

## Pyrrolizidine alkaloids – chemistry, biosynthesis, pathway, toxicity, safety and perspectives of medicinal usage

MARIOLA DREGER, MARZENA STANISŁAWSKA, ANNA KRAJEWSKA-PATAN,  
SEBASTIAN MIELCAREK, PRZEMYSŁAW ŁUKASZ MIKOŁAJCZAK,  
WALDEMAR BUCHWALD

The Branch of Medicinal Plants  
Institute of Natural Fibres and Medicinal Plants  
Libelta 27  
61-707 Poznań, Poland

\*corresponding author: mdreger@iripz.pl

### Summary

Pyrrolizidine alkaloids (PAs) are the class of secondary metabolites that evolved as a powerful tool in the plant defensive interactions against herbivores. The occurrence of PAs in the plant world is scattered in several unrelated botanic families with special abundance in Asteraceae, Boraginaceae and Fabaceae. Homospermidine synthase (HSS) was recognized as a key enzyme that catalyzes homospermidine formation from polyamines. The studies of HSS kinetic and gene sequence revealed that it is of polyphyletic origin and raised as a result of deoxyhypusine synthase (DHS) gene duplication. The ability of PAs production occurred independently at least four times in course of plant evolution. The PAs biosynthesis is tightly correlated with growth phase and biomass production. It is supposed that PAs biosynthesis is individually regulated in different lineages of plants. The PAs with a 1,2 unsaturated necine skeleton show toxic activity (hepatotoxicity, carcinogenicity, genotoxicity, teratogenicity and cytotoxicity). It is thought that pyrrolic esters formation during the detoxication process in the liver is the main mechanism of PAs toxicity. The pyrrolic esters are highly reactive and tend to bind rapidly with nucleophilic macromolecules including DNA and DNA-protein inducing hepatotoxicity or tumorigenicity. The problem of PAs toxicity cause the restrictions in the production and sale of herbal products. This review encompasses the present status of the pyrrolizidine alkaloids in the plants studies and summarize the topics of chemistry, biosynthesis, evolution including the involved genes, the factors affecting on biosynthesis, accumulation and toxicity of PAs.

**Key words:** pyrrolizidine alkaloids, metabolic pathways, biosynthesis, deoxyhypusine synthase (DHS), homospermidine synthase (HSS), toxicity

## INTRODUCTION

The pyrrolizidine alkaloids (PAs) are regarded as typical secondary metabolites that illustrate the chemically mediated plant-herbivore interactions. They play a key role in the plant defense against the attack of the insects, nematodes, mammals and other herbivores [1, 2]. The PAs derived from the plant are accumulated and utilized by some insects (the butterflies – Arctiidae; Danaidae; Ithomiinae and the beetles – Chrysomelidae) as the defense against predators and parasitoids [3-5] or for the production of male pheromones [6, 7]. The PAs produced by the grass endophytic fungus increase resistance to insect feeders, drought and inhibit the germination of seeds of the others plants [8, 9]. The main biological meaning of the PAs is a defense role and, although nowadays it has been intensively investigated, it is still not enough understood.

The occurrence of PAs in the plant world is scattered in several unrelated botanic families: Asteraceae, Boraginaceae, Fabaceae, Apiaceae, Convolvulaceae, Celastraceae, Proteaceae, Santalaceae, Sapotaceae, Ranunculaceae, Euphorbiaceae, Orchidaceae, Scrophulariaceae, and also Poaceae [10-14]. The most important herbal species originated from families: Asteraceae (*Tussilago farfara*, *Petasites* sp., *Senecio* sp., *Adenostyles alliariae*, *Eupatorium* sp.) and Boraginaceae (*Symphytum* sp., *Borago officinalis*, *Anchusa officinalis*, *Cynoglossum officinale*, *Echium* sp., *Heliotropium* sp., *Lithospermum* sp.) [15]. The plants containing PAs are estimated as 3% of total plants. The pyrrolizidine alkaloids are ones of the most common toxic compounds produced by plants. The cases of intoxication have been noted worldwide and toxicity effects of some PAs have been well documented in numerous publications. PAs show a special hepatotoxic activity [16, 17] and a long-term exposition may cause severe damages to heart, lungs, kidneys and lead to the death [18].

The problem of PAs toxicity led to the law restrictions in the sale of herbal products. According to the recommendation of E Commission [19], the allowed dose of PAs (with a 1,2 unsaturated necine skeleton) with their N-oxides cannot exceed 10  $\mu\text{g}$  per day in the oral administered herbal products (infusions and teas) and not more than 1  $\mu\text{g}$  in plant extract. The legislations of some countries are more restrictive, for instance in Austria any plant products containing senecionine or senkirkine can not be registered [20]. In Poland the recommendations of E Commission are expressed in the Ministry of Health Regulation from 2003 [21].

The law restrictions of PA content eliminated some traditional herbal products or significantly reduced use of them, for instance the comfrey or coltsfoot products – the crucial in the treatment of the upper respiratory tracts disorders and the caught.

This review encompasses the present status of the pyrrolizidine alkaloids in the plants studies and summarize the topics of chemistry, biosynthesis, evolution including the involved genes, the factors affecting on biosynthesis, accumulation and toxicity of PAs.

## CHEMISTRY AND TYPE OF PYRROLIZIDINE ALKALOIDS

Pyrrolizidine alkaloids constitute a large group of alkaloids containing the pyrrolizidine nucleus. The molecules comprises two five-membered rings which share a nitrogen atom at position 4. The base ring system is an amino alcohol (necine) that can be esterified with necic acids or alkaloid N-oxides (except from the otonecine-type alkaloids, see fig. 1) [22]. Necic acids are branched-chained mono- or di-carboxylic acids containing four to six carbon atoms and are typically unsaturated, hydroxylated or epoxidized. The esters can be divided into monoesters (C9), non-macrocyclic diesters (C7 and C9 positions) and macrocyclic diesters (linking C7 with C9) of a necine base. The necine can either be saturated or possess a double bond in the 1,2-position [15, 23].

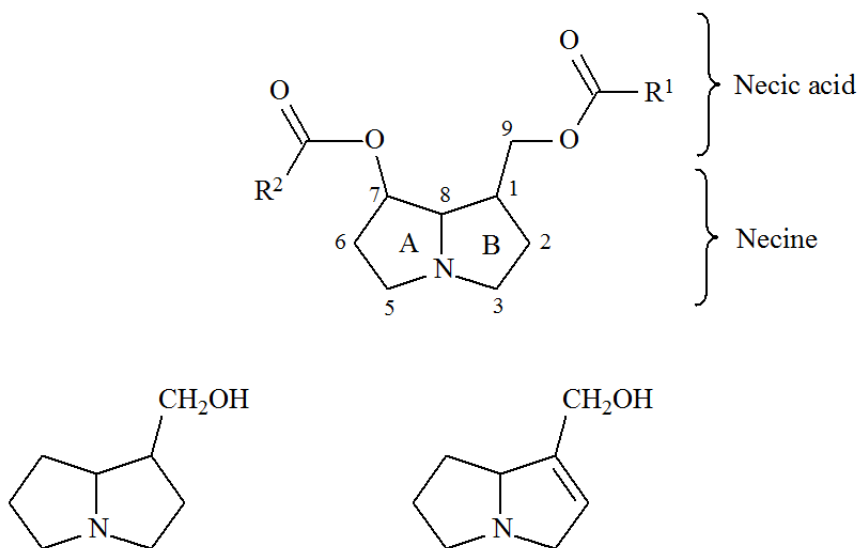


Figure 1. Pyrrolizidine alkaloid structure

In general, PAs have four bases: heliotridine-type, platynecine-type, otonecine-type and retronecine-type (fig. 2) [15, 24]. Retronecine and heliotridine are enantiomers at C7 position and have received the most attention [25]. For the majority of vertebrates PAs are very firmly hepatotoxic, pneumotoxic and genotoxic to insects. The hepatotoxic alkaloids have a 1,2-double bond in the pyrrolizidine ring and branched chain acids, esterifying a 9-hydroxyl and preferably also the 7-hydroxyl substituent, whereas in non-hepatotoxic PAs the necine base is saturated. The monoesters are the least toxic group, non-macrocyclic diesters have an intermediate toxicity, being about four times toxic as their respective monoesters and macrocyclic diesters are the most toxic compounds within the group of PAs [25]. On the other hand it is known that the pyrrolizidine alkaloids of the platynecine-type lacking the 1,2-double bond are not toxic.

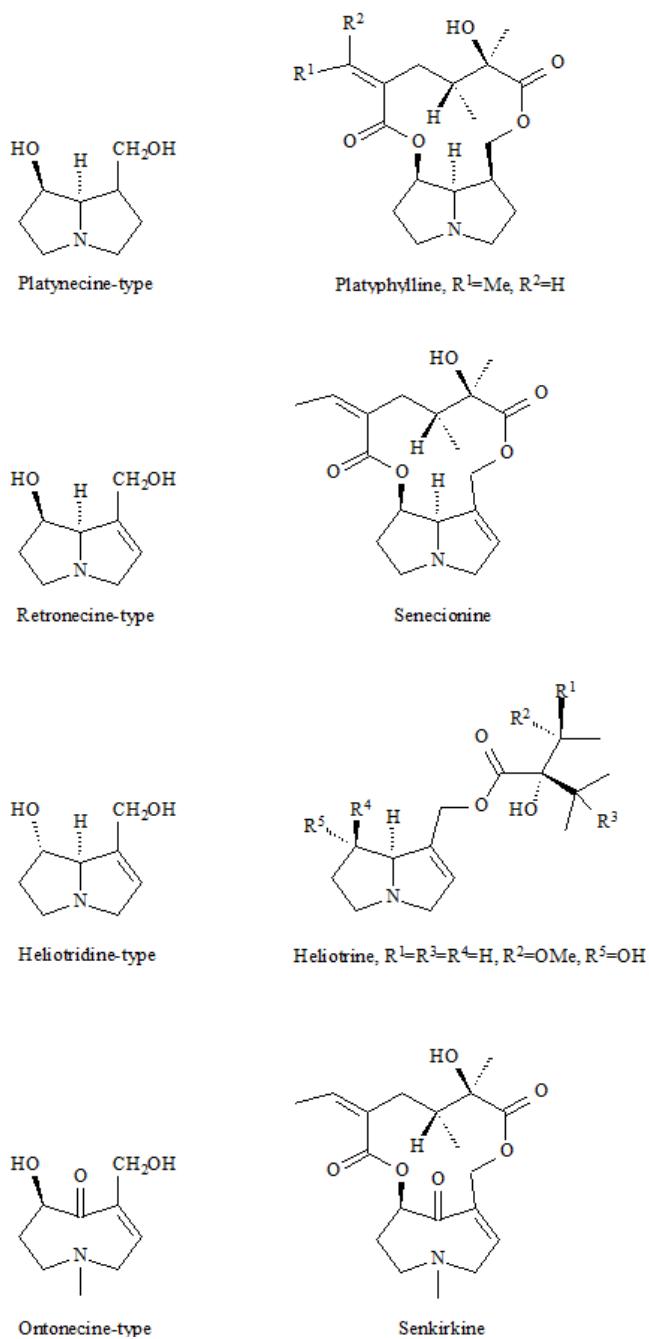


Figure 2. The base types of pyrrolizidine alkaloids and their examples

The pyrrolizidine alkaloids identified in the medicinal plants (families: Fabaceae, Boraginaceae and Asteraceae) are presented in table 1.

**Table 1.**

The pyrrolizidine alkaloids identified in plant species among main botanical families: Fabaceae, Boraginaceae and Asteraceae (data originated from the literature)

family	species	kind of pyrrolizidine alkaloids		
FABACEAE	<i>Crotalaria</i>	<i>juncea</i>	riddelline [26], seneciophylline [26]	
		<i>brevifolia</i>	integerrimine [26], usaramine [26]	
		<i>incana</i>	anacrotine [26], crotanecine [26], usaramine [26]	
		<i>quinquefolia</i>	monocrotaline [26]	
		<i>usaramoensis</i>	retrosine [26]	
		<i>fulva</i>	fulvine [26]	
BORAGINACEAE	<i>Alkana</i>	<i>trinctoria</i>	triangularine [15, 26], 7-angeloylretronecine [15], dihydroxytriangularine [2],	
		<i>officinalis</i>	clivorine [26], laburnine [15, 25], intermedine [15, 25], 7-acetyllycopsamine [25], acetyllyburnine [15, 25], lycopsamine [15, 25],	
		<i>officinalis</i>	thesinine- 4'-O-β-D-glucoside [27], intermedine [25], lycopsamine and their 7-acetyl derivatives [15], amabiline [15], thesinine [15], supinine [15]	
	<i>Cymoglossum</i>	<i>officinale</i>	heliosupine [15, 25, 26,28-30], heliosupine N-oxide [28-30], 12-acetylheliosupine [15], echinatine [15,28,29], 7-angeloylheliotridine [15, 28-30], 3'acetylheliosupine [28-30], viridiflorine [25, 28-30], trachelanthamine [25], 7-tigloylheliotridine [28-30], rinderine [28-30], 7-angeloylrinderine [28-30], 7-angeloylretronecine [28-30], 7-angeloyl-1-formyl-6,7-dihydro-5H-pyrrolizine [29], 7-angeloyl-9-(2-methylbutyryl)heliotridine [29], 7-angeloyl-9-(2,3-dihydroxybutyryl) heliotridine [29]	
			<i>furcatum</i>	lactodine-monoester alkaloid [31], viridinatine-diester alkaloid [31]
			<i>glomeratum</i>	(7S, 8R)-petranine [32], (7S,8S)-petranine [32], (7R,8R)-petranine [32], (7R,8S)-petranine-comprising tricyclic pyrrolizidine alkaloids subclass [32], 7-angeloylretronecine [32], 9-angeloylretronecine [32]
	<i>Echium</i>	<i>vulgare</i>	echimidine [29], retronecine [29], echihumiline [29], hydroxymyoscorpine [29], 3'acetylechimidine [29], 7- and 9-angeloylretronecine [29], 7- and 9-tigloylretronecine [29], 9-seneciolyretronecine [29], uplandicine [29], 7-(2-methylbutyryl)retronecine [29], 9-(2-methylbutyryl)-retronecine [29], 7-angeloyl-9-(2-methylbutyryl)retronecine [29], 7-tigloyl-9-(2-methylbutyryl)retronecine [29], 7-angeloyl-9-(2,3dihydroxybutyryl) retronecine [29]	

family	species	kind of pyrrolizidine alkaloids
BORAGINACEAE	<i>arborescens</i>	indicine [15, 25], 12-acetylindicine [15, 25]
	<i>indicum</i>	indicine [26, 30], heliotrine [26, 29, 30], supinine [26, 30], lasiocarpine [29], echinatine [30], europine [30], heleurine [30], lasiocarpine (N-oxide) [30], helindicine [30], acetyllasiocarpine [30], heliosupine [30], lycopsamine [33]
	<i>europaeum</i>	heliotrine [26], europine [34], lasiocarpine [34], supinine [35], heleurine [35], acetyllasiocarpine [35],
	<i>Lithospermum officinale</i>	lithosenine [15], 12-acetyllithosenine [15]
	<i>Myosotis scorpioides</i>	symphytine [15, 26], myoscorpine [15], scorpioidine [15], 7-acetylscorpioidine [15]
	<i>officinale</i>	echimidine [26, 28], heliosupine [15, 26], lasiocarpine [15, 26], symphytine [15, 26, 28], intermedine, lycopsamine and their 7-acetyl derivatives [15, 28], symlandine [15], symviridine [15, 28], myoscorpine [15], echinatine [15], N-oxide [15], heliotrine [15], viridiflorine [15]
	<i>tuberosum</i>	echimidine [15, 26], symlandine [15], anadoline [15]
	<i>caucasicum</i>	the N-oxide of echimidine [15, 26], asperumine [15, 26], echinatine [15, 26], lasiocarpine [15, 26]
	<i>uplandicum</i>	intermedine [26], lycopsamine [26], symlandine [26], symphytine [26], uplandicine [26]
	<i>asperum</i>	lasiocarpine [26], intermedine [15], echimidine [15], lycopsamine [15], symlandine [15], symviridine [15], heliosupine N-oxide [35], myoscorpine [15], echinatine [35], symphytine [15], asperumine [15], lasiocarpine [15], echumine [15], makrotomine [15], rousorine [15]
	<i>Symphytum x uplandicum</i> Nyman (syn. <i>S. peregrinum</i> Ledeb.)	intermedine and lycopsamine and 7-acetyl derivatives of these alkaloids [15], uplandicine [15], symlandine [15], symviridine [15] myoscorpine [15], symphitine [15], echimidine [15]
	<i>Onosma leptantha</i>	echihumiline [36], echihumiline N-oxide [36], new pyrrolizidine alkaloids: leptanthine (2), its corresponding N-oxide 1, and N-oxide of 3'-O-acetylechihumiline [36]
	<i>Adenostyles alliariae</i>	senecionine [15], seneciphylline [15], spartioidine [15]
ASTERACEAE	<i>cannabinum</i>	intermedine [15], lycopsamine [15], amabiline [15], supinine [15, 26], rinderine [15, 26], echinatine [15], viridiflorine [30], cynaustaline [30]
	<i>japonicum</i>	amabiline [30], echinatine [30], intermedine [30], lycopsamine [30], rinderine [30], suoinine [30], viridiflorine [30], integerrimine [30], hastacine [30],
	<i>Tussilago farfara</i>	senkirkine [15, 26, 30, 37], senecionine [15, 30], tussilagine [15, 26, 30], isotussilaginine [15, 30], tussilaginine [15], syneilesine [30], acetylsyneilesine [30], integerrimine [30], seneciphylline [30], neosenkirkine [30]

family	species	kind of pyrrolizidine alkaloids
ASTERACEAE	<i>Emilia sonchifolia</i>	senkirkine [15, 26], doronine [15], nilgirine [30], integerrimine [30]
	<i>bicolor</i> (Willd.)	senecinine [15, 38], integerrimine [38], seneciophylline [15, 38], jacobine [15,38], jacoline [38], jaconine [15, 38], retrorsine [38], otosenine [38]
	<i>vulgaris</i>	jacobine [26], retrorsine [15,26], riddelline [15, 26], senecionine [15,26], integerrimine [15], usamarine [15], seneciophylline [15], spartoidine [15]
	<i>nemorensis</i>	retronecine [15], triangularine [15], sarracine [15], saturated platynecine type such as 7-and 9-angeloylretronecine [15], fuchsisenecionine [15], 7-seneciylretronecine [15], retroisosenine [30], 6-, 7-, 9-angeloylplatynecine [30], platyphilline [15], nemorensine [15,30], bulgarsenine [15], senecionine [15], retroisosenine [15], doronenine [15, 30]
	<i>Senecio jacobaea</i>	jacobine [15, 26], senecionine [15, 26], retrorsine [15], senkirkine [26], senecivernine [15], integerrimine [15], usaramine [15], 21-hydroxyintegerrimine [15], riddelline [15], seneciophylline [15], spartoidine [15], jacoline [15], jaconine [15], jacozine [15] (Z)-erucifoline [15], (E)-erucifoline [15], acetylerucifoline [15]
	<i>platyphyllus</i>	platyphilline [26], seneciophylline [26]
	<i>brasiliensis</i>	integerrimine [26, 29], senecionine [29], retrorsine [29], usaramine [29]
	<i>aureus</i>	floridanine [15], otosenine [15], florosene [15], senecionine [35]
	<i>doronicum</i>	doronenine [15], bulgarsenine [15]
	<i>cineraria</i>	senecionine [39], jacobine [39], otosenine [39], 9-methyl-didehydroretronecine [39], 5,6-dihydro-7,9-dimethoxy-7H-pyrrolizidine [39], senecivernine [39], integerrimine [39], jacoline [39], jaconine [39], usaramine [39], florosene [39], doronine [39]
	<i>Petasites hybridus</i>	integerrimine [15, 26, 40], petasitenine [15, 26], senkirkine [15, 26, 29], senecionine [15, 29, 40], neopetasitenine [15], neoplatyphylline [15], isotussilagine [15], tussilagine [15, 29], seneciophylline [29]
	<i>spurius</i>	senkirkine [15], farfugine [15], isotussilagine [15], isotussilaginine [15], tussilagine [15]
	<i>japonicus</i>	petasinine [30], petasinoside [30], senecionine [30], integerrimine [30], seneciophylline [30]
	<i>Ligularia platyglossa</i>	petasitenine [30], neopetasitenine [30], senkirkine [30]
<i>dentate</i>	ligularizine [30], ligularinine [30], clivorine [26]	
<i>tsangchanensis</i>	O-acetylamataimine [42], O-acetylamataimine N-oxide [42]	
<i>hodgsonii</i>	clivorine [30,41], ligularine [30,41]	

## BIOSYNTHESIS

The PAs are synthesized from polyamines: putrescine and spermidine. Putrescine is a substrate in the secondary metabolism in plants, although, it is also a common polyamine in eukaryotes and bacterial. Spermidine is an universal cell-growth factor involved in many physiological processes in eucaryotes [43]. Both putrescine and spermidine derive from arginine. *Spermidine* is synthesized from putrescine and decarboxylated S-adenosylmethionine (*SAM*). An aminopropyl group is transferred from decarboxylated SAM to putrescine by spermidine synthase (SPDS) [44]. Putrescine may be produced from ornithine by ornithine decarboxylase (ODC) in animals' organisms. The plant roots are devoid of ODC, therefore, putrescine is synthesized via arginine-*agmatine* pathway [45]. The first pathway specific step of pyrrolizidine alkaloid biosynthesis is homospermidine formation catalyzed by homospermidine synthase (HSS). The aminobutyl moiety is transferred by HSS from spermidine in an NAD<sup>+</sup>-dependent reaction to putrescine [46-49]. This reaction has been shown in the root culture of *Senecio* species fed with radioactive precursors [50]. The necine base moiety is formed from homospermidine by oxidative deaminations and converted into senecionine [43]. The final products are N-oxide forms of alkaloids. If the oxidation of diamine is inhibited ( $\beta$ -hydroxyethylethylhydrazine-inhibitor)-homospermidine is accumulated until the inhibition is released [46].

The sites of synthesis may vary among species – in *Heliotropum indicum* (Boraginaceae) plants PAs are synthesized in shoots whereas in *Symphytum officinale* in roots only [51]. The first step of PAs biosynthesis in Asteraceae takes place in the roots [49, 50, 52, 53]. The next steps – the conversion of N-oxides into specific alkaloids take place in the underground parts of the plants – leaves and inflorescens [43]. The comparative studies on five *Senecio* species revealed that N-oxides senecionine is transformed into species-specific alkaloids patterns, the transformation takes places in shoots (leaves, stems and inflorescences) and may vary quantitatively and qualitatively among different organs. The transformation encompasses one- or two-step reactions (hydroxylation, dehydrogenation, epoxidation, acetylation etc.) and results in the unique bouquet of the pyrrolizidine alkaloids for the species. The PAs are spatially mobile and slowly allocated over the plant. They are mainly accumulated in the inflorescence (60% – 80%) in *Senecio* plants [10]. In *Phalenopsis* sp. orchids PAs are allocated in young organs but the highest concentration of them was found in flower buds [53].

The diversification in PAs accumulation in different organs may indicate a defensive strategy against herbivores. The distribution of PAs are highly plastic and dynamic, however, the final products do not underlie the turnover [10].

The studies on *Senecio* sp., *Symphytum* sp., *Petasites* sp. plants demonstrated the chemical diversity and genetic variation of chemotypes in different populations [39, 40, 54-57]. The detailed genetic studies on *Senecio jacobea* plants revealed large phenotypic variations in the total contents and relatively amounts of major



individual of PAs [58]. It suggests that natural selection may reinforce and consolidate variation in PAs concentration and composition in the different populations of *S. jacobea* [10]. Thus both the genetic and the ecological factors play main roles in variability and chemical variation between and within plant populations.

The structural similarities to pyrrolizidine alkaloids demonstrate the lolines – the compounds produced by grasses infected by endophytic fungal symbionts [9] that are suggested to increase tolerant for biotic and abiotic stress. Their chemical structures are necine ring containing tertiary amine. The lolines are insecticidal but not hepatotoxic for mammalian herbivores. The biosynthesis pathway of lolines are completely different – they are not made from polyamines like the rest of pyrrolizidine alkaloids [9], since lolines are formed from amino acids: L-proline and L-homoserine [59, 60]. The genetic studies confirmed the separated biosynthetic pathways [61, 62]. Nowadays, the lolines have been intensively studied and new clues for the biosynthesis and endophytic correlations are reported.

## EVOLUTIONARY ORIGIN OF PAs BIOSYNTHETIC PATHWAY

The HSS exhibits highly similarity to deoxyhypusine synthase (DHS) – an enzyme involved in the primary metabolism, which participates in post-translational activation of translation initiation factor 5A (eIF5A). DHS transfers aminobutyl moiety of spermidine like HSS but the acceptor of this moiety is not putrescine but factor 5A (eIF5A) [64-66]. This is two steps process: first aminobutyl moiety of spermidine is linked to a specific protein bound lysine residue forming deoxyhypusine. In the next step it is hydroxylated to hypusine activating eIF5A protein [14, 66]. The reaction is NAD<sup>+</sup> dependent and diaminopropane is released. The studies on hypusine formation revealed that eIF5A is essential for the cell proliferation, growth and survival [64, 67]. There are evidences indicating the various role of the eIF5A from translation to mRNA to nuclear protein export and also in germination, senescence and apoptosis [68-70]. The studies on DHS mRNA expression shows that this gene acts on plant growth, development and senescence [67, 71, 72]. The transgenic tobacco plants with virus-induced silencing of DHS gene exhibited the delay in natural senescence, bolting and fruiting, the increasing of biomass (only in lower humidity), decreasing the seeds yield and the lower chlorophyll content [70].

The studies on *Senecio vernalis* DHS and HSS recombinant revealed that HSS gene is a result of DHS gene duplication [14, 65, 66, 74]. The kinetic analyses of DHS and HSS established the identity of two enzymes with one exception: DHS is unable to bind the eIF5A factor. It has been shown that DHS was able to accept not only eIF5A but also putrescine and thus the formation of homospermidine [65, 66]. This side activity of DHS is interpreted as a remain of the past activity that was lost in evolutionary process. The homospermidine is found in almost all analyzed plant species but only in trace amounts [14]. This fact supports the Hughes' [75] evolutionary model that supposes the bifunctional activity period of an-

cestoral gene after duplication. Therefore, HSS is thought to be a duplicated gene of DHS that lost the ability to bind the eIF5A factor but retaining all properties including homospermidine formation [14]. The sequences encoding HSS and DHS are almost identical and highly conserved – the degree of amino acid identity in single plants is ranged between 70% (*Phalenopsis* sp. Orchids) and 90% (Asteraceae) [14, 76]. Only in Senecionae hss genes lost one of the six introns [12].

The plants that can produce PAs are scattered within angiosperms among many unrelated families. Moreover, the ability of PAs biosynthesis is also noted in monocotyledon – in a few species of Poaceae [13, 76]. This fact may suggest a very early origin of PAs biosynthesis from the common ancestor or the independently repeated evolution in the separated lineages. The phylogenetic studies demonstrated that duplication dhs gene had occurred in plant evolution at least four times – at the earliest in Boraginaceae, the monocot lineage and twice in Asteraceae (independently in subfamilies: Senecioneae and Eupatorieae) [12, 66]. The most recent studies on monocot lineages (Poaceae and Orchidaceae) suggest that gene duplication occurred early just before diversification of the separated lineages [76].

In their most recent work, Abdelhady and coworkers transformed tobacco plants with HSS of *Senecio vernalis* under the control of 35S promotor of cauliflower mosaic virus to simulate the probable evolutionary PAs biosynthesis pathway [77]. The results revealed the significant increase of homospermidine level (over 65-fold) in the transformed plants in comparison to the wild type plants. The drastic increase of homospermidine content effected on the putrescine and spermidine levels – the decrease from 20% and 80% was noted respectively while no changes in growth and phenotype of transformed plants were observed. The authors suggested several explanations: the reduced spermidine level was sufficient to maintain the base cellular physiological processes and the normal phenotype was kept. The another explanation postulates that homospermidine might be able to compensate the reduction of spermidine level and replace its functions. It has been documented that DHS is able to accept homospermidine instead of spermidine with the same efficiency [65, 66]. In the experiment the total content of spermidine and homospermidine in transgenic plants was almost identical to the wild type suggesting the substitution of these triamines. In the transformed plants the spermidine synthase competed with HSS for putrescine as the substrate that might explain the reduced level of this polyamine. Although HSS was active, the transgenic plants, did not produce pyrrolizidine alkaloids. The high level of homospermidine was not sufficient to initiate the next steps of PAs biosynthesis.

The recent view on the pathway evolution suggests that it is not a matter of genes duplication but rather it involves regulatory genes that control more than one pathway [77]. The authors postulate diamine oxidation as a candidate and suggest that it might be a “bottle neck” enzyme in PAs next steps of the biosynthesis. However, the studies on PAs biosynthesis and its evolutionary origin are still not enough to explain how the biosynthetic pathway is controlled and regulated as well as what factors effect on it.

## REGULATION OF PAS BIOSYNTHESIS

The PAs biosynthesis is a strictly regulated and coordinated process both developmentally and spatially [10, 12, 53]. It has been proved that different types of tissues involve HSS expression within Asteraceae family [49, 52, 68] and in *Phalenopsis* orchids [53]. In all cases HSS expression is restricted to specific cells or tissues dependent on their growth and developmental stage [14]. In Senecionae (Asteraceae), HSS is expressed exclusively in specialized cells in the cortex parenchyma and in endodermis of the roots [68]. It suggests that whole PA biosynthetic pathway takes place in roots and then PAs are transported by vascular tissues (the proximity of them and HSS express cells are striking). The HSS is expressed in *E. cannabinum* roots – in all cortex cells with exception of endodermis [49]. In the *Phalenopsis* plants, both the tips of the aerial roots and the young flower buds are the places of PAs biosynthesis [53]. The HSS is also expressed in young flower buds but just before opening the flowers the biosynthesis is switched off, despite the PAs level remains high. The same phenomenon was observed in *Eupatorium* plants – as soon as the developmentally growth stops and the flowers are open, HSS expression is switched off. In *Senecio vernalis* plants no developmentally regulation was observed. The regulation mechanism is supposed to ensure the constant PAs level in the biomass and PAs are synthesized as long as the biomass is produced [14]. These data support an idea of the individually regulated PAs biosynthesis in different lineages of the plants. Moreover, the regulatory elements controlling HSS expression have evolved independently and individually [14].

The developmentally regulated spatial expression pattern was also found in biosynthesis of tropane alkaloids and nicotine biosynthesis – the other important compounds in plants. A first enzyme in the nicotine-specific pathway – a putrescine N-methyltransferase (PMT) and isoflavone reductase are both expressed specifically in cortex and endodermis of the roots tips [78]. The alkaloids or their intermediates are moved from the synthesizing cells to neighbor tissues and transported *via* vascular system to accumulating cells [78]. The transport needs a special alkaloid carriers. An multidrug-resistance ATP-binding cassette (ABC) transporters associated with xylem parenchyma are involved in berberine and also in the others tropane alkaloids transport [43]. The translocation of N-oxides from roots to shoots *via* phloem needs the specific carriers responsible for the selective uptake and the accumulation in the vacuoles [79, 80]. The transporter system of the PAs has been not identified and characterized so far.

It is suggested that the feed stress stimulates the enhancement of PAs biosynthesis. The studies on alkaloids level in *Cynoglossum officinale* (Boraginaceae) and on *Senecio jacobea* (Asteraceae) are not equivocal [81-83]. In the experiments the PAs content was elevated after cutting off 50% of the leaves in intervals: 3, 6, 12, 24 and 48 hours. The PAs concentration decreased within 6-12 hours in the damaged *Senecio jacobea* plants while in *Cynoglossum officinale* was doubled compared

to control values within 24 hours [82]. The similar tests with *Cynoglossum officinale* did not confirm the inducible role of the damage stress [81]. The authors concluded the changes in PAs distribution over the plants resulted from transport of PAs within the plants and questioned the role of the damage stress in PAs induce. The study on *Senecio jacobea* plants (three genotypes) revealed the weak response to root damage on the PAs content in the shoot while the shoot damages caused the decrease of PAs level in the roots [83]. The authors assumed that allocation of the PAs depended on plant genotype. The changes in the composition of PAs was observed only in one genotype – the shoot damage increased erucifoline concentration in shoot and decreased senecionine level in the roots.

The main conclusion is that herbivory attack does not induce the enhancement of PAs production *de novo* but rather affects the allocation and the composition of alkaloids in the attacked plants.

The changes in concentration of different PAs in *Senecio jacobea* plants in response to nutrients addition were noted [84]. The decrease of PAs concentration (except jacobine) was noted while the nutrient supply was increasing. The total amount of PAs in whole plants was not influenced by nutrient supply. The authors explain the changes in PAs production as the effect of the biomass changes during the experiments.

Nowadays, the effect of rhizosphere fungal communities on PAs content and composition has been intensively studied. The report on *Senecio jacobea* indicated that plants with high jacobine level suppressed the growth of microbes by inducing a lower diversity of fungi in the rhizosphere compared to plants with low jacobine level [85]. The most recent study on *Jacobea vulgaris* (syn. *Senecio jacobea*) revealed that soil-type and soil-borne microorganisms influenced the composition of PAs in roots and shoots of the plants by changing the concentration of specific alkaloids [86]. The level of retrosine and retrosine N-oxide raised in the plants grown on soil inoculated with non-sterilized soil. The level of jacobine and jacobine N-oxide in plant's shoots was raised too. The strong negative effect on plant growth was found in the sterilized soil inoculated with their own microbial community. The results demonstrated that inoculation sterilized soil with only 5% of non-sterilized soil had a great impact on the plant growth and PAs composition but not a total PAs content.

There are limited reports on mutual relations between microorganism and plants and a role of microorganism in the synthesis of Pas. The intensive studies focused on these topics have been carried on.

## TOXIC ACTIVITY OF PYRROLIZIDINE ALKALOIDS

The presence of PAs in the herbal raw material is highly undesirable due to toxic activity of pyrrolizidine alkaloids. There are numerous reports concerning hepatotoxicity, carcinogenicity and genotoxicity of PAs [16, 87-94].

The pyrrolizidine alkaloids are responsible for human poisoning as a result of the consumption of contaminated food and medicinal plants (dietary supplements and teas). The intoxication cases have been noted worldwide: in Europe [18, 90, 91], Uzbekistan [95, 96], Tajikistan [97], Afghanistan [98, 99], India [100, 101], Africa [102, 103] and the USA [104-106]. The PAs have also caused economic damages to livestock: horses, cattle, sheep and others [107-110]. Moreover, the PAs have been found also in food products like milk, eggs, meat and honey [22].

More than 660 PAs have been identified and about half of them have been reported to cause liver damages [94]. In general, PAs that consist of unsaturated necine base are hepatotoxic, whereas those with saturated necine moiety are non-toxic. PAs require the metabolic activation in liver to exert their hepatotoxicity. The oxidation of the necine base to the pyrrolic ester (dehydropyrrolizidine) derivative is the first step leading to toxicity including cytotoxicity, hepatotoxicity, genotoxicity and tumorigenicity [93, 94]. Depending on the type of PA, the formation of pyrrolic esters proceeds in different manners. In the retronecine- and heliotridine-types it occurs by the hydroxylation of the necine base (at C-3 or C-7) and the next spontaneous dehydration. In the ontonecine – type of PAs, oxidative N-demethylation followed by spontaneous ring closure through the elimination of formaldehyde and then dehydration is needed to form pyrrolic esters [94]. The pyrrolic esters are highly reactive and tend to bind with nucleophilic macromolecules rapidly, inducing hepatotoxicity or tumorigenicity if they bind DNA leading to DNA-cross linking and DNA adducts formation [93]. The pyrrolic esters metabolites may bind with glutathione (GSH) and forming glutathione conjugates. This reaction is recognized as the self-defending mechanism preventing intoxication [93]. N-Oxidation (only in the retronecine- and heliotridine-types of PAs) is another metabolic intoxication pathway that generates N-oxides which can be reduced back to the parent PAs and then undergo metabolic activation to pyrrolic alcohols referred to as secondary toxic metabolites [94]. Both C-oxidation and N-oxidation reactions are metabolized by microsomal cytochrom P-450 (CYP) monooxygenases and CYP3A. The pyrrolic esters react in the site of formation in the hepatocytes. They can also pass to the adjacent sinusoids and react with endothelial cells of hepatic veins leading to hepatic Venous Occlusive Disease (VOD) and resulting in liver failure [91]. Sometimes pyrrolic esters can reach the lungs and heart and also bind with red blood cells causing lung damages [111]. Although there is no evidence that the pyrrolizidine alkaloids poisoning can cause liver cancer in humans [11], the animal tests demonstrated undoubtedly the tumorigenic activity of PAs [112-116]. The liver tumor is thought to be induced through the genotoxic mechanism mediated with DHP - derived DNA adduct formation. The reactive sites of the pyrrolic esters with DNA are C-7 or C-9 positions with the cyclic nitrogen in guanosine [117]. They are capable to bind to DNA and protein to form DNA cross-linking and DNA-protein cross-linking [118-120]. The DNA adducts formation is also thought to be responsible for mutagenicity and teratogenicity of PAs [119]. For example the dose dependent mutagenic effect was observed in *Drosophila melanogaster* assay

[121, 122]. The chromosome damages in human lymphocytes in *in vitro* study induced by senkirkine and tussilagine was demonstrated [92]. The inducible role of PAs in apoptosis of human cell lines was noted, too [123].

On the base of human PAs toxicity studies, WHO-IPCS [35] established that daily PAs intake of 10  $\mu\text{g}/\text{kg}$  bw may lead to veno-occlusive disease in humans. The Australian New Zealand Food Safety Authority (ANZFS) proposed a provisional tolerable daily intake (PTDI) of PAs in a dose of 1  $\mu\text{g}/\text{kg}$  b.w. per day [11]. The suggestion of ANZFS has been accepted and maintained by European Food Safety Authority in its 2007 report [25].

## CONCLUSIONS AND FUTURE PROSPECTS

Although the basis of PAs biosynthesis and its origin are known, the subsequent steps and especially the regulation mechanism are still poor understood. The searching and identification of new enzymes of PAs pathway are necessary to explain how it works and how it has evolved. In particular the studies of genes expression of the involved enzymes are crucial for the determining of the control and regulation system.

Without this knowledge and experimental data we are not able to control or use this promising system of safe pharmaceutical raw material production. Nowadays, much efforts are taken to eliminate or minimize the PAs content in the plants to obtain the safe raw material by searching and selection of low alkaloids plants lines [124, 125].

The manipulation of the acting factors involved in PAs production is an attractive idea as far as we will learn to control native gene expression in alkaloid biosynthesis. The missing gene of the PAs pathway is needed to complete as well as the specific signaling pathway. Moreover we have to know how it is integrated into general signaling cascades of the plant development and hormonal regulation [78]. It is necessary to know which alkaloid-specific transporters affect the accumulation and diversification of pyrrolizidine alkaloids and how they work. The role of the symbiotic microorganisms in the production of pyrrolizidine alkaloids and their relations with plants are next topics that might provide the new scope for searching and implementations. We still know very little about the mutual interactions between plants and microorganisms and their role in secondary metabolites productions. The co-evolution of plants and fungi/bacteria/insects suggests that some enzymes or intermediates of the metabolic pathways may be present also in microorganisms that colonize the plants. They might have evolved a detoxification system for antifungal and antibacterial toxins or might have evolved metabolic enzymes that catabolize plant secondary products for their nutrition [78]. The most known example is the degradation of salicylic acid by *Pseudomonas putida* salicylate hydrolase in transgenic *Arabidopsis* plants [126]. The idea of the exotic genes (enzymes) from various organisms that interact with

plants seems to be a promising in the plant engineering metabolic pathways. These exotic genes may be introduced into plants in order to reduce the level of accumulating alkaloids considerably or may be used in enzyme-linked assays to measure alkaloid content in plant extract [78]. The current study progress in genomic and molecular techniques create a hope that all enzymes from a given pathway will be cloned for several alkaloid classes. In future the results of studies focused on the metabolic pathways and regulation mechanism may enable designing and engineering of the plants which produce desirable alkaloids level or the plants that accumulate alkaloids in the proper phase of growth in the suitable organs.

Nowadays, the new pyrrolizidine alkaloids have been still identified and also have been synthesized to find the new compounds with promising pharmacological activities or the new important economical implementation. For example, the neuroprotective and antioxidant activities of ethyl acetate fraction extract from *Tussilago farfara* as the potential treatment in the neurodegenerative disorders were reported by Cho and coworkers [127]. The most current works indicate the new application of plants containing pyrrolizidine alkaloids: as a source of a novel effective antitripanosomal treatment against *Trypanosoma brucei brucei* [128] and also as the biological control tool against the root nematode management [2, 129].

The pyrrolizidine alkaloids are still fascinating and promising group of secondary metabolites. The future studies focused on their biosynthetic pathways and recognition of control mechanisms will allow to produce the valuable and safe raw material or to find the new applications for plants containing pyrrolizidine alkaloids.

## ACKNOWLEDGEMENT

This study was financed by the Ministry of Science and Higher Education under a research project no. NN405306236

## REFERENCES

1. Hartmann T, Ober D. Biosynthesis and Metabolism of Pyrrolizidine alkaloids in Plants and Specialized Insect Herbivores. *Topics Curr Chem* 2002; 209:207-43.
2. Thoden TC, Bopré M, Hallmann J. Pyrrolizidine alkaloids of *Chromolaena odorata* act as nematocidal agents and reduce infection of lettuce roots by *Meloidogyne incognita*. *Nematology* 2007; 9:343-9.
3. Rotschild M, Aplin RT, Cockrum PA, Edgar JA, Fairweather P, Lees R. Pyrrolizidine Alkaloids in arctiid moths (Lep.) with a discussion on host plant relationships and the role of these secondary plant substances in the Arctiidae. *Biol J Linn Soc* 1979; 12(4):305-26.
4. Bezzerides A, Yong TH, Bezzerides J, Hussein J, Ladau J, Eisner M, Eisner T. Plant-derived pyrrolizidine alkaloid protects eggs of a moth (*Uthesia ornatrix*) against a parasitoid wasp (*Trichogramma ostrinae*). *Proc Nat Acad Sci USA* 2004; 101(24):9029-32.
5. Lindigkeit R, Biller A, Buch M, Schibel H-M, Boppré M, Hartman T. The two faces of Pyrrolizidine Alkaloids: the Role of the Tertiary Amine and its N-Oxide in chemical Defense of Insects with Acquired Plant Alkaloids. *Eur J Biochem* 2004; 245(3):626-36.

6. Schulz S. Insect-Plant Interactions – Metabolism of Plant Compounds to Pheromones and Allomones by Lepidoptera and Leaf Beetles. *Eur J Org Chem* 1998; 1:13-20.
7. Bernays EA, Chapman RF, Hartmann T. A highly sensitive taste receptor cell for pyrrolizidine alkaloids in the lateral galeal sensillum of a polyphagous caterpillar, *Estigmene acrea*. *J Comp Physiol A* 2002; 188:715-23.
8. Bush LP, Wilkinson HH, Schardl CL. Bioprotective Alkaloids of Grass-Fungal Endophyte Symbioses. *Plant Physiol* 1997; 114:1-7.
9. Schardl CL, Grossman RB, Nagabyuru P, Faulkner JR, Malik UP. Loline alkaloids: currencies of mutualism. *Phytochemistry* 2007; 68(7):980-96.
10. Hartmann T. Chemical ecology of pyrrolizidine alkaloids. *Planta* 1999; 207:483-95.
11. Pyrrolizidine Alkaloids in Food. A toxicology Review and Risk Assessment. Technical Report. Series No 2. 2001 Australia New Zealand Food Authority:1-16
12. Reimann A, Nurhayati N, Backenköhler A, Ober D. Repeated evolution of the Pyrrolizidine Alkaloid-MediaTED Defense System in Separated Angiosperm Lineages. *The Plant Cell*. 2004; 16:2772-84.
13. Koulman A, Seelinger C, Edwards PJB, Fraser K, Simpson W, Johnson L, Cao M, Rasmussen S, Lane GA. E/Z-thesine –O-4'- $\alpha$ -rhamnoside, pyrrolizidine conjugates produced by grasses (Poaceae). *Phytochemistry*. 2008; 69:1927-32.
14. Ober D, Kaltenecker E. Pyrrolizidine alkaloids biosynthesis, evolution of a pathway in plant secondary metabolism. *Phytochemistry* 2009; 70:1687-95.
15. Roeder E. Medicinal plants in Europe containing pyrrolizidine alkaloids. *Pharmazie* 1995; 50:83-98.
16. Mattocks AR. Toxicity of pyrrolizidine alkaloids. *Nature* 1968; 217:723-8.
17. Furbee RB, Barlotta KS, Allen MK, Holstege CP. Hepatotoxicity Associated with Herbal Products. *Clin Lab Med* 2006; 26:227-41.
18. Roulet M, Laurini R, River L, Calame A. Hepatic veno-occlusive disease in newborn infant of a woman drinking herbal tea. *J Pediatr* 1988; 112:433-6.
19. Monography of E Commission Bundesanzeiger nr 138 z 27.07.1990. In: Rośliny Lecznicze w Fitoterapii. Research Institute of Medicinal Plants. Poznań 2000.
20. Bundesgesetzblatt 1994. Nr 555 z 21 lipca 1994, Verordnung 469:7.
21. Rozporządzenie Ministra Zdrowia z 30 maja 2003 r. w sprawie przedstawienia dokumentacji dołączanej do wniosku o dopuszczenie do obrotu produktu leczniczego. *Dz. U.* z 2003 nr 154 poz. 1506.
22. Coulombe RA. Pyrrolizidine alkaloids in foods. *Adv Food Nutr Res* 2003; 45:61-99.
23. Crews C, Berthiller F, Krska R. Update on analytical methods for toxic Pyrrolizidine alkaloids. *Anal Bioanal Chem* 2009. Published online: Springer.
24. Report on Carcinogens. Draft Background Document for Riddelliline. U.S. Department of Health and Human Services. Public Health Services. National Toxicology Program 2008.
25. European Food Safety Authority. Opinion the scientific panel on contaminants in the food chain on a request from the European Commission related to pyrrolizidine alkaloids as undesirable substances in animal feed. *The EFSA Journal* 2007; 447:1-51.
26. Haraborne JB, Baxter H, Moss GP. *Phytochemical Dictionary. A Handbook of Bioactive Compounds from Plants*. 2<sup>nd</sup> ed. 1999; 26:287-300.
27. Herrmann M, Joppe H, Schmaus M. Thesinine- 4'-O- $\beta$ -<sub>D</sub>-glucoside the first glycosylated plant pyrrolizidine alkaloid from *Borago officinalis*. *Phytochemistry* 2002; 60(4):399-402.
28. Stickele F, Seitz HK. The efficacy and safety of comfrey. *Public Health Nutrition* 2000; 3(4A):501-8.
29. Liddell JR. Pyrrolizidine alkaloids. *Natural Product Reports* 1998; 363-70.
30. Jiang Y, Fu PP, Lin G. Hepatotoxicity of naturally occurring pyrrolizidine alkaloids. *Asian Journal of Pharmacodynamics and Pharmacokinetics* 2006; 6(3):187-92.
31. Ravi S, Ravikumar R, Lakshmanan AJ. Pyrrolizidine alkaloids from *Cynoglossum furcatum*. *J Asian Nat Prod Res* 2008; 10(3-4):349-54.
32. Alali FQ, Tahboub YR, Ibrahim ES, Qanadil AM, Tawaha k, Burgess JP, Sy A, Nakanishi Y, Kroll DJ, Oberlies NH. Pyrrolizidine alkaloids from *Echium glomeratum* (Boraginaceae). *Phytochemistry* 2008; 69(12):2341-6.
33. Souza JSN, Machado LL, Pessoa ODL, Braz-Filho R, Overk CR, Yao P, Cordel GA, Lemos TLG. Pyrrolizidine alkaloids from *Heliotropium indicum*. *J. Braz Chem Soc* 2005; 16(6).
34. O'Dowd DJ, Edgar JA. Seasonal dynamics in pyrrolizidine alkaloids of *Heliotropium europaeum*. *Australian Journal of Ecology* 1989; 18:95-105.



35. WHO-IPCS (World Health Organisation-International Programme of Chemical Safety). Pyrrolizidine Alkaloids. *Environ Health Criteria* 1988; 80:1-345.
36. Kretsi O, Aligiannis N, Skaltsounis AL, Chinou IB. Pyrrolizidine Alkaloids from *Onosma leptantha*. *Helvetica Chimica Acta* 2009; 86(9):3136-40.
37. Bartkowski JP, Wiedenfeld H, Roeder E. Quantitative Photometric Determination of Senkirkine in *Farfara Folium*. *Phytochemical Analysis* 1998; 8(1):1-4.
38. Wiedenfeld H, Montes C, Tawil B, Contin A, Wynna R. Pyrrolizidine alkaloid level in *Senecio bicolor* (Willd.) Tod, ssp. *cineraria* (DC.) from middle Europe. *Pharmazie* 2006; 61(6):559-61.
39. Tundis R, Loizzo MR, Statti GA, Passalacqua NG, Peruzzi L, Menichini F. Pyrrolizidine alkaloids Profiles of the *Senecio cineraria* Group (Asteraceae). *Z Naturforsch* 2007; 62c: 467-72.
40. Chizzola R. The main pyrrolizidine alkaloids of *Petasites hybridus*: variation within and between populations. *Acta Horticulturae* 1993; 333:143-50.
41. Lin G, Rose P, Chatson KB, Hawes EM, Zhao XG, Wang ZT. Characterization of two structural forms of otonecine-type pyrrolizidine alkaloids from *Ligularia hodgsonii* by NMR spectroscopy. *Journal of Natural Products* 2000; 63(6):857-60.
42. Tan A, Wang Z, He H, Zhang M, Hao X. New Pyrrolizidine Alkaloids from *Ligularia Tsangchanensis*. *Heterocycles* 2003; 60(5):1195-8.
43. Facchini PJ. Regulation of Alkaloid Biosynthesis in Plants. Chapter 1. The Alkaloids. 2006; 63:1-44.
44. Graser G, Hartmann T. Biosynthesis of spermidine, a direct precursor of pyrrolizidine alkaloids in root cultures of *Senecio vulgaris* L. *Planta* 2000; 211:239-45.
45. Hartmann T, Sander H, Adolf R, Toppel G. Metabolic links between the biosynthesis of pyrrolizidine alkaloids and polyamines in root cultures of *Senecio vulgaris*. *Planta* 1988; 175:82-90.
46. Böttcher F, Ober D, Hartmann T. biosynthesis of pyrrolizidine alkaloids: putrescine and spermidine are essential substrates of enzymatic homospermidine formation. *Can J Chem* 1993; 72:80-85.
47. Ober D, Hartmann T. Homospermidine synthase, the first pathway-specific enzyme of pyrrolizidine alkaloid biosynthesis, evolved from deoxyhypusine synthase. *PNAS* 1999; 96: 14777-82.
48. Ober D, Harms R, Hartmann T. Cloning and expression of homospermidine synthase from *Senecio vulgaris*: a revision. *Phytochemistry* 2000; 55:305-9.
49. Anke S, Niemüller D, Moll S, Hänsch R, Ober D. Polyphyletic origin of pyrrolizidine alkaloids within Asteraceae. Evidence from differential tissue expression of homospermidine synthase. *Plant Physiology* 2004; 136:4037-47.
50. Sander H, Hartmann T. Site of synthesis, metabolism and translocation of senecionine N-oxide in cultured roots of *Senecio erucifolius*. *Plant Cell Tiss Org Cult* 1989; 18:19-31.
51. Frölich C, Ober D, Hartmann T. Tissue distribution, core biosynthesis and diversification of pyrrolizidine alkaloids of lycopsamine type in three Boraginaceae species. *Phytochemistry* 2007; 68:1026-37.
52. Hartmann T, Ehmke A, Eliet U, Borstel K, Theuring C. Sites of synthesis, translocation and accumulation of pyrrolizidine alkaloid N-oxides in *Senecio vulgaris*. *Planta* 1989; 177:98-107.
53. Anke S, Gondé D, Kaltenegger E, Hänsch R, Theuring C, Ober D. Pyrrolizidine Alkaloid Biosynthesis in *Phalenopsis* Orchids: Developmental Expression of Alkaloid-Specific Homospermidine Synthase in Root Tips and Young Flower Bud. *Plant Physiol* 2008; 148:751-60.
54. Macel M, Vrieling K, Klinkhamer PGL. Variation in pyrrolizidine alkaloid patterns in *Senecio jacobaea*. *Phytochemistry* 2004; 65:865-73.
55. Hartmann T, Ober D. Chemical diversity and variation of pyrrolizidine alkaloids of senecionine type: biological need or coincidence? *Planta* 1998; 206:443-51.
56. Trigo JR, Leal IR, Matzenbacher NI, Lewinson TM. Chemotaxonomic value of pyrrolizidine alkaloids in souther Brazil *Senecio* (Senecioneae: Asteraceae). *Biochemical Systematics and Ecology* 2003; 31:1011-22.
57. Huizing HJ, Gadella ThW, Kliphuis E. Chemotaxonomical investigations of *Symphytum officinale* polyploidy complex and *S. asperum* (Boraginaceae): The pyrrolizidine alkaloids. *Plant Systematics and Evolution* 1982; 40:279-92.
58. Vrieling K, De Vos K, Van Wijk CAM. Genetic analysis of concentration of pyrrolizidine alkaloids in *Senecio jacobaea*. *Phytochemistry* 1993; 32:1141-4.
59. Blankenship JD, Houseknecht JB, Pal S, Bush LP, Grossman RB, Schardl CL. Biosynthetic precursors of fungal pyrrolizidines, the loline alkaloids. *Chembiochem* 2005; 6:1016-22.

60. Faulkner JR, Hussaini SR, Blankenship JD, Pal S, Branan BM, Grossman RB, Schardl CL. On the sequence of bond formation in loline alkaloid biosynthesis. *Chembiochem* 2006; 7:1078-88.
61. Spiering MJ, Moon ChD, Wilkinson HH, Schardl CL. Gene clusters for insecticidal loline alkaloids in the grass-endophytic fungus *Neotyphodium uncinatum*. *Genetics* 2005; 169:1403-14.
62. Kutil BL, Greenwald C, Liu G, Spiering MJ, Schardl CL, Wilkinson HH. Comparison of loline alkaloid gene clusters across fungal endophytes: predicting the co-regulatory sequence motifs and evolutionary history. *Fungal Genet Biol* 2007; 44:1002-10.
63. Spiering MJ, Faulkner JR, Zhang DX, Machado C, Grossman RB, Schardl CL. Role of the LoP cytochrome P450 monooxygenase in loline alkaloid biosynthesis. *Fungal Genet Biol* 2008; 45:1307-14.
64. Park MH, Lee YB, Joe YA. Hypusine is essential for eukaryotic cell proliferation. *Boil Signals* 1997; 6:115-123.
65. Ober D, Hartmann T. Homospermidine synthase, the first pathway specific enzyme of pyrrolizidine alkaloid biosynthesis, evolved from deoxyhypusine synthase. *PNAS* 1999; 96:14777-82.
66. Ober D, Harms R, White L, Hartmann T. Molecular evolution by change of function: alkaloid-specific homospermidine synthase retained all properties of deoxyhypusine synthase except binding the eIF5A precursor protein. *J Biol Chem* 2003; 278:12805-12.
67. Park MH, Wolff EC, Folk JE. Is hypusine essential for cell proliferation. *Trends Biochem Sci* 1993; 18:475-9.
68. Moll S, Anke S, Kahmann U, Hänsch R, Hartmann T. Cell-specific expression of homospermidine synthase, the entry enzyme of the pyrrolizidine alkaloids pathway in *Senecio vernalis*, in comparison with its ancestor, deoxyhypusine synthase. *Plant Physiol* 2002; 130:47-57.
69. Hopkins M, Taylor C, Liu Z, Ma F, McNamara L, Wang TW, Thompson JE. Regulation and execution of molecular disassembly and catabolism during senescence. *New Phytol* 2007; 175:201-14.
70. Wang TW, Zhang CG, Wu W, Nowack LM, Madey E, Thompson JE. Antisense suppression of deoxyhypusine synthase in tomato delays fruits softening and alters growth and development. *Plant Physiol* 2005; 138:1372-82.
71. Ober D, Hartmann T. Deoxyhypusine synthase from tobacco. *J Biol Chem* 1999; 274:32040-7.
72. Wang TW, Lu L, Zhang CG, Taylor C, Thompson JE. Pleiotropic effects of suppressing deoxyhypusine synthase expression in *Arabidopsis thaliana*. *Plant Mol Biol* 2003; 52:1223-35.
73. Wang H, Ma R, Li R, Wang G, Wei J. Virus-induced silencing of a tobacco deoxyhypusine synthase gene. *Chinese Science Bulletin* 2005; 50:2707-13.
74. Ober D. Seeing double: gene duplication and diversification in plant secondary metabolism. *Trends Plant Sci* 2005; 10:444-9.
75. Hughes AL. The evolution of functionally novel proteins after gene duplication. *Proc R Soc Lond B Biol Sci* 1994; 256:119-24.
76. Nurhayati N, Gondè D, Ober D. Evolution of pyrrolizidine alkaloids in *Phalenopsis* orchids and other monocotyledons: Identification of deoxyhypusine synthase, homospermidine synthase and related pseudogenes. *Phytochemistry* 2009; 70:508-16.
77. Abdelhady M, Beuerele T, Ober D. Homospermidine in transgenic tobacco results in considerably reduced spermidine levels but is not converted to pyrrolizidine alkaloid precursors. *Plant Mol Biol* 2009; 71:145-55.
78. Hashimoto T, Yamada Y. New genes in alkaloid metabolism and transport. *Curr Opin Biotechnol* 2003; 14:163-8.
79. Von Borstel and Hartmann. Selective uptake of pyrrolizidine alkaloids by cell suspension cultures from pyrrolizidine alkaloid producing species. *Plant Cell Reports* 1986; 5:39-42
80. Ehmke A, von Borstel K, Hartmann T. Alkaloid N-oxides as transport and vacuolar storage compounds of pyrrolizidine alkaloids in *Senecio vulgaris* L. *Planta* 1988; 176:83-90.
81. Van Dam N, Vrieling K. Genetic variation in constitutive and inducible pyrrolizidine alkaloid level in *Cynoglossum officinale*. *Oecologia* 1994; 99:374-8.
82. Van Dam N, van der Meijen E, Verpoorte R. Induce responses in three alkaloid-containing plant species. *Oecologia* 1993; 95:425-30.
83. Hol WHG, Macel M, van Veen JA, van der Meijen E. Root damage and aboveground herbivory change concentration and composition of pyrrolizidine alkaloids of *Senecio jacobaea*. *Basic and Applied Ecology* 2004; 5:253-60.
84. Hol WHG, Vrieling K, van Veen JA. Nutrients decrease pyrrolizidine alkaloid concentration in *Senecio jacobaea* *New Phytologist* 2003; 158:175-81.

85. Kowalchuk GA, Hol WHG, van Veen JA. Rhizosphere fungal communities are influenced by *Senecio jacobaea* pyrrolizidine alkaloid content and composition. *Soil Biol Biochem* 2006; 38:2852-9.
86. Joosten L, Mulder PJJ, Klinkhamer PGL, van Veen JA. Soil-borne microorganisms and soil-type affect pyrrolizidine alkaloids in *Jacobaea vulgaris*. *Plant and Soil* 2009; 325:133-43.
87. Schoental R. Toxicology and Carcinogenic Action of Pyrrolizidine Alkaloids. *Cancer Res* 1968; 28:2237-46.
88. Hirono I, Mori H, Culvenor CC. Carcinogenic activity of coltsfoot, *Tussilago farfara* L. *Gann* 1976; 67:125-8.
89. Mori H, Sugie S, Yoshimi N, Asada Y, Furuya T, Williams GM. Genotoxicity of a Variety of Pyrrolizidine Alkaloids in the Hepatocyte Primary Culture-DNA Repair Test Using Rat, Mouse, and Hamster Hepatocytes. *Cancer Res* 1985; 45:3125-9.
90. Ridker PM, Ohkuma S, McDermott WV, Trey Ch, Huxtable RJ. Hepatic Venocclusive Disease Associated with the Consumption of Pyrrolizidine –Containing Dietary Supplements. *Gastroenterology* 1985; 88:1050-4.
91. Sperl W, Stuppner H, Gassner J, Judmaier W, Dietze O, Vogel W. *Eur J Pediatr* 1995; 154:112-6.
92. Kraus C, Abel G, Schimmer O. Studies on the Chromosome damaging Effect of Some Pyrrolizidine Alkaloids in Human Lymphocytes *in vitro*. *Planta Med* 1985; 51:89-91.
93. Fu PP, Xia Q, Lin G, Chou MW. Pyrrolizidine Alkaloids – Genotoxicity, Metabolism Enzymes, Metabolic Activation, and Mechanism. *Drug Metabol Rev* 2004; 36:1-55.
94. Jiang Y, Fu PP, Lin G. Hepatotoxicity of naturally occurring pyrrolizidine alkaloids. *Asian J Pharmacodyn Pharmacokin* 2006; 6:187-92.
95. Dubrovinski SB. The etiology of toxic hepatitis with ascites. In: Millenkov S.M. & Kizhaikin, Y, (ed.). *[Symposium on Toxic Hepatitis With Ascites,] Tashkent, 1952.* V.M. Molotov Medical Institute.
96. Khanin MN. The etiology of toxic hepatitis with ascites. *Arch Pathol USSR* 1948; 1:42-7.
97. Chauvin P, Dillon JC, Moren A. An outbreak of Heliotrope food poisoning, Tadjikistan, November 1992-March 1993. *Sante* 1994; 4:263-8.
98. Tandon HD, Tandon BN, Mattocks RR. An epidemic of veno-occlusive disease of the liver in Afghanistan. *Am J Gastroenterol* 1978; 72:607-13.
99. Mohabat O, Srivastava RN, Younos MS, Sediq GG, Merzad AA, Aram GN. An outbreak of hepatic veno-occlusive disease in Afghanistan. *Lancet* 1976; 2:269-71.
100. Tandon BN, Tandon HD, Tandon RK, Narendranathan M, Joshi YK. Epidemic of veno-occlusive disease in central India. *Lancet* 1976; 2:271-2.
101. Tandon BN, Krishnamurthy L, Koshy A, Tandon HD, Ramalingaswami V, Bhandari JR, Mathini MM, Mathur PD. Study of an epidemic of jaundice in North-West India, presumably due to toxic hepatitis. *Gastroenterology* 1977; 72:488-94.
102. Selzer G, Parker RGF. *Senecio* poisoning exhibiting as Chiari's syndrome: a report of 12 cases. *Am J Pathol* 1951; 27:885-907.
103. Ka AS, Michel G, Imbert P, Diakhaté I, Seye MN. Hepatic veno-occlusive disease in a five-year-old child in Senegal. *Med Trop* 2006; 65:514-5.
104. Stillman AS. Hepatic veno-occlusive disease due to pyrrolizidine (*Senecio*) poisoning in Arizona. *Gastroenterology* 1977; 73:349-52.
105. Huxtable RJ. Herbal teas and toxins; novel aspect of pyrrolizidine poisoning in US. *Perspect Biol Med* 1980; 24:1-14.
106. Bach N, Thung SN, Schaffner F. Comfrey herb tea-induced hepatic veno-occlusive disease. *The American Journal of Medicine* 1989; 87:97-9.
107. Knight AP, Kimberling CV, Stermitz FR, Roby MR. *Cynoglossum officinale* (hound's –tongue)-a cause of pyrrolizidine alkaloid poisoning in horses. *J Am Vet Assoc* 1984; 185:647-50.
108. Fletcher MT, McKenzie RA, Blaney BJ, Reichmann KG. Pyrrolizidine alkaloids in *Crotalaria taxa* from northern Australia: risk to grazing livestock. *J Agric Food Chem* 2009; 57:311-9.
109. Nobre VMT, Dantas AFM, Riet-Correa F, Barbarosa Filho JM, Tabosa IM, Vasconcelos JS. Acute intoxication by *Crotalaria retusa* in sheep. *Toxicon* 2005; 45:347-52.
110. Pass DA, Hogg GG, Russell RG, Edgar JA, Tence IM, Rikard-Bell L. Poisoning of chickens and ducks by pyrrolizidine alkaloids of *Heliotropium europium*. *Australian Veterinary Journal* 1978; 55:284-8.
111. Huxtable RJ. Activation and pulmonary toxicity of pyrrolizidine alkaloids. *Pharmacology and Therapeutics*. 1990; 47:371-389

112. Mattocks AR, Cabral JR. Carcinogenicity of some pyrrolic pyrrolizidine alkaloid metabolites and analogues. *Cancer Letters* 1982; 17:61-6.
113. Hirono I, Haga M, Fuji M, Matsuura S, Matsubara M, Nakayama M, Furuya T, Hikichi M, Takanashi H, Uchida E, Hosaka S, Ueno I. Induction of hepatic tumors in rats by senkirkine and synphytine. *J Natl Cancer Inst* 1979; 63:469-72.
114. Chan PC, Mahler JR, Bucher JR, Travlos GS, Reid JB. Toxicity and carcinogenicity of riddelline following 13 weeks of treatment to rats and mice. *Toxicol* 1994; 32:891-908.
115. Chan PC, Haseman JK, Prejean JD, Nyska A. Toxicity and carcinogenicity of riddelline in rats and mice. *Toxicol Lett* 2003; 144:295-311.
116. Culvenor CCJ. Estimated intakes of pyrrolizidine alkaloids by humans. A comparison with dose rats causing tumors in rats. *J Toxicol Environmental Health* 1983; 11:625-35.
117. Robertson KA. Alkylation of N2 in deoxyguanosine by dehydroretronecine, a carcinogenic metabolite of PA monocrotaline. *Cancer Res* 1982; 42:8-14.
118. Hinks JR, Kim HY, Segall HJ, Molyneux RJ, Stermitz FR, Coulombe RA. DNA cross-linking in mammalian cells by pyrrolizidine alkaloids: Structure-activity relationships. *Toxicol Appl Pharmacol* 1991; 111:90-8.
119. Fu PP, Xia Q, Lin G, Chou MW. Genotoxic Pyrrolizidine Alkaloids – Mechanism leading to DNA Adduct Formation and Tumorigenicity. *Int J Mol Sci* 2002; 3:948-64.
120. Xia Q, Chou MW, Edgar JA, Doerge DR, Fu PP. Formation of DHP-derived DNA adducts from metabolic activation of the prototype heliotridine-type pyrrolizidine alkaloid, lasiocarpine. *Cancer Lett* 2006; 231:138-45.
121. Clark AM. The mutagenic activity of some pyrrolizidine alkaloids in *Drosophila*. *Molecular General Gen* 1960; 91:74-80.
122. Yoon S, Mason JM, Valencia R, Woodruff RC, Zimmering S. Chemical mutagenesis testing in *drosophila*. IV. Results of 45 coded compounds tested for the national toxicology program. *Environmental Mutagenesis* 1985; 7:349-67.
123. Ji LL, Zhang M, Sheng YCh, Wang ZT. Pyrrolizidine alkaloid clivorine induces apoptosis in human normal liver L-02 cells and reduces the expression of p53 protein. *Toxicology in Vitro* 2005; 19:41-6.
124. Wawrosch Ch, Kopp B, Wiedenfeld H. Permanent monitoring of pyrrolizidine alkaloid content in micropropagated *Tussilago farfara* L. a tool to fulfill statutory demands for quality of coltsfoot in Austria and Germany. *ISHS Acta Horticulturae* 530. International Symposium on Methods and Markers for Quality Assurance in Micropropagation 2000.
125. Kopp B, Wawrosch C. EU-Sortenschutz Nr EU5559 vom 6.12.1999 für *Tussilago farfara* L. cv. "Wien".
126. Delaney T, Ukness S, Vernooij B, Fridrich L, Weymann K, Negrotto D, Gaffney T, Gut-Rella M, Kessmann H, Ward E. A central role of salicylic acid in plant disease resistance. *Science* 1994; 266:1247-50.
127. Cho J, Hyang MK, Ryu JH, Jeong YS, Lee YS, Jin Ch.. Neuroprotective and antioxidant effects of the ethyl acetate fraction prepared from *Tussilago farfara* L. *Biol Pharm Bull* 2005; 28(3):455-60.
128. Nibret E, Sporer F, Asres K, Wink M. Antitrypanosomal and cytotoxic activities of pyrrolizidine alkaloid-producing plants of Ethiopia. *J Pharm Pharmacol* 2009; 61:801-8.
129. Thoden TC, Hallmann J, Boppré M. Effect of plants containing pyrrolizidine alkaloids on the northern root-knot *Meloidogyne hapla*. *Eur J Plant Pathol* 2009; 123:27-36.

**ALKALOIDY PIROLIZYDYNOWE – CHEMIA, BIOSYNTETA, SZLAK METABOLICZNY, TOKSYCZNOŚĆ, BEZPIECZEŃSTWO I PERSPEKTYWY ZASTOSOWANIA W MEDYCYNIE**

MARIOLA DREGER, MARZENA STANISŁAWSKA, ANNA KRAJEWSKA-PATAN,  
SEBASTIAN MIELCAREK, PRZEMYSŁAW ŁUKASZ MIKOŁAJCZAK,  
WALDEMAR BUCHWALD

Oddział Roślin Zielarskich  
Instytut Włókien Naturalnych i Roślin Zielarskich  
ul. Libelta 27  
61-707 Poznań

\*autor, do którego należy kierować korespondencję: mdreger@iripz.pl

**Streszczenie**

Alkaloidy pirolizydynowe (PA) to metabolity wtórne, które stanowią potężną broń roślin stosowaną w reakcjach obronnych przeciwko roślinożercom. Występują w kilkunastu nie spokrewnionych ze sobą rodzinach botanicznych, szczególnie często wśród przedstawicieli Asteraceae, Boraginaceae i Fabaceae. Stwierdzono, że syntaza homospermidyny (HSS) jest kluczowym enzymem katalizującym powstawanie homospermidyny z poliamin. Badania kinetyki enzymu oraz sekwencji genów ujawniły jego polifiletyczne pochodzenie: powstał on w wyniku duplikacji genu syntazy deoksyhypuzyny (DHS). Zdolność do produkcji alkaloidów pirolizydynowych pojawiła się w toku ewolucji przynajmniej cztery razy niezależnie w różnych liniach rodowych. Biosynteza PA jest ściśle związana z fazą wzrostu i produkcją biomasy. Przypuszcza się, że biosynteza tych związków jest odmiennie regulowana (indywidualnie) w różnych grupach roślin produkujących alkaloidy. PA pochodzące od necyny z podwójnym wiązaniem w położeniu 1,2 ich estrów działają toksycznie: hepatotoksycznie, rakotwórczo, cytotoksycznie, teratogennie i mutagennie. Za główny mechanizm prowadzący toksyczności uważa się formowanie piroli PA (estry dihydropirilizydyny), które powstają w procesie detoksykacji w wątrobie. Bardzo reaktywne pirole wiążą się z dużymi cząsteczkami protein i kwasów nukleinowych, a także kompleksów DNA-białko, powodując działanie hepatotoksyczne i rakotwórcze. Problem toksyczności alkaloidów pirolizydynowych skutkuje ograniczeniami i restrykcjami w produkcji i sprzedaży produktów zielarskich zawierających rośliny produkujące PA.

Praca przedstawia obecny stan badań dotyczących alkaloidów pirolizydynowych i obejmuje takie zagadnienia jak: budowa chemiczna, biosynteza, ewolucja z uwzględnieniem powiązanych genów oraz czynniki wpływające na biosyntezę i gromadzenie, a także toksyczność PA.

**Słowa kluczowe:** alkaloidy pirolizydynowe (PA), szlaki metaboliczne, biosynteza, syntaza deoksyhypuzyny (DHS), syntaza homospermidyny (HSS), toksyczność