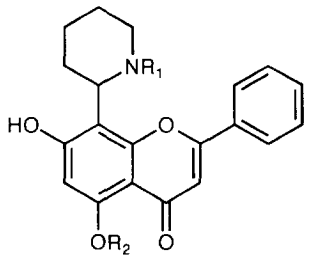
89 isoficine



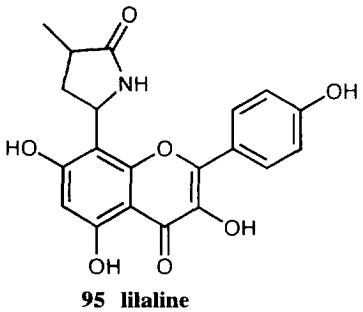
90 phyllospadine



91 vochysine



92 R_1=R_2=Me buchenavianine
93 R_1=Me, R_2=H demethylbuchenavianine
94 R_1=R_2=H *N,O*-didemethyl-buchenavianine



95 lilaline

complement of isoflavonoids known to occur in the intact root [142]. These consist of genistein (**2**), 2'-hydroxygenistein and their 7-*O*-glucosides, as well as their 6-, 8-mono- and 6,3'-diprenyl derivatives [142]. Rotenone (**4**) and deguelin (**6**), among other related rotenoids, have been reported to accumulate in a number of leguminous cultures [143–145]. The pattern of rotenoids produced in *Tephrosia vogelii* [146] seems to depend on the culture conditions, where the photomixotrophic cell line produced mainly rotenone and deguelin, and the heterotrophic cell line accumulated deguelin and its hydroxy derivative, tephrosin [146]. In veiw of the important roles of isoflavonoids (see below), further studies are urgently required in order to better understand the mechanisms involved in the regulation of their biosynthesis and accumulation *in vitro*.

BIOLOGICAL ACTIVITIES OF ISOFLAVONOIDS

The biological activities of isoflavonoids are quite diverse, and specific aspects of their antimicrobial, estrogenic and insecticidal activities, have previously been reviewed [e.g. 1, 15–17, 66, 146–152]. The simplest isoflavone genistein (**2**), which is the precursor of 5-hydroxyisoflavonoids, has been implicated in a variety of biological activities. These include allelopathic, estrogenic or proestrogenic, antihaemolytic, antioxidant and anticancer activities; as well as the inhibition of several enzymes, including catechol *O*-methyltransferase, DOPA decarboxylase, dopamine β-hydroxylase, histidine decarboxylase, and lipase [16, 17 and refs therein].

A number of isoflavonoids have recently been reported to act as signalling molecules in plant interactions with other organisms [153, 154 and refs therein]. Genistein and its 5-deoxy isomer daidzein, which are natural constituents of soybean roots, have been reported to be among the most powerful inducers of nodulation (*nod*) gene expression in *Bradyrhizobium japonicum* and *Rhizobium fredii*, whereas 4'-*O*-methylgenistein (biochanin A) is inactive [153, 155 and refs therein].

A number of simple isoflavones have recently been reported to act as fungal spore attractants. The zoospores

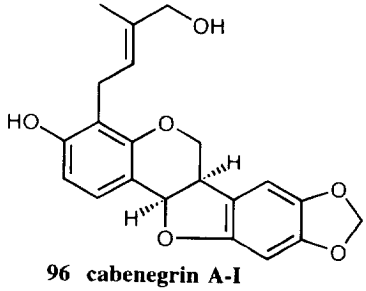
of *Aphanomyces euteiches*, a causal fungus of aphanomyces root rot of pea [156] have been reported to be attracted by prunetin (7-*O*-methylgenistein) excreted from the roots of pea [157]. Genistein and its *O*-methylated derivatives possess similar activity towards *Phytophthora sojae* [158]. Studies of the structure–zoospore attracting relationship revealed that the presence of a OH-4′ was not significant for activity, but that the OH-5 was essential for zoospore attraction, and methylation of the hydroxyl group markedly reduced the activity [159]. On the other hand, the zoospores of *A. cochlioides*, a causal fungus of spinach root rot, have been reported to be attracted not by prunetin but by a host-specific flavone, cochliophilin A (5-hydroxy-6,7-methylenedioxyflavone) which exudes from the roots into the rhizosphere and exhibits a potent attractance [160]. In addition, genistein acts as one of the phytoalexins of soybean, in response to infection by *Phytophthora megasperma* f.sp. *glycinea* [161], and causes a marked swelling of hyphal tips and an increase in the number of oogonia formed [162]. Furthermore, genistein and biochanin A have recently been reported to inhibit hyphal growth of the vesicular-arbuscular mycorrhizal (VAM) fungus, *Gigaspora margarita*, whereas flavonoids, other than isoflavones, are known to promote the development of VAM fungi [163]. However, recent work [164] indicates that colonization of alfalfa roots with the VAM fungus *Glomus versiforme* causes transient increases in the levels of the phytoalexin medicarpin, as well as increased transcript levels of phenylalanine ammonia-lyase and chalcone synthase, but not so for isoflavone reductase.

Recently, there has been considerable interest on the impact of flavonoids from vegetable foods on mammalian biology [165], especially the preventive effects of genistein on chronic diseases, including solid tumours [166–169], by inhibiting neovascularization (angiogenesis) [170]. In addition, genistein has been reported to inhibit tumour promoter-induced hydrogen peroxide formation [171], arrest cell cycle progression [172, 173], cause transient increase in cytoplasmic free calcium [174], and inhibit postreceptor effects of insulin [175]. In spite of the beneficial effect of genistein, however, it is known to be responsible for the undesirable astringent and bitter taste of edible soybean products [176]. Of particular interest, is the specific effect on osteoporosis of the synthetic isoflavone, ipriflavone (**98**) which suppresses bone resorption [177].

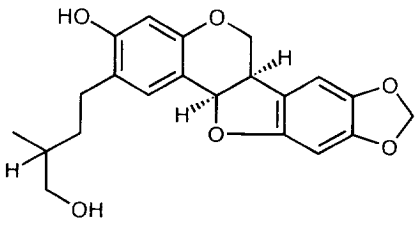
Simple isoflavones, especially genistein, may also be implicated in plant growth processes as potent inhibitors of polar auxin transport [178, 179], IAA oxidase [180], cell wall peroxidases invovled in coniferyl alcohol oxidation [181], as well as the inhibition of alcohol dehydrogenase [182] and tyrosine kinase [183–185 and refs therein]. In addition, genistein has been reported to act as an antidote against herbicides [186].

Other biologically active derivatives, specifically related to the complex isoflavonoid structures, are associated with the piscicidal and insecticidal rotenoids [66, 187, 188], complex isoflavonoid phytoalexins [80, 147, 149, 151, 189], insect feeding deterrents [76, 190], and two pterocarpans, cabenegrins AI (**96**) and AII (**97**) as antidotes against snake venoms [191]. The structural requirements for insecticidal activity of natural rotenoids are quite strict, and only some derivatives such as rotenone, dihydrorotenone and deguelin are practical insecticides [80, 149]. The fact that high insect feeding deterrence was generally associated with antifungal activity, suggests that isoflavonoids play a dual role in the Leguminosae, and are used by these plants as defensive agents against both insects and fungi [76, 148, 190]. Some complex isoflavonoids possessing antimutagenic activity are interesting as lead compounds for developing chemotherapeutic agents [192].

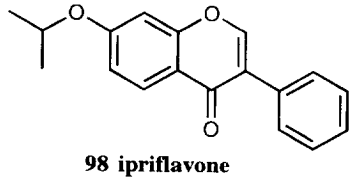
A further evidence for the ability of pathogens to suppress expression of the host defence response has recently been demonstrated. Two glycopeptide suppressors, supprescins A and B, have been purified from the spore culture filtrates of the pea pathogen, *Mycosphaerella pinoides*, and were shown to suppress accumulation of the pterocarpan phytoalexin, pisatin, in pea leaves



96 cabenegrin A-I



97 cabenegrin A-II



98 ipriflavone

treated with a fungal elicitor [193]. The mechanism of action seems to involve binding of the suppressor to putative receptors on the transmembrane signalling system associated with the host defence responses [194]. Finally, a recent study of the structure–activity relations of isoflavonoid phytoalexin biocides [195] indicated that their antifungal activity may be correlated with the relative acidity and number of hydroxyl groups per molecule. On the other hand, there was no correlation between lipophilicity and antibacterial activity of these phytoalexins against *Streptococcus faecium*, implying a different mode of action in bacteria [195].

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