



## Chapter 1

# INTRODUCTION

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**Abstract:** Secondary metabolites (SM) occur in plants in a high structural diversity. A typical feature of SM is their storage in relatively high concentrations, sometimes in organs which do not produce them or as inactive 'prodrugs' that are enzymatically activated in case of danger. Biochemical and physiological features of secondary metabolism are strongly correlated with the function of SM: SM are not useless waste products (as assumed earlier) but important tools of plants needed against herbivores, microbes (bacteria, fungi) and viruses. Some of the SM also function as signal molecules to attract pollinating arthropods or seed-dispersing animals. During more than 500 million years of evolution, plants have evolved SM with a wide variety of biochemical and pharmacological properties. Many SM interact with proteins (receptors, ion channels, enzymes, cytoskeleton, transcription factors), DNA/RNA and/or biomembranes. Some of the interactions with molecular targets are highly specific, others have pleiotropic properties. Potential modes of action are summarized. As a consequence of the pharmacological properties of SM, several of them are used in medicine to treat disorders and infections. Others are interesting in biotechnology as rational pesticides. Phytomedicine normally employs complex mixtures, as they are present in the producing plant, which may exert additive or even synergistic properties.

**Keywords:** secondary metabolites; ecological functions; herbivores; microbes; signal compounds; molecular modes of action; targets; phytomedicine

### 1.1 Ecological function of secondary metabolites

A typical trait of plants is the production of a high diversity of secondary metabolites (SM) (the number of identified substances exceeds 100 000 at present), including many nitrogen-free (such as terpenes, polyketides, phenolics, saponins and polyacetylenes) and nitrogen-containing compounds (such as alkaloids, amines, cyanogenic glycosides, non-protein amino acids, glucosinolates, alkalamides and peptides). In plants, several major SM, usually from different classes and biochemical pathways, are commonly accompanied by dozens of minor components. Complex mixtures, which

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differ from organ to organ, sometimes between individual plants and regularly between species, are the result.

These compounds are synthesized in plants in a tissue-, organ- and developmental-specific way by specific biosynthetic enzymes (Facchini and De Luca, 2008; Murata *et al.*, 2008). The corresponding genes are regulated accordingly and gene regulation shows all the complexity known for genes encoding enzymes of primary metabolism. It is a particular feature of SM that they are accumulated and stored in high concentrations in the plant organs important for survival and reproduction; SM levels of 1–3% dry weight are regularly seen. In general, hydrophilic compounds are stored in the vacuole, whereas lipophilic substances are deposited in resin ducts, laticifers, trichomes, oil cells, or in the cuticle. As sites of synthesis are not necessary, the sites of storage, long-distance transport by xylem, phloem or via the apoplast have been discovered in some instances (see *Biochemistry of plant secondary metabolism*, for a more detailed discussion).

Although SM were known to mankind for thousands of years (Mann, 1992; Roberts and Wink, 1998) and have been used as dyes (e.g. indigo, shikonin), flavours (e.g. vanillin, capsaicin, mustard oils), fragrances (e.g. rose oil, lavender oil and other essential oils), stimulants (e.g. caffeine, nicotine, ephedrine), hallucinogens (e.g. morphine, cocaine, scopolamine, tetrahydrocannabinol), insecticides (e.g. nicotine, piperine, pyrethrin, rotenone), vertebrate and human poisons (e.g. coniine, strychnine, aconitine, colchicine, cardiac glycosides) and even as therapeutic agents (e.g. atropine, quinine, cardenolides, codeine), their putative biological functions have been a matter of controversy.

Whereas most animals can run or fly away when attacked by a predator (Edmunds, 1974), or possess an immune system to protect them against invading microbes or parasites, these means are apparently not available for plants when molested by herbivores, microbes (bacteria, fungi) and even other plants competing for light, space and nutrients. In contrast to most animals, plants can replace the parts, which have been diseased, wounded or browsed. This capacity for open growth and regeneration, which is most prominent in perennials, allows a certain tolerance towards herbivores and microbes. A number of plants employ mechanical and morphological protection, such as thorns, spikes, glandular and stinging hairs (often filled with noxious chemicals), or develop an almost impenetrable bark (especially woody perennials); these features can be interpreted as antipredatory means (in analogy to weapons and shells in animals).

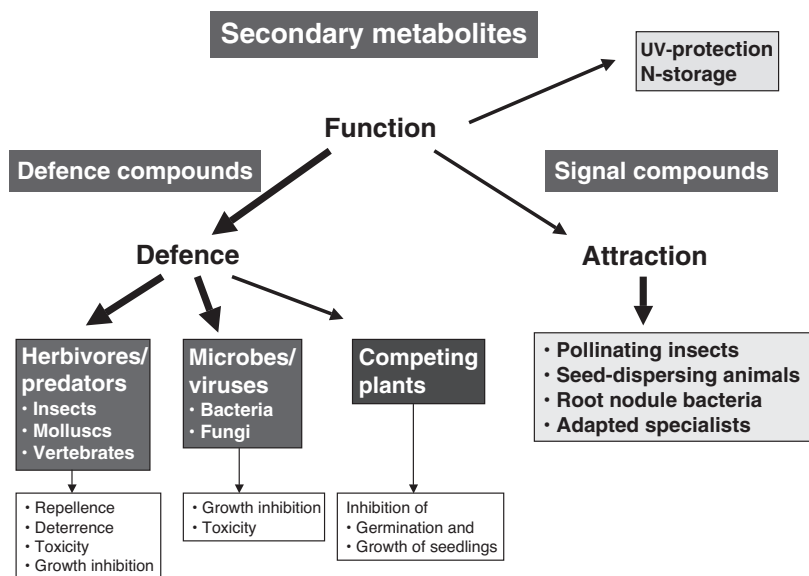
Sessile or slow-moving animals, such as sponges, nudibranch molluscs, corals (see Chapter 3 in this book) and amphibia (e.g. salamanders, poisonous frogs, toads) are infamous for their ability to produce a wide range of chemicals that are usually toxic (for reviews, see Braekman *et al.*, 1998; Proksch and Ebel, 1998). Some insects either produce SM themselves or sequester them from their host plants (for overviews, see Duffey, 1980; Blum, 1981; Bernays and Chapman, 1994; Eisner *et al.*, 2005). Zoologists have never

doubted that these compounds serve for chemical defence against predators. Surprisingly, the defence function of SM in plants has been and sometimes is still controversial.

It had often been argued that SM are waste products or have no function at all (Hartmann, 2007). This hypothesis fails to explain several observations: (1) waste products are characteristic and necessary for heterotrophic animals that cannot degrade their food completely for energy production. These organisms excrete waste products that are often rich in nitrogen (i.e. urea, uric acid). However, plants are essential autotrophs and, therefore, do not need elaborate excretory mechanisms. Furthermore, nitrogen is a limiting nutrient for plants. Consequently, the production of nitrogen-containing excretions, such as alkaloids, would be difficult to explain. In addition, alkaloids are often found in young or metabolically active tissues but not in dying or senescing cells, as would be expected according to the waste product hypothesis. (2) SM are often not inert end products of metabolism (an expected trait of waste products), but many of them can be metabolized by plant cells. For example, nitrogenous SM, such as alkaloids, non-protein amino acids, cyanogenic glucosides or lectins, are often stored in considerable quantities in leguminous seeds. During germination, a degradation of these compounds can be seen, indicating that their nitrogen is reused by the seedling. (3) Secondary metabolism is often highly complex and regulated in a tissue- and developmentally specific manner, which would be surprising for a waste product without function.

Alternatively, it was argued as long as 100 years ago by E. Stahl in Jena (Germany), that SM serve as defence compounds against herbivores (Hartmann, 2007). This hypothesis has been elaborated during recent decades (Fraenkel, 1959; Ehrlich and Raven, 1964; Levin, 1976; Swain, 1977) and a large body of experimental evidence supports the concept that follows (for reviews, see Wink, 1988, 1992, 1993a; 2003b; Harborne, 1993; Bernays and Chapman, 1994). Several SM have evolved for protection against viruses, bacteria, fungi, competing plants and, importantly, against herbivores (e.g. slugs and snails, arthropods and vertebrates). In addition, SM can serve as signal compounds to attract animals for pollination (fragrant monoterpenes, coloured anthocyanins or carotenoids) and for seed dispersal (reviewed by Cipollini and Levey, 1997) (Fig. 1.1). In several instances, both activities are exhibited by the same compounds: anthocyanins or monoterpenes can be insect attractants in flowers but are insecticidal and antimicrobial at the same time. This makes sense, since insects need to be attracted as pollinators, but should not eat the flowers. The pollinators are rewarded by nectar instead. In addition, some SM concomitantly exhibit physiological functions, for example they can serve as mobile and toxic nitrogen transport and storage compounds or ultraviolet-protectants. These multiple functions are typical and do not contradict their main role as chemical defence and signal compounds. If a trait can serve multiple functions, it is more likely to be maintained by natural selection. In this book, Chapters 2 and 4 review some of these aspects.

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**Figure 1.1** Ecological and physiological functions of plant secondary metabolites. (See Plate 1 in colour plate section.)

## 1.2 Presence of defence and signal compounds at the right time and place

In most plants, synthesis and accumulation of SM is regulated in space and time. As a rule, vulnerable tissues are defended more than old, senescing tissues. For example, it is usually observed that seeds, seedlings, buds and young tissues either sequester large amounts of a compound or actively synthesize them – ‘optimal defence theory’. Organs that are important for survival and multiplication, such as flowers, fruits and seeds, are nearly always a rich source of defence chemicals.

The specific localizations of SM make sense if their role as defence and/or signal compounds is accepted. Trichomes and glandular hairs are always on the surface of the plant; a herbivore cannot avoid direct contact with them if it tries to feed on the plant. If membrane-active terpenes reach their lips, tongue or mandibles, many herbivores can be deterred before they actually start feeding on the plant. Another example is the sequestration of high concentrations of SM in vacuoles, which are often positioned in a favourable site for defence, as many of them are stored in epidermal and subepidermal cells (Saunders and Conn, 1978; Kojima *et al.*, 1979; Matile, 1984; Wink *et al.*, 1984; Werner and Matile, 1985; Wink, 1992, 1997; Gruhnert *et al.*, 1994). If a small herbivore or microbe attacks such a plant, it will encounter a high SM concentration immediately at the periphery when wounding or entering the

tissue, which might deter further feeding. Compounds that are sequestered in resin ducts or laticifers are often under high pressure and readily squirt out when these elements become wounded. For a small herbivorous insect, this will be a dangerous situation, since these effluents will make their mandibles sticky. A few 'clever' beetles and caterpillars cut the veins of leaves upstream to the area on which they want to feed. The fluids emerge from the cuts but can no longer reach the parts downstream, which are eaten later (Dussourd and Eisner, 1987; Becerra, 1994).

Several defence compounds are transported via the phloem from the site of synthesis to other plant organs. Since the phloem is a target for many sucking insects, such as aphids, these insects encounter a high load of alkaloids in the plants producing them. For lupins, in which alkaloid-rich and almost alkaloid-free varieties (sweet lupins) are known, it could be shown that aphid generalists (e.g. *Myzus persicae*) sucked only on 'sweet' lupins but never on alkaloid-rich varieties, with high alkaloid contents (Table 1.1) in the phloem (Wink, 1992). Moreover, many other animals, from leaf miners (Agromyzidae) to rabbits (*Oryctolagus cuniculus*) showed a similar discrimination, in that alkaloid-rich plants were left alone, while 'alkaloid-free' cultivars were highly susceptible. The only exception is a specialized aphid, *Macrosiphum albifrons*, which lives on lupins and sequesters the dietary alkaloids, using them for defence against predators (Wink and Römer, 1986).

In general, a series of related compounds is found in each plant: often a few major metabolites and several minor components, which differ in the

**Table 1.1** Relationship between alkaloid content and percentage herbivory by aphids (generalists and specialists) and other herbivores

Species	Alkaloid content mg/g FW	Herbivory (%) by			
		<i>Myzus</i> spp.	Leaf miners	Rabbits	<i>Macrosiphum</i> <i>albifrons</i>
<i>Lupinus albus</i>					
var. <i>lucky</i>	<0.01	20	100	100	< 5
var. <i>lublanc</i>	<0.01	15	100	100	< 5
var. <i>multolupa</i>	0.03	15	100	80	<10
<b>Wild-type from Syria</b>	<b>2.0</b>	<b>0</b>	<b>&lt;1</b>	<b>&lt;10</b>	<b>100</b>
<b>Wild-type from Crete</b>	<b>2.2</b>	<b>0</b>	<b>&lt;1</b>	<b>n.d.</b>	<b>100</b>
<i>L. luteus</i>	0.01	100	n.d.	n.d.	n.d.
	0.25	50	n.d.	n.d.	n.d.
	0.7	< 1	n.d.	<5	0
<b><i>L. polyphyllus</i></b>	<b>1.0</b>	<b>0</b>	<b>&lt;1</b>	<b>&lt;5</b>	<b>80</b>
<b><i>L. angustifolius</i></b>	<b>1.5</b>	<b>0</b>	<b>&lt;1</b>	<b>&lt;10</b>	<b>100</b>

n.d., not determined; FW, fresh weight.

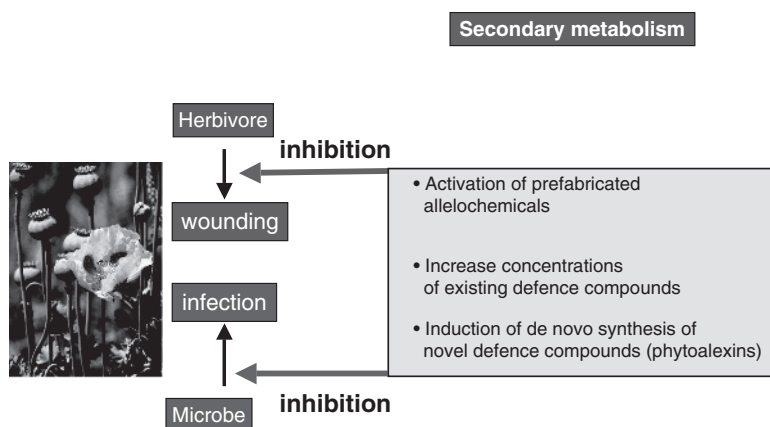
Source: After Wink and Römer (1986), Wink (1987a, 1988, 1992), Wink and Witte (1991).

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position of their functional groups. The profile usually varies between plant organs, within developmental periods and sometimes even diurnally, for example as shown for lupin alkaloids (Wink and Witte, 1984). Furthermore, marked differences can usually be seen between individual plants of a single population and even more so between members of different populations. This variation, which is part of the apparent evolutionary 'arms race' between plants and herbivores, makes adaptation by herbivores more difficult, since even small changes in chemistry can be the basis for a new pharmacological activity (for more details, see Chapter 2 in this book). Furthermore, mixtures can address a multitude of targets and might act additive or even synergistic. There is evidence, for example, that some SM with membrane activities can facilitate the uptake of polar substances and thus increase the bioavailability of allelochemicals (Wink, 2008a).

Defence against herbivores and pathogens is not necessarily constitutive. Research in recent decades has shown that wounding and infection triggers several events in plants. For example, wounding can lead to a decompartmentalization, thus releasing prefabricated defence chemicals and mixing them with hydrolyzing enzymes, such as  $\beta$ -glycosidase, myrosinase, nitrilase or alliinase (Matile, 1980) (Fig. 1.2; Table 1.2). Active allelochemicals are the result.

In other instances, it has been shown that the level of existing defence chemicals is increased substantially within hours or days after wounding or infection, for example nicotine in *Nicotiana tabacum* (Baldwin, 1994) or lupin alkaloids in *Lupinus polyphyllus* (Wink, 1983). After infection, in particular, new compounds with antifungal, antibacterial or herbivore-detering activities are made and sequestered; phytopathologists have termed these compounds 'phytoalexins' (Fig. 1.2). These compounds include, among others, several isoflavones, pterocarpans, furanocoumarins, chalcones and stilbenes.



**Figure 1.2** Examples of induced defence in plants. (See Plate 2 in colour plate section.)

**Table 1.2** Typical 'prodrugs' present in plants that are activated by wounding, infection or in the human body

Secondary metabolites of undamaged tissue	Active metabolite
Cyanogenic glucoside	Hydrogen cyanide
Glucosinolate	Isothiocyanate
Alliin	Allicin
Coumaroylglucoside	Coumarin
Arbutin	Benzoquinone
Salicin, methylsalicylate	Saligenin, salicylic acid
Gein	Eugenol
Bi-desmosidic saponins	Mono-desmosidic saponins
Cardiac glycosides with terminal glucose residues	Cardiac glycosides without terminal glucose residues
Cycasin	Methylazoxymethanol
Ranunculin	Protoanemonine
Tuliposide	Tulipalin
Crocetin	Safranal
Cucurbitacin glycosides	Free cucurbitacins

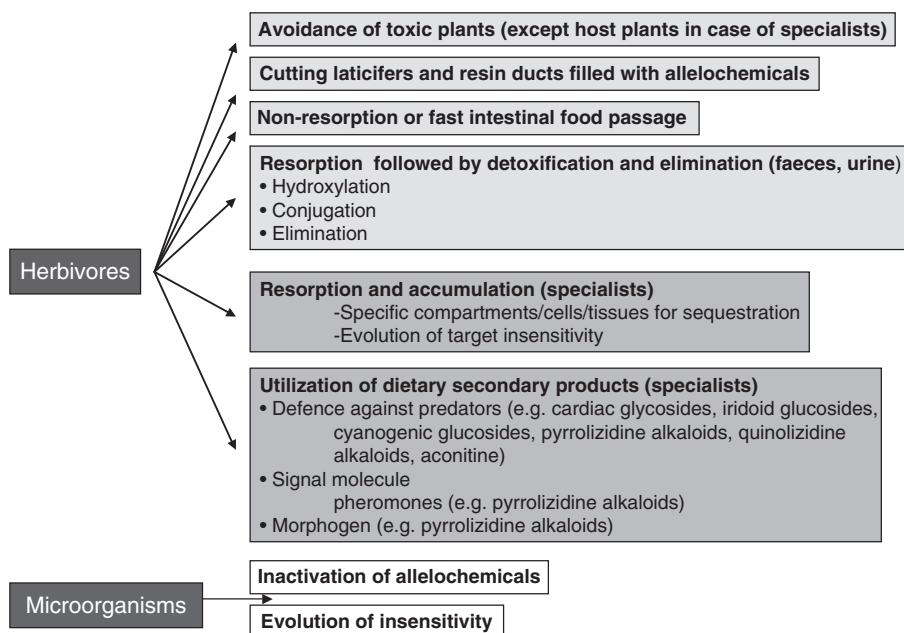
Many of these metabolites have antifungal properties, so that they are sometimes considered to be part of the specific antimicrobial defence system of plants. However, since most of these compounds also affect herbivores, the plant defence induced appears to be a more general phenomenon (see also Chapter 4 in this book).

Recent research has shown that elicitors, receptors, ion channels, salicylic acid and the pathway leading to jasmonic acid and methyljasmonate are important elements in converting the external signal into a cellular response (Creelman and Mullet, 1997).

The SM defence system works in general but a number of herbivores and microorganisms have evolved that have overcome the defence barrier (analogous to the situation in which some viruses, bacteria or parasites overwhelm the human immune system). In these organisms, a series of adaptations can be observed, allowing them to tolerate or even use the dietary defence chemicals (a schematic overview is presented in Fig. 1.3) (for reviews, see Ahmad, 1983; Brattsten and Ahmad, 1986; Rosenthal and Berenbaum, 1991/1992; Wink, 1993a; Bernays and Chapman, 1994; Brown and Trigo, 1995; Hartmann and Witte, 1995).

Several volatiles are produced by plants when wounded, including aldehydes, esters, amines or ethylene. It has been proposed that some of these volatiles can alert the defence system of neighbouring plants. In addition, they can attract predatory arthropods. A well-studied example is that of spider mites (*Tetranychus urticae*) on *Phaseolus lunatus* leaves. Volatiles from infested plants attract predatory mites (*Phytoseiulus persimilis*), which prey on the mites that induced the reaction in the first place (Dicke *et al.*, 1990; De

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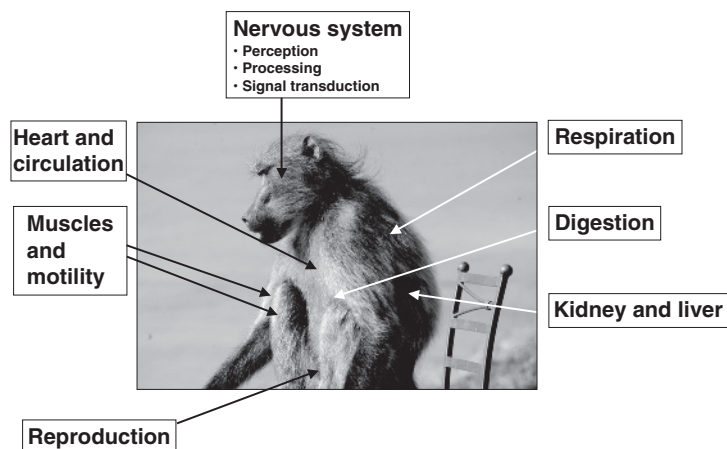
**Figure 1.3** Adaptations of specialist herbivores and pathogens. (See Plate 3 in colour plate section.)

Moraes *et al.*, 1998). It is likely that more tritrophic systems work in this way; many of them still await discovery.

Chemical defence is not only obvious in terrestrial ecosystems but also of major importance in the survival of marine organisms. In this book, Chapter 3 provides an overview of this exciting and rapidly growing research field.

### 1.3 Molecular modes of action of SM

If defence compounds inhibit the growth of microbes or herbivores or are otherwise toxic to them, they must interfere with the physiology and biochemistry of these organisms. A large body of pharmacological and toxicological literature clearly documents that these activities do exist (Wink, 1993a,b, 1999, 2000, 2007, 2008a; Teuscher and Lindequist, 1994). Typical organ systems that are often affected by SM in animals are schematically illustrated in Fig. 1.4. Typical for animals are nerves and muscles; it is not surprising that many SM, especially alkaloids, immediately modulate elements of neuronal signal transduction, neuromuscular signalling and muscle contractions. If these defence compounds are animal specific, plants have the advantage that these compounds are not toxic for them (as nerves and muscles are absent in plants). In case of defence compounds with a broader specificity or which also affects



**Figure 1.4** Targets for allelochemicals in animals. (See Plate 4 in colour plate section.)

targets in plants, special mechanisms are required in order to avoid autotoxicity, such as sequestration in specialized cells and tissues (see Vol. 2, Chapter 1).

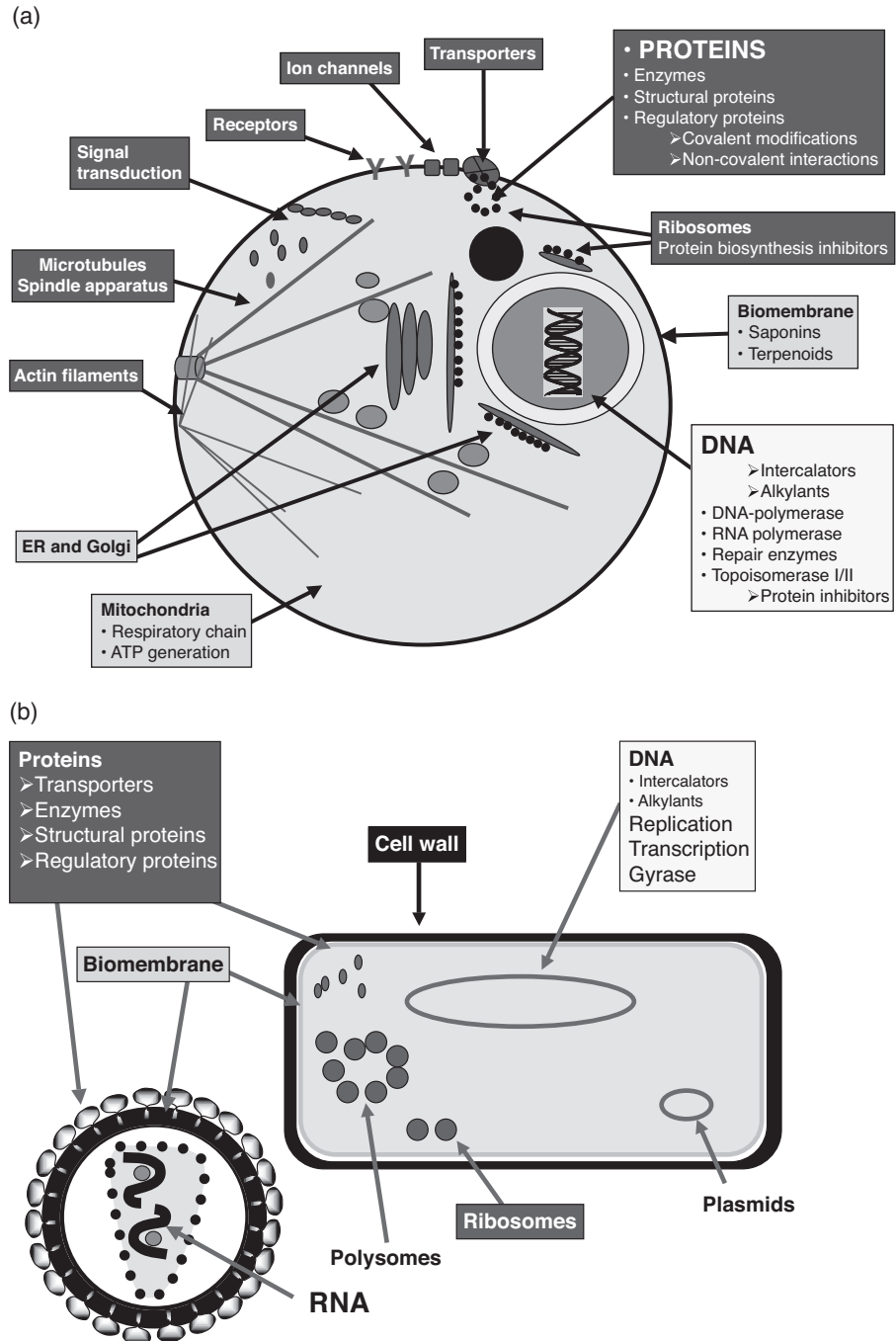
In many instances, the mechanisms, which underlie these effects, have been elucidated; often specific interactions with one or several of the molecular targets shown in Fig. 1.5 can be observed. It has been argued that some defence compounds have been shaped during evolution to specifically interact with particular targets in a process termed ‘evolutionary molecular modelling’ (Wink, 1997). In this book, Chapters 2 and 4 explore this topic in more detail.

SM or toxins negatively interfere with molecular targets in cells of herbivores or pathogens. Major cellular targets (Fig. 1.5; Table 1.3) include:

- The biomembrane
- Proteins (including receptors, ion channels, enzymes, transporters, regulatory proteins, structure proteins, cytoskeleton, mitotic spindle [microtubules], transcription factors, hormones)
- Nucleic acids (DNA, RNA)

Allelochemicals can act as agonists or antagonists at a given molecular target. If this happens to a crucial cellular target, severe negative consequences result. For example, if a compound is cytotoxic to individual kidney cells, the effect will usually damage the function of the kidney. Organ damage can be so serious that it leads to coma and death. Acute cytotoxins are those, which inhibit or kill cells with high rates of protein synthesis, such as liver cells. Therefore, such cells are a prime target for many cytotoxic substances. In contrast, chronic exposure to mutagenic substances does not lead to immediate death but can cause cancer or teratogenic effects (for details, see Chapter 2 in this book).

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**Figure 1.5** Molecular targets of defence chemicals in animal cells (a, b). (See Plate 5 in colour plate section.)

**Table 1.3** Interaction of representative secondary metabolites with molecular targets

<b>Target</b>	<b>Activity</b>	<b>Secondary metabolites (examples)</b>
Biomembrane	Membrane disruption Disturbance of membrane fluidity Inhibition of membrane proteins	Saponins Small lipophilic SM Small lipophilic SM
Proteins Changing protein conformations	Non-covalent bonding	Phenolic SM (phenylpropanoids, flavonoids, catechins, tannins, lignans, quinones, anthraquinones, some isoquinoline alkaloids)
	Covalent bonding	Isothiocyanates Sesquiterpene lactones, allicin, protoanemonine, furanocoumarins, iridoids (aldehydes), SM with aldehyde groups, SM with exocyclic CH <sub>2</sub> groups SM with epoxide groups SM with cyclopropane rings
Specific interactions	Inhibition of enzymes Modulation of regulatory proteins Inhibition of ion pumps Inhibition of microtubule formation Inhibition of protein biosynthesis Inhibition of transporters	Hydrogen cyanide from cyanogens, many structural mimics Phorbol esters, caffeine Cardiac glycosides; Vinblastine, colchicine, taxol, podophyllotoxin, Emetine, lectins Non-protein amino acids

(Continued)

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**Table 1.3** (Continued)

<b>Target</b>	<b>activity</b>	<b>SM (examples)</b>
DNA/RNA	Modulation of hormone receptors	Genistein, many other isoflavonoids
	Modulation of neuroreceptors	Many alkaloids, some non-protein amino acids
	Modulation of ion channels	Aconitine, many alkaloids; conotoxins, tetrodotoxin, saxitoxin, gonyautoxin,
	Modulation of transcription factors	Cycloparmine, hormone mimics
	Covalent modifications (alkylation)	Pyrrolizidine alkaloids, cycasine
	Intercalation	Aristolochic acids, Furanocoumarins, SM with epoxy groups
	Inhibition of DNA topoisomerase I	Planar, aromatic and lipophilic SM
	Inhibition of transcription	Sanguinarine, berberine, emetine, quinine, furanocoumarins; anthraquinones
		Camptothecin, berberine
		Amanitine

The **biomembrane** is affected by lipophilic or amphiphilic compounds (Table 1.3), which are widely present among terpenoids. These compounds will interact with the lipophilic inner core of biomembranes represented by phospholipids and cholesterol. They can form transient pores or may even solubilize biomembranes. These compounds can additionally interact with membrane proteins, such as ion channels, transporters and receptors, thereby influencing signal transduction and transport processes in cells and tissues. These interactions are widely non-specific. Therefore, membrane-active SM can promote cytotoxic effects in animal, bacterial and fungal cells, even in some enveloped viruses. Some saponins may facilitate the absorption of polar compounds (see discussion in Wink, 2008a).

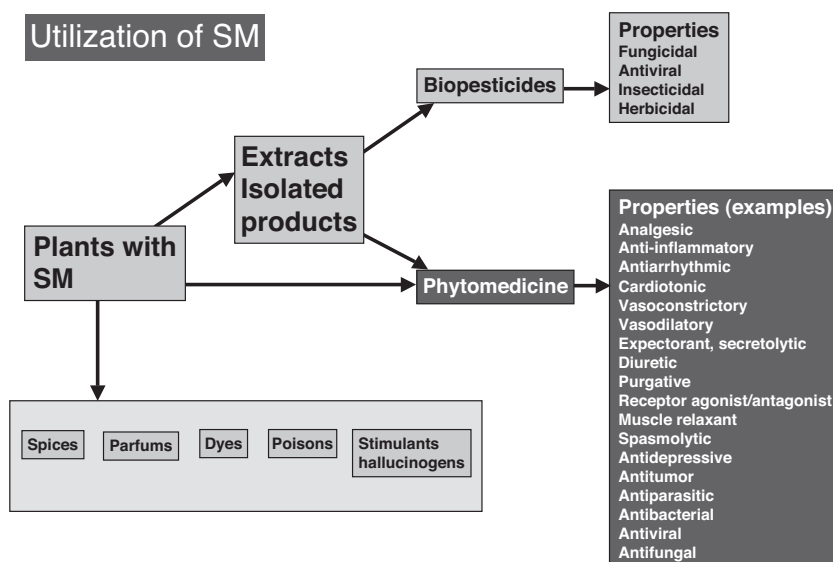
**Proteins** are the main players in cells, important for metabolism, structures, motility, cell division, gene regulation and communication. Proteins need to have the correct three-dimensional shape (conformation) in order to recognize their substrates, ligands and other protein partners. SM very often are capable of interfering with proteins (Table 1.3), especially by inducing conformational changes. These conformational changes can either activate (agonists) or inactivate (antagonist) a protein. We can distinguish between covalent and non-covalent interactions. Some SM have highly reactive functional groups, such as aldehydes, epoxides, sulfhydryls, exocyclic methylenes or cyclopropanes, which can make covalent bonds with functional groups of proteins (Table 1.3). Phenolic compounds carry phenolic hydroxyl groups that can dissociate under physiological conditions into negatively charged  $O^-$  groups. These  $O^-$  groups can form hydrogen bonds or more stable ionic bonds with positively charged amino acid residues (as present in lysine, arginine or histidine). A single of such non-covalent bonds is weak and hardly influences protein conformation. In polyphenols, we usually see several phenolic OH groups; together, they can react cooperatively and effectively induce conformational changes. Such interactions are likely to be the cause for many of the adverse effects of SM on herbivores, microbes and viruses (Wink and Van Wyk, 2008) (for details, see Chapter 2 in this book).

**Nucleic acids**, such as DNA, rRNA, mRNA and the corresponding enzymes for replication, transcription and repair, are another major target for allelochemicals. Nucleic acids can be modified by alkylation and intercalation (Table 1.3). These interactions with DNA can lead to point mutations, which can cause amino acid substitutions or frame shift mutations, that usually results in detrimental effects if the mutations are not repaired by repair enzymes (for details, see Chapter 2 in this book).

## 1.4 Biotechnology and utilization of SM

Since SM have evolved as compounds that are important for the fitness of the organisms producing them, many of them interfere with the pharmacological targets, which make them interesting for several biotechnological

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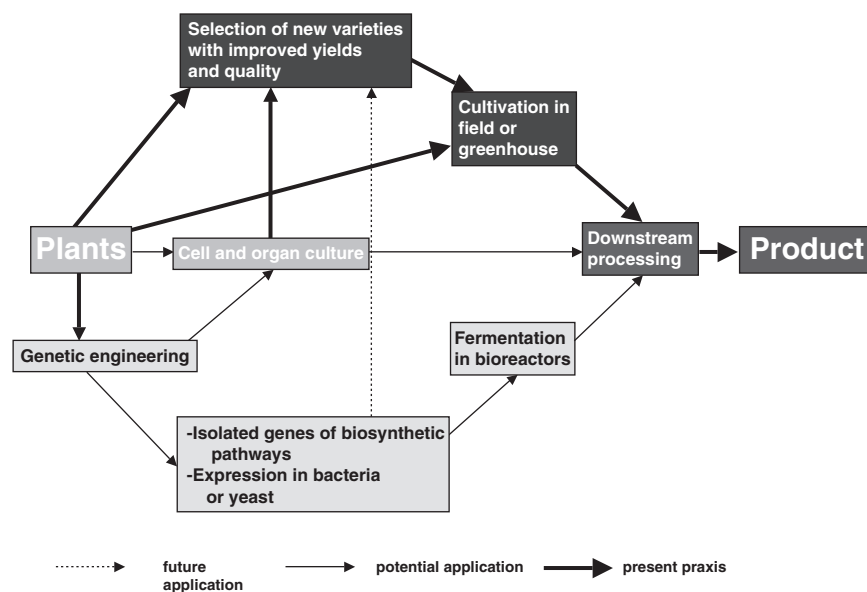
**Figure 1.6** Utilization of secondary metabolites (SM) in biotechnology. (See Plate 6 in colour plate section.)

applications (an overview is presented in Fig. 1.6). A main area is phytomedicine, and several thousand plants are in use worldwide to treat human ailments and diseases (Fig. 1.6). In addition to isolated substances with established pharmacological profiles (including potent antineoplastic drugs, such as the alkaloids vinblastine, vincristine or taxol) (Wink, 2007), complex extracts or even crude plant drugs are often used (Wink, 2008a). Controlled clinical studies have shown the efficacy of several, for example extracts from *Ginkgo biloba*, *Hypericum perforatum*, *Piper methysticum*, *Chamomilla recutita*, *Crataegus monogyna*, *Silibum marianum*, *Melissa officinalis*, *Mentha x piperita*, *Valeriana officinalis* (Wagner and Wiesenauer, 1995; van Wyk and Wink, 2004). The use of stimulants (such as caffeine, nicotine, ephedrine), fragrances (several essential oils), flavours (essential oils, capsaicin, piperine, etc.), natural dyes, poisons (strychnine) and hallucinogens (morphine, heroin, cocaine, tetrahydrocannabinol) is based on SM (Fig. 1.6). In this book, Chapters 4 and 5 explore this wide field in more detail.

Since many SM are insecticidal, fungicidal and phytotoxic, they may be used in agriculture as natural plant protectants. Before the advent of synthetic pesticides about 60 years ago, plant-derived insecticides (including nicotine, rotenone, quassin, ryanodine, pyrethrins and azadirachtins) were a common theme (Jacobson and Crosby, 1971; Wink, 1993b). Applications unequivocally showed that these natural insecticides worked. One ecological advantage, that is that SM are readily degraded in plants and in soil, is also their disadvantage and synthetic pesticides are more resistant and persistent.

Moreover, modern pesticides are usually more potent than biopesticides. On the other hand, plants are easy to grow and biopesticides could be a sustainable source of plant protectants for farmers in countries that do not have access to Western synthetic pesticides. Unfortunately, legislation does not favour mixtures of compounds to be used as pesticides; therefore, the development of biorational pesticides has to face many obstacles. Nevertheless, natural compounds do provide an underexplored alternative (for review, see Wink, 1993b).

As a consequence of these various applications, a world market for plant extracts and isolated SM exists, which exceeds 10 billion US dollars annually (Balandrin *et al.*, 1985). Therefore, it is a challenge for biotechnologists to find ways to produce these compounds in sufficient quantity and quality (Wildi and Wink, 2002; see Chapter 6 of this volume). The main and traditional way is to grow the respective plants in the field or in greenhouses and to extract the products from them (Fig. 1.7). For several species, new varieties have been selected with improved yields and quality. In this context, cell and organ culture are important techniques for *in vitro* propagation. In a few instances, genetic engineering of secondary metabolism has already had a direct influence; for example, when *Atropa belladonna* plants were transformed with the gene that encodes the enzymes converting L-hyoscyamine into L-scopolamine, new plants were generated which produced scopolamine as the major product (Hashimoto and Yamada, 1992). More often, flavonoid metabolism has been



**Figure 1.7** Strategies for the production of secondary metabolites. (See Plate 7 in colour plate section.)

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altered genetically, producing plants with different flower colours (Mol *et al.*, 1998).

It is a challenge for future research to isolate the genes of biosynthetic pathways and to express them either in transgenic plants or in microbes. If successful, recombinant bacteria or yeasts might be grown some day, which will produce valuable plant SM (Wink, 1989; Kutchan, 1995; Facchini, 2001) (Fig. 1.7); combinatorial biosynthesis might then be an open field. Using genes encoding enzymes for the biosynthesis of antibiotics, this strategy has already been successful (Katz and Hutchinson, 1992; Katz and Donadio, 1993; Mc-Daniels *et al.*, 1993). Interest in growing and manipulating microorganisms and plants in cell culture for commercial purposes (Verpoorte *et al.*, 2007; Oksman-Caldentey *et al.*, 2007) has given impetus to the study of the biosynthesis of alkaloids and other SM and, in particular, to the elucidation of the enzymes involved. It has also brought about renewed interest in the regulation of SM synthesis and in the location and means of sequestration of these substances within the plant. In recent years, attempts have been made to express the genes of alkaloid biosynthesis in microorganisms (Marasco and Schmidt-Dannert, 2007; Minami *et al.*, 2008; Wu and Chappell, 2008; Schäfer and Wink, 2009). Ultimately, it might be possible to produce valuable alkaloids from recombinant bacteria or yeast.

If the corresponding SM (both from plant or microbial origin) confer resistance to insects or pathogens, genetic transformation of susceptible crop plants could be another valuable avenue for exploitation.

For more than two decades, scientists around the world have tried to produce valuable SM in cell or organ cultures (for overviews, see Neumann *et al.*, 1985; Constabel and Vasil, 1987; Kurz, 1989; Charlwood and Rhodes, 1990). Whereas undifferentiated cell cultures have often failed to produce such a compound in reasonable yields, differentiated organ cultures (e.g. transformed root cultures) are often as active as the intact plant (Rhodes *et al.*, 1990; Wink *et al.*, 2005). Cell- and tissue-specific gene expression appears to control these processes. In this book, Chapter 6 addresses the production of SM *in vitro*. It is possible that genetic engineering may help to improve plant cell cultures as biotechnological production systems in the future.

### 1.5 Conclusions

Plant secondary metabolism is still an interesting and challenging field of scientific endeavour ranging from botany, plant physiology and biochemistry, chemistry, pharmacology and medicine to evolution, ecology and biotechnology. It is the aim of the two volumes (*Biochemistry of Plant Secondary Metabolism and Functions* and *Biotechnology of Plant Secondary Metabolites*) to highlight recent results and to stimulate young researchers to enter a field that looks back on a long history but nevertheless provides an interesting present-day research topic and will hopefully experience an exciting future.

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