16

Secondary metabolites fulfill specific ecological functions in plants

In addition to **primary** metabolites such as carbohydrates, amino acids, fatty acids, cytochromes, chlorophylls, and metabolic intermediates of the anabolic and catabolic pathways, which occur in all plants and where they all have the same metabolic functions, plants also produce a large variety of compounds, with no apparent function in the primary metabolism, and therefore are called **secondary** metabolites. Certain secondary metabolites are restricted to a few plant species where they fulfill specific ecological functions, such as attracting insects to transfer pollen, or animals for consuming fruits to distribute seeds, or as **natural pesticides** that act as defense compounds to combat herbivores and pathogens.

16.1 Secondary metabolites often protect plants from pathogenic microorganisms and herbivores

Plants, because of their protein and carbohydrate content, are an important food source for many animals, such as insects, snails, and many vertebrates. Since plants cannot run away, they have had to evolve strategies that make them indigestible or poisonous to protect them from being eaten. Many plants protect themselves by producing toxic proteins (e.g., amylase, proteinase inhibitors or lectins), which impair the digestion of herbivores (section 14.4). In response to caterpillar feeding, maize plants mobilize a protease that destroys the caterpillar's intestine. To secondary metabolites belong alkaloids (this chapter), isoprenoids (Chapter 17), and phenylpropanoids (Chapter 18), all of which include natural pesticides that protect plants against herbivores and pathogenic microorganisms. In some plants these natural pesticides amount to 10% of the dry matter.

Some defense compounds against herbivores are part of the permanent outfit of plants; they are **constitutive**. Other defense components are only synthesized by the plant after browsing damage (**induced defense**). Section 18.7 describes how acacias, after feeding damage, produce more tannins, thus making the leaves inedible. Another example, as described in section 15.7, is when plants damaged by caterpillars use the synthesis of scents (volatile secondary metabolites) to attract parasitic wasps, which lay their eggs in the caterpillars, thus killing them (**indirect defense**).

Microorganisms can be pathogens

Certain fungi and bacteria infect plants in order to utilize their resources for their own nutritional requirements. As this often leads to plant diseases, these infectants are called **pathogens**. In order to infect the plants effectively, the pathogenic microbes produce aggressive substances such as enzymes, which degrade the cell walls, or toxins, which damage the plant. An example is **fuscicoccin** (section 10.3), which is produced by the fungus *Fusicoccum amygdalis*. The production of compounds for infecting plants requires the presence of specific **virulence genes**. Plants protect themselves against pathogens by producing defense compounds that are encoded by specific **resistance genes**. The interaction of the virulence genes and resistance genes decides the success of the attack and defense.

When a plant is susceptible and the pathogen is aggressive, it leads to a disease, and the pathogen is called **virulent**. Such is termed a **compatible interaction**. If, on the other hand, the infecting pathogen is killed or at least its growth is very much retarded, this is an **incompatible interaction**, and the plant is regarded as **resistant**. Often just a single gene decides on compatibility and resistance between pathogen and host.

Plants synthesize phytoalexins in response to microbial infection

Defense compounds against microorganisms, especially fungi, are synthesized mostly in response to an infection (induced defense). These inducible defense substances, which are produced within hours, are called **phytoalexins** (*alekein*, Greek, to defend). Phytoalexins comprise a large number of compounds with very different structures such as isoprenoids, flavonoids, and stilbenes, many of which act as antibiotics against a broad spectrum of pathogenic fungi and bacteria. Plant root exudates contain bacteriostatic compounds such as cumaric acid, 3-indol propionic acid and methyl *p*-hydroxybenzoate that can render a plant resistant against pathogens. Plants produce for defense aggressive oxygen compounds such as superoxide radicals ($\bullet O_2^-$) and H_2O_2, as well as nitrogen monoxide (NO) (section 19.9), and enzymes, such as β-glucanases, chitinases, and proteinases, which damage the cell walls of bacteria and fungi. Also the emission of volatile metabolites is induced after pathogen attack, which directly or indirectly can alarm defense reactions in the plant or in plants in the neighborhood. The synthesis of these various defense substances is induced by so-called **elicitors**. Elicitors are often proteins excreted by the pathogens to attack plant cells (e.g., cell-degrading enzymes). Moreover, polysaccharide segments of the cell's own wall, produced by degradative enzymes of the pathogen, function as elicitors. But elicitors can also be fragments from the cell wall of the pathogen, released by defense enzymes of the plant. These various elicitors are bound to specific receptors on the outer surface of the plasma membrane of the plant cell. The binding of the elicitor releases signal cascades in which protein kinases (section 19.1) and signal substances such as salicylic acid (section 18.2) and jasmonic acid (section 15.7) participate, and which finally induce the **transcription of genes** for the synthesis of phytoalexins, reactive oxygen compounds, and defense enzymes (section 19.9).

Elicitors may also cause an infected cell to die and the surrounding cells to die with it. In other words, the infected cells and those surrounding it commit suicide. This can be caused, for instance, by the production of phenols of the infected cells to poison not only themselves but also their surrounding cells. This programmed cell death, called a **hypersensitive response**, serves to protect the plant. The cell walls around the necrotic tissue are strengthened by increased biosynthesis of lignin, and in this way the plant barricades itself against further spreading of the infection.

Plant defense compounds can also be a risk for humans

Substances toxic for animals are, in many cases, also toxic for humans. In crop plants, toxic or inedible secondary metabolites have been eliminated or at least decreased by breeding. This is why cultivated plants usually are more sensitive to pests than wild plants, thus necessitating the use of external pest control, which is predominantly achieved by the use of chemicals. Attempts to breed more resistant crop plants by crossing them with wild plants, however, may lead to problems, e.g., a newly introduced variety of insect-resistant potato had to be taken off the market because the highly toxic solanine content (an alkaloid, see following section) made these potatoes unsuitable for human consumption. In a new variety of insect-resistant celery cultivated in the United States, the 10-fold increase in the content of psoralines (section 18.2) caused severe skin damage to people harvesting the plants. This illustrates that natural pest control is not without risk.

A number of plant constituents that are harmful to humans (e.g., proteins such as lectins, amylase inhibitors, proteinase inhibitors, and cyanogenic glycosides or glucosinolates (dealt with in this chapter)) decompose when cooked. But most secondary metabolites are not destroyed in this way. In higher concentrations, many plant secondary metabolites are cancerogenic. It has been estimated that in industrialized countries more than 99% of all cancerogenic compounds that humans normally consume with their diet are plant secondary metabolites that are natural constituents of the food. However, experience has shown that the human metabolism usually provides sufficient protection against many harmful natural substances particularly at lower concentrations. As will be discussed in the following, plants also contain many compounds which are used as pharmaceuticals to combat illnesses.

16.2 Alkaloids comprise a variety of heterocyclic secondary metabolites

Alkaloids belong to a group of secondary metabolites that are synthesized from **amino acids** and contain one or several N atoms as constituents of **heterocycles**. Many of these alkaloids act as defense compounds against animals and microorganisms. Since alkaloids usually are bases, they are stored in the protonated form, mostly in the vacuole which is acidic. Since ancient times humans have used alkaloids in the form of plant extracts as poisons, stimulants, and narcotics, and, last but not least, as medicine. In 1806 the pharmacy assistant Friedrich Wilhelm Sertürner isolated morphine from poppy seeds. Another 146 years had to pass before the structure of morphine was finally resolved in 1952. More than 10,000 alkaloids of very different structures are now known. Their biosynthesis pathways are very diverse, to a large extent still not known, and will not be discussed here.

Figure 16.1 shows a small selection of important alkaloids. Alkaloids are classified according to their heterocycles. **Coniine**, a piperidine alkaloid, is a very potent poison in hemlock. Socrates died when he was forced to drink this poison. **Nicotine**, which also is very toxic, contains a pyridine and a pyrrolidine ring. It is synthesized in the roots of tobacco plants and is carried along with the xylem sap into the stems and leaves. Nicotine sulfate, a by-product of the tobacco industry, is used as a very potent insecticide (e.g., for fumigating greenhouses). There is no insect known to be resistant to nicotine. Genetically modified tobacco plants where the nicotine content

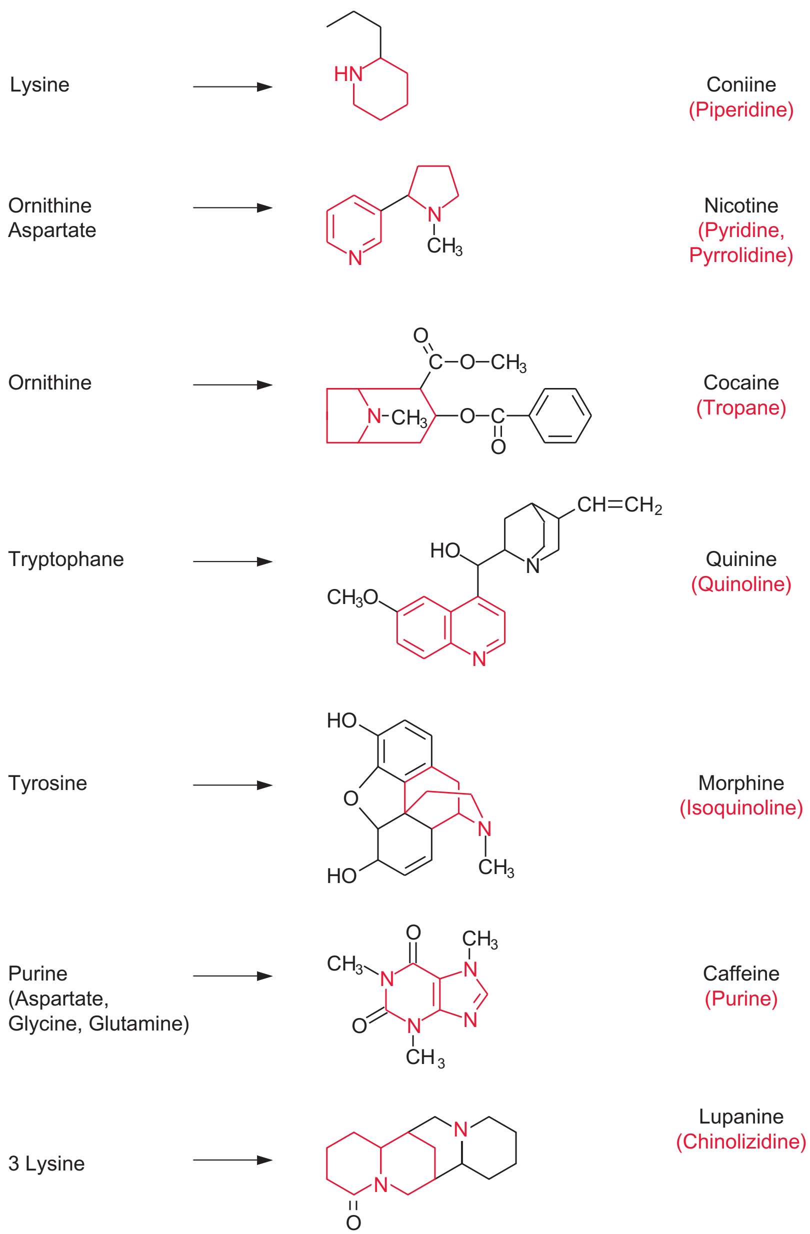

Figure 16.1 Some alkaloids and the amino acids from which they are synthesized. The heterocycles, after which the alkaloids are classified, are colored red; their names are given in brackets. A synthesis of coniine from acetyl CoA has also been described. Purine is synthesized from aspartate, glycine, and glutamine.

was decreased by 96% were shown to be highly infested by the caterpillar *Maduca sexta*. **Cocaine**, the well-known narcotic, contains **tropane** as a heterocycle, in which the N atom is a constituent of two rings. A further well-known tropane alkaloid is **atropine** (formula not shown), a poison accumulated in deadly nightshade (*Atropa belladonna*). In low doses, it dilates the pupils of the eye and is therefore used in medicine for eye examination. Cleopatra allegedly used extracts containing atropine to dilate her pupils to appear more attractive. **Quinine**, a quinoline alkaloid from the bark of *Chinchuna officinalis* growing in South America, was known by the Spanish conquerors to be an antimalarial drug. The isoquinoline alkaloid **morphine** is an important pain killer and is also a precursor for the synthesis of heroin. **Caffeine**, the stimulant of coffee, has purine as the heterocycle. **Chinolizidin alkaloids**, such as **lupinin** and **lupanin**, which primarily accumulate in varieties of lupines, are synthesized from three lysine molecules. Due to the toxicity of these compounds sheep frequently die in the autumn from eating too much lupine seed. **Pyrrolizidin** alkaloids, such as **senecionin** (formula not shown) are synthesized by plants to combat herbivores. These compounds, however, are not harmful to certain specifically adapted herbivores, which accumulate them and thus render themselves poisonous towards predators, parasitoids and pathogens.

In order to search for new medicines, large numbers of plants are being analyzed for their secondary metabolite contents. One result is the alkaloid **taxol**, isolated from the yew tree *Taxus brevifolia*, now used for cancer treatment. Derivatives of the alkaloid camptothezine from the Chinese "happy tree" *Camptotheca acuminata* are also being clinically tested as cancer therapeutics. The search for new medicines against malaria and viral infections continues. Since large quantities of pharmacologically interesting compounds often cannot be gained from plant material, attempts are being made with the aid of genetic engineering either to increase production in the corresponding plants or to transfer the plant genes into microorganisms in order to use the latter for production.

16.3 Some plants emit prussic acid when wounded by animals

Since **prussic acid** (**HCN**) inhibits cytochrome oxidase which is the final step of the respiratory chain, it is a very potent poison (section 5.5). Ten percent of all plants are estimated to use this poison as a defense strategy against being eaten by animals. The consumption of peach kernels, for instance, or bitter almonds can have fatal consequences for humans. Since plants also possess a mitochondrial respiratory chain, in order not to poison themselves, prussic acid is bound in a non-toxic form as **cyanogenic glycoside**, e.g., amygdalin (Fig. 16.2), which is present in the kernels and roots of peaches. The cyanogenic glycosides are stored as stable compounds in the vacuole. The **glycosidase**, which catalyzes the hydrolysis of the glycoside, is present in

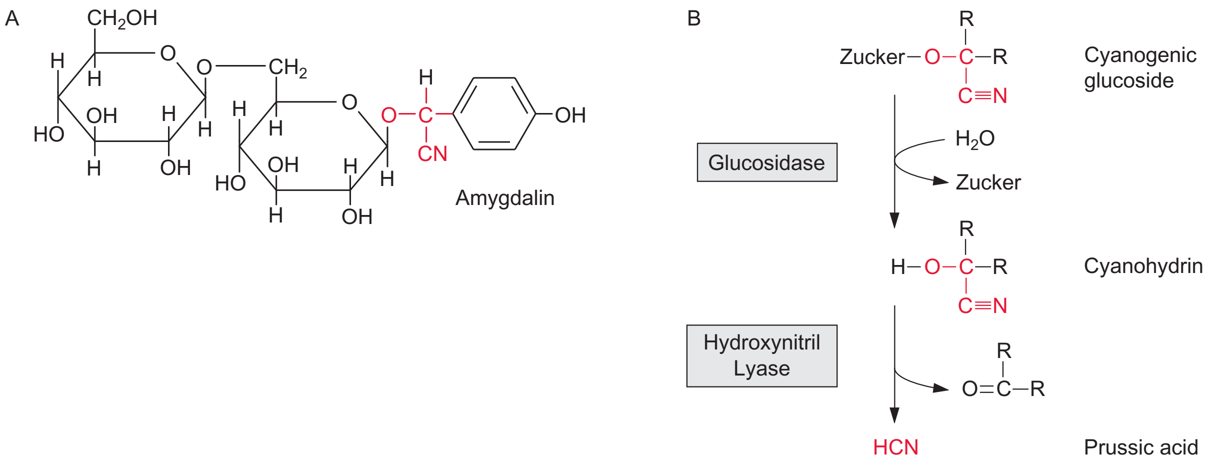

Figure 16.2 A. Amygdalin, a cyanogenic glycoside, accumulates in some stone fruit kernels. B. After the sugar residue has been cleaved off by hydrolysis, cyanohydrin is released from cyanogenic glycosides, which decomposes spontaneously to prussic acid and a carbonyl compound.

another compartment (cytosol). If the cell is wounded by feeding animals, the compartmentation is disrupted and the glycosidase comes into contact with the cyanogenic glycoside. After the hydrolysis of the glucose residue, the remaining cyanhydrin is very unstable and decomposes spontaneously to prussic acid and an aldehyde. A **hydroxynitrile lyase** enzyme accelerates this reaction. The aldehydes synthesized from cyanogenic glycosides are often very toxic. For a feeding animal, the detoxification of these aldehydes can be even more difficult than that of prussic acid. Due to the formation of the two different toxic compounds, cyanogenic glycosides are a very effective defense system.

16.4 Some wounded plants emit volatile mustard oils

Glucosinolates, also called mustard oil glycosides, have a similar protective function against herbivores as cyanogenic glycosides. Glucosinolates can be found, for instance, in radish, several cabbage varieties, and mustard. Cabbage contains the glycoside glucobrassicin (Fig. 16.3), which is synthesized from tryptophan. The hydrolysis of the glycoside by a **thioglucosidase** results in a very unstable product from which, after the liberation and rearrangement of the sulfate residue, an isothiocyanate, also termed **mustard oil**,

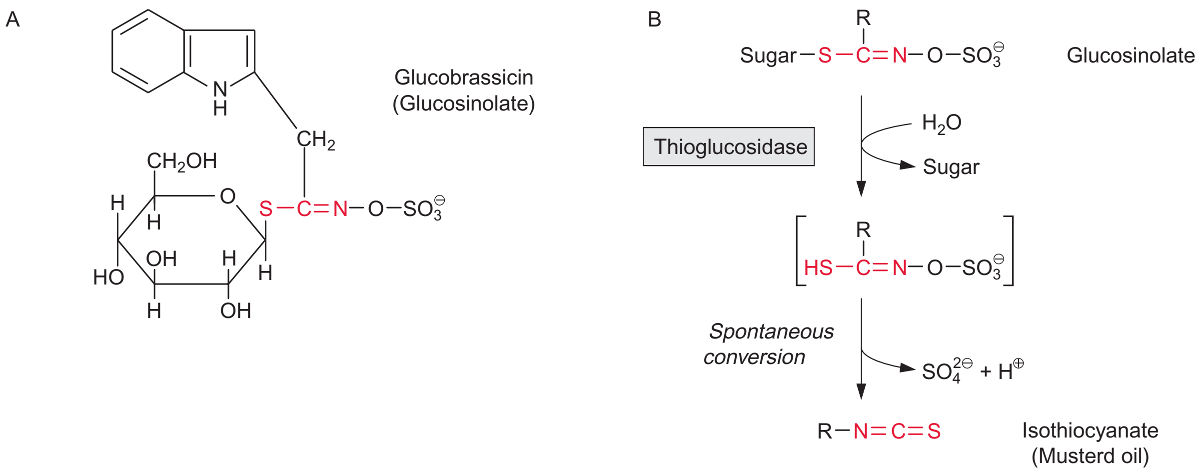

Figure 16.3 A. Glucobrassicin, a glucosinolate from cabbage. B. The hydrolysis of the glycoside by thioglucosidase results in an unstable product, which decomposes spontaneously into sulfate and isothiocyanate.

is spontaneously released. Depending on the cellular pH nitriles, thiocyanate and oxazolidin 3-thion can also be formed as glucosinolate products. Mustard oils are toxic in higher concentrations. As is the case of the cyanogenic glycosides, glucosinolates and the hydrolyzing enzyme thioglucosidase are also located in separate compartments of the plant tissues. The enzyme comes into contact with its substrate only after wounding. When cells of these plants have been damaged, the pungent smell of mustard oil can easily be detected (e.g., in freshly cut radish). The high glucosinolate content in early varieties of rape seed made the pressed seed unsuitable for fodder. Nowadays, as a result of successful breeding, rape seed varieties are cultivated without glucosinolate in the seeds and the pressed seeds are a valuable fodder due to their high protein content.

16.5 Plants protect themselves by tricking herbivores with false amino acids

Many plants contain unusual amino acids with a structure very similar to that of protein building amino acids (e.g., **canavanine** from Jack bean (*Canavalia ensiformis*), a structural analogue of **arginine** (Fig. 16.4)). Herbivores take up canavanine with their food. During protein biosynthesis, the arginine-transfer RNAs of animals cannot distinguish between arginine

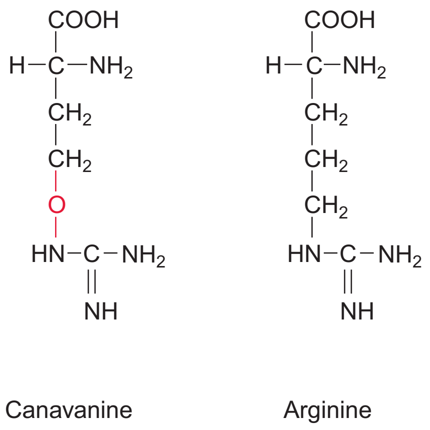

Figure 16.4 Canavanine is a structural analogue of arginine.

and canavanine and incorporate canavanine instead of arginine into proteins. This exchange can alter the three-dimensional structure of proteins, which then lose their biological function partially or even completely. Therefore canavanine is toxic for herbivores. In those plants which synthesize canavanine, the arginine transfer RNA does not react with canavanine, therefore it is not toxic for these plants. This same protective mechanism is used by some insects, which are specialized in eating leaves containing canavanine.

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