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Biological activities of lignans and stilbenoids associated with plant-insect chemical interactions

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Abstract

Lignans and biogenetically-related secondary metabolites derived from phenylpropanoid precursors play a significant role in the defence of plants against insects. They act largely as regulators of insect feeding, but in a few cases they can influence also specific physiological functions of insects. The antifeedant activities of a series of lignans are summarised and compared with previously published data. The compounds represent either natural substances isolated from plants or their chemically transformed structural analogues. The precise mode of action of such compounds is mostly unknown. One possible mechanism might be interaction with, and disruption of, the endocrine system, which is crucial for proper development of insects and is dependent on the action of moulting hormones (ecdysteroids). This hypothesis has been tested using the specific *Drosophila melanogaster* B_{II} cell line bioassay for ecdysteroid agonists and antagonists, in which the potency of the test compound reflects the affinity of binding to the ligand-binding site of the *D. melanogaster* ecdysteroid receptor. The activity data are evaluated in terms of a structure-activity relationship. To explore this phenomenon, the compounds were prepared and tested alongside ecdysteroid analogues and some insect ecdysis/metamorphosis-disturbing steroidal and non-steroidal natural compounds. Several phenylpropanoids, including lignans and stilbenoids (derived from resveratrol), were evaluated with promising results. The results indicate that such phenylpropanoid-derived compounds can possess ecdysteroid antagonistic activity, which could potentially influence insect development.

Introduction

Lignans and related phenolic compounds, biogenetically derived from phenylpropanoid precursors, form a large group of secondary metabolites with a remarkably rich structural variation and a large variety of biological functions and activities, not only in the producing organism itself, but also in other symbiotic or parasitic organisms. Previously, the main research effort has been directed at basic primary metabolites or other economically, pharmacologically or otherwise attractive natural products, rather than at unspecified secondary metabolites. In the last three decades the interest in studying secondary metabolites has grown, because of their great significance in ecological and phylogenetic relationships of organisms. The significance of plant secondary metabolites is complex and can be studied from various points of view (Hartmann, 1996). One of the preferred approaches is chemical ecology, i.e. the study of the chemical interactions between plants and other organisms vitally dependent upon plants, reflecting various host and guest relationships. The guest can be a parasite or a symbiotic partner, or sometimes just a competitor in the environment. There exist many types, and thus also many models, of such interactions. The best-known interactions are between plants and micro-organisms (e.g. phytoalexin formation), plants and plants (allelopathic interactions) and plant – insect interactions. The scope of specific chemical interactions between plants and

insects is very extensive and complex, reflecting the diversity of their co-evolution. Insects respond to a large range of plant chemical components, which may induce changes in behaviour, growth, development or reproduction. These represent a great variety of biological effects (Table 1), but even greater is the variety of chemical structures responsible for these effects.

Nearly all classes of low molecular weight natural substances can be found among regulators of insect-plant interactions, e.g. aliphatic substances, terpenoids, steroids, alkaloids, lignans and related phenylpropanoids (flavonoids, stilbenoids, coumarins) or simple phenolics, as well as various derivatives of these compounds, most frequently simple ethers, esters, glycosides or saponins. Such derivatives or more complicated conjugates can act directly, or can be first activated by release from the complex structure. Simple and volatile phenolics are important for insect behaviour and communication (Table 1, part A). Phenylpropanoids, such as lignans, flavonoids, rotenoids and coumarins are often allomones, mostly acting as feeding regulators. They can also act, however, as regulators of growth and development with antihormonal effects (Table 1, part B). Some lignans possess juvenile hormone-like activity (Bowers, 1968). On the other hand, ageratochromenes (precocenes) have antijuvenoid activity (Bowers, 1991). Lignans and stilbenoids can cause moulting disturbances (Garcia et al., 2000). Therefore, they have been tested in bioassays to assess their ecdysteroid antagonistic activity. Phytoecdysteroids, as well as any moulting modifiers or antihormonal plant substances, occupy an important place in the wide variety of compounds effective in plant-insect interactions (Table 1).

Table 1. Biologically active and ecologically significant plant substances effective in plant-insect chemical interactions

A. Regulators of behaviour and communication (with information effect)
1. Kairomones – attractants, arrestants, stimulants
2. Allomones – repellents, deterrents, antifeedants
3. Plant components and precursors of pheromones
B. Regulators of growth, development and reproduction (with physiological effect)
1. Juvenoids – plant analogues of insect juvenile hormones
2. Phytoecdysteroids – plant analogues of insect moulting hormones
3. Antihormonal substances and chemosterilants
4. Plant toxins and insecticides

Lignans and insect feeding preferences

Lignans represent one of the most abundant biogenetically related and, thus, structurally defined and characteristic groups of phenylpropanoids. They are formed by oxidative dimerisation of two phenylpropanoid units and are defined by the link in the side-chain between the two central (C-8, C-8′) carbon atoms. Thus, they display a limited number of structural types (Figure 1). Structural variability is, however, extended by the additional occurrence of one or two double bonds in the aliphatic part and of various substituents (mostly hydroxy, methoxy, methylenedioxy or glycosyl groups) on one or both aromatic rings and/or aliphatic carbon atoms. Even more variability is associated with the inherent stereochemical disposition at the several chiral centres of the molecule.

There are four stereochemical variants possible at the C-8/C-8′ link. However, natural lignans (especially types A-C) exist in reality only in two variants: as a less abundant *cis*-isomer (αH,αH) and a more abundant *trans*-isomer (αH,βH, in the sequence C-8, C-8′). The remaining two variants were found in only a very small number of compounds, so that they could be considered as artefacts (Harmatha et al., 1982). Type B has an additional chiral centre at C-7′, again predominantly with βH-configuration. The number of steric forms is increased in the case of dibenzocyclooctane lignans (type C), because of the presence of the additional C-2/C-2′ axis of chirality, stabilised by substituents and/or by an anelated cyclooctane ring. The steric arrangement of types D-F is more complicated from a formal point of view, but in principle is similar to types A and B. The decisive element, which influences the actual presence of certain stereospecific forms, is the stereoselective control of the C-8/C-8′ coupling step of biosynthesis in the presence of a specific dirigent protein (Davin and Lewis, 2000). Nevertheless, the stereospecific consequences considerably increase the original constitutive structural variability.

Further structural transformations of lignans (based on decarboxylations) expand the structural variability to several other related classes, such as the norlignans or conioids (Erdtman and Harmatha, 1979). All other side-chain carbon links form lignan-related structures, and the resultant compounds are generally classified as neolignans (Aires and Loike, 1990).

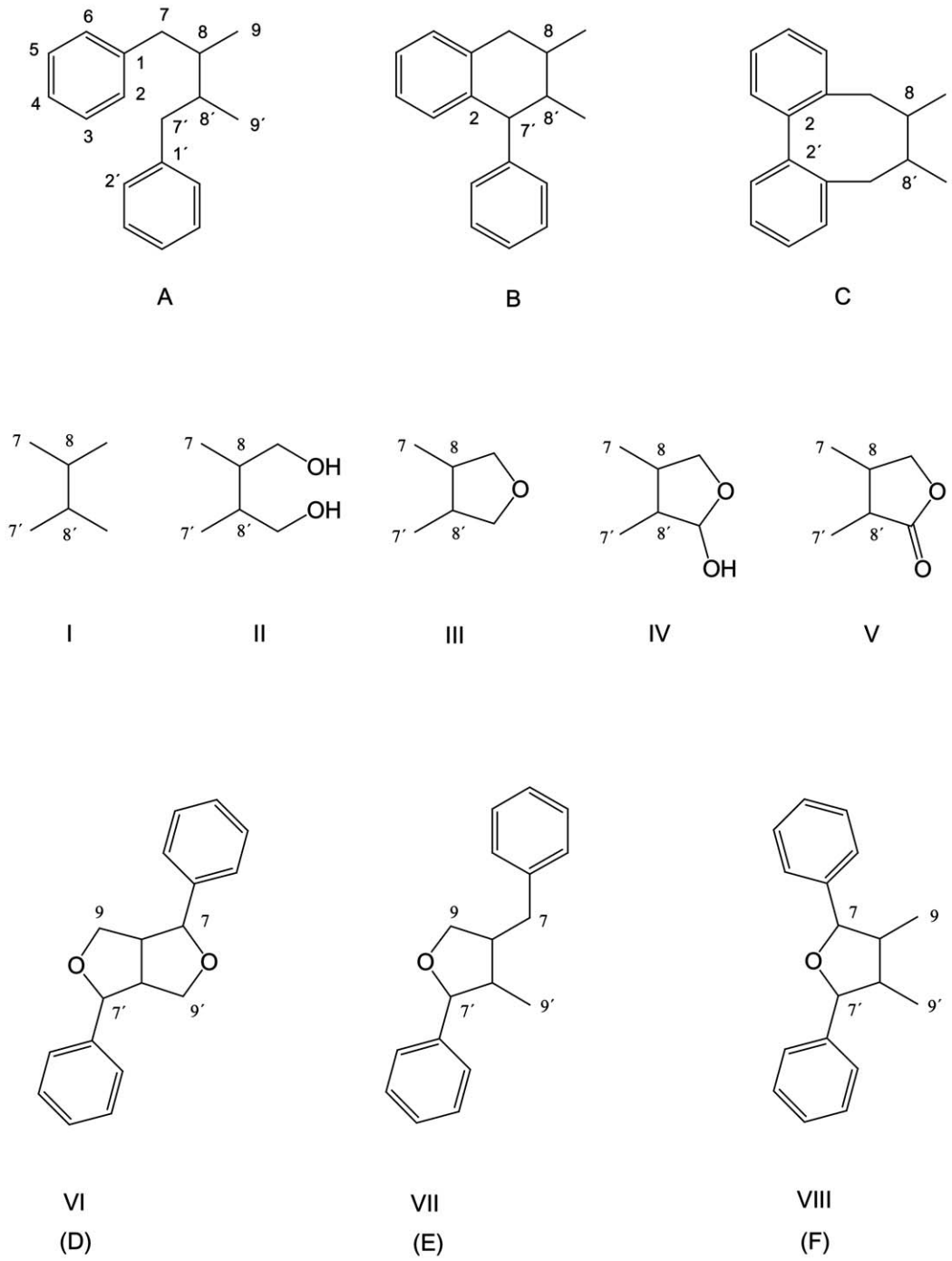

Figure 1. Types and forms of lignans. Types (**A – C**) derived from the mode and extent of phenolic coupling: *dibenzylbutane type* (**A**), *aryltetraline type* (**B**) and *dibenzocyclooctane type* (**C**). Forms **I – VIII** according to degree of oxidation. Forms **I-V** are relevant to all three types (**A-C**), forms **VI-VIII** are feasible only for type **A,** resulting from the epoxide formation at the 7,8; 7′,8′- and/or 8,9; 8′,9′and 9,9′ positions of the butane moieties. The nomenclature of respective forms mainly reflects their formation: non-oxidized i.e. *butane form* (**I**), hydroxy, i.e. *butanediol form* (**II**), cyclic ether i.e. *9.9′-epoxide form*, syn. *oxybutane form* (**III**), hemiacetal or *"lactol" form* (**IV**), butyrolactone or *butanolide form* (**V**), *bis-epoxide form*, syn. *bis-perhydrofurane* or *furofurane form* (**VI**), *7,9′or 7′-9 -epoxide form*s (**VII)** and *7,7′-epoxide form* (**VIII**). Forms **VI - VIII** can be regarded as independent structural types **D - F**, respectively.

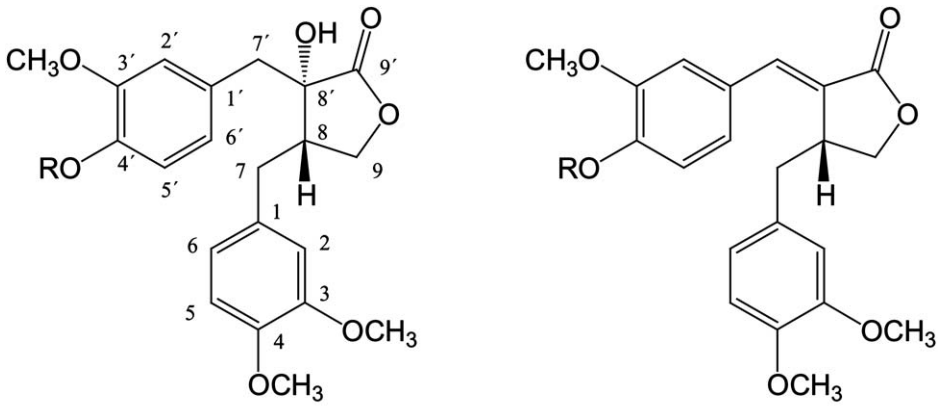

Figure 2. Lignans from *Leuzea carthanoides*.

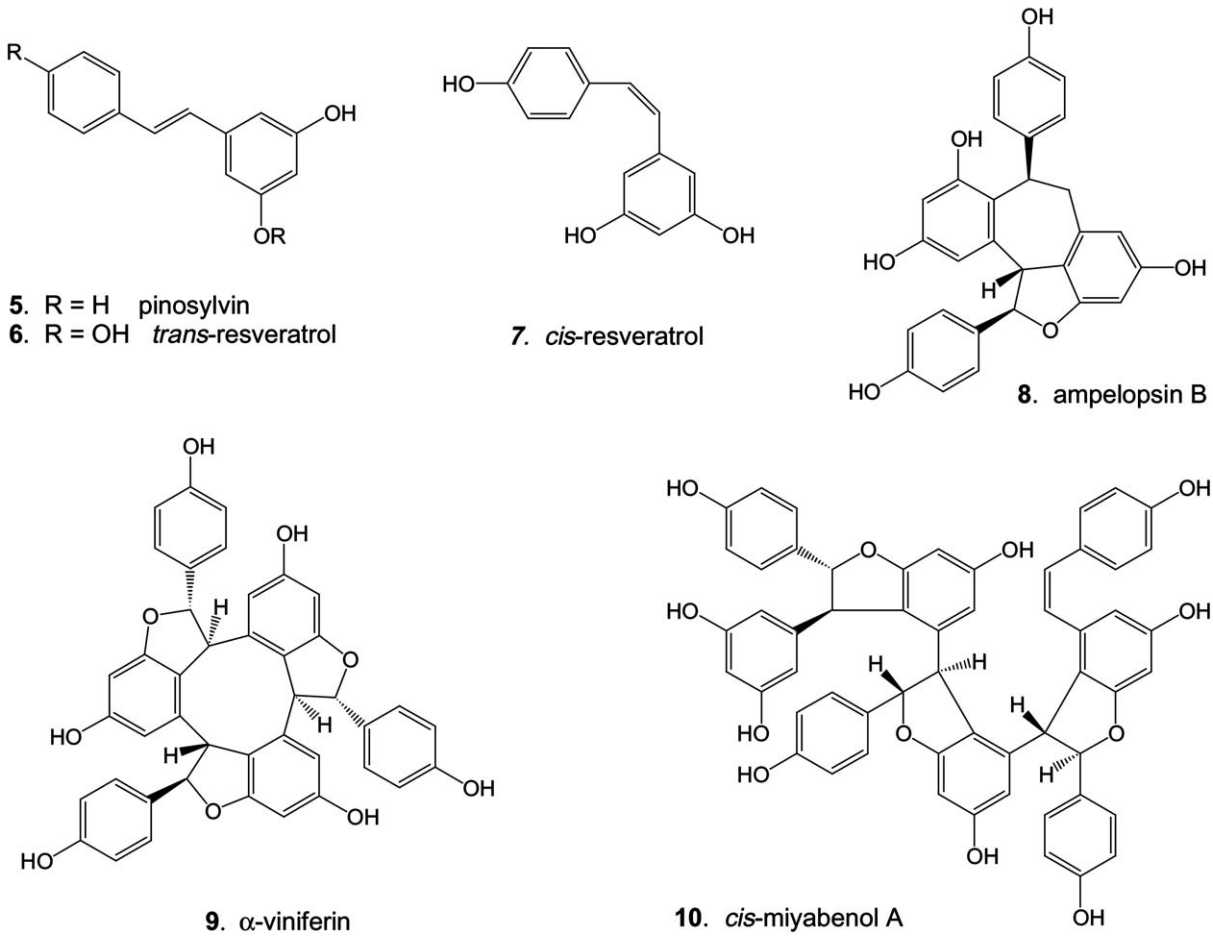

Figure 3. Stilbenoids tested in the B_{11}-bioassay (see Table 3).

Lignans have attracted much interest over the years and are still intensively investigated because of their wide occurrence in nature (mainly in the plant kingdom) and because of their wide range of biological effects (MacRae and Towers, 1984). There are many reports describing the effects of lignans on various organisms, including humans, at molecular, enzymatic, physiological, pharmacological and even clinical levels. However, less information is available on their function and role in the plants producing them. The major interest in this respect is directed at their ecological role, mainly concerning the co-evolution of plants and insects. Nevertheless, information concerning the effects of lignans on insects and their role in plant-insect interaction is still limited (MacRae and Towers, 1984; Nawrot and Harmatha, 1994; Bernard et al., 1995; Gang et al., 1997).

The first report about the activity of lignans against insects appeared in 1942 and concerned the synergistic action of sesamin, asarinin and pinoresinol with pyrethrum insecticides (Haller et al., 1942a, b). More synergistically active lignans and related phenylpropanoids were reported over the next few decades (see Table 2). Insecticide synergists of simple phenylpropanoid type and also of some lignans have been reviewed and discussed at a biochemical level, concerning synergism specificity and mechanism of resistance (Casida, 1970). Although many compounds with phenylpropanoid structures possess synergistic activity, they are inactive by themselves. However, since 1970 several lignans have been reported possessing inherent feeding regulating activity (Table 2). Recently, several papers appeared reporting equivalent or even better insecticidal activities also with structurally related neolignans, such as are licarin derivatives (González-Coloma et al., 1994), burchellin and licarin A (Garcia et al., 2000, Cabral et al., 2000b) or *Piper* neolignans (Chauret et al., 1996).

Certain structural features, especially the piperonyl (methylenedioxyphenyl) moiety of many lignans and simple phenylpropanoids, attracted specific interest (Neil, 1989; Bernard et al., 1995; Poplawski et al., 2000; Harmatha and Nawrot, 2002) and stimulated the design of synthetic insecticides or their synergists (Yamauchi and Taniguchi, 1991, 1992a, b; Xu et al., 2002). Synthetic piperonylbutoxide was found to be the most potent antifeedant (with absolutely the highest possible antifeedant activity coefficients) for storage insect pests (Harmatha and Nawrot, 2002) and is, thus, on a par with the activities of rotenone or coumarin (Nawrot et al., 1989; Harmatha et al., 1991).

The antifeedant activity of a series of lignan lactones, hemiacetals, ethers or alcohols, belonging to the diphenylbutane type of lignans (Figure 1: type A, forms II-V) was assessed on selected storage insect pests (Harmatha and Nawrot, 1984, 1988, 2002; Harmatha et al., 1991). The compounds represented either natural substances isolated from resistant plants (*Libocedrus yateensis* Guillaumin [Erdtman and Harmatha, 1979] and *Piper cubeba* L. [Jensen et al., 1993]), or chemically transformed structural analogues of natural lignans (Harmatha et al., 1982). Certain lignans and their glycosides were isolated from the medicinal plant *Leuzea carthamoides* DC [syn. *Rhaponticum carthamoides* (Wild) Iljin] (Harmatha et al., 2003). Most of the tested lignans showed strong antifeedant activity in the storage insect feeding assay (Nawrot et al., 1986a, b) and indicated even certain structure-activity relationships, which were discussed in these papers. However, the chemical mechanism of the antifeedant activity of lignans is so far only poorly understood. The fact that a wide variety of structures are effective (Harmatha and Nawrot, 1994) would indicate that several mechanisms may be involved. This is probably why only very few studies based on quantitative structure-activity relationship evaluations have been performed (Harmatha and Nawrot, 2002). Existing papers mainly discuss the importance of the structural type, or the character, position and variability of substituents in the activities, but not stereochemical aspects.

The precise mode of action of such compounds is largely unknown. One possible mechanism might be disruption of the arthropod steroid hormone endocrine system, which is crucial for the proper development of insects. This possibility can be explored by testing their biological effect and comparing it with the activities of ecdysteroids (Lafont et al., 2002) or ecdysis-disturbing compounds (Harmatha, 2000) in the *Drosophila* B_{II} cell line bioassay (Dinan et al., 1999a, b). The potency of the effect in this assay reflects the affinity of binding to the ligand-binding site of the *Drosophila melanogaster* ecdysteroid receptor. This cell line does not metabolise the ecdysteroids ecdysone, 20-hydroxyecdysone or ponasterone A. For other classes of compounds, the cells probably generate a simpler metabolic profile than would occur in insects *in vivo* and other factors, such as penetration, sequestration and excretion, will play a lesser or no role. Compounds which are agonistic in this assay (ecdysteroids, bisacylhydrazines) show characteristic defects *in vivo* associated with moulting;

Table 2. Biological activities of lignans with feeding deterrent and physiological effects and with synergistic action on insecticides

Compounds	Type-Form[a]	Biological activity	References
Sesamin, asarinin	(D) / VI	pyrethrum insecticides synergistic	Haller et al. (1942b)
Pinoresinol + deriv.	(D) / VI	pyrethrum insecticides synergistic	Haller et al. (1942b)
Hinokinin, hibalactone	A-V	synergistic	Yamashita et al. 1961
Sesamin, sesamolin	(D) / VI	low juvenoid morphogenetic activ.	Bowers 1968; Casida (1970)
Piperolignanolides	A-V, B-V	feeding inhibitory activity	Wada and Munakata (1970)
Hinokinin, taiwanin, pawlownin	A-V, (D) / VI	pyrethroid-synergistic effect	Matsubara (1972)
Deoxypodophyllic acid	B-I	toxic	Russel et al. (1976)
Deoxypodophyllotoxin	B-V	insecticidal	Kozawa et al. (1982)
Yatein	A-V	feeding deterrent	Harmatha and Nawrot (1984)
Yatein, cubebin + deriv. podophyllotoxin	A (II-V) B-V	feeding deterrent	Harmatha and Nawrot (1988, 2002)
Cubebin	A-IV	termite antfeedant, practical use	Křečková et al. 1988; Bloszyk et al. (1990)
Cubebin, sezamolin, epiyangambin + deriv.	A-IV (D) / VI	gut microsomal monooxygenasee activity	Bernard et al. (1989)
Cubebin, yatein	A-IV, V	antifeedant	Nawrot et al. (1991)
Haedoxan + sesquilignans	(D) / VI	insecticidal	Yamauchi and Taniguchi (1991, 1992a, b)
Various *Piper* lignans	A (II-V)	insecticidal	Boll et al. (1994)
Epimagnolins	(D) / VI	insect growth inhibitory	Miyazawa et al. (1994)
Cannabisin B, cannabisin D	B-I deriv.	termite antifeedant lignanamides	Lajide et al. (1995)
Gomisin B, gomisin N	C-I	insecticidal	Miyazawa et al. (1998)
Podophyllotoxin	B-V	insecticidal	Miyazawa et al. (1999)
Pinoresinol + deriv. podophyllotoxin	(D) / VI B–V	antifeedant, moulting inhibition	Cabral et al. (1999, 2000a)
Sesemin	(D) / VI	antifeedant	Srivastava et al. (2001)
Podophyllotoxin deriv.	B-V	insecticidal	Xu et al. (2002)
Tracheloside, carthamoside + their glucosides	A-V	feeding deterrent	Harmatha and Nawrot (2002)

[a]Types and Forms are shown in Figure 1

cessation of feeding, premature induction of moulting, failed moulting and death (Oberlander et al., 1995). Antagonistic compounds would be expected to cause delayed moulting and reproductive disruption *in vivo*. We prepared and tested in this bioassay several ecdysis-disturbing steroids and also non-steroidal natural compounds (Dinan et al., 2001a, b; Harmatha and Dinan, 1997, 2002; Harmatha et al., 2002). Members of several structurally diverse types of natural compounds were found to antagonise the action of 20-hydroxyecdysone in the *Drosophila* B_{II} cell line, e.g. cucurbitacins, withanolides, limonoids and various phenylpropanoids including lignans and stilbenoids (Dinan et al., 2001a). However, examples of non-ecdysteroidal natural compounds possessing agonist activity are, according to present information, rather rare in nature.

The activity of synthetic non-ecdysteroidal agonists of the phenolic bisacylhydrazine type (Dhadialla et al., 1998), as well as antagonistic stilbenes (e.g. *cis*-resveratrol) or simple phenylpropanoids (e.g. apiol), reported by Dinan et al. (1999b; 2001a, b), encouraged us to assess lignans, which are known mostly as insect antifeedants (Harmatha and Nawrot, 2002). Only a few representatives of the large variety of structural types and forms of lignans (Figure 1) have so

Table 3. EC_{50} values for selected lignans and stilbenoids in the B_{II} bioassay

Compounds	Type-Form[a]	Max. conc. [M]	Agonist activity (EC_{50}) [M]	Antagonist activity (EC_{50}) [M]	Cyto-toxicity	Reference
Lignans						
Yatein	A-V	10^{-3}	inactive	inactive	$\geq 2.5 \times 10^{-4}$	unpublished
Podophyllotoxin	B-V	10^{-4}	inactive	inactive	$\geq 10^{-5}$	unpublished
Cubebin	A-IV	10^{-3}	inactive	inactive	$>10^{-4}$	unpublished
Hinokinin	A-V	10^{-4}	inactive	inactive	at 10^{-4}	unpublished
Dihydrocubebin	A-II	10^{-3}	inactive	inactive	2.5×10^{-4}	unpublished
Deoxycubebin	A-III	10^{-4}	inactive	inactive	at 10^{-4}	unpublished
Trachelogenin (**1**)	A-V	10^{-4}	2.5×10^{-4}	inactive	–	unpublished
Carthamogenin (**3**)	A-V	10^{-4}	$\geq 2.5 \times 10^{-4}$	inactive	–	unpublished
Lignan glycosides						
Tracheloside (**2**)	A-V	10^{-4}	inactive	inactive	$\geq 5.0 \times 10^{-6}$	unpublished
Carthamoside (**4**)	A-V	10^{-4}	active at 10^{-5}	inactive	at 10^{-4}	unpublished
Stilbenoids						
trans-Resveratrol (**6**)	mono-	10^{-4}	inactive	inactive	–	Dinan et al. (2001b)
cis-Resveratrol (**7**)	mono-	10^{-2}	inactive	1.2×10^{-5}	$\geq 2.5 \times 10^{-4}$	Sarker et al. (1999)
Ampelopsin B (**8**)	di-	10^{-3}	inactive	3.3×10^{-5}	–	Keckeis et al. (2000)
α-Viniferin (**9**)	tri-	10^{-3}	inactive	1.0×10^{-5}	$>2.5 \times 10^{-4}$	Keckeis et al. (2000)
cis-Miyabenol C	tri-	2.5×10^{-4}	inactive	1.9×10^{-5}	$\geq 10^{-4}$	Meng et al. (2001)
Suffruticosol A	tri-	5×10^{-3}	inactive	5.3×10^{-5}	$\geq 10^{-3}$	Sarker et al. (1999)
Suffruticosol B	tri-	10^{-2}	inactive	1.4×10^{-5}	$\geq 2.5 \times 10^{-3}$	Sarker et al. (1999)
Suffruticosol C	tri-	5×10^{-3}	inactive	2.2×10^{-5}	$\geq 10^{-3}$	Sarker et al. (1999)
cis-Miyabenol A (**10**)	tetra-	5×10^{-4}	inactive	3.1×10^{-5}	at 5×10^{-4}	Meng et al. (2001)
Kobophenol B	tetra-	10^{-3}	inactive	3.7×10^{-5}	$\geq 2.5 \times 10^{-4}$	Meng et al. (2001)
Bisacylhydrazine						
RH-5992[b]		10^{-4}	5.3×10^{-7}	inactive	–	Dinan et al. (2001b)
Ecdysteroid						
20-hydroxyecdysone[b]		10^{-3}	7.5×10^{-9}	inactive	$\geq 10^{-4}$	Dinan et al. (1999a)

[a]for types and forms see Figures 1 and 3; [b]standard reference compounds

far been tested in the B_{II} bioassay, in order to learn more about their interaction with the ecdysteroid receptor. Most were inactive (Table 3). Only lignans from the medicinal plant *Leuzea carthamoides*, (Figure 2) trachelogenin (**1**), carthamogenin (**3**) and its glucoside, carthamoside (**4**), were found to be active as agonists at rather high concentrations ($\geq 10^{-4}$ M). All lignans were cytotoxic, which is in accord with their well-known pharmacological activity as cancerostatic agents (Aires and Loike, 1990).

It is known that the activity of lignans depends to a great extent on the type and position of substituents. At present, the lignans with hydroxyls in the *meta*-positions of the aromatic rings, which is characteristic for natural enterolignans, the mammalian gastrointestinal bacterial metabolites (Raffaelli et al., 2002), are of particular interest for testing. These lignans share similarities with several oestrogen-like substances (phyto-oestrogens), which displayed weak activity (Dinan et al., 2001b). The observed similarity between enterolignans and phyto-oestrogens has led to the idea (Harmatha, 2002) that they may also interact with the ecdysteroid receptor and, thus, serve as insect ecdysis-disturbing agents.

Stilbenoids as ecdysteroid receptor antagonists

One can presume that stilbenoids should be even better mimics of phyto-oestrogen activities than lignans. The results obtained in previous studies (see Table 3) have been already reported (Dinan et al., 1999b, 2001b;

Sarker et al., 1999; Keckeis et al., 2000; Meng et al., 2001). Stilbenoids promise even better qualifications than lignans as active ecdysteroid receptor ligands and useful ecdysis-disturbing agents. Such compounds are widely abundant in natural sources and can also be prepared in various simple synthetic ways. Special consideration in our case was given to structurally simple derivatives of pinosylvin (**5**) or resveratrols (**6** and **7**), which occur as phytoalexins in higher concentrations after a bacterial infection of their sources: pines, grapevines or grapes. Similarly interesting results, however, exhibited also their transformed complex dehydro-oligomers: dimeric analogues, e.g. ampelosin B (**8**), trimeric analogues, e.g. α-viniferin (**9**) or even tetrameric analogues, e.g. *cis*-miyabenol A (**10**). Their unrelated structures and large molecular shape (Figure 3), when compared to ecdysteroids (Lafont et al., 2002), but still a significant activity (Table 3), may allow new insights into ecdysteroid receptor mapping. It may help to explain unexpected effects of some other unusual large-molecular structural analogues at the receptor, e.g. dimeric ecdysteroids (Harmatha et al., 2002).

Leuzea carthamoides, the main source of the majority of our ecdysteroids (Píš et al., 1994; Vokáč et al., 2002), lignans (Harmatha et al., 2003) and *N*-phenylpropanoid serotonin conjugates (Pavlík et al., 2002), contains also a stilbenoid: (*E*)-3,3′-dimethoxy-4,4′-dihydrostilbene (Hajdu et al., 1998), as well as a series of flavonoids (Varga et al., 1990). Such compounds are able to modulate growth and reproduction in insects by direct or indirect interaction with the steroid hormone system. An example has been reported (Oberdörster et al., 2001) with a series of flavonoids. Although none of the tested flavonoids are ecdysteroid agonists in the reported assay, they significantly inhibited ecdysteroid receptor-dependent gene transcription, and in some cases they showed a synergistic effect with ecdysteroid in the reduction of cell growth. Results obtained for the same type of compounds (flavonoids) with the B_{II} bioassay displayed only weak antagonist activity (Dinan et al., 2001a).

All the reported information summarised above, as well as our own preliminary experiments, led us to explore at least four types of specifically designed and transformed compounds derived from plant substances: side-chain modified ecdysteroids, simple and complex stilbenoids, enterolignans and flavonoid-type phyto-oestrogens.

Natural non-ecdysteroid (ant)agonists possess predominantly moderate to low affinity for the receptor. However, they are often present at higher concentrations in plants than the specific high-affinity ecdysteroid ligands. This may be significant in plant-insect chemical interaction and be evolutionarily advantageous to plants. Moreover, most simple phenylpropanoids, stilbenoids and lignans can be prepared by total synthesis much more readily than any ecdysteroid. This is a good advantage for practical use of such compounds in plant protection strategies against insect pests.

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