1 Introduction

Plants produce a vast and diverse assortment of chemical compounds. These compounds can be classified as products or intermediates from primary and secondary metabolism. Primary metabolism is necessary for growth and development, is universal, uniform, and hardly changed during evolution. Examples of plant primary metabolites are simple carbonic acids and sugars, amino and nucleic acids (Croteau et al. 2000).

On the other hand, secondary metabolism is not necessary for growth and development but is essential for continued existence of a species within its environment (Hartmann 1985). Plant secondary metabolites serve as chemical signals that enable the sessile plant to respond to environmental cues. Secondary compounds can function in the defense against herbivores, pathogens, and competitors while others provide protection from sun radiation, aid in pollen and seed dispersal or can submit informations acting as pheromone like signals and phytohormones (Hartmann 1991, Harborne 1993).

Plant secondary compounds are derived from central primary metabolites and, based on their biosynthetic origins, they can be divided into three major groups: the terpenoids, the phenylpropanoids including phenolic compounds, and the alkaloids. Terpenoids originate from carbohydrate metabolism (carbonic acids and sugars) and functionally include toxins and feeding deterrents against herbivores like cardenolides or antibacterial, fungicide and viricidal mono- and sesquiterpenes in essential oils. In lower dosages, essential oil components are important to attract pollinators and seed dispersers. Those are visually attracted by many flavonoid pigments that belong to the phenolic compound group, e.g., anthocyanins can be responsible for red and blue, while chalcones count for yellow flower pigments. Besides this visual function, phenolic compounds like tannins, lignans, and flavonoids serve as defense against herbivores and pathogens. Moreover, lignins strengthen cell walls mechanically, and naphthaquinones like juglone have allelopathic activity and may adversely influence the growth of neighbouring plants.

The fascinating, chemical diverse group of alkaloids is synthesized from compounds of the amino and nucleic acid metabolism. These bitter-tasting, nitrogenous alkaloids protect plants from a variety of herbivorous animals, and many possess dramatic physiological effects on vertebrates including humans.

Most alkaloids are easily resorbed and many of them interfere with essential parts of the nervous system, acting on pre- and/or post-synaptic receptors, inactivating neurotransmitters, inhibiting the post-synaptic signal transduction pathways, or hampering a proper function of ion channels. Depending on the targeted nerve, this can result in paralysis of selected muscle tissues like skeleton muscles and heart muscles, or it can result in the paralysis of pain-conductive nerves providing an analgesic effect, or it can result in the paralysis of the central nervous system causing psychological effects. Moreover, some alkaloids interfere with the assembly or disassembly of essential structures within the cytoskeleton, hindering cell division and awarding some cytostatic effects while other alkaloids are able to alkylate DNA probably causing cancer (Hänsel and Sticher 2004).

The first identified alkaloid was morphine from the latex of opium poppy (*Papaver somniferum*). Until today, morphine is used in medicine as an analgesic and cough suppressant. However, excessive use of this drug can lead to strong addiction and, if overdosed, it provokes respiratory paralysis and death.

More than 12,000 alkaloids have been isolated since the discovery of morphine by the German pharmacist Fiedrich Sertürner in 1806 and the questions, how and why alkaloids are made by plants, have fascinated generations of researchers within the fields of biology, chemistry, and pharmacy.

1.1 Pyrrolizidine Alkaloids - Typical Compounds of Plant Secondary Metabolism

Pyrrolizidine alkaloids (PAs) are characterized as typical compounds of plant secondary metabolism (Croteau et al. 2000). PAs do fulfill the four essential demands on secondary compounds as proposed by Hänsel and Sticher (2004).

1. Differential distribution among limited taxonomic groups within the plant kingdom

PAs were discovered in more than 6,000 plant species (Chou and Fu 2006) distributed among certain unrelated Angiosperm taxa. More than 95% of the PA-producing species investigated so far belong to just five families (marked with a red disc in Figure 1.1) namely the Asteraceae (1), the Boraginaceae (2), the Apocynaceae (3), the Orchidaceae (4), and the Fabaceae (5). Further isolated occurrences were reported from single species of other families (marked with a pink disc in Figure 1.1) like the Convolvulaceae (6), the Santalaceae (7), the Sapotaceae (8), the Ranunculaceae (9), the Celastraceae (10) (reviewed by Hartmann and Witte, 1995), and recently from the Lamiaceae (11) discovered by Nawaz et al. (2000).

The scattered occurrence of PAs among the Angiosperms has been particularly puzzling and provoked the question whether the PA biosynthetic pathway was invented just once and lost several times during evolution or whether it evolved several times independently in distinct, separate lineages. To date, this question was answered in favour of an independent evolution. Homospermidine synthase (HSS, EC 2.5.1.44), catalyzing the first step in PA biosynthesis, was found to be of polyphyletic origin within the Angiosperms (Ober and Hartmann 1999b). Up to now, the HSS has been shown to be recruited at least four times independently, once early in the evolution of the Boraginaceae, once within the monocots, and twice within the Asteraceae separating the tribes of Senecioneae and Eupatorieae (Reimann et al. 2004).

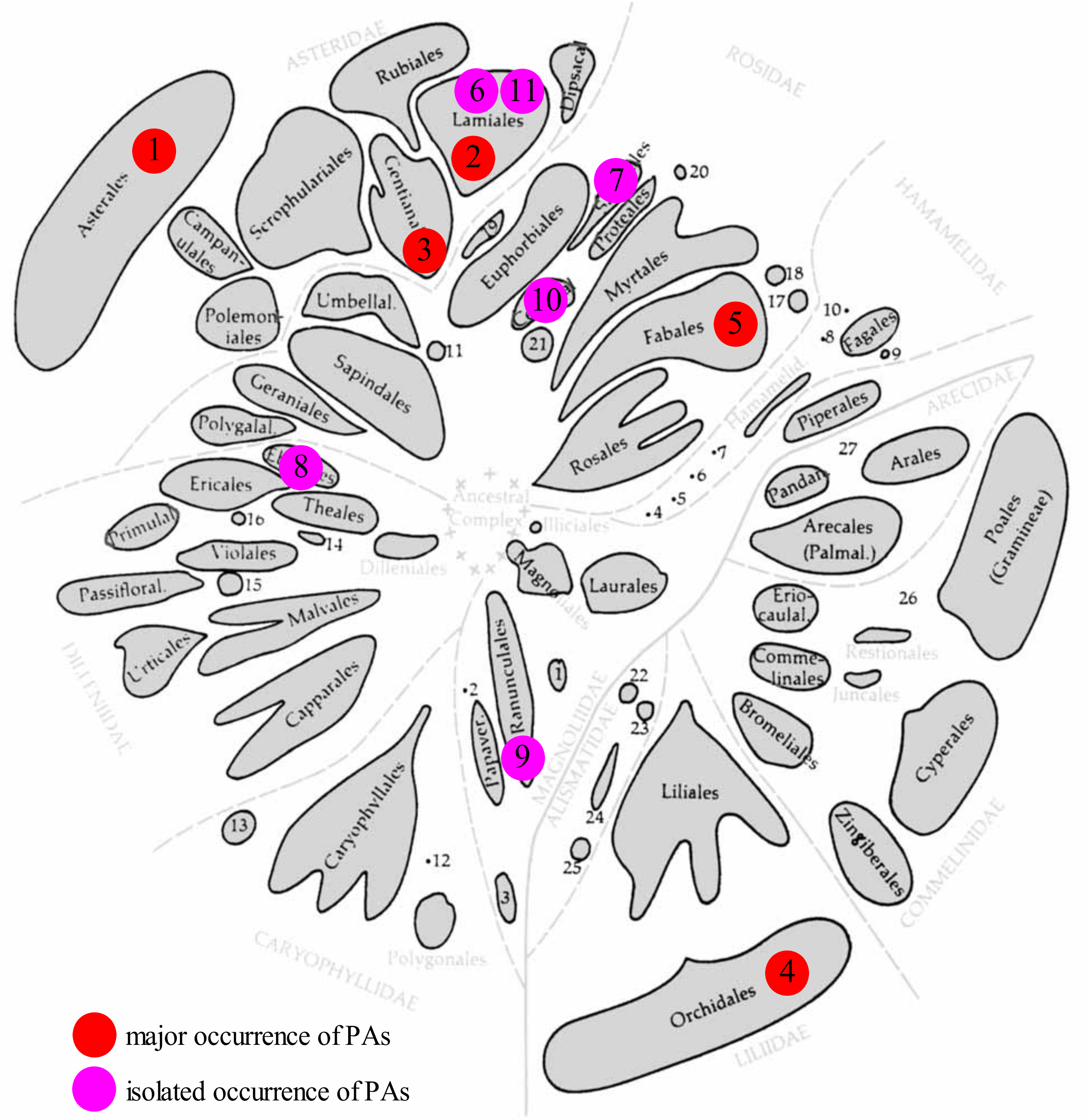

Figure 1.1: Major and isolated occurrences of pyrrolizidine alkaloids within the angiosperms, modified from Ober and Hartmann 1999b and Stebbins 1974: Asteraceae (1), Boraginaceae (2), Apocynaceae (3), Orchidaceae (4), Fabaceae (5), Convolvulaceae (6), Santalaceae (7), Sapotaceae (8), Ranunculaceae (9), Celastraceae (10), and Lamiaceae (11).

2. Great structural diversity and complexity

More than 660 different chemical structures of PAs are known so far (Jiang et al. 2006). Basically, plant PAs are composed of a "necine base" moiety that is esterified with one or more "necic acids" (Panel B of Figure 1.2, Crout 1966).

In 1995, the main PA-types (see Figure 1.2) comprising senecionine, triangularine, monocrotaline, lycopsamine, and phalaenopsine type PAs were described by Hartmann and Witte (1995) based on Culvenor's chemosystematic suggestions (Culvenor 1978). The growing number of discovered PA-structures as well as the increased understanding of the mechanisms involved in biosynthesis, biological activities and functions of PAs led to a new modified classification introduced by Hartmann (2006) which emphasizes the biosynthetic relationship within the necic acids:

I. Macrocyclic or open-chain diester types with eleven or more members including their related monoesters comprising senecionine (**S**), triangularine (**T**), and monocrotaline (**M**) types esterified with isoleucine derived necic acids (Panel I in Figure 1.2).

II. Lycopsamine (**L**) type PAs occurring as open-chain mono- and diesters, and macrocyclic triesters esterified with a unique C7 necic acid (Panel II in Figure 1.2).

III. Special type PAs characteristic to selected genera enclosing ipanguline (**I**) and phalaenopsine (**P**) types, occurring as mono- and open-chain di- and triesters with aryl, aralkyl, and rarely alkyl necic acids (Panel III in Figure 1.2).

The distribution of PA types within the Angiosperm families is shown in Table 1.1. Remarkably, the alkaloid pattern within the Asteraceae differs significantly between its two PA-producing tribes, the Senecioneae and the Eupatorieae. While the vast majority of PA types within the Eupatorieae belongs to the lycopsamine (L) type, PAs within the Senecioneae are exclusively found in class I with an accumulation of senecionine (S) types. These distinct alkaloid patterns are reflected in the finding that the homospermidine synthase (HSS) catalyzing the first step in PA biosynthesis was recruited independently within the Eupatorieae and the Senecioneae. Moreover, the inability of Senecioneae species to produce lycopsamine type PAs leads to the hypothesis that the HSS might not be the only enzyme specialized in PA biosynthesis. The formation of lycopsamine type PAs requires a second specialized enzyme that is important for the formation of the unique C7 necic acids characterizing the lycopsamine type PAs. Thus, this second specialized enzyme might not be present in the Senecioneae, the Orchidaceae, the Convolvulaceae (*Ipomoea*), Ranunculaceae, Celastraceae, and Lamiaceae but within the Eupatorieae as well as in the Boraginaceae, the Apocynaceae, the Santalaceae, the Sapotaceae, the Convolvulaceae (*Merremia*), and in the Fabaceae (*Laburnum anagyroides*).

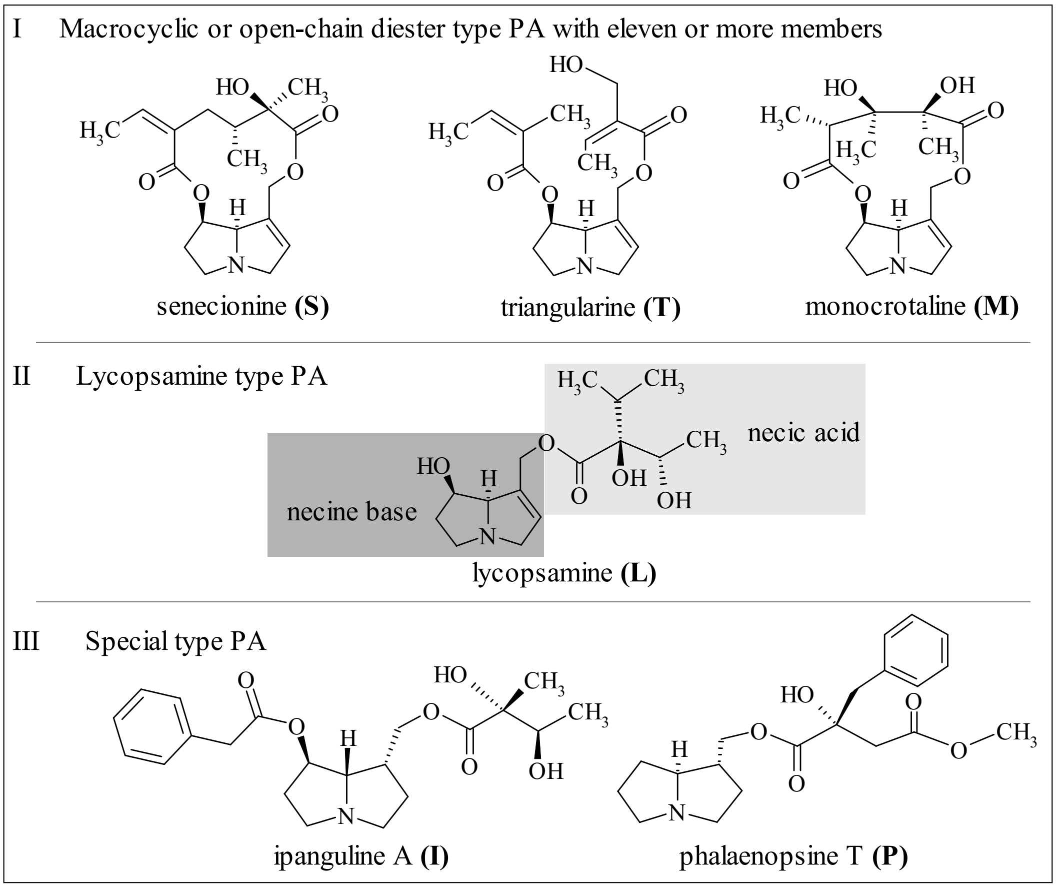

Figure 1.2: The three classes of pyrrolizidine alkaloids (PAs): **I.** Macrocyclic or open-chain diesters comprising the senecionine (S), triangularine (T), and monocrotaline (M) type PAs; **II.** Lycopsamine (L) type PAs; and **III.** Special type PAs enclosing the ipanguline (I) and phalaenopsine (P) type PAs.

family of PA occurrence	prevalent genera (Hartmann and Witte 1995)	distribution of PA types [%]						
		I			II	III		others
		S	**T**	**M**	**L**	**P**	**I**	
Asteraceae, tribe Senecioneae	*Senecio*	82	16	1	-	-	-	1
Asteraceae, tribe Eupatorieae	*Eupatorium*, *Ageratum*	8	5	-	87	-	-	
Boraginaceae	virtually all genera[1]	< 1	7	1	90	1	-	
Apocynaceae	*Parsonsia*	4	-	-	77	4	-	15
Orchidaceae	subfamily of Epidendroideae[2]	-	-	-	-	95	-	5
Fabaceae	*Crotalaria*	39	2	45	1	-	-	13
Convolvulaceae	*Ipomoea*, *Merremia*[3]	-	-	-	- 100	-	100 -	
Santalaceae	*Thesium*, *Amphorogyne*[4]	-	-	-	14	86	-	
Sapotaceae	*Planchonella*, *Minusops*	-	-	-	13	75	-	12
Ranunculaceae	*Caltha*, *Trollius*[5]	100	-	-	-	-	-	
Celastraceae	*Bhesa*	-	100	-	-	-	-	
Lamiaceae	*Ajuga*[6]	100	-	-	-	-	-	

[1] Röder (1995), [2] Frölich et al. (2006), [3] Mann et al. (1996),
[4] Thu Huong et al. (1998), [5] Liddell and Stermitz (1994), [6] Nawaz et al. (2000)

Table 1.1: The distribution of PA types within the Angiosperm families.

3. Characteristic supply with PAs depending on the developmental stage
PA biosynthesis in the Asteraceae is strictly coordinated to root growth (Hartmann et al. 1988, Sander and Hartmann 1989) and is terminated when flowers open (Anke 2004). PA backbone structures like senecionine *N*-oxide are formed constitutively and under high constraint. Their biosynthesis is not inducible by wounding nor by microbial attack (van Dam and Vrieling 1994, Tinney et al. 1998). Additionally, PA backbones are diversified via specific one- or two-step reactions such as hydroxylation, epoxidation, O-acetylation, or otonecine formation which proceed in a molecule position-specific and stereoselective manner (Hartmann and Dierich 1998). Any genetic variability affecting the PA transforming enzymes modifies the PA bouquet without affecting the overall quantity. Such a highly plastic system may indicate a powerful strategy in constitutive plant defense against differential herbivory (Hartmann 1999, Hagen 2003).

4. Biosynthesis in specialized tissues, accumulation in unique, defined patterns
PAs are synthesized in specific tissues (see Table 1.2) and are accumulated in cell vacuoles of various tissues (Ehmke et al. 1987) with up to 80% of total plant PA content. Such accumulation tissues are important for species or individual survival, e.g., buds, seeds or roots (Hartmann and Zimmer 1986). Translocation from tissues of biosynthesis to those of accumulation is enabled by PAs existing as hydrophilic *N*-oxides (Figure 1.3) which allows transport via phloems (Hartmann et al. 1989, Witte et al. 1990). A specific PA *N*-oxide carrier, responsible for selective uptake into the vacuoles, has been characterized (Ehmke et al. 1988). Phloem loading and unloading is still under investigation and predicted to be carrier-mediated since species, which do not produce PAs, are unable to translocate PAs via the phloem (Hartmann et al. 1989). Stored PAs are stably retained and show no metabolic turnover except for PA-protected seedlings from *Crotalaria scassellatii* using a PA-specific *N*-oxygenase to start the mobilization of the bound nitrogen utilized for growth and development (Chang 1997).

1.1.1 Toxicity of Pyrrolizidine Alkaloids and Role in Plant Protection

In mammals, PAs are hepatotoxic and carcinogenic (Mattocks 1972, Culvenor et al. 1976, Wiedenfeld and Röder 1984, Steenkamp et al. 2001). The toxic compound is formed after ingestion via bioactivation by microsomal liver cytochrome P450 monooxygenases producing unstable pyrrolic intermediates (Figure 1.3). These highly reactive intermediates are only formed when the alkaloid substrates display the following essential features (Winter and Segall 1989): C1-C2 double bond (boxed area A in the pro-toxic PA structure in Figure 1.3), esterification of the allylic hydroxyl group at C9 (boxed area B), and free or esterified hydroxyl group at C7 (boxed area C). The necic acids are cleaved off and the remaining nucleophilic necine base derived compound is able to alkylate DNA (Fu et al. 2004). The attacked liver develops necrosis, fibrosis, and eventually neoplastic growth. The loss of liver function means an overflow of brain toxic substances like ammonia and

families	organs of PA synthesis	tissues of PA synthesis[1]	organs of highest[2] PA accumulation
Asteraceae, tribe Senecioneae	roots	specific groups of endodermis + adjacent cortex cells opposite the phloem[3]	infl.[4] (90%)
Asteraceae, tribe Eupatorieae	roots	cortex of annually sprouted roots[5]	infl.[6] (90%)
Boraginaceae	Sof roots[7]	endodermis[8]	?
	Cof shoots[9], roots[8]	endodermis + pericycle[8]	?
	Hin shoots[7], leaves[8] + buds[8]	epidermis of shoots + leaves[8]	infl. (71%)[7]
Orchidaceae	aerial root tips	basic meristem[10]	buds[11] (52%)
	buds	epidermis[10]	
Fabaceae	roots and/or leaves and/or shoot tips[12]	nd	seeds[13]
Convolvulaceae	shoots[14], roots[15]	nd	young leaves + shoot tips (60%)[14]

[1] deduced from the localization of the first pathway specific enzyme, the homospermidine synthase (HSS), [2] PAs were found in all parts of the plants

[3] Moll et al. (2002), [4] Hartmann and Zimmer (1986), [5] Anke et al. (2004), [6] Biller et al. (1994), [7] Frölich et al. (2007), [8] Niemüller (2007), [9] Van Dam et al. 1995, [10] Anke (2004), [11] Frölich et al. (2006), [12] Nurhayati and Ober (2005), [13] Toppel et al.(1988), [14] Jenett-Siems et al. (1998), [15] Jenett-Siems et al. (2005)

Table 1.2: Plant organs and tissue of PA biosynthesis vary significantly among the PA producing families or even genera while plant organs of PA accumulation are preferentiably reproductive tissues. Abbreviations: infl. = inflorescence (includes buds, flowers + fruits), Sof = *Symphytum officinale*, Cof = *Cynoglossum officinale*, Hin = *Heliotropium indicum*, nd = not determined, ? = unfortunately, most phytochemical reports do not contain any quantitive information on PA levels or even specifications of the analyzed plant organs.