4. Allelochemicals in Plants

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Abstract. This chapter presents a brief coverage of a range of common plant allelochemical groups, and includes discussion of their structures, chemistry, distribution, ecology, bioactivity, biosynthesis, allelopathy, and mode of action where known.

4.1 Introduction

Allelochemicals are non-nutritional secondary metabolites produced by living organisms (i.e., plants) that have stimulatory or inhibitory effects upon the growth, health, behavior, or population biology of neighboring organisms (plants, insects, microbes, etc.). In plant–plant allelopathy, the more common inhibitor effects are visible upon such plant functions as respiration; photosynthesis; water balance and stomatal function; stem conductance of water; xylem element flux; membrane permeability; cell division and development; protein synthesis; and enzyme activity alteration. Within plants, allelochemicals may be distributed broadly among organs such as seeds, flowers, pollen, leaves, stems, and roots, or sometimes found in just one or two such locations. Their kingdom distribution is rather specific, with a particular class of allelochemical being often found confined to a limited range of plant families or genera, for example, the sesquiterpene lactones found in the Asteraceae. When released from plants, a potential allelopathic compound may first have to undergo some metabolic or environmental alteration in structure before it assumes biological activity. Alternatively, some bioactive compounds released from a plant may be rendered inactive by subsequent chemical transformations in the air, water, or soil. Allelochemicals exist in a vast array of seemingly disconnected structures and possess widely different modes of action. Not many of these modes of action are completely understood, but of those known, it appears that they can be quite divergent from the 30 or so presently known activity modes associated with artificial herbicides. The role played by allelochemicals is mostly interpreted now in an ecological way, usually in some means of plant defense against other plants, pests, or diseases. The standard modes of release for allelochemicals are volatilization, residue decay, leaching, or root exudation. Such exudation has been somewhat overlooked in the past history of allelopathic science, with most attention devoted to the more visible, above-ground aspects of plants. However, the central significance of underground communication and transfer between neighboring plants has received more attention and doubtless will give rise to new endeavors to elucidate allelopathic chemistry and mechanism (Bais et al. 2001). Laboratory bioassays for phytotoxicity/allelopathy are becoming more standardized so that effective comparisons can be made between the allelopathic potentials of different plants (Belz and Hurle 2004). A cautionary comment upon the use of the word "allelochemical" is also warranted. Strictly speaking, this term should really only be applied to those substances whose combat role between donor and receiver plants has been firmly established by quality experimental evidence (especially in genuine field circumstances), and not simply that a plant-derived compound shows toxicity toward some (perhaps irrelevant) other plant during in vitro bioassay. Of course phytotoxicity is a necessary condition for allelopathy, but other criteria must also be fulfilled in order to make the term meaningful. Nevertheless, this chapter has followed the use of the term allelochemical in this broader, less proven way according to much of the published literature. The context of the original information is usually sufficient for the reader to use his/her judgment on the status of the compound.

The deepening of our understanding of allelochemicals in plants holds much promise for widening our knowledge of plant ecosystems. Allelopathy is being increasingly recognized as a contributing explanation for such things as plant dominance, succession, formation of communities, climax vegetation, crop productivity, and exotic plant invasion. Indeed, the quality series of investigations carried out by Bais et al. (2003a) on the exotic invasive effectiveness of spotted knapweed (*Centaurea maculosa*) in the western USA represents the type of integrated study involving ecological, physiological, biochemical, cellular, and genomic sciences that serves as a good example among allelopathy scientists today.

Allelopathy holds out hope for improvements in crop production through such means as discovering eco-friendly herbicides with new sites of action, harmless to crops but toxic to weeds, and without formation of dangerous residues. Finally, the genetic manipulation of crop germplasm to produce crops which carry inwardly all the desirable features of a successful competitor to their weeds, is a strongly motivating goal which is presently being pursued around the world.

4.2 Glucosinolates: Chemistry and Ecology

4.2.1 Structure

The glucosinolates (GSL's) are a chemical class of sulfur-containing, naturally occurring secondary metabolites (β-D-thioglucoside-N-hydroxysulfates) found mainly in the plant order Capparales, and possessing an organic, anionic form usually balanced by cations such as sodium or potassium. Each compound contains a C=N bond capable of E-Z geometrical isomerism and generally accepted as being the Z-isomer on the basis of X-ray crystallographic analysis of one typical representative, sinigrin (Fig. 1). Every member of this class has sulfate attached to the N atom, a thioglucose moiety attached to the C=N carbon, and a varied side chain (R) also attached to the same carbon, thus serving to differentiate individual class members (Brown and Morra 1995; Fahey et al. 2001; Warton et al. 2001).

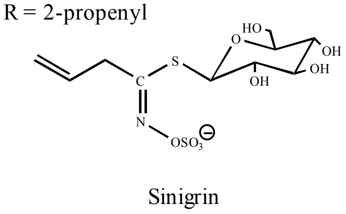


Fig. 1. Sinigrin (2-propenylglucosinolate)

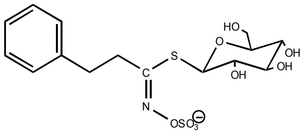
There are at least 120 known GSL's which Fahey et al. (2001) have divided into ten groups on the basis of similarity in structure in the R moiety. These group names and some representative structures are illustrated in Table 1.

Table 1. The ten glucosinolate structural types

Group Number	Glucosinolate Group Type	Chemical Name of a Group Representative (R)
1	Sulfur-containing side-chains	2-(methylthio)ethyl
2	Aliphatic, straight chain	n-pentyl
3	Aliphatic, branched chain	1-methylethyl
4	Olefins, or alcohols	2(R)-2-hydroxy-3-butenyl
5	Aliphatic straight and branched chain alcohols	1-ethyl-2-hydroxyethyl
6	Aliphatic straight chain ketones	5-oxoheptyl
7	Aromatic	benzyl
8	Hydroxyalkyl benzoates	2-benzoyloxy-1-ethylethyl
9	Indoles	4-hydroxyindol-3-ylmethyl
10	Others	e.g. 3-methoxycarbonylpropyl

Those GSL's containing a sulfur atom in the side chain in various states of oxidation comprise the largest single group (39 compounds), but those with such chiral centers as sulfinyl, like the majority of other chiral carbon centers within glucosinolates, have an (as yet) unknown stereochemistry. The most extensively studied GSL's are a group of 19 from *Brassica* vegetables whose side chains (R) contain such structures as the indole ring, benzene rings, and ω-methylthioalkyl chains, typical examples of which appear in Fig. 2.

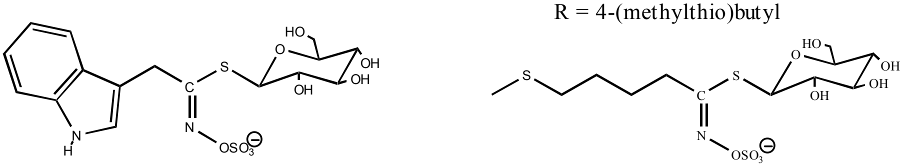
R = 2-phenylethyl



Gluconasturtiin

R = indol-3-ylmethyl

R = 4-(methylthio)butyl



Glucobrassicin

Glucoerucin

Fig. 2. Some common glucosinolates from *Brassica* species

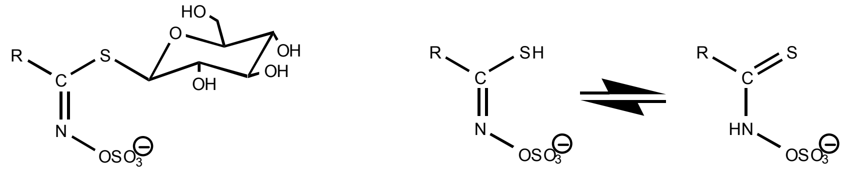
4.2.2 Distribution and Ecology

The first isolation of GSL's goes back to the 1830's when the compounds sinalbin (4-hydroxybenzylglucosinolate) and sinigrin (2-propenylglucosinolate) were obtained from white mustard (*Sinapsis alba*) and black mustard (*Brassica nigra*) respectively. Glucosinolates are now known to be distributed across 16 plant families of dicotyledonous angiosperms (Fahey et al. 2001) with the Brassicaceae containing all ten structural groups and about 96 individual compounds. While the Brassicaceae contains more than 350 genera and 3000 species, GSL's have been found in more than 500 species from other families. The Capparaceae contain eight structure groups and about 19 compounds, while the Resedaceae contain four structure groups and about 11 GSL compounds. With some common overlap, the remaining 13 plant families account for the present total of 120 different GSL structures, some of which have long been known for their allelopathic properties.

The genus *Brassica,* containing numerous edible vegetable crops such as cabbage, choy, kohlrobi, broccoli, swede, turnip, cauliflower, kale, calabrese, Brussels sprouts, and oil crop canola (rapeseed, *Brassica napus*), has been the focus of much chemical attention concerning GSL's (Rosa et al. 1997). Various studies have been conducted on the allelopathic effects of *Brassica* allelochemicals in connection with the growth of lettuce, barnyard grass, and wheat (Oleszek 1987); (Bialy et al. 1990); with growth of velvetleaf (Wolf et al. 1984); and the impact of allyl isothiocyanate released from *Brassica napus* (Choesin and Boerner 1991). The shredded leaves from some *Brassica* species have also been examined for their allelopathic effect upon *Pythium ultimum* and *Rhizoctonia solani* (Charron and Sams 1999). GSL's have been shown to be taken up by a specific carrier system and transported by phloem, so that the internal distribution of GSL's within a plant is usually broad, with most major tissues of stems, leaves, roots, and seeds carrying a proportion of the total. Plant age adversely affects the quantities available, especially after having reached the later vegetative stage. Older plants may contain as little as 1 μmol/g fresh weight, while young sprouts of broccoli (*Brassica oleracea*) can contain up to 100 μmol/g fresh weight. For a given plant organ, variability in GSL type can be quite diverse. For example, with the seeds of radish (*Raphanus sativus*) 4-methylsulfinyl-3-butenylglucosinolate is the main compound, whereas in mustard seed (*Brassica juncea*) allylglucosinolate predominates, while in seeds of cabbage (*Brassica oleracea*) allyl and 2-hydroxy-3-butenylglucosinolate are dominant (Brown and Morra 1997). Environmental factors play a role in determining levels of GSL's in growing plants and may also influence distribution within a plant. Such factors as pest attack, soil fertility, and wounding exert significant effects. Antagonistic interactions between GSL's and bacteria, nematodes, fungi, and herbivores are also well documented in the literature (Fahey et al. 2001). Interestingly, the model genome plant *Arabidopsis thaliana* has been shown to contain 23 different GSL's, which is very high compared to most other plants (Hogge et al. 1988; Haughn et al. 1991). Additionally, as there is a strong interest in controlling the levels of GSL's in crops to improve pest resistance and nutritional value, genes identified in the *A. thaliana* genome program will greatly assist strategies to control GSL's in related *Brassica* crops (Chen and Andreasson 2001). Benefits to allelopathy have already emerged from the growing *A. thaliana* genome information in a study of spotted knapweed (*Centaurea maculosa*) conducted by Bais et al. (2003a) who integrated such data with ecological, physiological, biochemical, and cellular approaches.

4.2.3 Chemistry

Like the cyanogenic glucosides, the initial step in the biosynthesis of GSL's proceeds by hydroxylation of nitrogen in a precursor aminoacid, with subsequent aldoxime formation by decarboxylation. Further steps follow to bring about side-chain modifications and glucone conjugation through the sulfur atom introduced by cysteine. The GSL's are stable water-soluble anions which become easily converted into biologically active degradation products (Fig. 3) such as thiocyanates, isothiocyanates, nitriles, epithionitriles, and oxazolidine-2-thiones, by the enzymatic action of myrosinase (EC 3.2.3.1), a glycoprotein which exists in physical separation from the GSL substrates until such time as the plant cells are broken by wounding, chewing, freeze-thawing, or bruising (Fahey et al. 2001). It is mainly the isothiocyanates (ITC's) which are considered the active allelochemicals arising from the GSL precursors. After the initial hydrolytic cleavage of the sugar moiety by the β–thio-glucosidase, the GSL-aglycone then spontaneously loses the sulfate group to form an unstable intermediate which proceeds to form the abovementioned degradation products. One of the intermediate's major decomposition pathways is via a Lossen rearrangement to form an isothiocyanate, often as the major product (Warton et al. 2001). ITC's interact with proteins irreversibly by attacking their sulfhydryl, disulfide, and amino groups and will therefore destroy enzymes and act as biocides (Brown and Morra 1997).



General glucosinolate structure
Intermediate after myrosinase action

General isothiocyanates and oxazolidinethiones
General nitriles, thiocyanates, and epithionitriles

Fig. 3. Degradation products from glucosinolates after enzymatic action by myrosinase

4.2.4 Allelopathy

The glucosinolates are considered the precursors of the true allelochemicals possessing the interesting phytotoxicity. Complete GSL anions are water soluble but out of several which have been tested for bioactivity, only 3-indolyl-methylglucosinolate has demonstrated plant growth inhibition (Brown and Morra 1997). Most attention has been given to the ITC's which tend to be the most bioactive and form in the larger amounts during GSL degradation. Thus Tsao et al. (2000) assert that allyl isothiocyanate is considered the actual cause of sinigrin's biological activity. Because a number of the common short side-chain GSL's give rise to low molecular weight isothiocyanates after degradation, these ITC's are relatively volatile and can thus permeate soil pores with some ease. This process has been termed "biofumigation" (Angus et al. 1994; Warton et al. 2001). Activity in the gas phase for allyl isothiocyanate has been demonstrated by germination inhibition of cucumber, dandelion, barnyard grass, soybeans, carrot, and wheat at concentrations near 1 ppm in sealed containers (Brown and Morra 1997). In one study by Brown and Morra (1995, 1996), 22 volatile GSL products made up of ITC's and nitriles were identified in the headspace above *Brassica napus* seed meal, root, leaf and stem tissues, and were shown to be the causative agents for inhibited germination of test seeds in bioassay.

Because glucosinolate-rich plants give rise to active phytotoxins such as ITC's after mechanical damage to cells, green manure mulching (Krishnan et al. 1998), (Petersen et al. 2001) has been one effective way to suppress weed growth without need of artificial herbicides. The volatile ITC's (e.g. butyl-ITC) which possess quite short half-lives in soil, are thought to act as biofumigants on weed seeds within the gas-filled soil pores, while the non-volatile, longer half-life group (e.g. 2-phenylethyl-ITC) which is less susceptible to hydrolysis, continue their herbicidal action within soil solution.

From Petersen's et al. (2001) work using turnip-rape mulch, it appears that all ITC's have the same mode of action and that the primary biological targets are the enzymes of glycolysis and respiration (Drobnica et al. 1977).

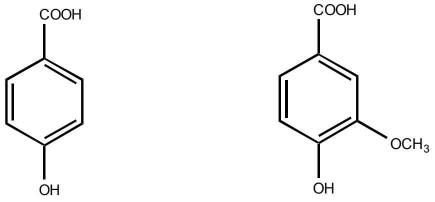
4.3 Phenolic Compounds: Biosynthesis, Distribution, and Chemoecology

4.3.1 Introduction

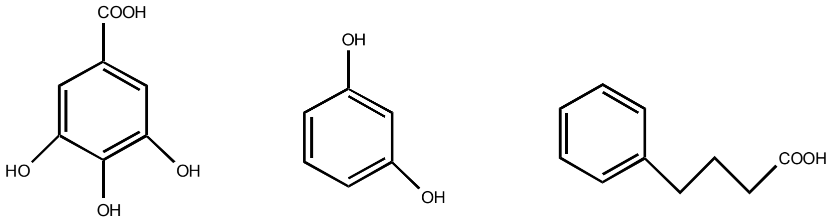
Within the context of allelopathy, the term "phenolic compounds" has a loose meaning, but is generally thought of as containing a range of compound types that include structures such as simple aromatic phenols, hydroxy and substituted benzoic acids and aldehydes, hydroxy and substituted cinnamic acids, coumarins, tannins, and perhaps a few of the flavonoids. In this chapter, allelopathic flavonoids will be treated separately in the final Section 4.7.2 headed "Other Compounds". An important subset of the phenolics, known as phenolic acids, describes a small range of water-soluble, aromatic, carboxylic group-containing phenols (such as salicylic acid), which are very broadly distributed. Certainly, as a group of allelochemicals, the phenolics have received by far the greatest attention, with a voluminous supporting literature implicating them in some allelopathic activity (Einhellig 2004). Yet in spite of this great body of data, there are properties of this group which do not properly fit the "concept" of an allelochemical. For example, these types of compounds are among the most widely distributed of all plant substances and the least specific in action. Cinnamic acids are considered to be universally present in all higher plants, and benzoic acids have been identified in all angiosperms so far examined (Dalton 1999). They are a common and normal part of most soils. The accumulated evidence points to phenolics as playing a significant role in allelopathy, and a number of reviews on their biochemistry and ecology have been written (Inderjit 1996). However, in the vast majority of cases, these compounds appear as a mixture and not a single substance, and what contribution is made to allelopathy by phenolic compounds is probably never due to a single substance (Einhellig 2004). The different compounds have variable toxicity, have similar modes of action, and attack cellular function at more than one site. Thus they do not display a typical herbicidal response. Their individual concentrations in soil (micromolar) are usually rather lower than what appears by pure chemoassay (0.1–1 mM) to be necessary for significant activity. The weak phytotoxicity they do exhibit seems to be dependent upon additive effects from the individuals in the mixture. Some researchers have sought to invoke the notion of synergism in an attempt to explain the apparent effectiveness of such low-concentration mixtures, but so far no one has been able to duplicate a synergistic mixture of the required activity level. The ordinary natural abundance of phenolics in soils as a standard part of the organic matter is imagined by some, under certain soil conditions, to be able to differentiate the growth of different plants within a neighborhood, yet to date, no experiment has been published which provides conclusive proof of phenolics allelopathy under typical field conditions (Dalton 1999; Huang et al. 2003).

4.3.2 Structure and Distribution

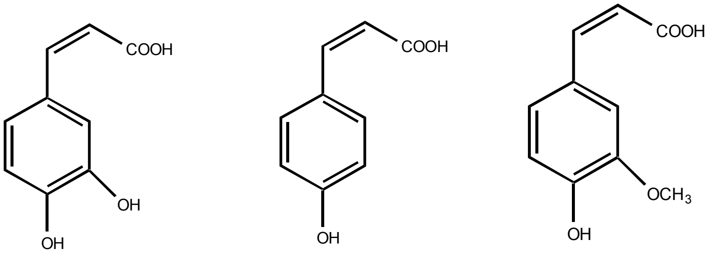
Of the scores of compounds which belong to this phenolics group, a range of typical structures is displayed in Fig. 4.



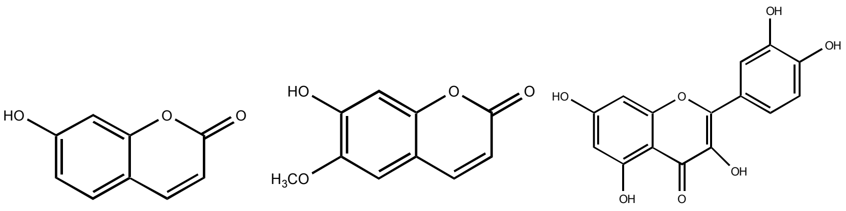
p-Hydroxybenzoic acid Vanillic acid Salicylic acid



Gallic acid Rescorcinol 4-Phenylbutyric acid



Caffeic acid p-Coumaric acid Ferulic acid



Umbelliferone Scopoletin Quercetin

Fig. 4. Typical structures among the allelochemical phenolics

Because the phenolics comprise the bulk of the structural matrix of plants and exist in soils through plant decomposition, there exists a continuous ecological cycle of uptake, synthesis, transformation, exudation, and decay involving these substances (Dalton 1999). Soil solutions of free phenolic acids come from plant leachates, root exudates, and bound-form microbial transformations of debris and litter. Loss of free phenolics arises from soil sorption, oxidation, microbial transformation, polymerization, and plant root uptake. Soil microbe action is believed to be the major influence upon the soil solution concentration of phenolics (Blum 2004b). Some workers have perceived simple phenolics as being so readily recycled and metabolized by rhizosphere and soil microorganisms, that naming the substances as allelochemicals can be quite misleading, for their presence can lead to inhibition, to no effect, or even to stimulation of plant growth, depending upon their concentrations and chemical state.

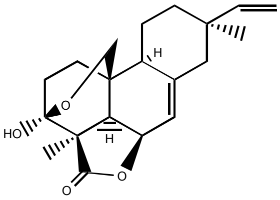
4.3.3 Biosynthesis

Phenolic compounds arise from the shikimic and acetic acid (polyketide) metabolic pathways. The phenylpropanoid group of secondary metabolites (e.g. ferulic acid) recognized by their aromatic ring with a 3-carbon side-chain, is generated via phenylalanine as intermediate, and includes the O-heterocyclic form usually called coumarins (e.g. scopoletin, Fig. 4). Phenylpropanoid compounds play a role in their esterified and glycosidated precursor forms in cell wall lignin production. Certain phenolic acids (e.g. salicylic acid) also play a role in plant physiology, and act as intermediates in acquired disease resistance (Einhellig 2004). The metabolic transformations of phenolics through microbial action are comprehensively discussed by Inderjit et al. (1999). Blum (2004b) concisely describes the bioconversion by fungi of ferulic acid ultimately into carbon dioxide and water, via such intermediate compounds as vanillic acid, protocatechuic acid, β-carboxymuconic acid, β-oxo-adipic acid, acetic and succinic acids.

4.3.4 Chemoecology and Mode of Action

The actual involvement of phenolics in allelopathic interactions is still controversial despite a vast literature linking them to allelopathic effects (Dalton 1999). There is still no definitive proof of phenolics affecting plant growth under field conditions. Despite the name applied to this group, the *un-substituted* skeleton structures such as simple benzoic and trans-cinnamic acids show greater toxicity than the hydroxylated derivatives (Einhellig et al. 1982). It is not surprising that there appears to be no structure/activity relationship within this group.

Other characteristics of the phenolics are that their inhibitory action is dependent upon concentration (Einhellig 2004); both their primary and secondary effects are readily reversible once they are removed or depleted from the plant rhizosphere (Blum 2004b); and it is soil concentration and not root uptake that appears to be the ultimate determinant of inhibition (Lehman and Blum 1999). They exert multiple physiological effects resulting in a generalized cytotoxicity, often by non-specific permeability changes to cell wall membranes, and do not seem to have a primary target site as do most herbicides (Einhellig 2004). The phenolics may well be working together with much lower concentrations of other more phytotoxic substances passed over unnoticed in the usual complex mixture isolated from plant parts or exudates (Rimando et al. 2001). For example, the composition of rice exudates has been studied for over ten years but despite the presence of phenolic acids in exudates, it is believed that these substances can not explain the experimental observations in allelopathic rice cultivars (Olofsdotter et al. 2002). Later work by Kato-Noguchi (with others) (2002a; 2002b; 2003a; 2003b; 2004) has shown that the trace compound momilactone B (Fig. 5) is probably the true rice allelochemical, perhaps assisted by phenolic action. The natural activity level of momilactone B is as low as 3 micromolar. Additional information on phenolic modes of action in relation to such matters as cell membrane effects, water relationships, phytohormone and enzyme effects, photosynthesis, respiration, and the flow of carbon in plants, can be found in the comprehensive treatment on phenolics by Einhellig (2004).

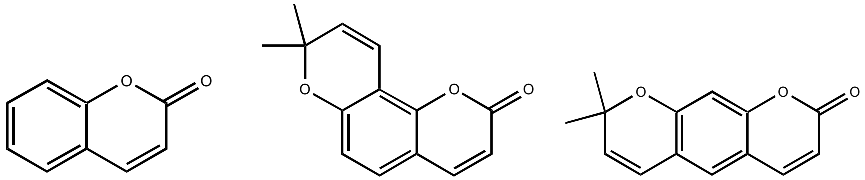


Momilactone B

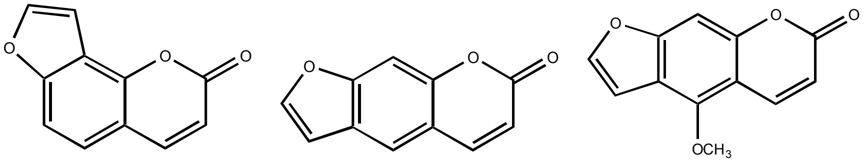
Fig. 5. The probable diterpenoid allelochemical source of rice allelopathy

The coumarins (as phenylpropanoids) are lactones of a 1,2-benzopyran structure, and are also usually considered a sub-group of the phenolics, with activities very similar to the cinnamic acids, though with some occasional differences. Many coumarins are oxygenated at their 7-position, and have been prenylated by (5-carbon) 3-methylbut-2-enyl units from the mevalonic acid bio-pathway. This mix of biosynthetic paths leads to a vast range of furano- and pyrano- fused-ring coumarins (Fig. 6) possessing an amazing range of bioactivities (Sardari et al. 2000).

Scopoletin, umbelliferone (Fig. 4) and esculetin (6,7-dihydroxycoumarin) make up the most cited group of coumarins linked to allelopathy. As for the apparent additive effect already noted among the phenolic acids, coumarin effects can similarly be enhanced by the presence of a phenolic acid. For example, Korableva et al. (1969) report that scopoletin is more effective as a growth retardant when used in combination with caffeic acid, than when used alone, and Einhellig (1996) reports that a combination of coumarin (umbelliferone), phenolic acid (salicylic acid), and flavonol (rutin) also possesses a stronger effect in combination. While the phenolic acids do not show evidence of being able to influence cell division, compounds such as scopoletin and coumarin have been reported as decreasing mitosis (Avers and Goodwin 1956), and also inhibiting certain enzyme actions (Podbielkowski et al. 1996).



Coumarin Seselin Xanthyletin



Angelicin Psoralen Bergapten

Fig. 6. Typical bioactive coumarins

4.4 Terpenoids: Volatile Allelochemicals

4.4.1 Introduction and Structure

As a chemical class, the terpenoids are distinguished by their origin from the biochemical pathway via mevalonic acid and isopentenyl pyrophosphate (a basic C-5 building unit) which combines with itself to produce C-10 monoterpenes, C-15 sesquiterpenes, C-20 diterpenes, and C-30 triterpenes. Many of the over 24,000 different compounds now known are of a cyclic unsaturated type and carry oxygen at various functional sites, e.g. aldehydes, ketones, ethers, alcohols, and lactones. Stereo-isomers are very common and may possess very different physico-chemical properties from each other. Terpenoid natural functions are very diverse and have been recorded as signal molecules, allelochemicals, phytoalexins, pheromones, visual pigments, photoprotective agents, membrane constituents, and reproductive hormones. A characteristic feature of the C-10 terpenoids is their high volatility and significant odors.

For more than 75 years it has been known that the monoterpenes of essential plant oils have had strong inhibitive effects upon plant growth and seed germination, and that these effects may proceed through the vapor phase. For example, the monoterpene 1,8-cineole (Fig. 7) suppresses the mitochondrial activity of *Avena fatua* coleoptiles and reduces the rate of oxidative phosphorylation (Fischer 1991). The review by Fischer (1986) in a major work of that day published on allelopathy, described mono- and sesqui-terpenes as plant germination and growth regulators, and listed 14 monoterpenes associated with phytotoxicity, including, borneol, pulegone (Fig. 7), camphor (Fig. 7), 1,8-cineole (Fig. 7), limonene (Fig. 7), camphene, and

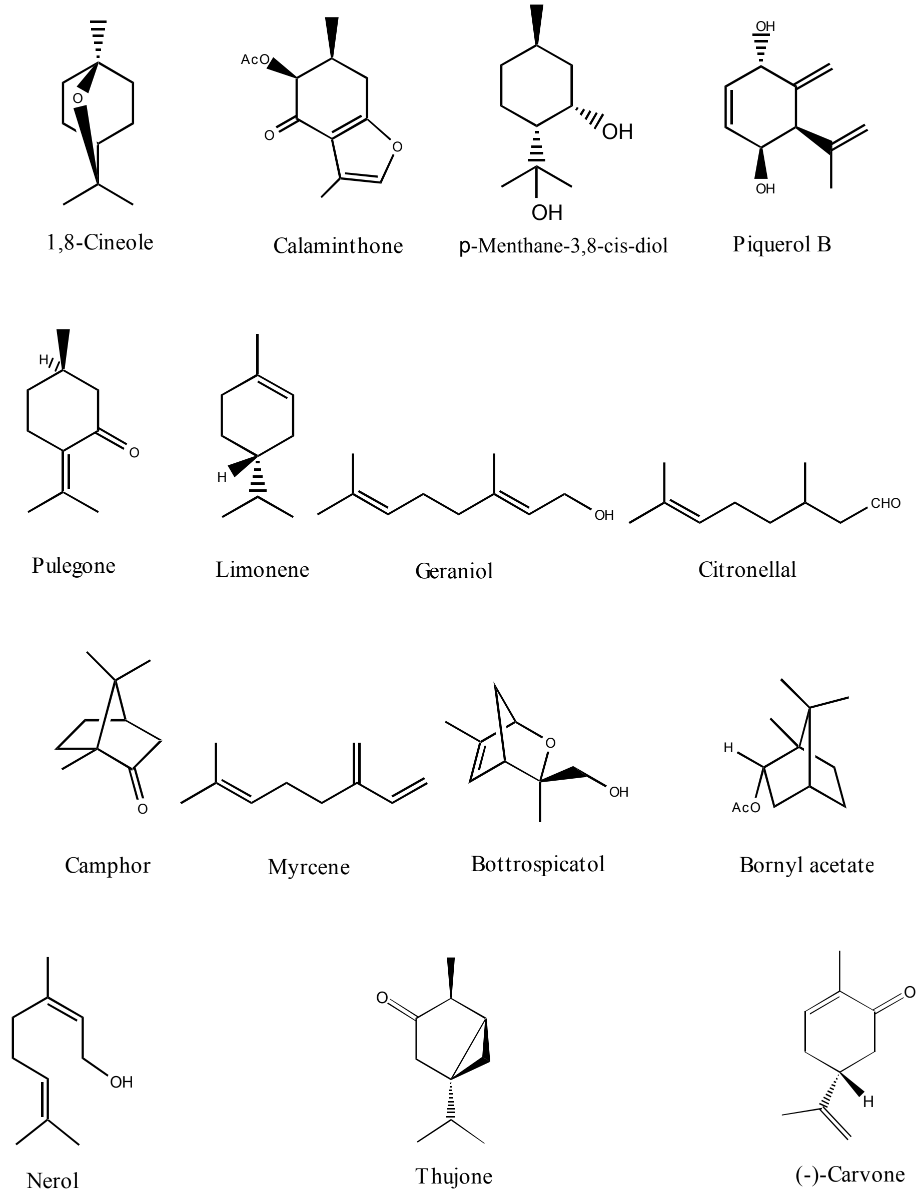


Fig. 7. Typical monoterpenoids

p-cymene. Some workers have at times inferred the phytotoxic action of a particular monoterpenoid within an essential oil, but given that these compounds occur naturally as mixtures, it is now thought that terpenoid toxicity is most probably due to a joint action by two or more compounds combining their activities. Thus in their examination of the effects of the essential oils of rosemary (*Rosmarinus officinalis*), savory (*Satureja montana*), and thyme (*Thymus vulgaris*), against seed germination of radish (*Raphanis sativus*), Angelini et al. (2003) found that the pure principal components of each oil (cineole/borneol; carvacrol; thymol respectively) were not as inhibitive as the essential oil mixture. Some have even suggested a synergistic interaction between oil mixture components, but a recent and comprehensive examination of these ideas by Vokou et al. (2003) with measurements upon germination and growth of *Lactuca sativa* using 47 different monoterpenoids and 11 pairs (in five different proportions) of such compounds, would seem to deny any great significance for the role of synergy in monoterpenoid toxicity. While some degree of synergy did appear for certain pairs, the level of growth inhibition was not comparable to that of a single highly active compound.

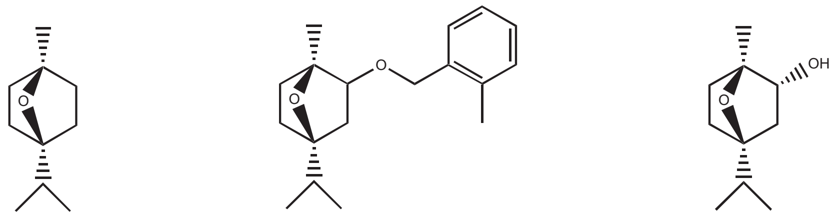
The direct effect of volatile substances from tomato (*Lycopersicon esculentum*) leaves which included such terpenoids as á-terpineol, linalool, thymol, and geraniol (Fig. 7), upon the dry weight of *Lactuca sativa*, was to reduce that weight by 34% (Kim and Kil 2001). GC/MS analysis of the essential oil from tomato plant leaves revealed at least 40 different compounds, of which about 20% were terpenoids. Again, it would be difficult to attempt to assign this toxicity to any one compound.

4.4.2 Monoterpenoid Chemistry and Mode of Action

While it had been long believed that the monoterpenoids were essentially hydrophobic compounds, more recent investigations have actually shown that the terpene hydrocarbons were low (10–20 ppm) in solubility, but that ethers, ketones, and alcohols showed surprisingly high water solubility, e.g., (saturated solutions at room temperature): camphor (11.3 mM) (Fig. 7); borneol (4.2 mM); piperitone (16.8 mM); pulegone (9.2 mM) (Fig. 7); α-terpineol (12.9 mM); menthol (2.7 mM) – concentrations which are higher than those normally used in germination and growth bioassays (Fischer 1991). In fact, Reynolds (1987) has reported comparative phytotoxic effects of aqueous solutions of a series of open chain, cyclic, and bicyclic terpenes for their activity against the indicator species lettuce. Reynolds found that the unsaturated hydrocarbons such as myrcene (Fig. 7), limonene (Fig. 7), and *p*-cymene required concentrations in excess of 2 mM to cause 50% germination reduction. The open chain, the cyclic, and the bicyclic alcohols (e.g., nerol (Fig. 7), terpinen-4-ol, and borneol) were significantly more active, while greatest bioactivity was associated with unsaturated ketones such as thujone (0.022 mM) (Fig. 7) and piperitone (0.075 mM). The impact of stereochemistry was also observed in these studies, whereby the lettuce germination mechanism was affected by (+)-carvone at 0.052 mM, but only by the (–)-carvone isomer (Fig. 7) at the higher 0.38 mM value. Experiments by Mucciarelli et al. (2001) on the essential oil from peppermint (*Mentha* × *piperita* L.) measuring root and mitochondrial respiration of etiolated seedlings of cucumber (*Cucumis sativus* L.), showed that total oil mixture was effective for 50% inhibition at 324 ppm and 593 ppm respectively, and that (+)-pulegone (Fig. 7) was the most toxic compound with 0.08 mM and 0.12 mM concentrations required respectively for 50% inhibition of root and mitochondrial respiration. Also in this work, an interesting positive correlation was found for (+)-pulegone, (–)-menthone, and (–)-menthol between water solubility and respiratory inhibition.

An interesting comparative study by Vokou et al. (2003) which lists the level of bioactivity against indicator species *Lactuca sativa* for seed germination and seedling growth for 47 different monoterpenoids revealed some interesting findings. First, a range of seven different chemical classes were among the chosen terpenoids: hydrocarbons, alcohols, aldehydes, ketones, ethers, acetates, and phenols. For germination inhibition, the alcohol terpinen-4-ol was the most inhibitory compound, while the ketones pulegone (Fig. 7), dihydrocarvone, and carvone (Fig. 7) were next in effectiveness. As overall classes, ketones were most inhibitory, followed by aldehydes, ethers, alcohols, and phenols, with acetates and hydrocarbons least inhibitory. In respect of *L. sativa* seedling growth, 24 compounds from across all chemical classes except acetates demonstrated strong inhibition, with the highest activities shown by citronellol, linalool, isopulegol, citronellal (Fig. 7), myrtenal, carvone (Fig. 7), and dihydrocarvone. Acetates and hydrocarbons were the least active, although one hydrocarbon [(+)-3-carene] did show strong inhibition. Second, non-oxygenated compounds are less active than oxygenated ones (established in the literature already), but if the oxygen is part of an acetate function then the activity is largely lost. Third, straight chain terpenoids are about as active as cyclic ones. Fourth, aromatic terpenoids do not appear to have any greater germination inhibitory power than their more saturated analogs. Finally, there does not appear to be any obvious structure/activity relationship among the monoterpenoids, and the influence of chirality for a given isomer is usually insignificant.

Despite the vast array of known terpenoid structures, very little use has so far been made of them as lead structures for herbicides (Vaughn and Spencer 1993), and very little is so far known of their modes of action. Probably the best understood mode of action is that of 1,4-cineole. Both 1,8-cineole (Fig. 7) and 1,4-cineole (Fig. 8) are strong growth inhibitors, but only the 1,8- isomer inhibits all stages of mitosis. The cineoles are commonly found as components of essential oils from aromatic plants such as *Artemisia* spp. and *Eucalyptus* spp. The 1,4-isomer causes growth abnormalities in shoots, unlike the 1,8-compound (eucalyptol), and so despite the great similarity of structure, the two compounds have different modes of action. The molecular target of 1,4-cineole has been determined to be a critical nitrogen-mobilizing enzyme asparagine synthetase (Romagni et al. 2000). The commercial herbicide cinmethylin (Fig. 8) has a structure based upon that of 1,4-cineole. The benzyl ether group acts to reduce compound volatility and to promote formation (through metabolization of the cinmethylin proherbicide) of the more active *cis*-2-hydroxy-1,4-cineole phytotoxin (Fig. 8) (Duke and Oliva 2004). This hydroxycineole under bioassay against asparagine synthetase activity demonstrated an I_{50} value of 0.03 ìM, more than ten times lower than that of the parent 1,4-cineole.



1,4-Cineole Cinmethylin cis-2-Hydroxy-1,4-cineole

Fig. 8. The bicyclic terpenoid lead structure (1,4-cineole) and its commercial herbicide analogs

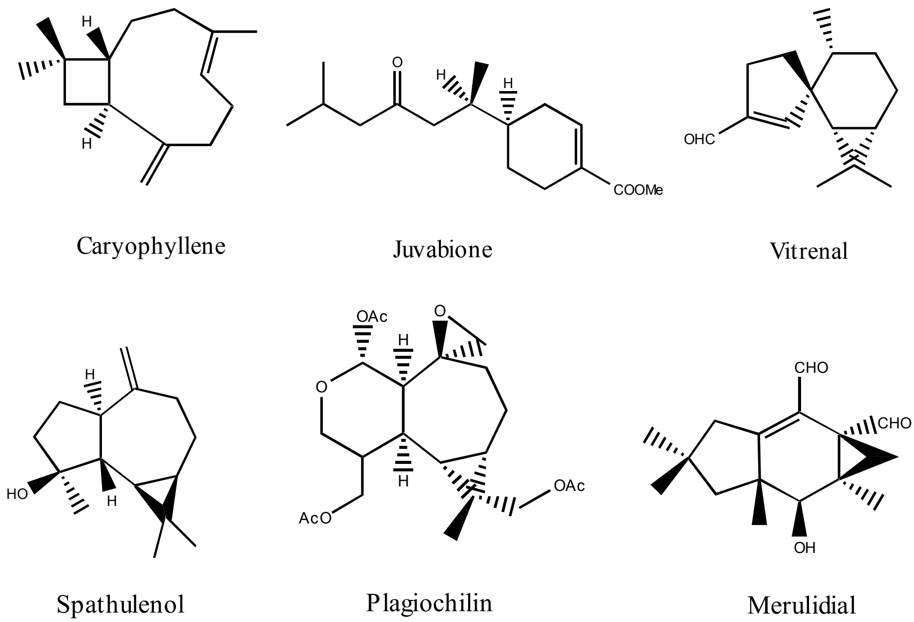
From an allelopathic perspective, the nature of soil transformations upon terpenoid structures holds considerable interest. The fate of both bioactive and inactive terpenes within the rhizosphere soil has received a modest level of investigation, but only little with an allelopathic purpose (Inderjit et al. 1999). However, Nishimura et al. (1983) found that (–)-*cis*-carveol is transformed into (+)-bottrospicatol (Fig. 7) by soil *Streptomyces bottropensis* and that the metabolite was active against lettuce seed germination. This field deserves more investigation in the future.

4.4.3 Sesquiterpenoid Activity

Many C-15 sesquiterpenoids and metabolized derivatives are bioactive across a wide range of organisms and deserve at least a brief survey in relation to phytotoxicity and possible allelopathic effects.

The sesquiterpenoids often accompany the monoterpenes within essential oils, but there are much greater numbers of the sesquiterpenoids. These C-15 compounds are biosynthetically derived from their common farnesyl pyrophosphate intermediate, and because of their higher molecular weights, are found in the less volatile oil fractions. Some typical sesquiterpenoids are depicted in Fig. 9.

Some of the ecological roles found for sesquiterpenoids include hormonal defense, pollination attraction, herbivory defense, antecedent activity, phytoalexins defense, and pheromonal mimicking (Harborne 1991). While the large sub-group of sesquiterpene lactones (> 5000) seems to have drawn the most attention for their bioactivity, the non-lactone group has demonstrated some limited levels of phytotoxicity. For example, the liverwort *Plagiochila ovalifolia* was found to contain plagiochilin (Fig. 9), which at the 50 ppm level, strongly inhibited the growth of rice seedlings. Structure/activity studies pointed to the acetyl hemiacetal group as being necessary for activity (Matsuo et al. 1981). A more recent example of sesquiterpenes showing putative allelopathic activity is that of the two geometric isomers *cis, trans*- and *trans*, *trans*-xanthoxin (Fig. 10) which were extracted with methanol from the leaves of *Pueraria thunbergiana,* where they were present at concentrations of 51.4 and 72.5 ng/g fresh weights respectively. The xanthoxins inhibited the root growth of cress (*Lepidium sativum*) seedlings at lowest concentrations of 0.3 ìM (*cis, trans*) and 3.0 ìM (*trans, trans*), while the 50% inhibitions of cress roots were measured at 1.1 and 14.0 ìM respectively (Kato-Noguchi 2003c). Very little is presently known about sesquiterpenoid modes of action or molecular target sites.


Caryophyllene Juvabione Vitrenal

Spathulenol Plagiochilin Merulidial

Fig. 9. Typical sesquiterpenoids

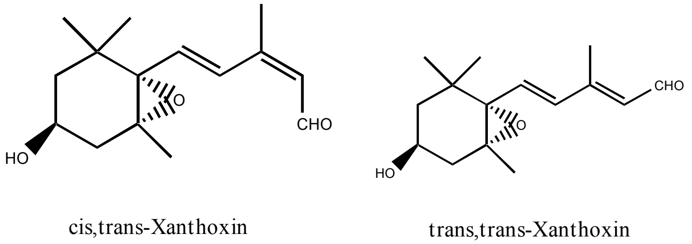
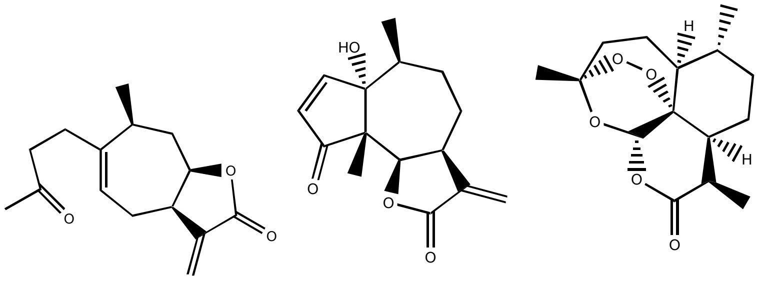

cis,trans-Xanthoxin trans,trans-Xanthoxin

Fig. 10. Sesquiterpenoid allelochemicals from *Pueraria thunbergiana*

The much larger group of sesquiterpenoid lactones (SL's) mostly carry an α-methylene-γ-lactone moiety, and are found in over 15 plant families, especially in the Asteraceae (Fig. 11).



Tomentosin Parthenin Artemisinin

Fig. 11. Typical growth regulating sesquiterpenoid lactones (SL)

The SL's also often contain such additional groups as epoxides, esters, unsaturated aldehydes and ketones which have good alkylating properties, and so may easily react with biological nucleophiles such as the thiol groups of plant enzymes. This gives the SL's a broad range of biological activities, including plant growth regulation and phytotoxicity (Fischer 1991).

Some useful examples of phytotoxic SL's include tomentosin, parthenin, and artemisinin (Fig. 11).

Tomentosin is a lactone isolated from the seeds of *Iva axillaris* (poverty weed) by Spencer et al. (1984) which demonstrates a capacity to inhibit both growth and germination of velvet leaf (*Abutilon theophrasti*), a major agricultural weed. Parthenin was studied by Fischer et al. (1989) at levels of 1, 10, and 100 ìM for its action on sorghum, which it was able to significantly inhibit from germination, even at 1 ìM. Such high activity levels are above many other naturally occurring inhibitor compounds. Parthenin's potential allelopathic and herbicidal properties were further explored by Batish et al. (2002) for its effect upon two weedy species *Avena fatua* and *Bidens pilosa*. Parthenin inhibited the root and shoot length and seedling dry weight of both weeds, and continued to do so even when the weeds were grown in soil amended with different concentrations of parthenin. A reduction in chlorophyll content in the growing seedlings was also noticed. The authors recommended further exploration of parthenin as a potential herbicide for future weed management studies. Artemisinin (Fig. 11) is a sesquiterpenoid lactone obtained from annual wormwood (*Artemisia annua*) and possesses an unusual peroxide moiety. Besides being highly active against malarial parasites, artemisinin is strongly phytotoxic, but its mode of action is still unknown despite a series of studies by different groups (Duke and Oliva 2004).

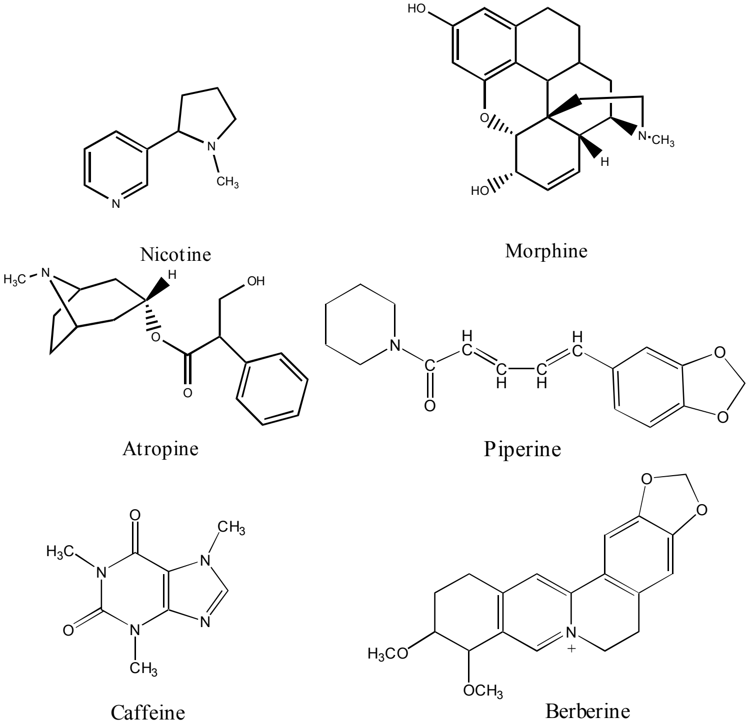
4.5 Alkaloids: Chemistry and Ecology

4.5.1 Introduction

One group of secondary metabolites known from ancient times and given the name "alkaloids" by Meissner, the apothecary from Halle in 1819, because of their alkali-like behavior during extraction from plant sources using aqueous acid, today numbers in excess of 12,000 compounds, of which only some 600 have been examined for their biochemical properties (Wink 1998). These substances are usually cyclic or polycyclic, and are mostly derived through biosynthetic pathways which begin with a natural aminoacid. They therefore always contain at least one nitrogen atom and will often demonstrate the basicity of an amine. Their chemical structures are very diverse and no core fragment can represent the class. Roberts and Wink (1998) state that it is possible to recognize four main groups:

a) alkaloids derived from various aminoacids, e.g. ornithine; lysine; histidine; tryptophan,
b) purine alkaloids, such as the xanthine caffeine,
c) aminated terpenes, such as aconitine (diterpene) and solanine (triterpene), and
d) polyketide alkaloids, e.g. coniine, where nitrogen is introduced into a polyketide skeleton.

While each separate alkaloid can be given a full systematic name, it is much more convenient to refer to them by their trivial names, often related to their original plant source. Some common examples would be the compounds displayed in Fig. 12.


Nicotine

Morphine

Atropine

Piperine

Caffeine

Berberine

Fig. 12. Typical plant alkaloids

Plant alkaloids are found widely distributed in the families Fabaceae, Apocynaceae, Asteraceae, and Borginaceae, and often fulfill the role of plant defensive agent against fungi, viruses, herbivores, microorganisms, and competing plants. Some plant species are especially insecticidal because of a range of defensive alkaloids they produce, including such example compounds as strychnine, ephedrine, piperine (Fig. 12), nicotine (Fig. 12), and gramine (Fig. 13). The insecticide nicotine has been used commercially over a long period as protection against commercially important insect pests, while the alkaloid caffeine (Fig. 12) is also quite insecticidal and is probably the most widely distributed compound of this class (Blum 2004a). Such alkaloids as morphine (Fig. 12), berberine (Fig. 12), ergotamine, coniine (Fig. 13), quinine (Fig. 13), and colchicine (Fig. 13) among a group of at least 50 tested compounds possess phytotoxicity, acting to inhibit germination and/or seedling growth in neighboring plants.

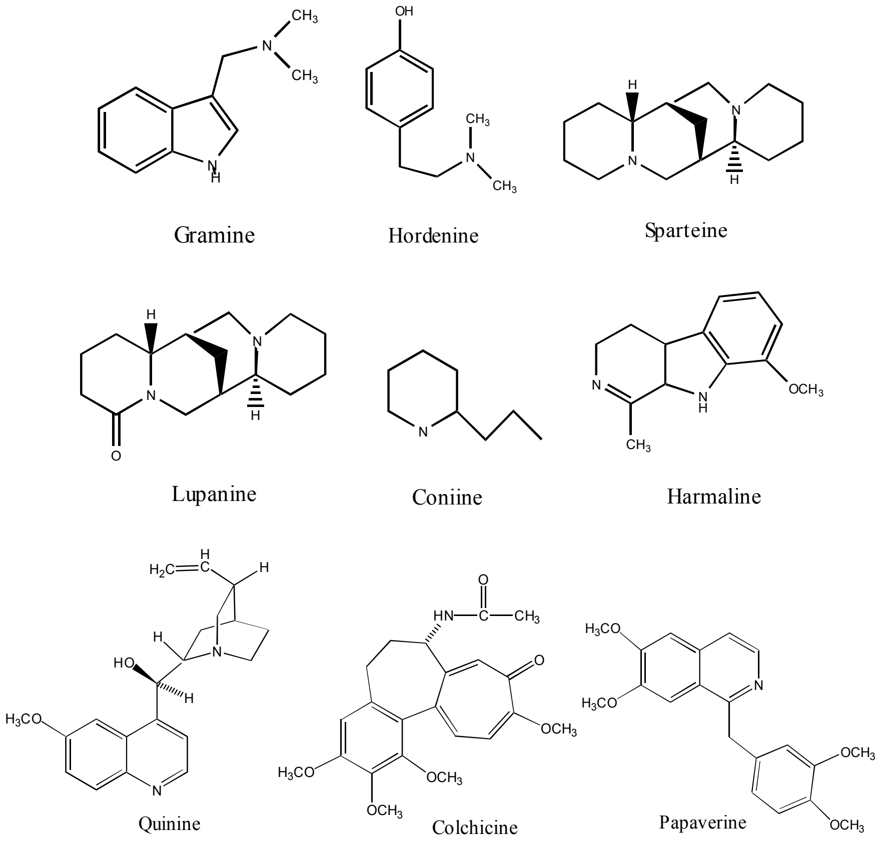


Fig. 13. Some alkaloids which demonstrate phytotoxicity

4.5.2 Modes of Allelopathic Action

While a high proportion of phytotoxic alkaloids attacks more than one plant target site at a time, it is known that the range of targets includes key enzyme functions, photosynthesis, respiration, transcription, protein synthesis, membrane stability, signal transduction, electron transport, and replication (Wink and Latz-Bruning 1995). Thus, a variety of alkaloids binds to or intercalates with DNA or DNA/RNA processing enzymes and can disrupt replication or transcription, e.g. berberine (Fig. 12) and quinine (Fig. 13). Lupanine (Fig. 13) and tubulosine interfere with protein synthesis, while disruption of biomembranes and transport processes is carried out by berbamine and tetrandrine (Blum 2004a). These types of molecular processes are manifested in the outcome from plant bioassays, such as the impact of the four alkaloids colchicine (Fig. 13), harmaline (Fig. 13), papaverine (Fig. 13), and salsoline upon the root growth of *Lepidium sativum*, where 0.01% solutions of these substances reduced radicle length to around 50% or less of the control (Wink and Latz-Bruning 1995). Likewise, in more recent work on the sensitivity of the same test plant *L. sativum* towards the growth inhibiting alkaloids 3′′′′-oxo-juliprosopine and secojuliprosopinal isolated from the leaves of mesquite (*Prosopis juliflora* (Sw.) DC.), the respective I_{50} values were found to be 0.4 mM and 0.5 mM (Nakano et al. 2004).

4.5.3 Examples of Allelopathic Action

Conclusive experimental evidence linking plant alkaloid production with significant allelopathic field activity remains small.

The alkaloids scopolamine and hyoscyamine were recognized as present in the leachates from seeds of thornapple (*Datura stramonium*) which was studied for its allelopathic potential. Both these compounds were isolated from the soil at a field site overgrown with this plant (Lovett et al. 1981). Levitt and Lovett (1984) showed later that the inhibitory effect of thornapple seeds and leachate on *Helianthus annuus* seedlings remained for a period of eight months in a black earth soil under field conditions.

Two more detailed and specific cases among the alkaloid allelochemicals will be described as examples of plant–plant allelopathy.

The first example consists of the work of the Lovett group at Armidale, Australia, (see e.g. Lovett and Hoult 1995) who, from about 1987–1995 investigated self-defense in the crop barley (*Hordeum* spp), based upon some earlier exploration by Overland (1966). These workers found that living barley plants, hydroponically grown, released the alkaloids gramine (Fig. 13) and hordenine (Fig. 13) from their root systems. The alkaloids were identified and quantified by HPLC (Hoult and Lovett 1993), and their phytotoxicity against white mustard (*Sinapsis alba*) confirmed in chemoassay (Lovett et al. 1989) and biological assay. By four days after germination, barley exudate of hordenine had reached 48 ppm, a concentration which gave significant reduction in radicle length on test plant *S. alba*. The gramine in barley exudate maximized at 22 ppm and also contributed to radicle reduction in *S. alba*. Release of these allelochemicals was observed over a growth period of 75 days (Liu and Lovett 1993a). In a control hydroponics solution in which barley had not been grown, no affect on the bioassay test species was observed. Radicle length response by white mustard to the different doses of each alkaloid followed the classic curve depicting hormesis at very low concentration (a stimulation above control) followed by growing levels of inhibition as the concentration of allelochemical increased. Physical examination by electron microscopy of the root tips of *S. alba* after subjection to the alkaloids, evidenced the same type of cellular disruption as is commonly observed in such cells under other types of stress (Liu and Lovett 1993b). Significantly, these workers have also demonstrated that at 250 ppm, gramine is also able to severely inhibit the growth of white mustard in soil. A genetic investigation (Lovett et al. 1994) into hordenine production by 43 various barley lines (ancestral, landraces, middle eastern, and modern) under two types of environmental conditions, found that environmental conditions during growth had more influence over alkaloid production than did genetic factors. Highest hordenine levels were measured at 327 ìg/g dry weight barley root.

The second example relates to the quinolizidine alkaloids, prominent among legumes. The quinolizidine alkaloids (QA) biosynthesis from aminoacid lysine via cadaverine to representatives sparteine (Fig. 13) and lupanine (Fig. 13) occurs within the green above-ground plant parts, and is thus regulated by light (Wink 2004). The seeds of such plants can be especially rich in alkaloids and may reach levels of 8% by dry weight. Within lupins, QA's have been measured at flowers 4%; fruit 3.9%; pollen 4.7%; leaf 4%; epidermis 6% dry weight; phloem 5 mg/mL; and xylem 0.05 mg/mL. The various bioactivities of QA's consist of such properties as inhibiting the multiplication of potato-X virus and the growth of bacteria and fungi; having repellant, toxic, or mutagenic actions toward nematodes, cows, snails, aphids, bees, beetles, and locusts; and phytotoxicity towards germination and growth of certain plants (Wink 1983 and 1985). The role of these alkaloids appears to be one of general plant defense. The effects of QA's observed against plants are probably due to their interference with membrane integrity and with protein synthesis.

4.6 Hydroxamic Acids: From Among the Benzoxazinoids

4.6.1 Structure

The allelochemicals often called "cyclic hydroxamic acids" are actually a small part of the broader group of naturally occurring benzoxazinones which possess the 2-hydroxy-2H-1,4-benzoxazin-3-(4H)-one skeleton as shown in Fig. 14 (Sicker and Schulz 2002).

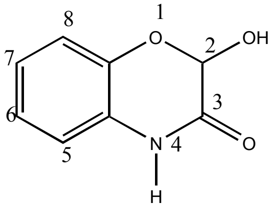


Fig. 14. The 2-hydroxy-2H-1,4-benzoxazin-3-(4H)-one skeleton

Whilst certain variable levels of bioactivity have been found among a range of the broader benzoxazinone group, it is those particular members which possess the special labile combination of cyclic hemiacetal unit (at position 2) and a hydroxamic acid component (the N of the heterocyclic ring at position 4 carries an hydroxyl group) together, which are recognized as the key bioactive compounds for allelopathy (Fig. 15).

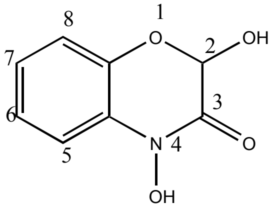
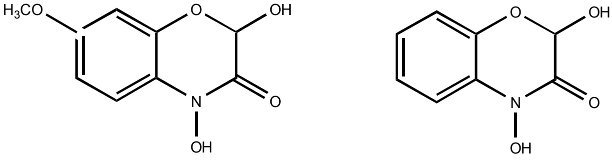


Fig. 15. The labile, bioactive, cyclic hydroxamic acid skeleton

Two prominent allelochemicals of this hydroxamic acid class are the compounds known mostly by their acronyms as DIMBOA and DIBOA (Fig. 16).

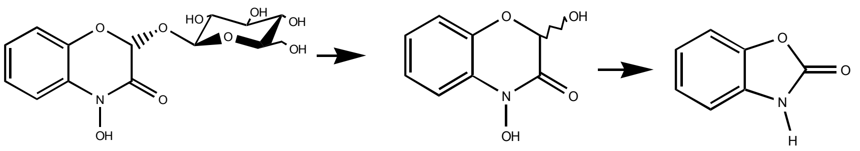


(a) DIMBOA (b) DIBOA

(a) DIMBOA [2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-(4H)-one],
(b) DIBOA [2,4-dihydroxy-2H-1,4-benzoxazin-3-(4H)-one]

Fig. 16. Prominent hydroxamic acid allelochemicals

The benzoxazinones (6-membered heterocyclic ring) are a sub-group of the wider class of natural products with the name benzoxazinoids whose members also embrace the structural group called benzoxazolinones (5-membered heterocyclic ring), some of which arise from the natural degradation of benzoxazinones. The first such benzoxazolinone (BOA) to be recognized in nature was from rye, where an elevated level of resistance towards pathogenic fungi was noticed. Soon after, BOA was realized to be the degradation product of DIBOA, which in its turn was formed from its stable, non-active 2-β-D-glucoside by enzymatic hydrolysis (Virtanen and Hietala 1960) (Fig. 17).

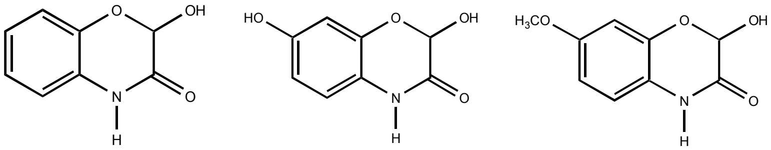


(a) DIBOA-Glc (b) DIBOA (c) BOA

(a) (2R)-2-β-D-glucopyranosyloxy-4-hydroxy-2H-1,4-benzoxazin-3(4H)-one [DIBOA-Glc],
(b) 2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one [DIBOA], (c) Benzoxazolin-2(3H)-one [BOA]

Fig. 17. Biosynthetic pathway in rye to BOA

All the natural reported glycosides of the benzoxazinoids have a D-glucose moiety [Glc-] at the 2-position with a (2R)-2-β-linkage. The only other known hydroxamic acids are DIM$_2$BOA (2,4-dihydroxy-7,8-dimethoxy-2H-1,4-benzoxazin-3(4H)-one) and TRIBOA (2,4,7-trihydroxy-2H-1,4-benzoxazin-3(4H)-one) from *Zea mays* (Sicker and Schulz 2002). One natural derivative of a hydroxamic acid (HDMBOA) 2-hydroxy-4,7-dimethoxy-2H-1,4-benzoxazin-3(4H)-one from maize and wheat still shows high bioactivity and lability like the free hydroxamic acids (Friebe 2001). All the benzoxazinone glucosides are biologically inactive. However, if they possess the hydroxamic acid-like structure after hydrolysis through disruption of the plant cell, they become bioactive in the aglucone form (Mizutani 1999). The range of natural benzoxazinones which have a simple hydrogen atom attached to the N atom at position 4 (the lactams) such as HBOA, DHBOA, or HMBOA, do not display significant bioactivity (Fig. 18).



HBOA DHBOA HMBOA

Fig. 18. Natural benzoxazinones of the lactam type

4.6.2 Distribution and Ecology

Benzoxazinone compounds (usually conjugated with glucose) occur naturally within the plant families Acanthaceae, Poaceae, Ranunculaceae, and Scrophulariaceae (Sicker and Schulz 2002), and hold a special place of interest because such bioactive compounds appear to act as defense chemicals for their host against a range of attacking organisms such as insects, microbes, and other plants (Niemeyer 1988; Wilkes et al. 1999). This property has stimulated wide interest in enhancing such ability within important crops such as maize, wheat, and rye which carry these compounds. The stable, inactive benzoxazinone glycoside can undergo a two-step degradation, first through a glucosidase hydrolysis to the active heterocyclic aglycone – a process enabled by plant cell injury, then through a simple chemical ring contraction to the benzoxazolinone, during which time chemical attack occurs on key enzymes of the attacking organism. For example, in maize, the enzymatic release of the active DIMBOA in response to attack by insects occurs within 30 minutes from injury. The content of DIMBOA in maize shoots has been measured at 1–10 mmol/kg fresh weight (Sicker and Schulz 2002), so that moderately high quantities are available for defense. Known occurrences for the glucosides of common benzoxazinones include the species HM_2BOA in *Zea mays*, HMBOA in *Secale cereale*, DHBOA in *Coix lachryma jobi*, HBOA in *Blepharis edulis*, HDMBOA in *Triticum aestivum*, DIM_2BOA in *Zea mays*, DIMBOA in *Triticum aestivum*, and DIBOA in *Consolida orientalis*.

The hydroxamic acids have been found in plant roots as well as aerial parts, and also in the exudates from selected cereals but appear to be absent from the seed (Niemeyer and Perez 1995). Compound levels usually increase quickly after germination, reaching a maximum in just a few days and then more slowly decrease with increasing plant age (Copaja et al. 1999; Burgos et al. 1999; Cambier et al. 2000). Absolute levels will also depend upon the cultivar and environmental growth conditions (Niemeyer 1988; Wu et al. 2001a; Friebe 2001).

4.6.3 Chemistry and Biosynthesis

Biosynthesis of plant benzoxazinoid compounds depends on the tryptophan pathway from anthranilic acid to indole, where the specialized production of the secondary pathway is capable of forming indolin-2-one. Further monooxygenase hydroxylations to 3-hydroxyindolin-2-one and then to 4-deoxy-DIBOA, form the lactam precursor of DIBOA at the end of this biosynthetic process (Mizutani 1999; Desai et al. 1996). The methoxyl group of related DIMBOA stems from various C-1 sources such as methionine, glycine, and glycerate (Niemeyer 1988).

Because of the hemi-acetal function at position 2 on the benzoxazolone ring, there is a natural equilibration between this form and the open-ring hydroxy-aldehyde structure (Fig. 19). Such tautomerism leads to incapacity to isolate either pure (2R)- or (2S)- forms. For DIMBOA (Fig. 16), the spontaneous ring contraction to MBOA (6-methoxybenzoxazolin-2-(3H)-one) occurs with overall loss of formic acid, probably through an intermediate isocyanate (Niemeyer 1988), and with a half-life in broken maize cells of about one day. The presence of a 7-MeO- group in DIMBOA enhances the reactivity of its heterocyclic ring above that for the unsubstituted DIBOA. Heating of an aqueous solution of DIMBOA is sufficient to convert it to MBOA. In China, roots from the plant *Coix lachryma jobi* L. (var. ma-yuen Stapf) known to contain the anti-inflammation agents MBOA and DIBOA have been applied as therapy for neuralgia and rheumatism (Sicker and Schulz 2002).

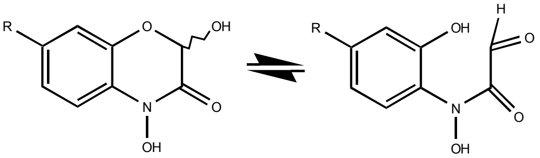


Fig. 19. Tautomeric equilibrium in the 2-hydroxybenzoxazolones

4.6.4 Structure/Activity and Mechanism of Molecular Action

In contrast to other common classes of acetal glycosides known to the chemical literature, the feature of the natural benzoxazinones is the presence of a nitrogen atom within the hemiacetal ring, which imparts an amount of instability to the aglucone, leading to the characteristic bio-activity. The evidence to date favours the need for both a 2-hydroxy and a 4-hydroxy group to be present on the benzoxazinone ring (Hashimoto and Shudo 1996), in which case, according to one model, a multi-centered cationic electrophile can form after metabolic O-acylation and heterolytic cleavage of the N-O bond (Sicker and Schulz 2002). The Sicker group (2002) have themselves proposed a possible alternative mechanism based upon the creation of a 3-formylbenzoxazolinone formed by dehydration of the cyclic hydroxamic acid aglucone. Such an electrophilic formyl group donor would be a strong interactor with typical nucleophilic centers in the enzymes of other organisms (Friebe 2001).

4.6.5 Allelopathy

Benzoxazinoid-containing plants have been known for quite some time to have significant allelopathic potential, and important crop types like rye have been closely investigated for their phytotoxic effects on other plants, especially weeds, as this effect might possibly be tailored to become one ingredient of a weed management system (Friebe 2001; Barnes and Putnam 1987). This allelopathic effect of rye on the weeds crabgrass and barnyard grass was attributed to the DIBOA isolated, together with its less bioactive degradation product BOA. Because the benzoxazinoids can be released from plant residues, the strategy of mulching has been often used as a weed control. Some crop cultivars of different species can exude benzoxazinoids from their roots directly into soil from the living plant. Considerable attention has been directed towards the development of crops (e.g. wheat) which may only require low applications of herbicide to control weeds (Wu et al. 2000; Wu et al. 2001b; Wu et al. 2002; Huang et al. 2003). Another class of less active allelochemicals is often found mixed with hydroxamic acids in plant root exudates, viz., the phenolic acids. Growing evidence suggests that these two classes of compounds may work together to generate an allelopathic effect greater than either class on its own (Huang et al. 2003). Hydroxamic acids have also been found in weeds such as quackgrass and barnyard grass (Pheto 1993). Quackgrass is an aggressive perennial and its allelopathic effect has been described using the growth of cress in a root exudate recirculating system (Schulz et al. 1994) where the phytotoxicity was at least partly attributed to the DIBOA content. Strongly evidential molecular-level explanations for allelopathic effects are rare in the literature, but a small body of useful data is beginning to be built. One such notable example is the study by Friebe et al. (1997) who showed that 0.25 mM solutions of DIBOA or DIMBOA inhibited the crucial enzyme H^+-ATPase which acts as the electrogenic pump of the plasma membrane maintaining a proton gradient required for the transport of ions and molecules, and thus is tied closely to a plant's root growth. Many allelopathic bioassays are based upon root growth inhibition (or stimulation) and therefore offer an indirect measure of allelochemical action upon key enzymes such as that above. The Friebe et al.

(1997) study performed dose-response measurements using both DIBOA and BOA over the range 0–5 mM and measured both radicle elongation and ATPase activity. They found remarkable parallelism between the two separate response measurements, even to the extent of observing the same typical allelochemical stimulation of oat root (*Avena sativa*) growth at low dose concentrations coinciding with corresponding stimulated ATPase activity.

Six naturally occurring benzoxazolin-2(3H)-ones have been recorded in a total of seven plant species, although all of these compounds have been reported as present in *Zea mays* (Sicker et al. 2004). Besides BOA (Fig. 17) and MBOA, there are also DMBOA (6,7-dimethoxybenzoxazolin-2(3H)-one), 4-ABOA (4-acetylbenzoxazolin-2(3H)-one), 5-Cl-MBOA (5-chloro-6-methoxybenzoxazolin-2(3H)-one), and 4-Cl-DMBOA (4-chloro-6,7-dimethoxybenzoxazolin-2(3H)-one)). While the first three have been shown to have a glucosidic origin, the biosynthetic source for the latter three has not yet been established. Some compounds like BOA and MBOA have demonstrated bioactivity against pests, and show phytotoxicity against a range of weeds and crops (Sicker et al. 2004). However, it appears that for compounds such as BOA, its phytotoxic ability can be overcome in some plants (such as oats) because they possess ability to detoxify it (Perez 1990). BOA absorbed from aqueous solution into oat roots, was found to be enzymatically converted to 6-hydroxy-BOA and thence to inactive BOA-6-O-glucoside. Direct glucosylation of the ring nitrogen was also an additional pathway for detoxification within oats (Wieland et al. 1998). One final and interesting fact about BOA (and MBOA) is its relatively easy conversion by soil bacteria, such as *Acinetobacter calcoaceticus*, *Waksmania aerata*, and *Pseudomonas iodina* into simple 2-aminophenol, which in air undergoes a dimeric oxidation to the dark red compound 2-amino-3H-phenoxazin-3-one (Fig. 20), also known independently as the antibiotic questiomycin A from *Streptomyces thioluteus*. This aminophenoxazinone demonstrated higher phytotoxicity than its precursor BOA against such weeds as barnyard grass, and therefore the allelopathic effect of plant mulch rich in BOA (such as rye) must be seen to be enhanced by this extension of soil metabolic processes (Gagliardo and Chilton 1992).

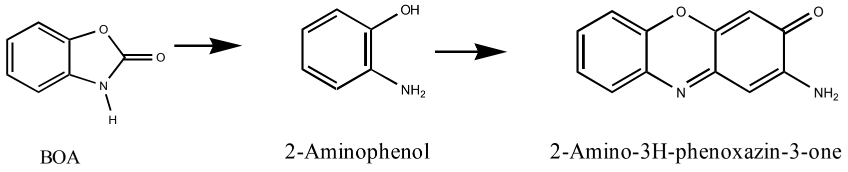


Fig. 20. Bacterial and aerial transformation of the benzoxazinoid BOA to questiomycin **A**

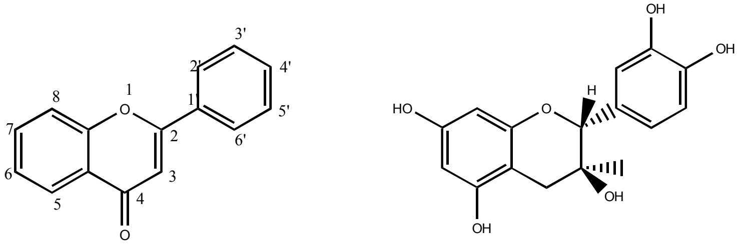
4.7 Other Compounds

4.7.1 Introduction

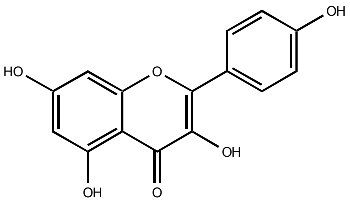
The above classes of allelochemicals by no means cover all that is presently known about plant allelochemicals. New discoveries of fresh plant–plant systems are being constantly made and published, revealing an ever-widening list of different structural types demonstrating phytotoxicity, and where it can be reasonably proved, mediating a true allelopathic effect. In this section, an attempt is made to introduce some of the common smaller groups of compounds associated with allelopathy.

4.7.2 Flavonoids

The flavonoids are a large group of compounds having a central (flavone) structure as depicted by typical examples in Fig. 21. Flavonoids are widely distributed in the plant kingdom, especially among the woody species.



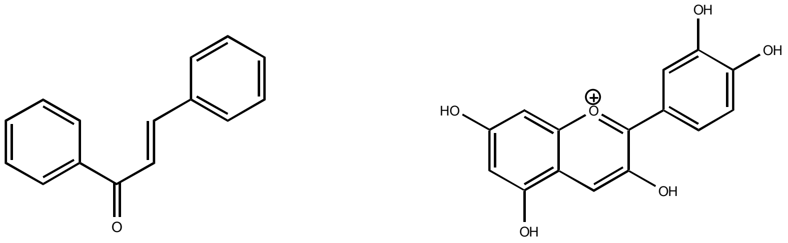
Flavone (+)-Catechin



Kaempferol

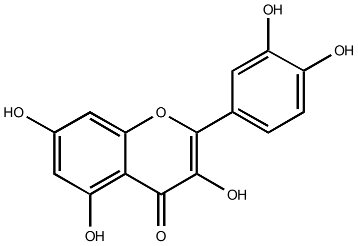
Fig. 21. Typical flavonoids

Only a small group has been shown so far to be implicated in allelopathy. The ring structure of flavonoids is such as to readily adopt the formation of a stable free radical, which can easily lead to the formation of dimers and oligomers and condensed tannins, from among which, a few phytotoxic compounds are known. The biosynthetic origin of the flavonoids arises from a shikimic acid metabolite (such as *p*-coumaric acid) acting as a starter acid for a triplicate acylation with malonyl-CoA to form a chalcone intermediate (Fig. 22), which then undergoes a simple cyclizing 1,4-conjugate addition by phenolic oxygen on one aromatic ring to the enone system, thereby creating the 6-ring oxygen heterocycle of a flavonoid. This central oxygen heterocycle is easily oxidized to the aromatic heterocycle characterizing the flavones (yellow to orange colors), while further dehydration of this ring system leads to the oxonium ion heterocycle of the anthocyanidins (reds and blues), e.g. cyanidin (Fig. 22). These flavonoid derivatives constitute many of the vivid colors seen in flowers, fruits, and autumn leaves.



Chalcone

Cyanidin



Quercetin

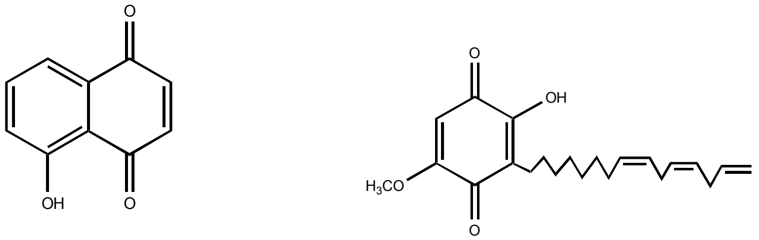
Fig. 22. Compounds related to the flavonoids

Such flavonoids as quercetin (Fig. 22) and its 3-O-rhamnoside (quercitrin), isolated from the leaves of the noxious perennial weed *Pluchea lanceolata* have been shown to inhibit the growth of the legume asparagus bean (*Vigna unguiculata*) at concentrations of 10^{-4} to 10^{-3} molar (Inderjit and Dakshini 1995). The allelopathic potential of *Cistus ladanifer* has also been examined because its root exudate was found to contain the flavone apigenin-4′-(O)-methyl and the flavonol kaempferol-3,7-di(O)-methyl which at concentrations of 0.5 and 1.0 mM respectively, could inhibit seedling growth of the herb *Rumex crispus* (Chaves et al. 2001).

The flavan-3-ols are one of the most prominent of the phytotoxic flavonoids, and among these, catechin (3,5,7,3′,4′-pentahydroxy-2-phenylbenzopyran) has received much attention (Fig. 21). In this structure, the 2-phenyl substituent and the 3-hydroxy substituent are *trans* to each other. The free radical scavenging and antioxidant ability of the flavan-3-ols has been recorded (Feucht and Treutter 1999) and their role recognized as first-aid molecules limiting cellular damage from free radicals formed in stress situations. The redox action of catechin itself is stated by Erdei et al. (1994) as being the reason for its inhibition of membrane-bound H^+-ATPase. In a review of the small amount of allelopathy literature on flavonoids, Einhellig (2004) states that the flavonoids are the second most active class of allelochemicals inhibiting mitochondrial oxygen uptake, and that only the quinone class was more effective in this role. Work by Moreland and Novitsky (1987) led them to conclude that flavonoids act primarily as electron transport inhibitors through perturbation of the mitochondrial inner membrane. In a series of recent papers by Bais and co-workers (e.g. Bais et al. 2003a,b; Weir et al. 2003), the unusually high level of toxicity of (–)-catechin was explored against other flavonoids with a range of weeds. Thus Bais et al. (2003b) assayed the flavonoids (–)-catechin, naringenin, kaempferol (Fig. 21), quercetin (Fig. 22), (–)-epicatechin, (+)-epicatechin, and (±)-dihydroquercetin against germination, root and shoot length in the weeds *Centaurea maculosa*, (spotted knapweed), *Centaurea diffusa* (diffuse knapweed), *Linaria dalmatica* (dalmatian toad flax), and *Kochia scoparia* (kochia), together with crop plants wheat and tomato. They found that the minimum inhibitory concentrations for bioassay for all flavonoids were about 250 lg/mL, except for (–)-catechin, which had a value five times less. This confirmed further their earlier findings that the phytotoxic root exudate from invasive *C. maculosa* contained (±)-catechin, and that the (–)-isomer was the one with potency. Some interesting structure/activity observations were also made in this work, in that the phytotoxic (–)-catechin and (+)-epicatechin both have a 2S configuration, and that the 2,3-*trans* relationship between substituents in (–)-catechin appears to provide additional potency. In the Weir et al. (2003) catechin studies, the relationship between concentration of (–)-catechin within root exudate of spotted knapweed (*Centaurea maculosa*) and the capacity of that weed to displace native plant communities was examined. These researchers measured a 5-week growth curve to determine the variation of (–)-catechin concentration in knapweed exudates as the plant matured and discovered that catechin concentration rapidly grew to a maximum in the first two weeks then slowly tapered off to less than 50% of that maximum after a further three weeks. While some test plants were significantly inhibited in growth with as little as 50 lg/mL of catechin, the donor weed itself (*C. maculosa*) did not show any significant effects until catechin reached 600 ìg/mL or more, thereby indicating that under natural field conditions, secretion by the weed does not occur at levels which are autotoxic. The follow-up studies by Bais et al. (2003a) which examined the mechanism by which (–)-catechin helped spotted knapweed to be such a successfully invasive species in North America, subsequently revealed that this allelochemical triggers a wave of reactive oxygen species (ROS) initiated at the root meristem, which leads to a Ca^{2+} signaling cascade triggering genome-wide changes in gene expression, finally leading to death of the root system. When 200 ìg/g soil (dry weight) of (–)-catechin was added to natural field soil in pots, the germination of two native North American grasses (*Festuca idahoensis* and *Koeleria micrantha*) was severely reduced. This series of studies on the invasive effectiveness of spotted knapweed in western USA is one of the best published studies on allelopathy in the literature, and admirably integrates data from ecological and other sources.

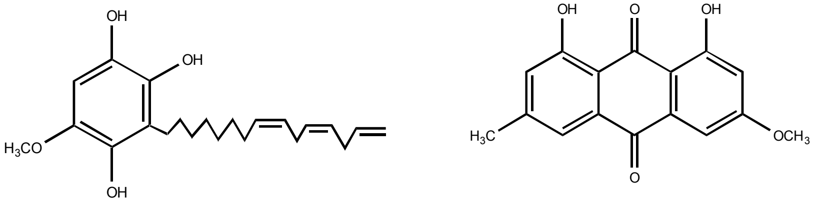
4.7.3 Quinones

While natural quinones are widespread in the plant kingdom, very few have been studied for their implication in allelopathy. Nevertheless, numerous references to the allelopathic phenomenon in the literature will have cited a classic situation involving the quinone known as juglone (Fig. 23), an exuded, amber-colored compound from the black walnut tree (*Juglans nigra*) reported by Massey (1925). Massey observed that walnut caused wilting and dying of alfalfa, tomato, and potato. Davis (1928) associated the observed phytotoxicity from walnut with the compound 5-hydroxy-1,4-naphthoquinone (juglone) and reported its toxicity against alfalfa and tomato. Some of the more significant plant quinones that have been associated with allelopathy are shown in Fig. 23.



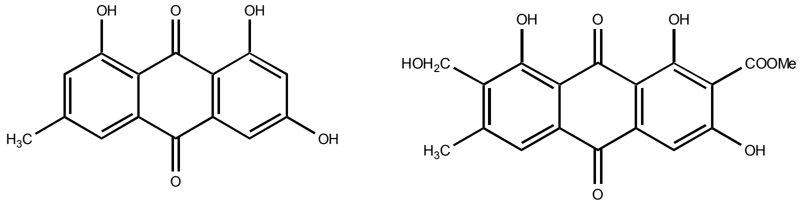
Juglone

Sorgoleone - quinone form



Sorgoleone - hydroquinone form

Physcion



Emodin

Rhodocladonic acid

Fig. 23. Examples of phytotoxic quinones

Among these quinones, juglone has the longest history of study (Massey 1925); (Lee and Campbell 1969); (Rietveld 1983); (Duroux et al. 1998); (Hejl and Koster 2004). It occurs as the colorless, non-toxic, reduced form (hydrojuglone) inside living tissue. Contact with atmospheric oxygen immediately oxidizes hydrojuglone to its toxic quinonoid form (Rietveld 1983). High phytotoxicity at micromolar levels has been reported for juglone against a large number of plant species (Hejl and Koster 2004). Juglone concentration levels for activity have been variously described as 10 ppm for inhibition of growth in *Alnus glutinosa*, and of germination in

Rudbeckia hirta, and *Lactuca sativa*; 1.3 ìM for inhibition of key enzyme *p*-hydroxyphenylpyruvate dioxygenase; and 2 ìM for increased oxygen uptake in isolated soybean and corn mitochondria (Hejl et al. 1993). Several earlier studies on the mechanism by which juglone exerts its toxicity, strongly suggested that it disrupts the electron transport functions in mitochondria and chloroplasts, but while Hejl and Koster (2004) agree that the symptoms of such processes are apparent in juglone-affected plants, they do not see this activity as the prime mechanistic action, but as a secondary outcome induced by juglone's action on H^+-ATPase in root cells, which induces loss of water uptake and hence stomatal closure with reduction of photosynthesis.

Another quinone which has received heavy attention for its allelopathic nature is sorgoleone (Fig. 23). This compound was first reported as an isolate from the root exudate of *Sorghum bicolor* (Netzley and Butler 1986) but is also available from other related sorghum species. It is quite hydrophobic and can be easily extracted from roots by methylene chloride. Across a number of sorghum cultivars, a remarkable 76–99% of root exudate consisted of sorgoleone-type compounds (Weston and Czarnota 2001); (Kagan et al. 2003), with some cultivars yielding as much as 15 mg/g fresh root weight. Studies across various laboratories point strongly to sorgoleone as being a potent inhibitor of photosynthesis with site of inhibition within the PSII complex. Sorgoleone has been shown to be quite phytotoxic towards velvetleaf, crabgrass, and barnyardgrass. A concentration-dependent growth reduction using sorgoleone in soil was observed against *Amaranthus retroflexus*, *Lactuca sativa*, and *Portulaca oleracea*, while fresh weight and shoot length of *Abutilon theophrasti*, *Cassia obtusifolia*, and *Portulaca oleracea* were reduced at sorgoleone concentrations of 40 ppm or less. However, crabgrass (*Digitaria sanguinalis*) and green foxtail (*Setaria viridis*) were little affected at such dilute concentrations (Weston and Czarnota 2001). Strictly speaking, the actual exudate from *Sorghum bicolor* does not consist just of the quinone sorgoleone with a C-15 side-chain, but also small amounts of other similar quinones with C-17 or C-5 side-chains (saturated and unsaturated) and with small differences on the ring moiety, such as ethoxyl replacing methoxyl, or acetyloxy replacing hydroxyl groups. However, the PSII inhibiting abilities of these minor quinones were very similar to that of the main structure, suggesting that all these sorgoleone congeners contribute to the overall allelopathy of the sorghum plant (Kagan et al. 2003). Like juglone, sorgoleone also has a reduced hydro-form (Fig. 23) (Fate et al. 1990) which is exuded from sorghum roots along with the main quinone, but is very easily oxidized to the active quinonoid structure. In recent times Dayan et al. (2003) have unraveled the biosynthetic pathway for sorgoleone biosynthesis using C^{13} NMR techniques. Both the C-15 side-chain and the quinone head of this molecule are derived from acetate units, but each moiety is biosynthesized in a different subcellular compartment. A 5-pentadecatriene resorcinol intermediate has been recognized as being the product of a polyketide synthase, and this alkylresorcinol methylated by an O-methyltransferase (using S-adenosylmethionine) then dihydroxylated by a P450 monooxygenase to produce the final hydrosorgoleone structure.

Other quinones demonstrating activity are the anthraquinones such as physcion, emodin, and rhodocladonic acid (Fig. 23). The latter two compounds have been isolated from lichen sources and appear to have an adverse effect upon the PSII system of plants. The first two compounds have been implicated in the allelochemical interactions of *Polygonum sachalinense* (Inoue et al. 1992), while bioactivities of emodin and physcion on target species *Lactuca sativa, Amaranthus viridus, and Phleum pratense* have been measured at 10–100 ppm (Macias 1995). Four-month-old defoliated leaves of *P. sachalinense* were found to contain large amounts of emodin (213 mg/kg) and physcion (180 mg/kg dry wt.), and the soil levels of these two quinones at the center of a *P. sachalinense* community were measured at 55 and 30 mg/kg dry wt. respectively, large enough to inhibit growth of all three test species.

4.7.4 Polyacetylenes

The biological activity of this small group of compounds mainly from the Compositae family has been reviewed by Towers and Wat (1978) who found them toxic to organisms of various types. Russian knapweed (*Centaurea repens*) has been shown by Stevens (1986) to contain at least five polyacetylenes, the structure of which at least one (Fig. 24) was shown to be very phytotoxic.

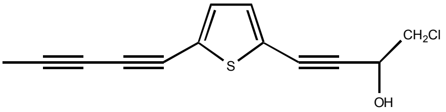
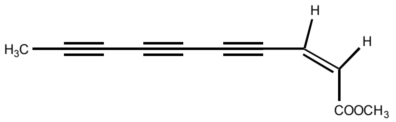


Fig. 24. A thiophene-containing phytotoxic polyacetylene found in the roots of *Centaurea repens*

A few other phytotoxic polyacetylenes are known, such as those from the roots of the Asteraceae, of which the plant *Solidago altissima* is one. From this species, a C-10 compound, *cis*-dehydromatricaria ester (*cis*-DME) has been isolated (Kobayashi et al. 1980; Lam et al. 1992; Inoguchi et al. 2003) and shown to possess strong growth inhibitory effects on other plants (Kobayashi et al. 1980; Tsao and Eto 1996). While *cis*-DME (Fig. 25) is considered an allelochemical of *S. altissima,* its biosynthesis and mode of action are still unknown.



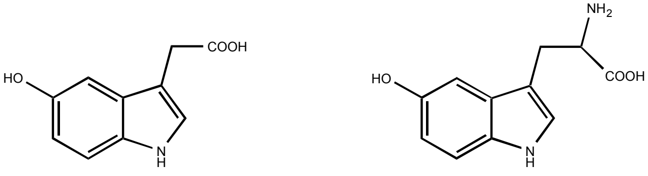
cis-Dehydromatricaria ester

Fig. 25. The allelopathic polyacetylene of *Solidago altissima* – *cis*-DME

4.7.5 Miscellaneous

New allelopathic compound discoveries from plant sources over the past 20 years of allelopathic science have proceeded at an escalating pace, particularly since general access to powerful mixture-separation and spectroscopic analysis instruments, such as GC/MS/MS; LC/MS/MS; HRMS; FTIR; HPLC-PDA; ^{1}H and ^{13}C-NMR has become available. These techniques have enabled plant scientists and natural products chemists to work with the often very small amounts of substance available in the complex mixtures obtained from plant and soil sources in order to determine the chemical structure of bioactive components present. Four representative studies along these lines across the years 1989 to 2003 are summarized below.

Hagin (1989), working on a base of earlier enquiries into the source of allelopathic activity belonging to the highly competitive perennial grass-weed quackgrass (*Agropyron repens* L. Beauv.), carried out quackgrass extractions and used bioassay-guided separations to eventually isolate and identify two key compounds responsible for inhibiting growth in other plants. These allelochemicals were identified as 5-hydroxyindole-3-acetic acid (5-HIAA) and 5-hydroxytryptophan (5-HTP) (Fig. 26), using TLC, HPLC, MS, UV, and IR, analysis techniques. The effects of these two auxin-like compounds were examined against test plants corn and kidney bean over the range 10^{-5} to 10^{-11} M. 5-HIAA stimulated corn root growth at levels below 10^{-6} M but had no effect on shoot growth. 5-HTP stimulated corn root growth with a maximum at 10^{-8} M and shoot growth with a maximum at 10^{-9} M. 5-HIAA had no effect on kidney bean shoot growth in the range 10^{-11} to 10^{-5} M, but became inhibitory at values above 10^{-4} M. It inhibited kidney bean root growth at all the tested levels.



5-Hydroxyindole-3-acetic acid

5-Hydroxytryptophan

Fig. 26. Allelopathic (stimulatory/inhibitory) compounds from quackgrass

Work published by Yu and Matsui (1993) described the isolation of a novel allelochemical from the root exudate of cucumber (*Cucumis sativus* L.) grown hydroponically, by using a recycled exudate trapping system. The phytotoxic compound they discovered was the simple molecule *para*-thiocyanatophenol (Fig. 27), whose structure was recognized by GC/MS and ^{1}H-NMR after solvent extraction from the XAD-4 trapping resin. This compound isolated from the cucumber exudate solution was established at 0.7 mg/g exudate from the growth over 80 days of three seedlings. Thiocyanatophenol markedly reduced the uptake of nutrient ions by cucumber seedlings, and exhibited an ED_{50} for lettuce roots of approximately 0.1 mM.

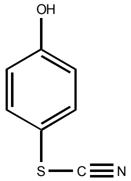
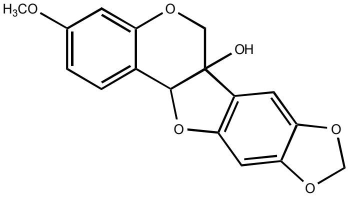


Fig. 27. The thiocyanate allelochemical found in cucumber exudates

The chemical structure of a phytotoxic compound extracted with methanol from pea (*Pisum sativum* L.) shoots and which may play an important allelopathic role in pea residues, was determined recently by the efforts of Kato-Noguchi (2003d), who used MS, IR, and ^{1}H-NMR techniques. The compound, pisatin, has the structure shown in Fig. 28, and demonstrated its toxicity in chemoassay by reducing the growth of cress (*Lepidium sativum* L.) with an ED_{50} as low as 10 ìM.



Pisatin

Fig. 28. Pisatin, a phytotoxin from *Pisum sativum* (pea)

A rather surprising outcome from studies carried out by Kamo et al. (2003) on acetone extracts from the leaves and stems of the legume hairy vetch (*Vicia villosa* Roth) was the discovery of the commercially manufactured nitrogen fertilizer and herbicide cyanamide (H_2NCN) as a phytotoxic natural product. That the cyanamide was indeed a proper metabolite within this legume was confirmed by the fact that 9-day-old seedlings grown without nutrients contained 40 times as much cyanamide as non-germinated seeds. Lettuce seedling hypocotyl length was used as the bioassay to direct the chromatographic fractionation of vetch extract, and while ^{1}H-NMR, ^{13}C-NMR, and IR spectrometries were used for identification of the allelochemical, the simplicity of this molecule gave relatively little spectroscopic data, so that comparison with authentic cyanamide was of considerable help. Quantitative estimation of the cyanamide content in the hairy vetch plants gave a value of 130 lg/g fresh weight.

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