Quinolizidine and Pyrrolizidine Alkaloid Chemical Ecology – a Mini-Review on Their Similarities and Differences

Michael Wink
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Received: 27 February 2018 / Revised: 13 July 2018 / Accepted: 30 July 2018 / Published online: 6 August 2018

Abstract
This mini-review summarizes over 40 years of research on quinolizidine (QAs) and pyrrolizidine alkaloids (PAs). Emphasis is on the chemical ecology of both groups of alkaloids, which serve as general defense compounds against herbivores for the plants producing them. For QAs and PAs, a number of insects (aphids, moths, beetles) have acquired tolerance. These specialists store the alkaloids and use them as defense chemicals against predators. In some PA sequestering moths, the adaptation is even more intricate and advanced. PAs can function as a morphogen to induce the formation of male coremata, inflatable organs that dissipate pheromones. In these insects, PAs are additionally used as a precursor for male pheromones. Female moths utilize their own PAs and those obtained from males via the spermatophore as nuptial gift, to transfer them to the eggs that thus become chemically protected. Novel genomic technologies will allow deeper insights in the molecular evolution of these two classes of alkaloids in plant-insect interactions.

Keywords Quinolizidine alkaloids · Pyrrolizidine alkaloids · Chemical ecology · Lupins · Insect herbivores · Plant-herbivore interactions

Introduction

It is a trivial observation that plants cannot run away when they are attacked by an herbivore. Neither do they have an immune system to defend themselves against invading microbes (Wink 1988). However, over the course of more than 400 million years of evolution, plants evolved complex bioactive defence and signalling molecules in order to survive in an environment with a multitude of enemies and competitors (Wink 2003). The compounds conferring these defence functions are often referred to as Plant Secondary Metabolites (PSM; Hartmann 2007). PSM not only serve as defence molecules; some of them are used by plants to attract pollinating and fruit-dispersing animals. In addition, some PSM are of direct use for plants as nitrogen storage compounds, antioxidants and UV protectants (Hartmann 2007; Harborne 2014).

Flowering plants and insects are closely connected. With the beginning of the Tertiary, we see a phylogenetic radiation of flowering plants and concomitantly of insects. Many insects are pollinators, others are herbivorous. Within the herbivorous insects, we can distinguish between polyphagous herbivores, which feed on many plant species; oligophagous species, which select a few host plants; and monophagous species, which are closely adapted to individual hostplants which produce a certain class of PSM (Ali and Agrawal 2012; Mason and Singer 2015). The fact that polyphagous species are not adapted to a single class of PSM, does not mean they do not possess mechanisms to deal with plant toxins. Common mechanisms found in many polyphagous insect herbivores are detoxification by generic detoxification enzymes, e.g. Cytochrome P450 (CYP) enzymes, or direct elimination of PSM by excretion via so-called ABC-transporters. Some herbivores harbour or even cultivate symbiotic intestinal microorganisms, which can degrade or inactivate toxic PSM (Pennisi 2017). A number of monophagous insects not only tolerate the toxic PSM of their host plant, but actively store them in their bodies (Boppré 1984; Cogni et al. 2012; Hartmann 2004; Laurent and Braekman J-C Daloze 2005; Macel 2011;
aminopentanal forms a Schiff base with the aldehyde function of a second and third molecule of 5-aminopentanal. In a complex reaction, in which the amino group of 5-aminopentanal forms a Schiff's base with the aldehyde function of a second and third molecule of 5-aminopentanal, a tetracyclic quinolizidine ring skeleton is generated which can be converted into sparteine or lupanine (Wink and Hartmann 1978; Wink et al. 1979). After lupanine has been hydroxylated to 13-hydroxylupanine, ester alkaloids can be generated by a tigloyl-CoA: 13-hydroxylupanine O-tigloyl transferase (Wink and Hartmann 1982a). An S-adenosyl-L-methionine: N-methyltransferase can transfer methyl groups to free nitrogen atoms in some QA, such as cytisine (Wink 1984). At that time, our evidence came from enzyme assays but failed to identify the corresponding genes. Now, 30 years later, we have genomic and transcriptomic tools to address the old questions again (Frick et al. 2017). So far, genes for LDC, cadaverine oxidase, and tigloyl-CoA: 13-hydroxylupanine O-tigloyl transferase have been identified. Presently, we analyse transcriptomes from QA producing plants, generated by RNASeq, to identify additional genes involved in QA synthesis, storage and transport.

According to our experimental data, the QA biosynthesis takes place in the chloroplast, which is also the site of lysine formation (Wink and Hartmann 1982b). QA synthesis is regulated by light and follows a diurnal rhythm. After synthesis, QAs are exported from the chloroplast into the cytoplasm and are actively sequestered in the vacuole. The necessary transport across the tonoplast is catalysed by an ATP-dependent proton antiporter (Mende and Wink 1987). Since plants have several hundred of ABC transporter genes it needs to be analysed if our QA transporter is a true H+ antiporter or an ABC transporter. Apparently, ABC transporters play a role for the transport of QAs from the phloem to the seeds (Frick et al. 2017).

After synthesis, QAs are exported from the leaves to other organs, such as stems, flowers and fruits. In stems and leaves, alkaloids are mainly stored in epidermal cells. The transport takes place in the phloem and not the xylem (review in Wink 1992). We had postulated that the transfer from the phloem into the growing seeds also requires an alkaloid transporter, which was identified recently (Frick et al. 2017). If such a transporter would be genetically inactivated, the breeding of lupin varieties with high alkaloid levels in the green parts, but low levels in the seeds, would be become feasible. Such lupins would retain their resistance against herbivores (see below), but would produce sweet seeds, suitable for animal and human nutrition.

Transport and accumulation occur in a diurnal cycle (Wink 1992). Alkaloid levels are highest during flowering and fruit formation. In annual species, most of the alkaloids are translocated into the seeds whereas the senescent aerial parts have very low levels. In wild lupins, seed alkaloid contents can be between 2 and 8% of dry weight (Wink 1992). After germination, QA levels drop, because QAs are metabolized and their nitrogen serves to build amino acids. Therefore, one additional function of QAs is that of a nitrogen storage. Alkaloid levels are not static but are regulated by environmental influences, such as heat, drought, high temperatures and herbivory. Artificial wounding can enhance QA contents by a factor of four with a couple of hours (Wink 1992). In lupins, growing along altitudinal gradients in the Rocky Mountains, lupins from high altitudes have lower alkaloid contents than those of lower elevations (Wink et al. 1995).

**Quinolizidine Alkaloid Diversity in Lupins**

QAs are typical PSM of papilionoid legumes (Wink et al. 2010), among them the species-rich genus *Lupinus*. The genus...
Lupinus belongs to the tribe Genisteae within the subfamily Papilionoideae of the family Fabaceae. More than 300 Lupinus species are known. We have used DNA sequences of chloroplast genes (rbcL) and nuclear genes (ITSI, ITSII) to reconstruct the molecular phylogeny of the genus Lupinus (Käss and Wink 1997). About 13 species occur in southern Europe, northern and eastern Africa, where this genus probably evolved. From the Old World two emigrations into the New World took place less than 15 million years ago. About 35 species now colonize the eastern lowlands of South America whereas more than 250 species evolved after several radiations in the Rocky Mountains and the Andes (Käss and Wink 1997; Nevado et al. 2016).

Lupins employ QAs, saponins, isoflavones and protease inhibitors against herbivores and saponins, phenolics and terpenoids against microbial infections. We developed a solid-liquid extraction procedure using Extrelut columns and dichloromethane as a solvent to obtain QA extracts suitable for further analyses (Wink 1993). QAs (and PAs) are volatile as free bases, thus the complex mixtures can be separated by high resolution capillary gas-liquid chromatography (GLC). For the identification of the individual alkaloids, the combination of GLC with mass spectrometry (GLC-MS) is the method of choice. Kovats retention indexes and mass spectral data allow the identification of most QAs (details in Wink 1993; Wink et al. 1995).

Using GLC-MS we were able to profile the alkaloid composition of more than 70 lupins and several other legumes from Europe, North America, Mexico and South America so far (Wink et al. 1995). It has been argued by chemotaxonomists that the chemical profiles of plants could help to reconstruct their evolutionary relationships. Knowing the molecular phylogeny of lupins, we could show that similarity of QA profiles are a poor indicator of a common descent. The genes of QA formation appear to be present in all legumes and it seems to be a matter of gene regulation which genes are switched on or off (Wink 2003; Wink et al. 2010). Furthermore, QA profiles often differ more between organs than between species, which would make the profiles an even more ambiguous taxonomic marker.

The Role of Quinolizidine Alkaloids in Plants

Lupin alkaloids are toxic to a number of insects, slugs and vertebrates. Furthermore, they are active against fungi (mildew), bacteria and even viruses and could be used as a natural pesticide (Wink 1992). QAs should thus be important for lupins as defence chemicals against herbivorous animals; in nature, alkaloid-rich lupins are usually left untouched by general herbivores (Wink 1992). QAs have evolved as neurotoxins, which target acetylcholine receptors and Na+/K+ ion
channels (Schmeller et al. 1997). QAs can bind to either the nicotinic acetylcholine receptor or the muscarinic acetylcholine receptor, probably as agonists (Wink 2000). Some individual QA interfere with dopamine, GABA, NMDA and alpha-2 neuroreceptors. Sparteine and lupanine are inhibitors of sodium and potassium channels (Wink et al. 1998). Both interactions with neuroreceptors and ion channels qualify these alkaloids as potent neurotoxins.

However, a few specialists have evolved counter adaptations, such as the lupin aphid, Macrosiphon albifrons, which loves alkaloid-rich lupins (Wink 1992). This aphid is apparently able to tolerate and to store QA-although we do not know the underlying mechanisms. Aphids rich in QA are apparently protected against predators (Wink and Römer 1986). Aposematically coloured larvae of the pyralid moth Uresiphita reversalis feeds on QA-rich Teline plants. Larvae actively take up QAs and store them in their integument. QAs are not transferred to pupae and imagines but end up in the cocoon silk of the pupae (Wink et al. 1991). As expected, non-specialised aphids avoid QA-rich lupins but select alkaloid-poor sweet lupin cultivars. Also other animals discriminate between bitter and sweet lupins and prefer the latter (Wink 1992). This illustrates the role of QAs as defences against a broad range of herbivores.

Occurrence and Biosynthesis of Pyrrolizidine Alkaloids

PAs occur in several families and are the main group of alkaloids in Boraginaceae, in the subtribe Senecioninae (Asteraceae) and in the tribe Crotalarieae (Fabaceae) (Hartmann and Witte 1995; El-Shazly and Wink 2014). The precursors of PAs are the amino acids ornithine and arginine, which produce the diamine putrescine. One unit of putrescine and spermidine are converted to homospermidine by homospermidine synthase. Homospermidine is deaminated and via a reactive aldehyde converted to the necine base skeleton. The necine base forms esters with small organic acids and generates cyclic PAs (retroconicine and otonecine type) and open-ringed PAs (heliotridine type) (Fig. 2) (Hartmann and Witte 1995).

Chemical Ecology of Pyrrolizidine Alkaloids

PAs are among the alkaloids, which are often stored by specialised insects. The role and fate of PAs have been most widely explored, thanks to the curiosity of Thomas Hartmann and Tom Eisner who explored dozens of PA sequestering arthropods (Eisner et al. 2007; Hartmann 1999; Hartmann and Witte 1995). As compared to QAs, the chemical ecology of PAs is far more intricate and advanced.

Arctiid moths are known to sequester PAs and cardiac glycosides. I personally came into contact with this topic through Dietrich Schneider, who had discovered the strange relationship between Creatonotus moths and PAs (Schneider et al. 1982; Boppré 1986). The hairy caterpillars of Creatonotus gangis and C. transiens prefer plant material with PAs. They even appear to be ‘addicted’ to PAs, because they will eat filter paper impregnated with pure PAs, when offered. We suggested that PAs induce a very strong feeding stimulus, similar to the behaviour of humans towards addictive drugs (Wink 2018).

For a couple of years, PAs were studied in Braunschweig by the Hartmann lab (Lindigkeit et al. 1997) and our lab in Munich, Mainz or Heidelberg (von Nickisch-Rosenegk et al. 1990; von Nickisch-Rosenegk and Wink 1993; Wink and Schneider 1988, 1990). Studies implied that PAs actively enter the larval body. In most plants, PAs are present as polar PA N-oxides that cannot pass biomembranes by simple diffusion. We discovered that epithelial transporter proteins can transport the polar PAs into the haemolymph (Wink and Schneider 1988). An alternative mechanism is also plausible: In the gut, PAs are reduced to the more lipophilic free base, which can pass the membranes by simple diffusion. In the haemolymph, however, PAs must be re-oxidised to PA N-oxides to avoid them from diffusing back (Lindigkeit et al. 1997; Wang et al. 2012). The PAs do not remain in the haemolymph but are sequestered into the larval integument (Egelhaaf et al. 1990; von Nickisch-Rosenegk et al. 1990; von Nickisch-Rosenegk and Wink 1993), where they apparently serve for chemical defence against predators (Martins et al. 2015).

The PA story becomes more complex, if we look closer into male and female larvae of Creatonotus after metamorphosis into adult insects: In female caterpillars, PAs will be stored partly in the integument, but a larger proportion is transferred to the eggs, which thus gain chemical defence (von Nickisch-Rosenegk et al. 1990). PAs thus function as a nuptial gift for the defense of the eggs; this phenomenon has also been described for other arctiids Uetheisa ornatrix and Cosmosoma myrodora (Bezzerides and Eisner 2002; Cogni et al. 2012; Conner et al. 2000; Gonzalez et al. 1999). Male moths of Creatonotus show impressive corematas (hairy, inflatable sacks at the abdomen). They are inflated during courtship to dissipate pheromones attracting female partners. Dietary PAs exhibit morphogenetic properties, i.e., they induce the formation of coremata. If a male caterpillar is reared on a PA-free diet, it will form only very small coremata as adult male (Boppré 1986; Schneider et al. 1982). The more PAs were ingested by a male caterpillar, the larger the corema become (von Nickisch-Rosenegk et al. 1990). Co-evolution was apparently even more intricate in this system (Schneider et al. 1982). As mentioned before, the coremata releases
pheromones to attract females. The pheromones of PA-sequestering arctiids consist of hydroxydanaidal (and others), which originate from dietary PAs (Boppré 1986; Schulz et al. 1993, 1998; Wink et al. 1988). Apparently, female moths are attracted by males with a fragrant PA perfume. And for good reason: the spermatophore of males contains dietary PAs, which were donated as a nuptial gift during copulation to the female. These nuptial PAs also end up in the eggs. Males can thus enhance the fitness of their offspring by transferring chemical defences.

Hydroxydanaidal, occurring in many PA plants is also a signal for other PA insects (Bogner and Boppré 1989). It was demonstrated that arctiid caterpillars have taste receptor neurons which are dedicated to the perception of PAs and PA-N oxides (Bernays et al. 2002, 2003). In vertebrates, PAs bind to serotonin receptors (Schmeller et al. 1997). We do not know, if this is also the case of insect serotonin receptors, which are involved in the regulation of feeding, food choice and sleep. Serotonin receptors are expressed in the brain but also in the intestinal tract of animals. Serotonin is involved in the regulation of appetite, mood and emotion, sleep, sexual activity, pain, learning and memory (Vleugels et al. 2015). As serotonin agonist often induce euphoria and hallucinations in vertebrates, we can only speculate that maybe also insects react to serotonin receptor agonists (Wink 2018). Possibly, this may explain the ‘addictive’ behaviour of arctiid moths towards PAs, described above. This is an open question, which needs to be addressed experimentally.

**Conclusion**

Both QAs and PAs are neurotoxins which are used by the plants producing them as chemical defense compounds against predators. However, a few insects have developed resistance to QAs and PAs. In fact, they do not only tolerate them, but store and utilize them as acquired defence chemicals against predators. In some moths, the interrelationship between PAs and insects is more complex and advanced. In these specialist species, PAs serve as morphogens for corema induction, as precursors for pheromones and as nuptial gifts. Despite many years of research by Thomas Hartmann, his students and colleagues, many details of the biochemistry and molecular biology of these adaptations need to be explored in future studies. New technologies, such as Next Generation Sequencing of DNA and RNA may open novel possibilities to gain a better understanding of the evolution and chemical ecology of these alkaloids.
Acknowledgements

Thomas Hartmann was my PhD supervisor at the Technical University of Braunschweig between 1977 and 1980. From 1980 to 1985 he was a mentor for my Post-Doc studies leading to my habilitation in 1985. I am always thankful to Thomas Hartmann that he had lured me away from zoology into botany and pharmaceutical biology for my PhD studies. What both of us did not know at that time, was that we would develop a serious interest in chemical ecology and the functional aspects of plant alkaloids and various trophic levels. After 40 years of endeavour, I am happy to say that this deviation was worth it.

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