1 Introduction: biochemistry, role and biotechnology of secondary metabolites
Michael Wink

1.1 Introduction

A characteristic feature of plants is their capacity to synthesize an enormous variety of low molecular weight compounds, the so-called secondary metabolites (SMs). Although only 20–30% of higher plants have been investigated so far, several tens of thousands of SMs have already been isolated and their structures determined by mass spectrometry (electron impact [EI]-MS, chemical ionisation [CI]-MS, fast atom bombardment [FAB]-MS), nuclear magnetic resonance ($^1$H-NMR, $^{13}$C-NMR) or X-ray diffraction (Harborne, 1993; DNP, 1996). In Table 1.1, an estimate of the numbers of known secondary metabolites is given. Representative structures are presented in Figure 1.1.

<table>
<thead>
<tr>
<th>Type of secondary metabolite</th>
<th>No. *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen-containing</td>
<td></td>
</tr>
<tr>
<td>Alkaloids</td>
<td>12000</td>
</tr>
<tr>
<td>Nonprotein amino acids (NPAA-s)</td>
<td>600</td>
</tr>
<tr>
<td>Amines</td>
<td>100</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>100</td>
</tr>
<tr>
<td>Glucosinolates</td>
<td>100</td>
</tr>
<tr>
<td>Without nitrogen</td>
<td></td>
</tr>
<tr>
<td>Sesquiterpenes**</td>
<td>3000</td>
</tr>
<tr>
<td>Monoterpenes**</td>
<td>1000</td>
</tr>
<tr>
<td>Diterpenes**</td>
<td>1000</td>
</tr>
<tr>
<td>Triterpenes, steroids, sapoains**</td>
<td>4000</td>
</tr>
<tr>
<td>Tetraterpenes**</td>
<td>350</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>2000</td>
</tr>
<tr>
<td>Polyacetylenes</td>
<td>1000</td>
</tr>
<tr>
<td>Polyketides</td>
<td>750</td>
</tr>
<tr>
<td>Phenylpropanoids</td>
<td>500</td>
</tr>
</tbody>
</table>

*approximate number of known structures.
**total number exceeds 22000 at present.
1.2 Biosynthesis

Despite the enormous variety of secondary metabolites, the number of corresponding basic biosynthetic pathways is restricted and distinct. Precursors usually derive from basic metabolic pathways, such as glycolysis, Krebs cycle or the shikimate pathway. A schematic overview is presented in Figures 1.2 and 1.3. Plausible hypotheses for the biosynthesis of most SMs have been published (for overviews see Luckner, 1990; Conn, 1981; Bell and Charlwood 1980; Mothes et al., 1983; Dey and Harborne, 1997) that are based, at least, on tracer
experiments. For pathways leading to cyanogenic glycosides, glucosinolates, some alkaloids and nonprotein amino acids (NPAAs), amines, flavonoids and several terpenes, the enzymes which catalyze individual steps have been identified. In pathways leading to isoquinoline, indole, pyrrolidine and tropane alkaloids, flavonoids, coumarins, NPAAs, mono-, sesqui- and triterpenes, some of the genes which encode biosynthetic enzymes have already been isolated and characterized (Kutchan, 1995; Kutchan et al., 1991; Saito and Murakoshi, 1998). Whereas, earlier this century, it was argued that secondary metabolites arise spontaneously or with the aid of nonspecific enzymes, we now have good evidence that biosynthetic enzymes are highly specific in most instances and most have been selected towards this special task (although they often derive from common progenitors with a function in primary metabolism). As a consequence, final products nearly always have a distinct stereochemistry. Only the enzymes that are involved in the degradation of SMs, such as \( \beta \)-glucosidases, esterases and hydrolases, are less substrate-specific.
Tetraeterpenes

\[ \text{\textbeta-carotene} \]

Anthraquinones

\[ \text{emodine} \]

Polyynes

\[ \text{cicutoxin} \]

Flavonoids

\[ \text{quercetin} \]

Isoflavonoids

\[ \text{genistein} \]

Anthocyanidins

\[ \text{malvidine} \]

Phenylpropanoids

\[ \text{rosmarinic acid} \]

Figure 1.1 (Continued).
Figure 1.2 Main pathways leading to secondary metabolites. Abbreviations: IPP, isopentenyl diphosphate; DMAPP, dimethyl allyl diphosphate; GAP, glyceraldehyde-3-phosphate; NPAAs, nonprotein amino acids; AcCoA, acetyl coenzyme A.
Some SMs are produced in all tissues but their formation is generally organ-, tissue-, cell- and often development-specific. Although, in most instances, details have not been elucidated, it can be assumed that the genes of secondary metabolism are also regulated in a cell-, tissue- and development-specific fashion (as are most plant genes that have been studied so far).

Sites of biosynthesis are compartmentalized in the plant cell. While most biosynthetic pathways proceed (at least partially) in the cytoplasm, there is evidence that some alkaloids (such as coniine, quinolizidines and caffeine), furano-coumarins and some terpenes (such as monoterpenes, diterpenes, phytol and carotenoids that are formed in the pyruvate/glyceraldehyde phosphate pathway) are synthesized in the chloroplast (Wink and
Hartmann, 1982; Roberts, 1981). Sesquiterpenes, sterols and dolichols are produced in the endoplasmic reticulum or cytosolic compartment. A schematic overview is presented in Figure 1.4. Connine and amine formation has been localized in mitochondria (Wink and Hartmann, 1981; Roberts, 1981) and steps of protoberberine biosynthesis in vesicles (Aman et al., 1986). Hydroxylation steps are often membrane-bound and the endoplasmic reticulum (ER) is the corresponding compartment, as is also probable for the synthesis of other lipophilic compounds.

The biosyntheses of the major groups of SMs have been reviewed in the present volume: alkaloids (including betalains) by M. Roberts and D. Strack in Chapter 2; cyanogenic glycosides, glucosinolates and NPAAs by D. Selmar in Chapter 3; phenylpropanoids, lignin, lignans, coumarins, furocoumarins, tannins, flavonoids, isoflavonoids and anthocyanins by M. Petersen, D. Strack and U. Matern in Chapter 4; mono-, sesqui- and diterpenes by J. Gershenzon and sterols, cardiac glycosides and steroid saponins by W. Kreis in Chapter 5.

1.3 Transport, storage and turnover

Water soluble compounds are usually stored in the vacuole (Matile, 1978, 1984; Boller and Wiemken, 1986) (Table 1.2) whereas lipophilic substances are sequestered in resin ducts, laticifers, glandular hairs, trichomes, thylakoid membranes or on the cuticle (Wiermann, 1981) (Fig. 1.5).

As mentioned previously, most substances are synthesized in the cytoplasm, the ER or in organelles and, if hydrophilic, they are exported to the vacuole. They have to pass the tonoplast, which is impermeable to many of the polar secondary metabolites. For some alkaloids and flavonoids, a specific transporter has been described, which pumps the compounds into the vacuole (Fig. 1.4). The proton gradient, which is built up by the tonoplast-residing adenosine triphosphatase (ATPase), is used as a driving force (by a so-called proton antiport mechanism) (Deus-Neumann and Zenk, 1984; Mende and Wink, 1987). Alternatively, diverse trapping mechanisms (e.g. isoquinoline alkaloids by chelidonic acid or meconic acid in the latex vesicles of Chelidonium or Papaver, respectively) can also help to concentrate a particular compound in the vacuole. Moreover, conjugation of secondary metabolites with glutathione in the cytoplasm (Martinoia et al., 1993; Li et al., 1995) and subsequent transportation by an ATP-dependent transporter into the vacuole has been proposed for xenobiotics and some SMs that can be conjugated (for reviews see Wink, 1993, 1997).

Lipophilic compounds will interfere not only with the biomembranes of microbes and herbivores but also with those of the producing plant. In
Figure 1.4 Compartmentation of biosynthesis and sequestration. Abbreviations: SM, secondary metabolite; GS-SM, conjugate of SM with glutathione; NPAAs, nonprotein amino acids; ATP, adenosine triphosphate; ADP, adenosine diphosphate.
### Table 1.2 Examples for vacuolar sequestration of secondary metabolites (Wink, 1997)

<table>
<thead>
<tr>
<th>Phenolics</th>
<th>Terpenoids</th>
<th>Oligosaccharides</th>
<th>Nitrogen-containing compounds (excluding alkaloids)</th>
<th>Alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthocyanins</td>
<td>Isoflavone malonyl glycosides</td>
<td>Gentianose</td>
<td>Cyanogenic glycosides (linamarin)</td>
<td>Ajmalicine</td>
</tr>
<tr>
<td>Bergenin</td>
<td>Kaempherol 3,7-O-glycoside</td>
<td>Gentiobiose</td>
<td></td>
<td>Atropine</td>
</tr>
<tr>
<td>Coumaroyl-glycosides (esculin)</td>
<td>Orientin-C-glycosides</td>
<td>Stachyose</td>
<td>Cyanogenic glycosides (linamarin)</td>
<td>Nicotine</td>
</tr>
<tr>
<td>Flavonol-glycosides</td>
<td>Pterocarpan malonyl glycosides</td>
<td></td>
<td></td>
<td>Berberine</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>Quercetin-3-triglucoside</td>
<td></td>
<td>Cyanogenic glycosides (linamarin)</td>
<td>Betaine</td>
</tr>
<tr>
<td>7'-Glucosyl-pleurostimnin</td>
<td>7-Rhamnosyl-6-hydroxyluteolin</td>
<td></td>
<td>Cyanogenic glycosides (linamarin)</td>
<td>Betalains</td>
</tr>
<tr>
<td>Isoflavonone malonyl glycosides</td>
<td>Shikimic acid</td>
<td></td>
<td>Cyanogenic glycosides (linamarin)</td>
<td>Capsaicin</td>
</tr>
<tr>
<td>Sinapylglycosides</td>
<td>Tricin 5-glucoside</td>
<td></td>
<td>Cyanogenic glycosides (linamarin)</td>
<td>Catharanthine</td>
</tr>
</tbody>
</table>

| Oleanolic acid (3-O-glucuronide) | Cardiac glycosides (lanatoside A, C; purpureaglycoside A) | Saponines (avenacosides) |

| Glucosinolates                   | Noscapine                                        | Polyamines                             |
|                                  | Papaverine                                       | (S)-Reticuline                         |
|                                  | Polyamines                                        | Sanguinarine                           |
|                                  | Scopolamine                                       | (S)-Scoulerine                         |
|                                  | Senecionine-N-oxide                               | Serpentine                             |
|                                  | Serpentine                                        | Solaridine                             |
|                                  | Thebaine                                          | Vindoline                              |

In order to avoid autotoxicity, plants cannot store these compounds in the vacuole but usually sequester them on the cuticle, in dead resin ducts or cells, which are lined not by a biomembrane but by an impermeable solid barrier (Fig. 1.5).

In many instances, the site of biosynthesis is restricted to a single organ, such as roots, leaves or fruits, but an accumulation of the corresponding products can be detected in several other plant tissues. Long distance transport must take place in these instances. The xylem or phloem are likely transport routes but an apoplastic transport can also be involved.
Figure 1.5 Storage compartments for hydrophilic and lipophilic compounds. Abbreviation: NPAAs, nonprotein amino acids.
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Table 1.3 summarizes the evidence for xylem and phloem transport of some SMs.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Xylem</th>
<th>Phloem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinolizidine alkaloids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pyrrolizidine alkaloids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Acosamine</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Polyhydroxy alkaloids (swainsonine)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glucosinolates</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Nicotine</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Tropane alkaloids</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Storage can also be tissue- and cell-specific (Guern et al., 1987). In a number of plants, specific idioblasts have been detected that contain tannins, alkaloids or glucosinolates. More often, SMs are concentrated in trichomes or glandular hairs (many terpenoids in Labiatae, Asteraceae), stinging hairs (many amines in Urticaceae) or the epidermis itself (many alkaloids, flavonoids, anthocyanins, cyanogenic glycosides, coumarins, etc.) (Wiermann, 1981; Wink, 1993, 1997; Wink and Roberts, 1998). Flowers, fruits and seeds are usually rich in SMs, especially in annual plants. In perennial species, high amounts of SMs are found in bulbs, roots, rhizomes and the bark of roots and stems.

Several SMs are not end-products of metabolism but are turned over at a regular rate (Barz and Köster, 1981). During germination, in particular, N-containing SMs, such as alkaloids, NPAAs, cyanogenic glycosides and protease inhibitors, are metabolized and serve as a nitrogen source for the growing seedling (Wink and Witte, 1985). Carbohydrates (e.g. oligosaccharides and lipids) are also turned over during germination. Concentrations of some SMs, such as quinolizidine alkaloids, nicotine, atropine, monoterpenes and phenylpropanoids, vary diurnally; an active interplay between synthesis and turnover is involved in these instances. Turnover of SMs is readily seen in cell suspension cultures (for reviews see Barz and Köster, 1981; Wink, 1997).

It is well-established that profiles of SMs vary with time, space and developmental stage. Since related plant species often show similarities in the profiles of their SMs, they have been used as a taxonomic tool in plant systematics (Harborne and Turner, 1984). However, profiles of closely-related plants quite often differ substantially or those of unrelated plant groups show strong similarities; this clearly shows that SM patterns are
not unambiguous systematic markers but that convergent evolution and selective gene expression are common themes. In the present volume, Chapter 6 by M. Wink and P. Waterman summarises the evidence for and against the use of SMs in chemotaxonomy.

1.4 Costs of secondary metabolism

Analogous with other proteins in cells, the enzymes involved in the biosynthesis and transport of SMs show a regular turnover. This means that messenger ribonucleic acid (mRNA) must be regularly transcribed and translated into proteins, even for constitutive compounds. Both transcription and translation require a substantial input of energy in terms of adenosine triphosphate (ATP). Furthermore, the biosynthesis itself is often costly, demanding ATP or reduction equivalents, i.e. nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH₂). In order to exhibit their function as defence or signal compounds, allelochemicals need to be present in relatively high concentrations at the right place and time. Many secondary metabolites are synthesized in the cytoplasm or in cell organelles (Fig. 1.4) but are stored in the vacuole. Energy for the uphill transport across the tonoplast and/or for trapping the metabolite in the vacuole is provided by a H⁺-ATPase. If special anatomical differentiations (ducts, gland cells, trichomes) are needed, the formation and maintenance of these structures is also costly. As a consequence, both biosynthesis and sequestration (and the corresponding transcription and translation of related genes and mRNAs) are processes which require substantial amounts of ATP; in other words, it must be costly for plants to produce defence and signal compounds (a schematic overview is presented in Fig. 1.6).

1.5 Role of secondary metabolites and their application in biotechnology

The biosynthesis of secondary metabolites exhibits a remarkable complexity. Enzymes are specific for each pathway and are highly regulated in terms of compartmentation, time and space. The same is true for the mechanisms of accumulation or the site and time of storage. In general, we find that tissues and organs which are important for survival and multiplication, such as epidermal and bark tissues, flowers, fruits and seeds, have distinctive profiles of secondary metabolites, and secondary compounds are abundant in them.
All these processes and the corresponding means and structures necessary to express these traits are costly in terms of ATP and NAD(P)H, so it would be highly unlikely that secondary metabolites were waste products or had no function at all, as has been suggested in the older literature. Costly traits without function or advantage usually do not survive in evolution, as plants expressing these traits should perform less well then plants without them. As these metabolites are maintained and diversified in an astounding fashion, it must be assumed that these traits are indeed important, even if their functions are not evident.

During the past few decades, experimental and circumstantial evidence has made it clear that secondary metabolites do indeed have functions that are vital for the fitness of a plant producing them. Main roles are

- Defence against herbivores (insects, vertebrates)
- Defence against fungi and bacteria
- Defence against viruses
- Defence against other plants competing for light, water and nutrients
• Signal compounds to attract pollinating and seed dispersing animals
• Signals for communication between plants and symbiotic microorganisms (N-fixing Rhizobia or mycorrhizal fungi)
• Protection against UV-light or other physical stress

In order to fulfil these functions, the structures of secondary metabolites have been shaped during evolution so that they can closely interact with molecular targets in cells and tissues or other physiological features in animals or microorganisms. Quite often structures of secondary metabolites resemble endogenous substrates, hormones or neurotransmitters and can thus mimic a response at the corresponding molecular targets. The process leading to these structure similarities could be termed 'evolutionary molecular modelling'.

There is hardly a target in animals or microorganisms for which a natural product does not exist. Thus plants provide a wide array of bioactive substances. This is the reason why so many natural products in biotechnology, pharmacy, medicine and agriculture can be used in so many ways. Using substances that are already known or looking for new ones, hitherto undiscovered compounds or the corresponding genes encoding the genes for their biosynthesis, can be discovered in plants living in deserts or rain forests (bioprospection or gene prospection).

Secondary metabolites often interfere with more than a single molecular target, which is advantageous for the producer, as a toxin might be more efficient if it knocks out two targets instead of one. Furthermore, it will be more difficult for a herbivore or microbe to develop resistance to such a compound, as concomitant resistance at two targets would be required. Plants usually produce a complex mixture of compounds, each of which has its own set of biological activities, which make these mixtures even more powerful as means of defence and protection.

Because of this evolutionary logic, most plants are able to withstand various threats from herbivores, microbes, and the physical environment. Exceptions are many agricultural crops which have been optimised for yield and quite often, their original lines of defence have been selected away, as these metabolites were unpalatable or toxic for humans or its livestock.

The role and function of secondary metabolites as well as their potential biotechnological applications are the topic of volume III of Annual Plant Reviews, 'Functions of Plant Secondary Metabolites and their Exploitation in Biotechnology'.
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