Plant Natural Products

Synthesis, Biological Functions and Practical Applications

Herwig O. Gutzeit and Jutta Ludwig-Müller

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CONTENTS

Cover

Related Titles

Title Page

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Preface

Chapter 1: Biosynthesis and Chemical Properties of Natural Substances in Plants

- 1.1 Selected Classes of Secondary Metabolites
- 1.2 Evolution of Natural Compounds in Plants
- 1.3 Biotechnological Applications

Chapter 2: Function of Natural Substances in Plants

- 2.1 Secondary Compounds in Primary Metabolism
- 2.2 Development
- 2.3 Abiotic Stress
- 2.4 Symbioses
- 2.5 Defense
- 2.6 Allelopathy

Chapter 3: Biological Effects on Microorganisms and Animals

- 3.1 Coevolution
- 3.2 Effects on Vertebrates: An Overview

Chapter 4: Metabolism and Toxicity of Natural Substances in Mammals

- 4.1 Metabolism of Natural Substances in Mammals
- 4.2 Toxicity

Chapter 5: Examples of Physiological Effects of Natural Substances Present in Food and in Medicinal Plants
- 5.1 Are Antioxidants Good for Health?
- 5.2 Endocrine Effects of Natural Substances
- 5.3 Interference with Neural Functions

Chapter 6: Nature's Drugstore for a Healthy Life
- 6.1 Biologically Active Food and Food Components
- 6.2 Natural Compounds in Pharmacological Research
- 6.3 The Problem of Complexity: Analysis and Consequences

Further Reading

Index

End User License Agreement

List of Tables

1. Table 1.1
2. Table 1.2
3. Table 1.3
4. Table 1.4
5. Table 1.5
6. Table 1.6
7. Table 1.7
8. Table 1.8
9. Table 1.9
10. Table 1.10
11. Table 2.1
12. Table 2.2

13. Table 2.3
14. Table 2.4
15. Table 2.5
16. Table 2.6
17. Table 2.7
18. Table 2.8
19. Table 2.9
20. Table 2.10
21. Table 2.11
22. Table 2.12
23. Table 2.13
24. Table 2.14
25. Table 2.15
26. Table 3.1
27. Table 3.2
28. Table 4.1
29. Table 4.2
30. Table 4.3
31. Table 4.4
32. Table 4.5
33. Table 4.6
34. Table 5.1
35. Table 5.2
36. Table 5.3
37. Table 6.1

List of Illustrations

1. Figure 1.1
2. Figure 1.2
3. Figure 1.3
4. Figure 1.4
5. Figure 1.5
6. Figure 1.6
7. Figure 1.7
8. Figure 1.8
9. Figure 1.9
10. Figure 1.10
11. Figure 1.11
12. Figure 1.12
13. Figure 1.13
14. Figure 1.14
15. Figure 1.15
16. Figure 1.16
17. Figure 1.17
18. Figure 1.18
19. Figure 1.19
20. Figure 1.20
21. Figure 1.21
22. Figure 1.22
23. Figure 1.23
24. Figure 1.24

25. Figure 1.25
26. Figure 1.26
27. Figure 1.27
28. Figure 1.28
29. Figure 1.29
30. Figure 1.30
31. Figure 1.31
32. Figure 1.32
33. Figure 1.33
34. Figure 1.34
35. Figure 2.1
36. Figure 2.2
37. Figure 2.3
38. Figure 2.4
39. Figure 2.5
40. Figure 2.6
41. Figure 2.7
42. Figure 2.8
43. Figure 2.9
44. Figure 2.10
45. Figure 2.11
46. Figure 2.12
47. Figure 2.13
48. Figure 2.14
49. Figure 2.15

50. Figure 2.16
51. Figure 2.17
52. Figure 2.18
53. Figure 2.19
54. Figure 2.20
55. Figure 2.21
56. Figure 2.22
57. Figure 2.23
58. Figure 2.24
59. Figure 2.25
60. Figure 2.26
61. Figure 2.27
62. Figure 2.28
63. Figure 2.29
64. Figure 2.30
65. Figure 2.31
66. Figure 2.32
67. Figure 2.33
68. Figure 2.34
69. Figure 2.35
70. Figure 2.36
71. Figure 2.37
72. Figure 2.38
73. Figure 2.39
74. Figure 2.40

75. Figure 2.41
76. Figure 2.42
77. Figure 2.43
78. Figure 2.44
79. Figure 3.1
80. Figure 3.2
81. Figure 3.3
82. Figure 3.4
83. Figure 3.5
84. Figure 3.6
85. Figure 3.7
86. Figure 3.8
87. Figure 3.9
88. Figure 3.10
89. Figure 3.11
90. Figure 3.12
91. Figure 3.13
92. Figure 3.14
93. Figure 3.15
94. Figure 3.16
95. Figure 3.17
96. Figure 3.18
97. Figure 3.19
98. Figure 3.20
99. Figure 3.21

00. Figure 4.1
01. Figure 4.2
02. Figure 4.3
03. Figure 4.4
04. Figure 4.5
05. Figure 4.6
06. Figure 4.7
07. Figure 4.8
08. Figure 4.9
09. Figure 4.10
10. Figure 4.11
11. Figure 4.12
12. Figure 4.13
13. Figure 4.14
14. Figure 4.15
15. Figure 4.16
16. Figure 4.17
17. Figure 4.18
18. Figure 4.19
19. Figure 4.20
20. Figure 5.1
21. Figure 5.2
22. Figure 5.3
23. Figure 5.4
24. Figure 5.5

25. Figure 5.6
26. Figure 5.7
27. Figure 5.8
28. Figure 5.9
29. Figure 5.10
30. Figure 5.11
31. Figure 5.12
32. Figure 5.13
33. Figure 5.14
34. Figure 5.15
35. Figure 5.16
36. Figure 5.17
37. Figure 5.18
38. Figure 5.19
39. Figure 5.20
40. Figure 5.21
41. Figure 5.22
42. Figure 5.23
43. Figure 5.24
44. Figure 5.25
45. Figure 5.26
46. Figure 5.27
47. Figure 5.28
48. Figure 5.29
49. Figure 5.30

50. Figure 5.31
51. Figure 5.32
52. Figure 5.33
53. Figure 5.34
54. Figure 5.35
55. Figure 5.36
56. Figure 5.37
57. Figure 5.38
58. Figure 5.39
59. Figure 5.40
60. Figure 5.41
61. Figure 6.1
62. Figure 6.2
63. Figure 6.3
64. Figure 6.4
65. Figure 6.5
66. Figure 6.6
67. Figure 6.7
68. Figure 6.8
69. Figure 6.9
70. Figure 6.10
71. Figure 6.11
72. Figure 6.12
73. Figure 6.13
74. Figure 6.14

75. Figure 6.15

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Preface

All organisms synthesize biologically active organic compounds that confer a selective advantage for the respective species. Natural substances play a central role in the evolutionary struggle for survival and hence Nature has invented a plethora of organic substances with different activities that, for example, help to cope with stressful situations, permit communication with other organisms, or deter enemies. We feel that this important interdisciplinary topic should be represented in student courses of biochemistry, pharmacology, and, of course, biology. The book is based on a lecture course on the biology of natural substances, which is part of the curriculum for biology students at the TU Dresden.

The book covers a wide range of different aspects concerning the importance of secondary metabolites for the plants in their respective ecological niche. We discuss, for example, the biosynthesis of biologically active substances, their role in the development of plants, and their interaction with beneficial organisms as well as pathogens, and present examples of the communication between plants and animals feeding on the plants. The toxicity of some substances in vegetarian food is of concern and a toxicological assessment is essential. For this reason, we outline basic principles of toxicological tests with relevant natural compounds. The public discussion about health-promoting substances addresses important scientific questions that are not easy to answer. In the chosen examples of natural substances affecting human health, we summarize the results of intensive research and illustrate the complexity of the elicited reactions. Estrogenic compounds and hallucinogenic drugs exemplify the perils and promises of natural compounds. Are some compounds in our food disease-preventing? In the book, we discuss current attempts to answer this important question. Finally, the inspiration of Nature for medicinal chemistry will be briefly addressed.

The focus of the book is on plant secondary metabolites but the reader is occasionally reminded that animals and bacteria also contain most interesting compounds. In view of the vast published literature on the subject, we had to resist the temptation to address every interesting aspect concerning the biology of natural substances. Rather, we present current concepts of general biological mechanisms and illustrate the essential interdisciplinary approach in the functional analysis of natural substances. We hope that the reader will share our enthusiasm for the subject and will find this book stimulating and an incentive for further studies.

Dresden	***Herwig O. Gutzeit***
February 2014	***Jutta Ludwig-Müller***

1
Biosynthesis and Chemical Properties of Natural Substances in Plants

The number of known so-called “secondary metabolites” (also referred to as “natural products”) that have been discovered to date is increasing at a constant rate. Yet, it is not only plants (as described in this book) that produce these bioactive compounds; rather, other organisms such as bacteria, fungi, sponges, as well as animals, are also capable of synthesizing a plethora of these metabolites.

Whilst some of these metabolites are discussed in Chapter 4 and 5, a large number remain undiscovered. Moreover, secondary metabolites often possess interesting pharmacological properties, and therefore their characterization is very important. It should not be forgotten that plants synthesize these compounds as part of their own survival strategies, typically as defense compounds or as signals for pollinators or symbionts. In addition, recent evidence has pointed to additional roles for secondary metabolites in plant development. Although the term “secondary metabolites” perhaps infers a less important role for these compounds than those involved in primary metabolism, this is not the case. In fact, many essential and nonessential compounds in this group are found in plants, and even so-called “nonessential materials” can play a role in a plant's responses against abiotic and biotic stress. In this situation, the deletion of a biosynthetic pathway would cause damage to the plant, even if the pathway was not needed under favorable conditions. Interest in the secondary metabolites of plants was further increased when more sensitive analytical instruments became available, as well as genome sequence data for many plant species. Together, these tools allow the details of biosynthetic pathways to be investigated, an example being biotechnological manipulation. Notably, attention also began to be focused on the evolutionary aspects of secondary metabolite synthesis.

In general, secondary metabolites occur as complex mixtures (Figure 1.1). Their biosynthesis can be influenced by a variety of factors during development, in addition to stress, which makes the determination of their complete pattern essentially impossible. Whilst secondary metabolites can occur in the tissues as active compounds, they can also be synthesized as inactive compounds that must be transformed into active products; such metabolites that pre-exist are known as “phytoanticipins.” Compounds that are biosynthesized under stress conditions are typically not detectable in unstressed tissues; when they are synthesized after the invasion of plants by various pests these metabolites are termed “phytoalexins.”

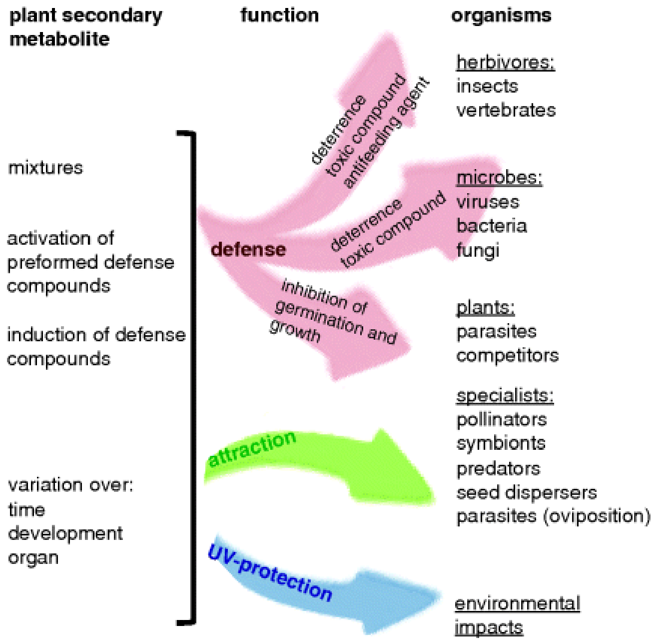


Figure 1.1 Correlation between secondary metabolites, their occurrence, and various functions. Secondary metabolites occur always as complex mixtures, and each plant has a specific set of compounds. The composition varies over time, development, and in different tissues and organs. In addition, the metabolites can be inactive and must be metabolized to the active form. Others will be produced only in response to biotic stress factors and other stress situations. The latter have a function in deterring a variety of herbivores, but can also attract insects for pollination.

Secondary metabolites play a variety of roles in the interaction of plants with their environment, but they may also be involved in plant development. They can function in defense mechanisms against herbivores such as nematodes, insects or vertebrates, as well as against microbes and competing plants. Metabolites may also play important roles in the attraction of symbionts, such as insects for pollination or zoophagous insects in multitrophic (when more than two organisms are involved) defense mechanisms. Animals that are required for seed-dispersal purposes are attracted by color and aroma compounds; however, some specialist insects and vertebrates have developed mechanisms to cope with the toxic effects of these compounds, and may generate an ecological niche in recognizing a specific host plant for either feeding or oviposition. Abiotic signals also contribute to the specific patterns of secondary metabolites; for example, some compounds are good protectors against ultraviolet (UV) light and photo-oxidation (i.e., oxidation reactions induced by light).

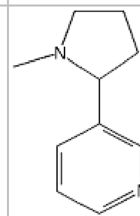
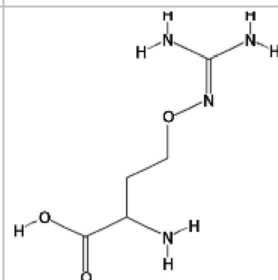
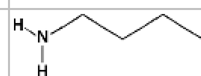
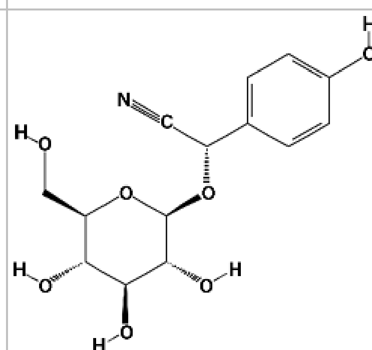
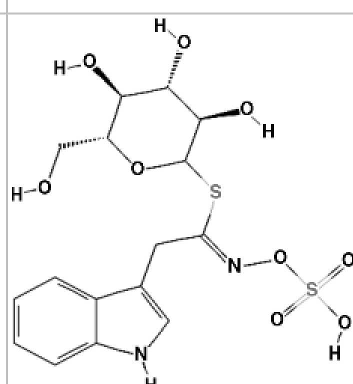
In order to understand the complexity of these compounds it is essential to have a good knowledge of their biosynthesis and the regulation of genes that encode the enzymes involved in syntheses, under changing conditions. A brief description of the biosynthesis of selected compounds is provided in the following sections of this chapter, but for additional information relating to the enzymes and their biochemistry, the reader is referred to respective textbooks on plant physiology and plant biochemistry.

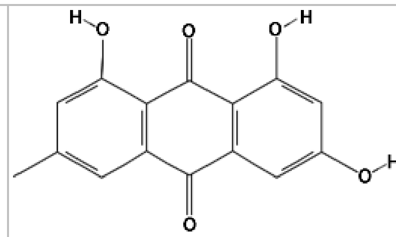
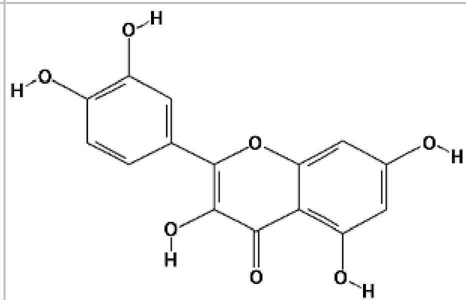
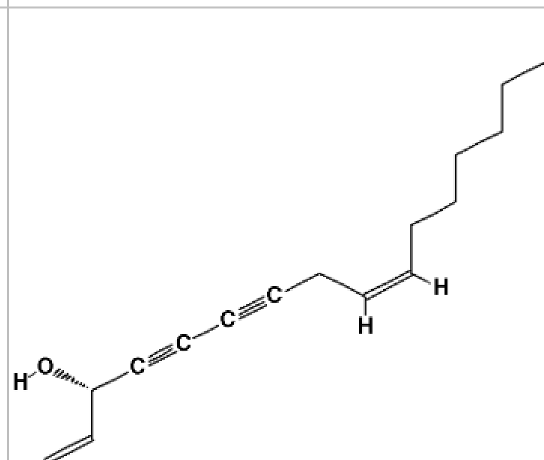
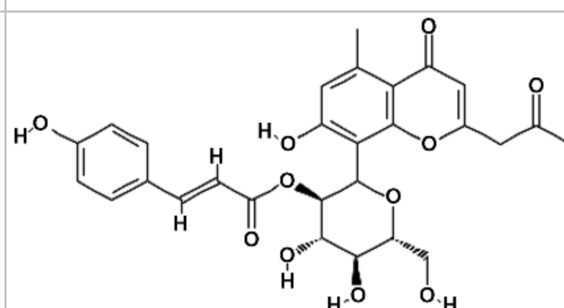
1.1 Selected Classes of Secondary Metabolites

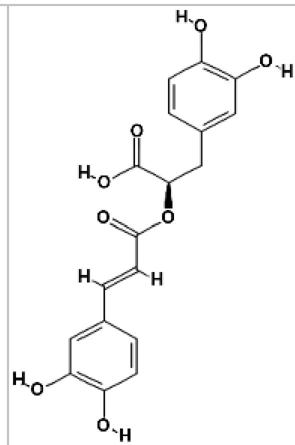
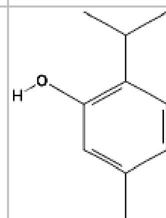
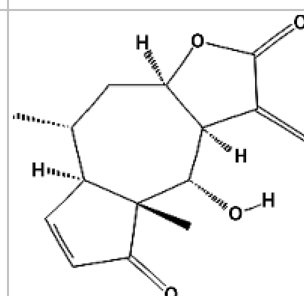
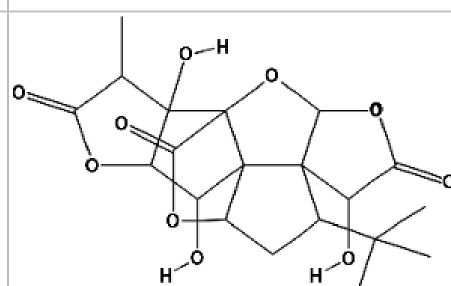
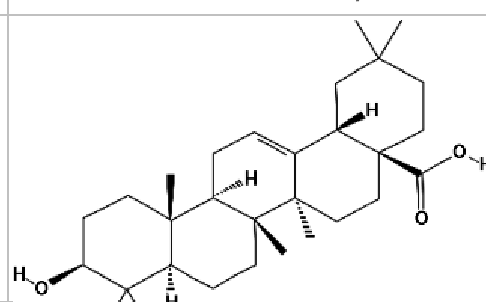
1.1.1 Occurrence and Compartmentation

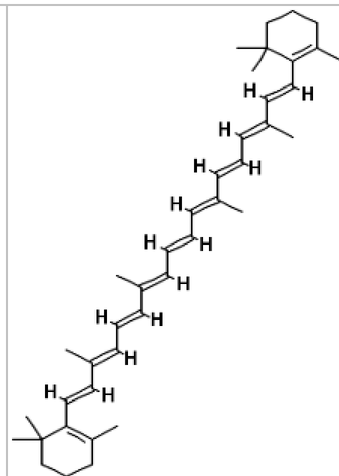
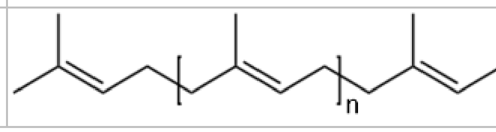
The patterns of secondary metabolites will differ depending on the species, the developmental stages, and the stress situations occurring in that species. The differences in pattern are the result of the metabolite biosynthesis, and the regulation of genes encoding the biosynthetic enzymes, transcription factors and transport proteins (see Sections 1.1.3 and 1.1.4). It is therefore difficult to generalize the metabolic patterns for a given plant species. The occurrence of some metabolites can be confined to a certain plant species, such as betalains in the Caryophyllales, glucosinolates in the Brassicales (with few exceptions), and polyacetylenes mainly in the Asteraceae and Apiaceae families. In the latter case, the compounds are important as bioactive substances in food crops (see Chapter 6) such as carrot (*Daucus carota*), celery (*Apium graveolens*) and fennel (*Foeniculum vulgare*). A compound from the Asteraceae *Carlina acaulis* has a strong antimicrobial activity and is also toxic for trypanosomes. Based on their chemical structures, the secondary metabolites can be placed into different classes (Table 1.1).

Table 1.1 The major classes of secondary metabolites based on their chemical structures. Chemical structures are from The PubChem Project.

Class of compound	Example	Structure
N-containing		
Alkaloids	Nicotine	
Nonproteinogenic amino acid	Canavanine	
Amines	Butylamine	
Cyanogenic glycosides	Dhurrin	
N- and S-containing		
Glucosinolates	Glucobrassicin	
Without N		

Class of compound	Example	Structure
Anthraquinones	Emodin	
Flavonoids	Quercetin	
Polyacetylenes	Falcarinol	
Polyketides	Aloeresin	

Class of compound	Example	Structure
Phenylpropanoids	Rosmarinic acid	
Terpenes		
Mono-	Thymol	
Sesqui-	Helenaline	
Di-	Gingkolide	
Tri-	Oleanolic acid	

Class of compound	**Example**	**Structure**
Tetra-	ß-Carotin	
Poly-	Rubber	

The synthesis of secondary metabolites can occur in all plant organs, including the roots, shoots, leaves, flowers, fruit, and seeds. Some metabolites are stored in specific compartments, which may be either whole organs or specialized cell types. Within these compartments the concentration of toxic secondary metabolites may be very high, so that they can exert an efficient defense against herbivores. For example, the glandular trichomes are often rich in toxic compounds, and some pathways – an example being the synthesis of the labdane diterpene Z-abienol in tobacco – occur exclusively in these trichomes. The glandular trichomes of peppermint leaves also contain large amounts of aromatic oils. The latex of *Euphorbia* is a good example of a toxic mixture of compounds being stored in specialized cells, the laticifers (Figure 1.2).

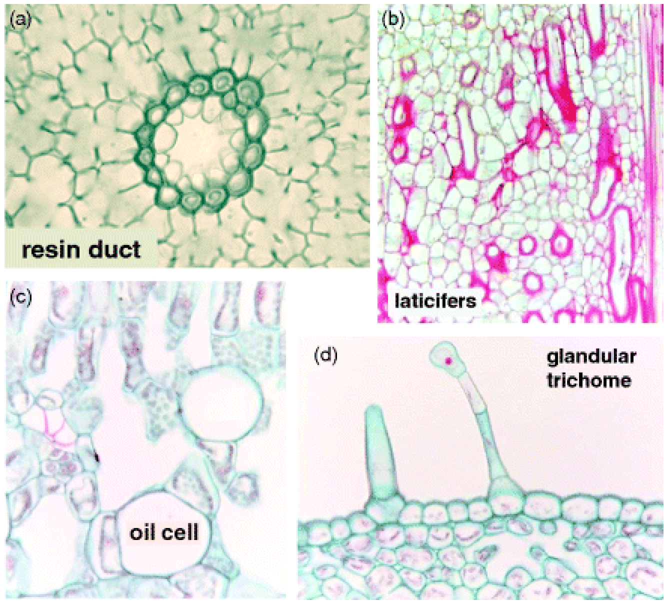


Figure 1.2 Some examples of secondary metabolite-containing plant compartments or cells. (a) Resin duct; cross-section through the needle of a *Pinus* species; (b) Laticifers; longitudinal section through the shoot of an *Euphorbia* species; (c) Oil cells; cross-section through a magnolia (*Magnolia* sp.) leaf; (d) Trichome; section through a foxglove (*Digitalis* sp.) leaf with glandular trichome. Image (a) from Bernd Schulz and Jutta Ludwig-Müller, Technische Universität Dresden, Germany; images (b–d) from James D. Mauseth, University of Texas, USA.

Lipophilic compounds can be found in the membranes of organelles or in the endoplasmic reticulum (ER), although specific storage vesicles have been reported for some compounds, such as the alkaloid berberin. Hydrophilic compounds are stored preferentially in vacuoles in the inner parts of the organelles and in the cytosol, as well as in the extracellular space (apoplast). Biosynthesis can occur in the cytosol, organelles and the ER, whereas transcriptional control takes place in the nucleus.

Some metabolites occur only in specialized subcellular compartments. In such cases, the tissue- and cell-specific localizations will depend on the solubility of the compound, notably whether it is lipophilic or hydrophilic (Table 1.2). Most hydrophilic compounds accumulate in vacuoles, and are typically present as inactive precursor substances that must be activated either chemically and/or enzymatically; this is the case for cyanogenic glycosides and glucosinolates. Antimicrobial tannins, which exert their protective effects in the extracellular space, are located in the apoplast, while the laticifers contain both hydrophilic and lipophilic compounds. The metabolite patterns are species-specific, but all are toxic; if human skin comes into contact with a latex extract, a severe irritation results. Lipophilic storage compartments also contain antimicrobial and/or defense compounds. Metabolites located in the membranes of the plastids and mitochondria are necessary for primary metabolism, an example being electron-transport chains and light absorbance. Plants secrete volatile compounds either to attract insects for pollination purposes, or zoophagous insects to defend themselves against herbivorous insects (Figure 1.1; see also Chapter 2). Plants may also deliver nonvolatile compounds into the soil, thereby altering the rhizosphere (termed allelopathy; see Section 2.6) that leads to an inhibition of the growth of other, competing, plants.

Table 1.2 Tissue-specific and subcellular compartmentation of hydrophilic and lipophilic compounds.

Characteristics	Storage compartment		Class of compounds
	Tissue	**Cell**	
Hydrophilic	Laticifer		Some alkaloids Nonproteinogenic amino acids Digitalis glycosides Cyanogenic glycosides
		Vacuole	Many alkaloids Nonproteinogenic amino acids Saponins Glycosides Flavonoids Anthocyanidins Betalains Tannins Cyanogenic glycosides Glucosinolates Amines
		Apoplast	Tannins
		Cytoplasm	Most hydrophilic compounds during their biosynthesis
		Nucleus	Flavonoids
Lipophilic	Trichome		Terpenoids
	Resin duct		Flavonoids Terpenoids
	Laticifer		Diterpenes Quinones Flavonoids Polyterpenes
	Oil cell		Anthraquinones Terpenoids

Characteristics	Storage compartment		Class of compounds
	Tissue	**Cell**	
		Cuticula	Wax Flavonoids Terpenoids
		Mitochondrion	Alkaloids (*Conium*)
		Plastid	Terpenes Alkaloids (coniin, quinolizidine, coffein)
		Specialized vesicles	Alkaloids (protoberberin)
		Plastid membrane	Ubiquinones
Tetraterpenes			
		Endoplasmic reticulum	Lipophilic substances during enzymatic hydroxylation steps

The process of compartmentation has three important implications:

1. The plant itself may not be in contact with the toxic compounds, because it is only after wounding or attack by other organisms that the metabolites will be released from the storage compartments.
2. The biosynthetic pathways are also compartmentalized, but this necessitates the involvement of transport processes for metabolite movement.
3. The target molecules for the secondary metabolites may be present in all compartments; however, the target is not necessarily colocalized with an active compound.

A few cellular targets will be discussed in the following section (see Figure 1.3), and further descriptions are provided in Chapters 5 and 6, with details of the compounds' biological activities. Many compounds that are used to protect plants against microbes and herbivores exert their biological activities outside the plant tissues; however, if these compounds were to exert any toxic effect on the plant, their compartmentation would provide protection against self-poisoning.

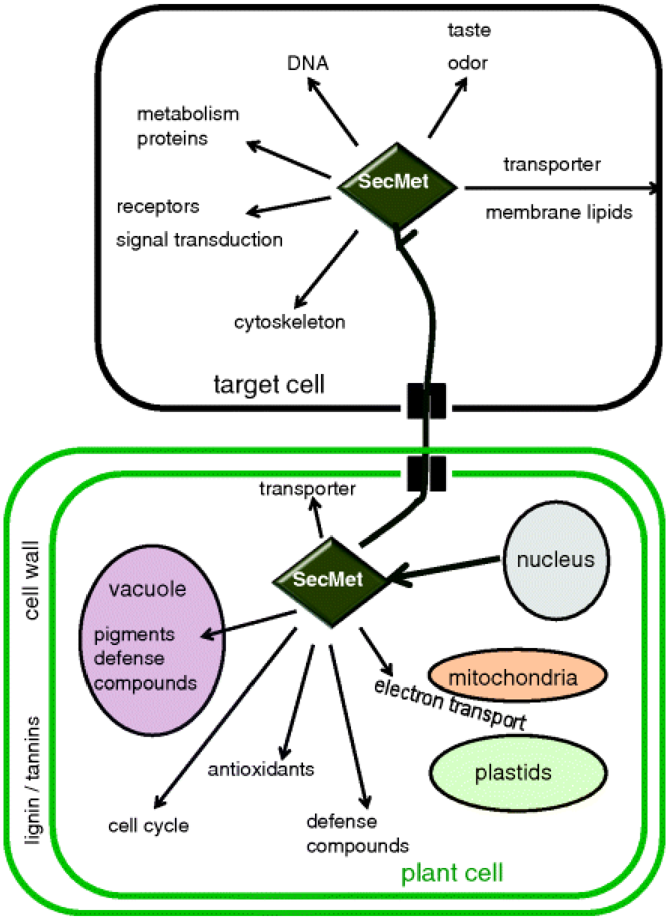


Figure 1.3 Cellular and molecular targets for secondary metabolites in plants and other organisms. In the plant cell (green), secondary metabolites (SecMet) are synthesized and can exert their effects in different compartments, such as the electron-transport chain in organelles, transporters at the plasma membrane, as antioxidants, and as fortification elements in the cell walls. They are stored in vacuoles or cell wall compartments as coloring or defense compounds. In the cells of the target organisms (brown), into which the metabolites must be transported, the targets can be in all compartments. Target molecules encompass transporters and channels, enzymes and proteins, nucleic acids, the cytoskeleton, receptor molecules and signal transduction chains, as well as membrane lipids.

Membrane targets can be found at the plasma membrane, ER or Golgi apparatus, where the functions of molecules such as transporter and channels, and also of membrane receptors, can be altered. The electron-transport chains of both plastids and mitochondria may also serve as targets, and plant compounds may inhibit their function. In addition, the structural proteins of the cell wall and of enzymes may be affected, while the cytoskeleton, with its component proteins myosin and tubulin, may be a target for secondary metabolites and result in an altered cell cycle (see Figure 2.5). Within the nucleus and the organelles, desoxyribonucleic acid (DNA) is a target for interaction with secondary metabolites; it is well known that some molecules