

INDUCTIVE SUBSTANCES IN FABACEAE: PHYTOALEXINS OR ANTIOXIDANTS?

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ABSTRACT

Comparison of the structure of inductive and constitutive isoflavonoids of Fabaceae reveal a stronger trend towards the biosynthesis of compounds endowed with more potent antioxidant properties to characterize the former metabolic category. The function of inductive isoflavonoids should thus defense of plant tissue against injury-stimulated oxidative burst.

Key words: Isoflavonoids, phytoalexins, Fabaceae

INTRODUCTION

According to their current definition phytoalexins are antibiotic compounds generated in plants by elicitation of latent biosynthetic routes through infection by microorganisms (virus, bacteria, fungi). However, comparative studies so far have failed to provide unequivocal structure-bioactivity relationships (Stoessl, 1986). Besides abiotic agents (nutrients, light and temperature, mechanical or chemical aggression, genetic manipulation) frequently elicitate identical compounds, now called stress metabolites (Dicosmo & Towers, 1984), from the same species (Stoessl, 1985). In order to avoid *a priori* association of phytoalexins or stress metabolites with any conceivable function they are here designated inductive substances.

It is generally assumed that inductive substances can be elicited in all angiosperm families (Harborne, 1986). Nevertheless the vast majority was registered for species of the Fabaceae. In this family most inductive substances are flavonoids, i.e. maintain a close biosynthetic relationship with the major group of constitutive compounds. This was

to be expected. Indeed it has already been show that the formation of inductive substances does not proceed haphazardly, but that their formation results through a process regulated by the activation of genes leading to accumulation of mRNA corresponding to specific enzymes (Stoessl, 1985). Hence inductive and constitutive substances are formed *via* identical precursors. Nevertheless, the two metabolic categories are frequently represented by different compounds, and it was anticipated that their structural comparison would clarify the yet uncertain function of the so called phytoalexins in plants, or at least in Fabaceae.

MATERIALS AND METHODS

The data acquisition regarding to different categories of substance structures for the elaboration of Tables, of basic on the Chemical Abstract service until December 2000 and, in special cases on specific sources (Ingham, 1982; Dewick, 1984; Brooks & Watson, 1985; Ebel, 1986;

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Bisby, 1994).

Calculation of the occurrence number of substances into was taken into account the differences in their structures, without considering how many times into was registered for the plant genus.

The rate inductive isoflavonoids / constitutive isoflavonoids (Table 1) for each structural type was calculated by the number of occurrences (NO) of these isoflavonoid representatives according to the expression:

$$I/C = \frac{\text{NO inductive isoflavonoid}}{\text{NO constitutive isoflavonoid}}$$

The rates prenylated inductive/ non-prenylated inductive, prenylated constitutive/ non-prenylated constitutive and prenylated inductive / prenylated constitutive – isoflavonoid (Table 2) were calculated as recorded above.

The average of oxy-substituent groups of inductive-isoflavonoids and constitutive-isoflavonoids (Table 3) was calculated according to the expression:

$$\overline{NG}_o = \frac{\sum NO \cdot NG_o}{\sum NO}$$

\overline{NG}_o = average numbers of oxy-groups
 NO = occurrences numbers
 NG_o = numbers of oxy-groups ($2 \leq NG_o \leq 7$)

The average number of free phenolic hydroxyls per molecule (Table 4) was calculated as above:

$$\overline{NOH}_f = \frac{\sum NO \cdot NOH_f}{\sum NO}$$

\overline{NOH}_f = average numbers of free phenolic hydroxyls per molecule
 NOH_f = number of free phenolic hydroxyls ($0 \leq NOH_f \leq 4$)

RESULTS AND DISCUSSION

Constitutive flavonoids and isoflavonoids are widespread in Fabaceae. Nevertheless, while phytoalexins flavonoids have been described with extreme rarity (the reference work by Ingham (1982) cites only one compound, the flavanone liquiritigenin), phytoalexin isoflavonoids have been mentioned frequently (the same reference work registers 89 compounds, a number which consultation of the more literature increases to 130). Even among the five isoflavonoid subclasses strong selectivity can be discerned, i.e. proportionally more compounds were described as phytoalexins passing from isoflavones (I/C proportion 0.26) *via* coumestans (I/C 0.43), isoflavanones (I/C 0.56) and isoflavans (I/C 0.59) towards pterocarpans (I/C 1,32) (Table 1). Trends towards the preferential acquisition of inductive, rather than constitutive, isoflavonoids of the all five subclasses include stronger prenylation and feebler oxygenation associated with stronger hydroxylation.

The proportion of prenylated isoflavonoids *versus* non-prenylated ones becomes gradually larger, the stronger the trend towards formation of inductive compounds of the pertinent isoflavonoid subclass (variation from isoflavones to pterocarpans PI/NI 0.27 to 0.96). The inverse phenomenon occurs in the constitutive category (PC/NC 0.70 to 0.23). The considerable tendency for prenylation of inductive *versus* prenylated constitutive derivatives which increases gradually for isoflavones (PI/PC 0.13) towards pterocarpans (PI/PC 3.43) (Table 2).

Substitution by oxy-groups in inductive isoflavonoids, irrespective of the particular subclass, is consistently less pronounced than in constitutive derivatives. The extent of this trend can be gauged by measurement of the ratios of the average number of substituents on inductive *versus* constitutive compounds ($\overline{NG}_{o/I/C}$ 0,88±0.06) (Table 3). In contrast substitution by free phenolic hydroxyls is somewhat more conspicuous in inductive isoflavonoids than in constitutive ones.

Tabela 1. Número de isoflavonóides em Fabaceae.

Table 1. Numbers of isoflavonoids in Fabaceae.

	T	I	C	I/C
Isoflavones	138	33	126	0.26
Coumestans	29	10	23	0.43
Isoflavanones	45	18	32	0.56
Isoflavans	47	20	34	0.59
Pterocarpan	74	49	37	1.32

T...Total I...Inductive compounds C... Constitutive compounds

Tabela 2. Número de isoflavonóides prenilados e não prenilados em Fabaceae.

Table 2. Numbers of prenylated and non-prenylated isoflavonoids in Fabaceae.

	PI	NI	PI/NI	PC	NC	PC/NC	PI/PC
Isoflavones	7	26	0.27	52	74	0.70	0.13
Coumestans	4	6	0.67	6	17	0.35	0.67
Isoflavanones	9	9	1.00	11	21	0.52	0.82
Isoflavans	8	12	0.67	8	26	0.31	1.00
Pterocarpan	24	25	0.96	7	30	0.23	3.43

PI...Prenylated Inductive

NI... Non-prenylated Inductive

PC...Prenylated Constitutive

NC...Non-prenylated Constitutive

Tabela 3. Número de isoflavonóides com 2 a 7 oxi-substituição em Fabaceae.

Table 3. Numbers of isoflavonoids with 2 to 7 oxy-substituents in Fabaceae.

	2		3		4		5		6		7		NG _o		I/C
	I	C	I	C	I	C	I	C	I	C	I	C	I	C	
Isoflavones	8	10	16	46	9	56	-	12	-	2	-	-	3.03	3.60	0.84
Coumestans	6	8	4	10	-	4	-	1	-	-	-	-	2.40	2.91	0.82
Isoflavanones	0	3	5	4	13	21	-	4	-	-	-	-	3.72	3.81	0.98
Isoflavans	0	1	11	7	9	16	-	6	-	3	-	1	3.45	4.18	0.83
Pterocarpan	12	12	29	15	4	9	4	1	-	-	-	-	2.79	2.97	0.94

I...Inductive compounds C...Constitutive compounds NG_o...Average numbers of oxy-groups per molecule

Tabela 4. Número de isoflavonóides com 0 a 4 hidroxilas fenólicas livres.**Table 4.** Numbers of isoflavonoids with 0 to 4 free phenolic hydroxyls.

	NOH _F												I/C
	0		1		2		3		4				
	I	C	I	C	I	C	I	C	I	C	I	C	
Isoflavones	3	20	8	33	7	34	9	29	5	11	2.16	1.83	1.18
Coumestans	-	3	4	11	4	7	2	2	-	-	1.80	1.35	1.33
Isoflavanones	-	4	2	6	4	8	8	9	4	5	2.22	2.16	1.03
Isoflavans	-	2	9	9	8	11	3	12	-	-	1.70	1.97	0.86
Pterocarpan	2	12	18	18	25	13	4	-	-	-	1.63	1.01	1.61

I...Inductive compounds
C...Constitutive compounds
NOH_F ...Average numbers of free phenolic hydroxyls per molecule

This trend is revealed through the ratios of the average number of hydroxyls on inductive *versus* constitutive compounds, $\overline{\text{NOH}}_F$ I/C 1.235 ± 0.375 , or excluding the aberrant result on isoflavans, $\overline{\text{NOH}}_F$ I/C 1.32 ± 0.29 , or considering only the case of pterocarpan, the most important subclass of inductive isoflavonoids, $\overline{\text{NOH}}_F$ I/C 1.61 (Table 4).

Although constitutive flavonoids are ubiquitous in plants, and hence also occur profusely in Fabaceae, where they show considerable structural diversity, only very few representatives were so far reported to be inductive metabolites. In contrast, constitutive isoflavonoids are of rather restricted occurrence and much less numerous. Nevertheless they form the richest class of inductive substances in Fabaceae, and possibly even in angiosperms (Gotlieb *et al.*, 1993). The reason for this paradox is suggestive. While only special flavonoids give, by enolization or by oxidation, vinyllogues of biphenyl. Such compounds can lead to stable phenoxyl radicals which, by virtue of scavenging deleterious radicals and suppressing oxidative reactions, may serve an important antioxidant function in preserving physiological integrity of cells exposed to air.

Differences in antioxidant potency occurs equally within isoflavonoid categories. Clearly, inductive representatives tend to possess stronger

antioxidant potential than constitutive ones. The fact transpires through the simple numerical comparison of both categories of compounds with respect to structure of their skeletons as well as nature and number of their substituents. First, with respect to skeletons, increasingly higher I/C ratios characterize isoflavones (possessing only sp²-carbons), coumestans (also only sp²-carbons, but greater planarity), isoflavanones (two sp³-carbons), isoflavans (three sp³-carbons) and pterocarpan (also three sp³-carbons, but greater trend towards planarity). Moreover, with respect to nature of the substituents of these metabolic groups, prenyl substitution becomes more frequent for inductive isoflavonoid subclasses in the same order, i.e. from isoflavones, *via* coumestans, isoflavanones and isoflavans towards pterocarpan. Indeed, prenylation enhances the susceptibility of phenols to oxidation. Lastly inductive isoflavonoids in Fabaceae are less oxygenated and relatively richer in free phenolic hydroxyls, than constitutive ones, additional reasons for their relative ease of oxygen capture and hydrogen loss.

CONCLUSION

Injury weakens plant tissue and stimulates a

rapid oxidative burst (Apostol, 1989). Thus it becomes highly probable that this process should indeed elicitate the enhancement of the antioxidant potency of secondary metabolism. However, this attribution of a general *raison d'être* to inductive isoflavonoids does of course not mean that one or another of these compounds cannot, eventually, display phytoalexin activity.

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