Alkaloids Derived From Ornithine: Pyrrolizidine Alkaloids

13

Helmut Wiedenfeld

Contents

Abstract

Pyrrolizidine alkaloids (PAs) occur in about 5% of all flowering plants. They are esters of a basic moiety ("Necine") and esterifying acids ("necic acids"). Up to now, about 500 different PAs are described. PAs can be hazardous to man and domestic animal. They can show cancerogenic, mutagenic, teratogenic, and fetotoxic properties. Many intoxications by PAs are described. The level of the toxicity of each single PA is dependent from its concrete chemical structure. This chapter gives an overview about the chemistry, biosynthesis, and toxicity of PAs.

Keywords

Biosynthesis • Chemical structure • Intoxications • Pyrrolizidine alkaloids • Toxicity and structure

H. Wiedenfeld
Pharmazeutisches Institut der Universität, Bonn, Germany
e-mail: wiedenfeld@uni-bonn.de; unc600@uni-bonn.de

K.G. Ramawat, J.M. Mérillon (eds.), *Natural Products*,
DOI 10.1007/978-3-642-22144-6_9,

1 Introduction

The term "pyrrolizidine alkaloids" (PAs) is used for all ester compounds of the hydroxy and/or dihydroxy and/or hydroxymethyl derivatives from pyrrolizidine (hexahydro-1*H*-pyrrolizine) or from 1,2-dehydro-pyrrolizidine (2,3,5,7a-tetrahydro-1*H*-pyrrolizine).

PAs occur in 13 plant families and in about 5% of all flowering plants. Toxic PAs can be found in six plant families: Apocynaceae, Boraginaceae, Asteraceae (Compositae), Leguminosae (Fabaceae), Ranunculaceae, and Scrophulariaceae. These PAs are hepatotoxic, genotoxic, teratogenic, carcinogenic, and pneumotoxic.

Toxic PAs are only those derived from the unsaturated moiety 1,2-dehydropyrrolizidine and are mainly esters of the aminoalcohols (= necines) retronecine, heliotridine, or the untypical aminoalcohol (no bicyclic five-membered system, but an eight-membered monocycle) otonecine. Some few toxic PAs are esters from crotanecine. Of minor toxicity are the esters of the 1-hydroxymethyl-1,2-dehydropyrrolizidine supinidine.

A very large range of pyrrolizidine alkaloids can theoretically be obtained by combining the known necines and the esterifying acids (= necic acids). So far, more than 500 alkaloids have been found and their structures determined. With the exception of the approximately 35 otonecine alkaloids that cannot form N-oxides, if the N-oxides of these alkaloids are taken into consideration, more than 900 structures are known.

The first intoxication was reported by Gilruth who found that a chronic liver disease in livestock was caused by *Senecio jacobaea* [1, 2]. It was further shown that other *Senecio* ssp. as well as *Crotalaria* ssp. led to the same disease [3]. Already in 1920, it was proven that a widespread chronic liver disease in humans was caused by grain contaminated with seeds of *Senecio* ssp. [4, 5]. Several severe intoxications, all caused by the contamination of food (mainly bread), were reported: In the 1950s, severe intoxications in the former USSR were found to be caused by seeds and dust of *Heliotropium lasiocarpum* which contaminated grain [6, 7]; similarly, the same species was the reason for the intoxication of 4,000 people in Tadjikistan [8, 9]. The severest incident was observed in the 1970s in Afghanistan where about 8,000 people were affected from a wheat contamination by seeds of *Heliotropium popovii*, subsp. *gillianum*; more than 3,000 died [10, 11].

It can be assumed that seeds and dust from PA-containing plants are a major source for a human PA exposure [12, 13]. In industrialized countries, grain cleaning methods reduce the PA contamination under a level where acute intoxications can occur, but the dust components are still remaining and there are indications that diseases such as cirrhosis, cancer, and pulmonary arterial hypertension are due to a long-time exposure of low doses of PAs [14].

Besides this, medicinal plants are a further source of a PA intoxication [15–18]. Especially herbal preparations (so-called bush-teas) were found to be the reason for different liver diseases observed in Jamaica and the West Indies as well as in Africa in the 1950s [19–21].

PA intoxications by herbal preparations are mainly observed in developing countries where the use of traditional medicines is common; however, in the last

three decades also, in industrial countries like the USA, the UK, Switzerland, Austria, and Germany PA intoxications were reported due to the increased and uncontrolled use of herbal medications. In the 1980s, hand in hand with the so-called green wave, several practitioners claimed that herbal medicine would show only benefits without undesirable side effects which led to several fatal intoxications, mainly in infants who show a higher susceptibility to PA than adults.

Other foods can also be contaminated with PAs and are, therefore, a potential hazard for humans. Milk was shown to be a source for a PA intoxication in a case where the milk-producing animals had access to PA containing feed (hay, silage) [22–30]. Human milk from women exposed to pyrrolizidine alkaloids has caused veno-occlusive disease in neonates and infants [31]. Honey has become of increased importance as it could be shown that, in commercial products, levels of PAs were found which exceeded tolerable values. Here, pollen seems to be the pathway of contamination [31–37].

Recently, in Germany it was found that salads (especially ready-packed rocket salads (*Eruca sativa*), sold in supermarkets) and salad mixtures can be contaminated with PA containing plants, mainly *Senecio vulgaris*, a typical weed of field crops [38].

1,2-dehydropyrrolizidine esters and their N-oxides are carcinogenic, mutagenic, genotoxic, fetototoxic, and teratogenic to varying degrees. The toxicity level of each single PA is dependent on its chemical structure and also physical properties such as lipophilicity, hydrophilicity, and pharmokinetics. PAs prior to metabolic activation show a more or less low acute toxicity, but in vivo they undergo a metabolic toxification process in the liver, which is, as a result, the first target organ for the toxicity. This toxication process is well investigated.

PAs are considered to be toxic contaminants and have no well-recognized uses on their own. In the 1970s, the PA indicine-*N*-oxide was found to show antitumor activities, but on account of severe toxic side effect (especially observed in children) its use was no longer justified [39–41].

1.1 Chemical Structures of PAs

PAs are esters and consist of two parts: the basic aminoalcohols ("necines") and one or more acids ("necic acids") that esterify the OH groups of the necines [42–44].

Necines consist of a five-membered bicyclic ring system with a bridgehead nitrogen and – at least – a hydroxymethyl group at position 1 (Fig. 13.1). They can occur as saturated systems or possess a double bond in position 1,2. Most of the PAs show a further OH group at position 7. Further hydroxylation can take place at positions 6 and 2; in the case of saturated PAs a few molecules show hydroxylation at position 1 and in one case the (+) isomer of isoretronecanol (R_3 = ß-H; α-hydroxymethyl at position 1) is reported. Chiral carbons can be at positions 1 and 2 (saturated necines) and 6,7,8 (all necines). Mainly all PAs belong to the 8-α series (only two times the 8-ß position is reported). In nature, the PAs mainly occur in their *N*-oxide form (water-soluble; transport form).

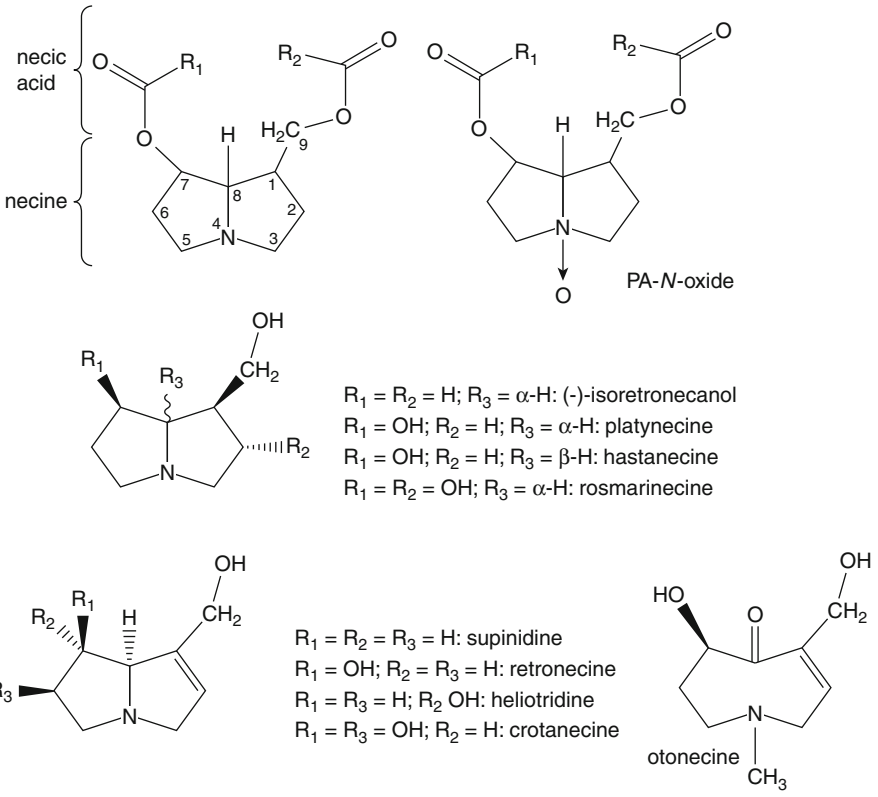


Fig. 13.1 Structures of necines

An untypical necine can be found in the otonecine PAs: This necine occurs not in form of a bicycle but as a 1-methylazocan-5-one. On account of the results by X-ray analysis, it could be found that there exists a transannular binding between carbonyl and the nitrogen leading to the same behavior as the typical bicycle PAs. All PAs showing a double bond in position 1,2 act as natural toxins.

Necic acids are structurally highly variable. Besides the simple acetic acid they consist mainly of 5–10 carbon atoms. They can form mono-, di-, or macrocyclic diesters and can show more or less branched carbon chains. The carbon atoms can bear different functional groups like hydroxyl, alkoxy, epoxy, and carboxyester groups. Double bonds at different positions are also possible. On account of this situation a theoretically high number of different unique structures are possible, including a large range of stereo isomers [42–44].

Necines with only one OH group can only form PA monoesters like supinine. If there exists a second OH group both can be found: mono as well as diesters as demonstrated for lycopsamine and its acetyl derivative or as in lasiocarpine. These examples demonstrate the variability of the stereochemistry (Fig. 13.2).

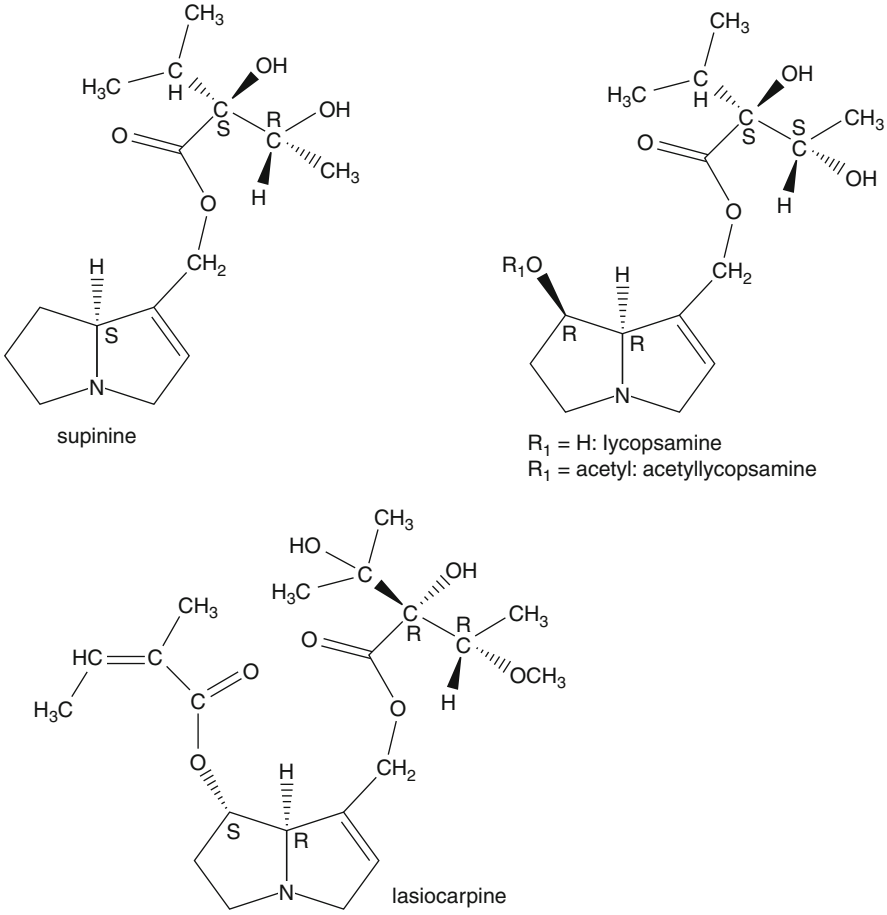


Fig. 13.2 Structures of PA mono- and diesters

With dicarboxyclic acids, the double esterification leads to macrocyclic diesters which can occur as 11–13-membered ring systems. A typical example for an 11-membered PA is monocrotaline, the main PA occurring in *crotalaria* species, or crispatine. Most of the PAs show a 12-membered ring system; this can be found, for example, in senecionine or senkirkine, both contained in many *senecio* species. A typical 13-member ring is demonstrated in the PAs doronenine (also isolated from *senecio*) and in madurensine (from *crotalaria*). Very rare is the 14-member macrocycle: It is found in the PA parsonsine (from *parsonsia*-species); this structure is possible on account of a further ester group within the necic acid chain. It is interesting to mention that until now in all cases of macrocyclic diester PAs only the 7-ß-OH isomer (mainly retronecine) is described and not the α-isomer (heliotridine) (Fig. 13.3).

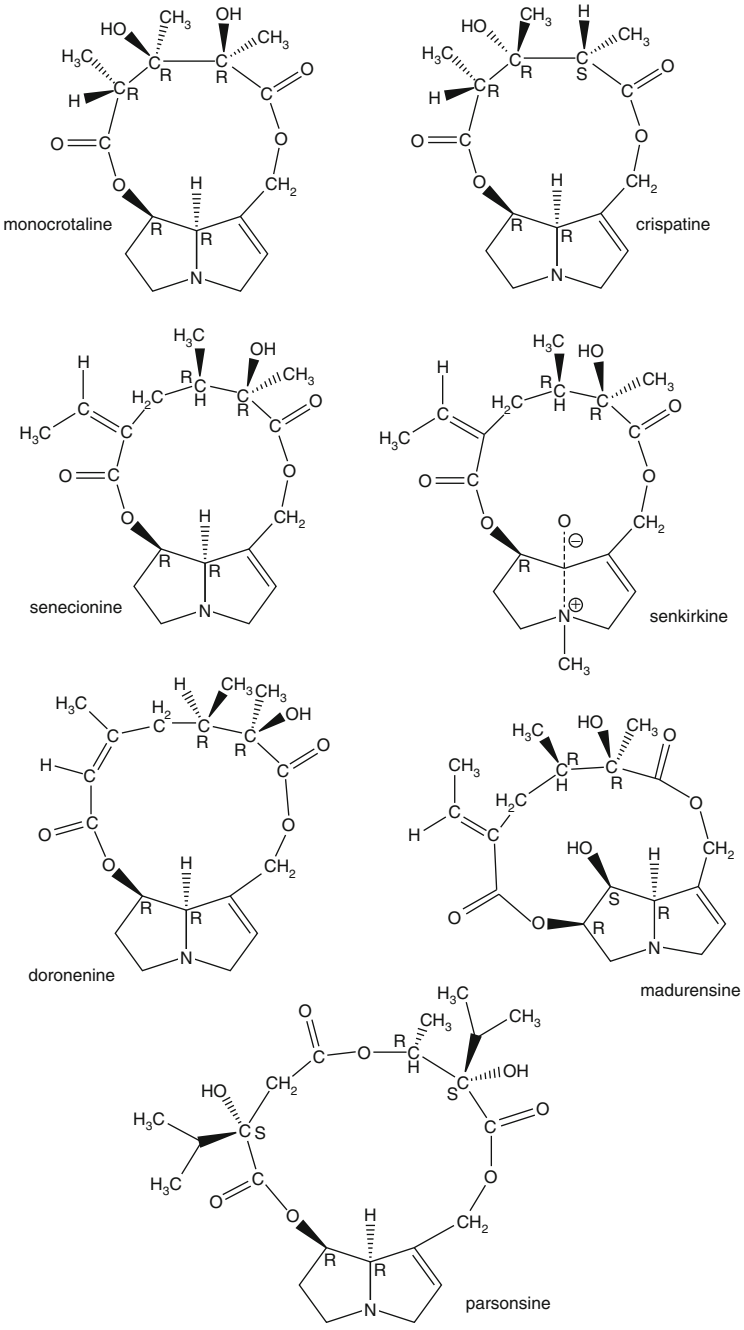


Fig. 13.3 Structures of macrocyclic PA

Theoretically, an innumerable amount of different structures is possible. So far, more than 500 PAs have been isolated and their structures described.

1.2 Biosynthesis

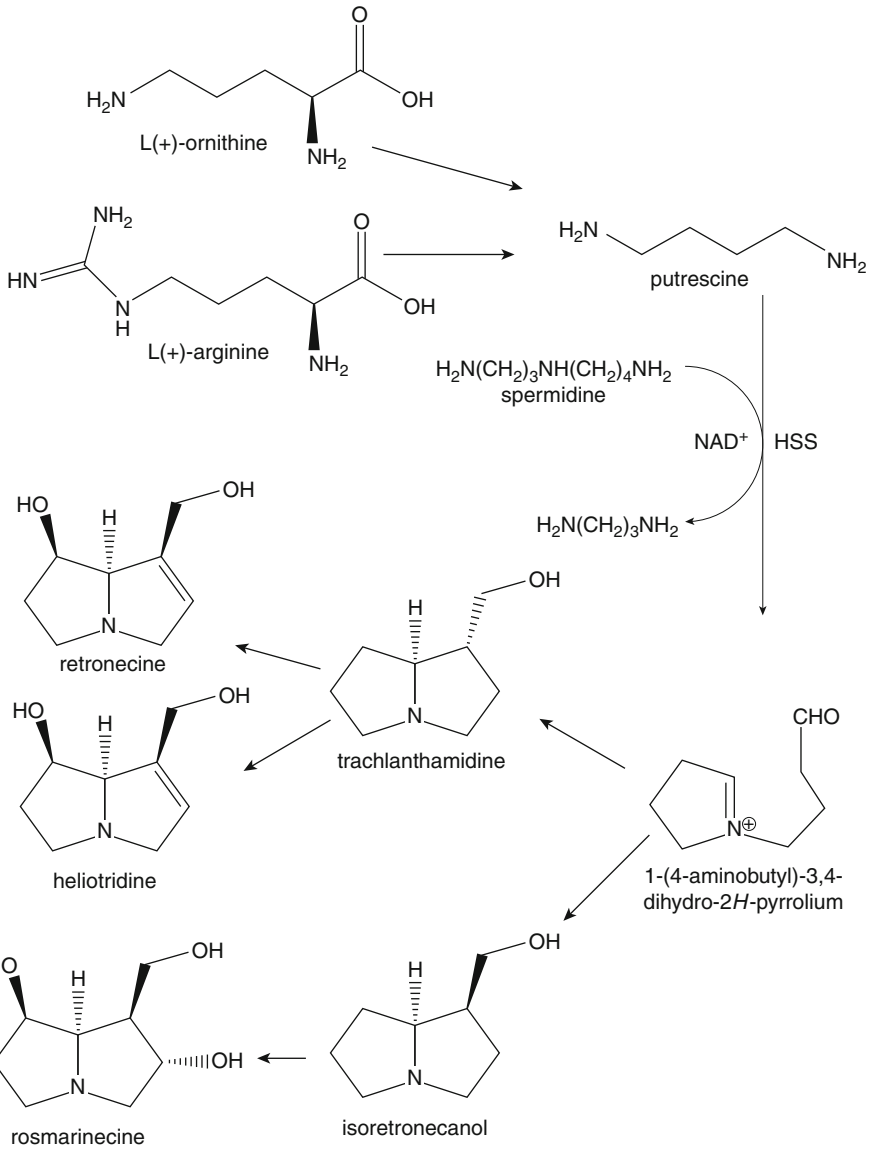
The first experiments concerning the biosynthesis of PAs were done in the 1960s: [2-^{14}C]ornithine, [1-^{14}C]acetate, and [1-^{14}C]propionate were fed to *Crotalaria* species [45]. Similarly, labelled ornithine was incorporated into the alkaloids from *Senecio* species [46, 47]. In all cases, ornithine was incorporated specifically into the basic moiety retronecine, whereas acetate and propionate could be found in the acidic parts of the alkaloids. Bale and Crout used a double isotope technique for a comparison between ornithine and arginine [48] showing that ornithine is the more efficient precursor than arginine. It could be found that ornithine and arginine were incorporated into retronecine via putrescine: Experiments gave evidence for the formation of putrescine from argine in *Heliotropium* species, whereas in *Crotalaria* and *Senecio* species, the main pathway to putrescine is from ornithine [49, 50]. Further experiments came to the conclusion that retronecine is derived from two molecules of putrescine, which can be formed from ornithine or arginine [51, 52]. The use of ^{13}C-^{15}N double-labelled putrescine gave evidence for a further symmetrical intermediate which was then found to be homospermidine. This was proven by feeding experiments with labelled homospermidine which was incorporated into the retronecine moiety of the PAs from *Senecio isatideus* [53–55]. It could be shown that a PA-special enzyme – the homospermidine synthase (HSS) – is essentially involved in the biosynthesis of homospermidine [56–58]. This HSS is responsible for the transfer of the aminobutyl moiety of spermidine into putrescine leading to the symmetric triamine homospermidine; this reaction is NAD^+ dependent. Via the 1-(4-aminobutyl)-3,4-dihydro-2*H*-pyrrolium salt the necines trachelanthamidine and further retronecine were generated [59].

Besides, it was shown that trachelanthamidine as well as retronecine were both efficient precursors for the necine otonecine, the base moiety of the PA emiline from *Emilia flammea* [60].

Trachelanthamidine as well as isoretronecanol can be generated from the imminium salt. There exists evidence that the saturated necine rosmarinecine is generated via isoretronecanol and that trachelanthamidine is the precursor for retronecine as well as heliotridine [61]. Isoretronecanol has been shown to be also the precursor of those PAs which have the untypical 8*β* stereochemistry (instead of 8α) as found in the PAs from *Cynoglossum australe* [62] (Scheme 13.1).

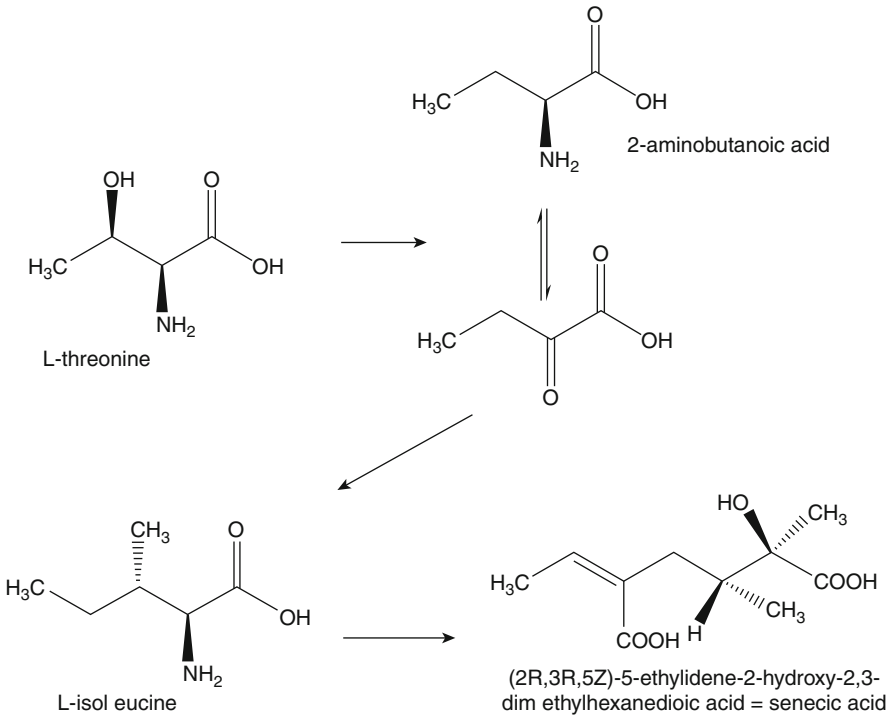
Whereas the biosynthesis of the necines (Scheme 13.1) seems to be clear and intensively investigated, fewer reports can be found for the necic acids. Still some aspects have to be investigated.

In 1966, labelled L-threonine and L-isoleucine were observed to be incorporated into the seneciphyllic acid [63]. Similarly, it was described that labelled DL-valine is a precursor of echimidinic acid [64]. The five-membered angeloylic acid is



Scheme 13.1 Biosynthetic pathway of necines

found often as a structure part in PAs. L-isoleucine was shown to be a precursor of the angelate component in PAs [65]. Crout et al. reported that in general C_{10} necic acids are derived from isoleucine [66, 67]. This was demonstrated in case of seneciphyllic acid and senecic acid, both forming 12-membered macrocyclic PAs. Monocrotalic acid, the acidic part of monocrotaline – a 11-membered macrocycle – was reported to be built from L-isoleucine and L-threonine [68].



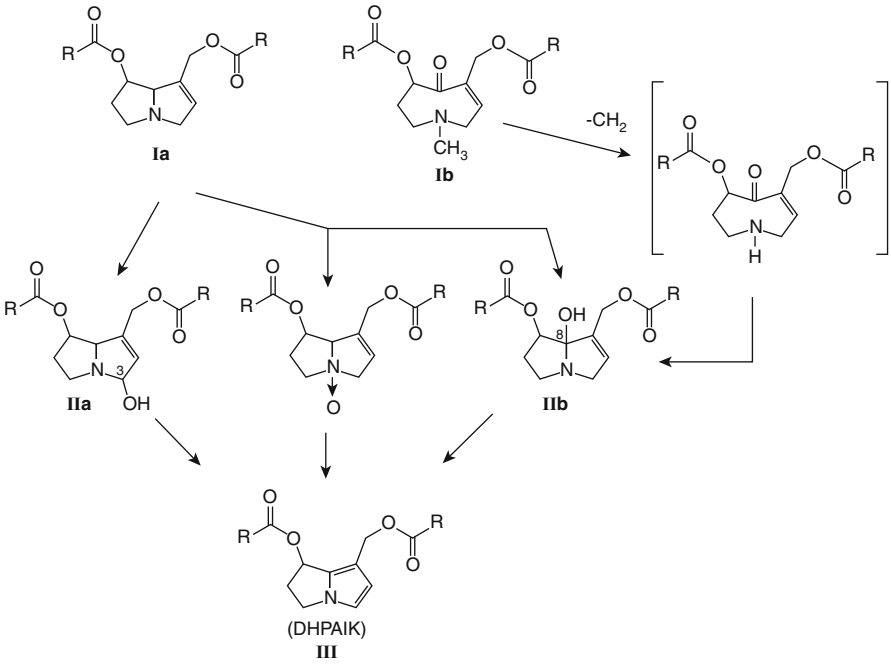
Scheme 13.2 Biosynthetic pathway of senecic acid

Similar findings were reported for the acidic part in the PA strigosine [69] and for angeloylic and tigloylic acid [70]. In the case of the acidic part in the PA monocrotaline, it was shown that not acetate, mevalonate, or glutarate (as reported earlier) are involved but it is only formed via isoleucine [71]. Stereospecific aspects were also studied in the case of senecic and isatinecic acid [72, 73]. The biosynthesis of trichodesmic acid was studied and it was shown that one part of the C_{10} acid was formed by (2*S*)-isoleucine or its biosynthetic precursor (2*S*)-threonine and the other C_5 unit from (2*S*)-leucine or (2*S*)-valine [74]. The complete labeling pattern of senecic acid (the acidic part in the PAs rosmarinine and senecionine) was studied by NMR experiments and it was stated that the biosynthesis of this acid is processed via two molecules of isoleucine (Scheme 13.2) [75].

1.2.1 Metabolic Toxication of PAs

PAs are ester alkaloids derived mainly from the necines retronecine and otonecine. They are carcinogenic, mutagenic, genotoxic, fetotoxic, and teratogenic.

PAs themselves show a more or less low acute toxicity but in vivo they undergo a three-step metabolic toxication process in the liver, which is, as a result, the first target organ for the toxicity.



Scheme 13.3 Metabolism of PAs to Pyrrols

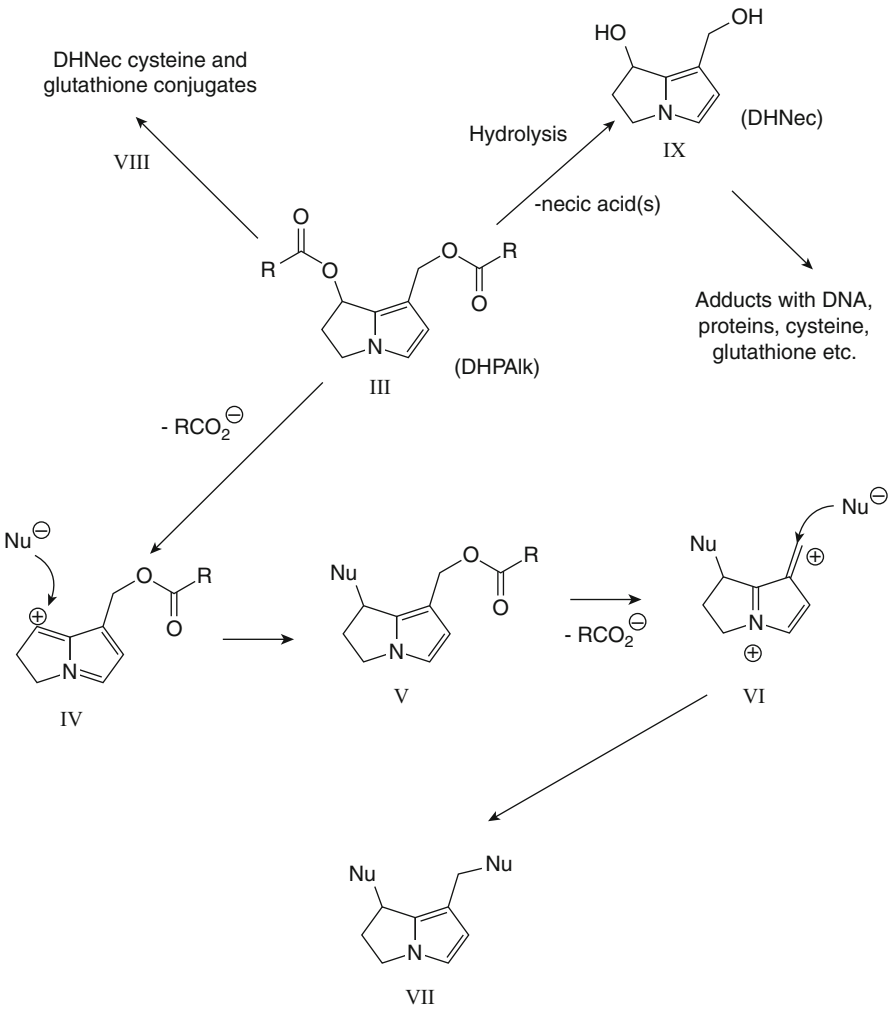
This toxication process is well investigated [12, 75–81].

After oral uptake and absorption of the PAs (Scheme 13.3: Ia and Ib), a hydroxyl-group is introduced adjacent to the nitrogen atom in the necine (position 3 or 8) by the cytochrome P-450 monoxogenase enzyme complex in the liver (Scheme 13.3: IIa and IIb).

These hydroxyl PAs (OHPAs) are unstable and undergo a rapid dehydration to the dehydropyrrolizidine alkaloids (DHPAlk; Scheme 13.3: III). This dehydration results in a second double bond in the necine followed by spontaneous rearrangement to an aromatic pyrrole system III.

PAs occur mainly as their N-oxides in the plants and these cannot be directly converted to the OHPA, but on oral ingestion they are reduced by the gut enzymes or the liver microsomes and NADH or NADPH to the free bases and therefore they show equal toxicity to that of the free bases [82–87].

Otonecine-type PAs (Scheme 13.3: Ib) are metabolized to the OHPAs [80, 88, 89]. These otonecine-PAs possess a methyl function at the nitrogen and a quasi keto function at the bridge carbon 8. After hydroxylation of the N-methyl group it is lost as formaldehyde leaving a NH-function which undergoes condensation with the C8 keto group to produce product IIb (Scheme 13.3) which spontaneously dehydrates to the DHPAlk III.



Scheme 13.4 Metabolism of the dehydropyrrolizidine alkaloids

The metabolites III are able to generate stabilized carbonium ions (Scheme 13.4: IV and VI) by loss of hydroxy groups or ester functions as hydroxyl or acid anions. These carbonium ions can react rapidly with nucleophiles (Scheme 13.4: VII).

In the case of necine-diesters (as shown in Ia and Ib, Scheme 13.3), typical of *Senecio* species, the formation of the reactive carbonium ions is facilitated because the necic acid groups provide good leaving groups that facilitate rapid formation of the carbonium ions IV and VI in high yield. Where one of the hydroxy groups at C7 or C1 of the necine is not esterified, formation of the carbonium ions is not so spontaneous. In these cases, the carbonium ions are most readily formed after protonation of the hydroxyls and loss of H_2O [90].

The metabolites IV and VI react rapidly with nucleophilic groups of proteins and the amino groups of the bases in nucleosides like DNA and RNA which leads to abnormal functions showing finally the veno-occlusive disease (VOD) in which the veins are narrowed. Typical macrocyclic diester PAs (like senecionine, seneciphylline, retrorsine, and senkirkine which are PAs commonly found in *Senecio* species) have been shown to produce liver damage due to cross-linking of DNA [84, 91–99]. In case of PA monoesters (e.g., derived from the necine supinidine which lacks a C7 hydroxyl, Fig. 13.2), cross-linking is not possible and they show a lower toxic potential. It has also been shown that the nucleophilic activity at C7 is higher than at C9 resulting in the primary nucleophilic attack at C7 followed by an attack at C9 (Scheme 13.4) [43].

As shown, the DHPAlks can also react with SH groups found in more soluble components like glutathione and cysteine (Scheme 13.4: VIII). High levels of glutathione and cysteine therefore reduce the toxic potential of PAs [88, 100–102].

Furthermore, hydrolysis can take place where the DHPAlks (Scheme 13.4) yield dehydronecine alcohols (DHNecs) (Scheme 13.4: IX) which are more water soluble and less reactive like the DHPAlks but still display a moderate level of alkylating activity [103, 104]. This higher water solubility and lower reactivity can lead to the escape from the liver tissue and subsequent reaction in other organs [12, 105, 106]. DHNecs like dehydroretronecine and dehydroheliotridine have also been shown to produce rhabdomyosarcoma, skin, liver, and lung tumors [24, 107–110].

1.2.2 Detoxication of PA

As well as the metabolic activation, detoxication of PAs also occurs in vivo: Hydrolysis of the ester bonds in PAs from type Ia or Ib by esterases leads to necic acids and to the free necines. Both are nontoxic products and – on account of their higher water solubility – can be renally excreted. The rate of hydrolysis is dependent on the level of steric hindrance of the ester linkages; and it has been shown that the more highly branched necic acids are more resistant to hydrolysis [43, 111]. This means that macrocyclic diesters with more complex acid moieties are more hazardous on account of their lower rate of hydrolytic detoxication.

The N-oxides of PAs (the form occurring most commonly in plant sources) are highly water soluble and can therefore be renally excreted. Besides their natural occurrence, N-oxidation of PAs also takes place in the liver and can be seen as a detoxication process (Scheme 13.3) [76, 78, 82, 112, 113]. However, it has been shown that the N-oxides – besides excretion – can be converted by dehydration or by acetylation followed by elimination of acetic acid to the DHPAlk (Scheme 13.3: III) [43, 90].

In conclusion, it can be stated that the toxicity level of different PAs in non-ruminants is dependent on the following three aspects:

- The efficiency of metabolic activation to form the key intermediate III (Scheme 13.3)
- The efficiency of ester hydrolysis to form nontoxic and water-soluble necines and necic acids
- The efficiency of N-oxidation and excretion via urine

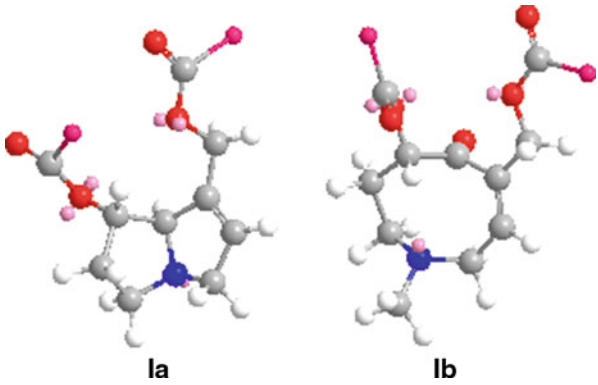


Fig. 13.4 *X-rays of Ia and Ib*

1.2.3 Structure and Toxicity

As mentioned, the key fragment didehydropyrrolizidine (e.g., III) is also generated from pyrrolizidine alkaloids of the otonecine type (e.g., Ib, Scheme 13.3).

These otonecine derivatives do not show a C8-N bond but possess a keto function at C8 and a methyl group at the nitrogen atom. Surprisingly, these seco alkaloids are of identical toxicity as the PA of type Ia (1,2-dehydro-retronecine and heliotridine esters).

On the other hand, for energetic reasons, these seco compounds should occur in a different necine conformation than type Ia PA: The missing C8-N bond should lead to a stable eight-membered macrocycle which generally prevents the metabolization via an intermediate to IIb (Scheme 13.4).

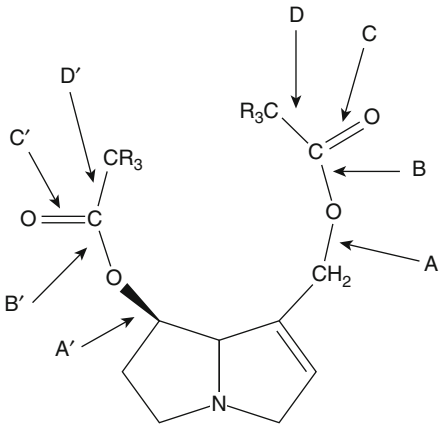
Molecular modeling experiments prove this assumption and show the energy minimized structure as depicted in Fig. 13.3 (energy minimization: Chem 3D ultra; V. 10.0; Cambridge Soft).

Interpretation of the X-ray structure analysis data helps to explain the identical toxicity of PA of type Ia and Ib: In all nine otonecine-type alkaloids measured to date, a necine conformation was found which is identical to those found in those having the C8-N bond (type Ia).

Both the distances between C8 and N are similar and equal values can be found for the plane angles built between plane C1-C3-N-C8 and plane C7-C5-N-C8 ($\sim$125°). This indicates that the seco 1,2-dehydroesters do not exist in the optimal, energy minimized form (Ib, Fig. 13.4) but are of an equal conformation to PA of type Ia, which finally enables the metabolization as shown in Scheme 13.3.

Furthermore, X-ray data show that the dedihydro metabolites (e.g., III, Scheme 13.3) derived from 1,2-dehydrodiester have a high toxic potential compared with the low or missing toxicity of the monoesters of retronecine or heliotridine. As already mentioned, a possible detoxification mechanism is the hydrolysis of the ester bindings by esterases and the subsequent building of dehydronecine (IX, Scheme 13.4) which – due to their higher water solubility – can be easily excreted renally.

Fig. 13.5 Bonds in PAs



On the other hand, the metabolization to C7 as well as to C9 carbonium ions (especially the speed rate of this metabolization) and the subsequent reaction with nucleophiles are seen as a key step concerning the level of toxicity. The concrete binding situation concerning the ester function can be found analyzing the X-ray data. Interpretation of the bond lengths shown in Fig. 13.5 leads to the following results:

In pyrrolizidine alkaloids of type Ia and Ib (1,2-dehydropyrrolizidine diesters) the C-O bonds A and A´ occur in a normal range of 1.45 Å; the following C-O bonds B and B´ are considerably shortened, whereas the keto functions C and C´ show a moderate shortening. The C-C bonds D and D´ show – similar to A and A´ – normal values of about 1.54 Å.

In contrast to these findings, the data for monoesters with retronecine or heliotridine give evidence of a different situation: Here, the A and A´ (C9 as well as C7) bonds are elongated and the corresponding keto bonds C or C´ are of nearly ideal length (1.21 Å).

These results show that in 1,2-dehydro diesters, the ester functions are a conjugated system leading to the conclusion that the bonds C9-O and C7-O (= A and A´) are the target breaking points within the molecule, which makes possible a quick and easy metabolization and further reaction with nucleophiles as described in Scheme 13.4.

Contrary to that, in PA of 1,2-dehydro monoesters, more stable ester bonds are found (no conjugation), which leads to the fact that the building of the didehydrometabolites is more difficult and time consuming. In this case, a hydrolysis by esterases can take place in higher amounts what leads to the detoxification via dehydronecines, which explains the missing or low toxicity of 1,2-dehydro monoester.

1.2.4 Metabolism of 1,2-Saturated Pyrrolizidine Alkaloids

Metabolism of saturated pyrrolizidine alkaloids (necic acid esters of platynecine, hastanecine, rosmarinecine and isoretronecanol (Fig. 13.1)) by mammals has not yet been extensively studied, in part because the saturated ester alkaloids and their necines do not display mammalian toxicity.

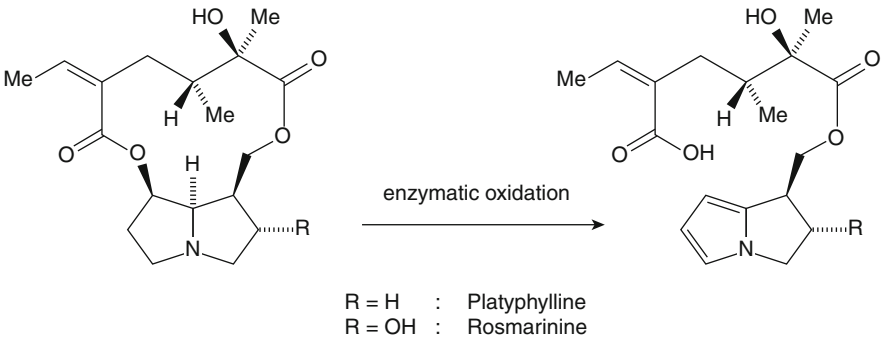


Fig. 13.6 Metabolism of saturated PAs

While 1,2-dehydropyrrolizidine alkaloids are metabolized by liver P-450 isozymes into hazardous dihydropyrrolizines (e.g., III) with a pyrrolic A ring, saturated pyrrolizidine alkaloids produce nontoxic, metabolites [79, 111]. The saturated alkaloids platyphylline and rosmarinine (Fig. 13.6), for example, are converted by liver microsomes into pyrrolic metabolites with an aromatic B-ring [79]. These are devoid of biological alkylating properties and are nontoxic.

2 PA Toxicity in Humans

PA poisoning of humans can be described by three dose-related levels: acute, subacute, and chronic. These levels can be progressive resulting in irreversible chronic toxic effects [12, 99, 106, 110, 114–116].

On account of the low toxicity of the PAs themselves, acute poisoning has been reported only in very rare cases; it occurs only in infants and neonates due to their higher susceptibility for a PA poisoning. It is characterized by hemorrhagic necrosis, hepatomegaly, and ascites; death is caused by liver failure [12, 106, 110, 115].

Subacute levels are characterized by hepatomegaly and recurrent ascites; endothelial proliferation and medial hypertrophy leading to an occlusion of hepatic veins, resulting in the so-called veno-occlusive disease (VOD) which can be seen as a characteristic histological sign for PA poisoning [12, 99, 106, 110, 115]. The VOD causes centrilobular congestion, necrosis, fibrosis, and liver cirrhosis, the end-stage of chronic PA intoxication.

As well as the liver VOD, other organs can be affected by PAs. It has been shown that the pyrrolic metabolites (DHPAlks and DHNecs) can escape from the liver into pulmonary arterioles where they can produce damages similar to the VOD changes in the liver [115]. It could be shown that from 62 tested PAs all can produce (dose-dependent) lung lesions [111] and it is speculated that pulmonary damage results from long-term and low-level exposure to PAs [12, 115].

PA intoxication in humans is not only related to the amount and the duration of the exposure but also to age and gender: Males react more sensitive than females,

and fetuses and children (especially neonates or infants) show the highest sensitivity to PA poisoning: In 2003, it was shown that the daily uptake of ~ 7 μg PA (from a herbal spice containing comfrey) during pregnancy did not show a toxic effect in the mother's liver but damaged the fetal liver in such a way that the newborn child died after 2 days [117].

It has also been observed that cofactors can exacerbate the PA poisoning: liver damaging agents, bacterial, or viral infections but also medical drugs like barbiturates or metals like copper or mycotoxins like aflatoxins can increase the severity and likelihood of PA liver damage [42, 118–122].

There is a large number of reports in the literature about different liver diseases (mainly VOD) possibly connected with PA poisoning. But in most cases, the connection could not be proven because the outbreak of the liver disease and a possible ingestion of PA-containing material were often separated by a long time period.

3 Conclusion

PAs are widespread in the plant kingdom. They occur in a great variety of different structures. They show no physiological and/or pharmacological properties, but they are of importance on account of possible severe side effects. These side effects are dependent on their chemical structures and range from low or missing toxicity up to strong severe and hazardous problems. PAs themselves are more or less nontoxic but undergo a metabolic toxication process. As a result cancerogenic, mutagenic, teratogenic, and fetotoxic effects can be observed. Many episodes are reported describing intoxications in humans, sometimes even epidemic. These cases were caused mainly by contaminations of food and feed by PA-containing plant material or by the uncontrolled use of herbal medical preparations. Therefore, to avoid those contaminations, special efforts should be made on a global scale to improve food control management.

References

1. Gilruth JA (1903) Hepatic cirrhosis affecting horses and cattle (so-called "Winton disease"). New Zealand Department of Agriculture, 11th annual report. pp 228–279
2. Gilruth JA (1904) Hepatic cirrhosis due to ragwort (*Senecio jacobaea*). Div Vet Sci New Zealand, Dept Agric Bull 9:252–254
3. Bull LB, Dick AT (1959) The chronic pathological effects on the liver of the rat of the pyrrolizidine alkaloids heliotrine, lasiocarpine, and their N-oxides. J Pathol Bacteriol 78:483–502
4. Willmott FC, Robertson GW (1920) Senecio disease or cirrhosis of the liver due to *Senecio* poisoning. Lancet 196:848–849
5. Steyn DG (1933) Poisoning of human beings by weeds contained in cereals (bread poisoning). Onderstepoort J Vet Sci Anim Ind 1:219–266
6. Bourkser GV (1947) On the question of the aetiology and pathogenesis of toxic hepatitis with ascites (heliotrope toxicosis). Hyg Sanit 6:24–26
7. Milenkov SM, Kizhaikin Y (1952) Toxic hepatitis with ascites. In: Proc. Sympos V M Molotov Med Inst, Tashkent

8. Chauvin P, Dillon J-C, Moren A (1994) Épidémie d' intoxication alimentaire á l' héliotrope, Tadjikistan, Novembre 1992-Mars 1993. Cahiers Santé 4:263–268
9. Mayer F, Lüthy J (1993) Heliotrope poisoning in Tadjikistan. Lancet 342:246–247
10. Tandon HD, Tandon BN (1975) Epidemic of liver disease – Gulran district, Herat Province, Afghanistan, Alexandria, World Health Organization, Regional Office for the Eastern Mediterranean (Assignment report No. EM/AFG/OCD/001/RB)
11. Mohabbat O, Srivastava RN, Younos MS, Sediq GG, Menzad AA, Aram GN (1976) An outbreak of hepatic veno-occlusive disease in north-western Afghanistan. Lancet 2:269–271
12. International Programme on Chemical Safety (IPCS) (1989) Pyrrolizidine alkaloids health and safety guide. Health and Safety Guide No. 26. WHO, Geneva, 1989
13. ANFFA (2001) Pyrrolizidine alkaloids in food. A toxicological review and risk assessment. Tech report series No 2ANZFA, Canberra
14. Edgar JA (2003) Pyrrolizidine alkaloids and food safety. Chem Austral 70:4–7
15. Roeder E (1995) Medicinal plants in Europe containing pyrrolizidine alkaloids. Pharmazie 50:83–98
16. Roeder E (2000) Medicinal plants in China containing pyrrolizidine alkaloids. Pharmazie 55:711–726
17. Roeder E, Wiedenfeld H (2009) Pyrrolizidine alkaloids in medicinal plants of Mongolia, Nepal and Tibet. Pharmazie 64:699–716
18. Roeder E, Wiedenfeld H (2009) Pyrrolizidine alkaloids in plants used in the traditional medicine of Madagascar and the Mascarene islands. Pharmazie 66:637–647
19. Bras G, Jelliffe DB, Stuart KL (1954) Veno-occlusive disease in liver with nonportal type of cirrhosis, occurring in Jamaica. Arch Path 57:285–300
20. Bras G, Berry DM, György P (1957) Plants as aetiological factor in veno-occlusive disease of the liver. Lancet 2:960–962
21. Bras G, Brooks SEH, Watler DC (1961) Cirrhosis of liver in Jamaica. J Pathol Bacteriol 82:503–511
22. Schoental R (1959) The chemical aspects of seneciosis. Proc R Soc Med 53:284–288
23. Dickinson JO, Cooke MP, King RR, Mohamed PA (1976) Milk transfer of pyrrolizidine alkaloids in cattle. J Am Vet Med Assoc 169:1192–1196
24. Johnson WD, Robertson KA, Pounds JG, Allen JR (1978) Dehydroretronecine-induced skin tumours in mice. J Natl Cancer Inst 61:85–89
25. Dickinson JO (1980) Release of pyrrolizidine alkaloids into milk. Proc West Pharmacol Soc 23:377–379
26. Goeger DE, Cheeke PR, Schmitz JA, Buhler DR (1982) Toxicity of tansy ragwort (*Senecio jacobaea*) to goats. Am J Vet Res 43:252–254
27. Lüthy J, Heim TH, Schlatter CH (1983) Transfer of [^{3}H] pyrrolizidine alkaloids from *Senecio vulgaris* L. and metabolites into rat milk and tissues. Toxicol Lett 17:283–288
28. Candrian U, Lüthy J, Graf U, Schlatter CH (1984) Mutagenic activity of the pyrrolizidine alkaloids Seneci-phylline and senkirkine in Drosophila and their transfer into rat milk. Food Chem Toxicol 22:223–225
29. Molyneux RJ, James LF (1990) Pyrrolizidine alkaloids in milk: thresholds of intoxication. Vet Hum Toxicol 32:S94–S103
30. Hoogenboom LA, Mulder PP, Zeilmaker MJ, van den Top HJ, Remmelink GJ, Brandon EF, Klijnstra M, Meijer GA, Schothorst R, Van Egmond HP (2011) Carry-over of pyrrolizidine alkaloids from feed to milk in dairy cows. Food Addit Contam Part A 28:359–372
31. Roulet M, Laurini R, Rivier L, Calarme A (1988) Hepatic veno-occlusive disease in newborn infant of a woman drinking herbal tea. J Pediatr 112:433–436
32. Deinzer ML, Thomson PA, Burgett DM, Isaacson DL (1977) Pyrrolizidine alkaloids: their occurrence in honey from tansy ragwort (*Senecio jacobaea* L.). Science 195:497–499
33. Culvenor CCJ, Edgar JA, Smith LW (1981) Pyrrolizidine alkaloids in honey from *Echium plantagineum* L. J Agric Food Chem 29:958–960

34. Edgar JA, Roeder E, Molyneux RJ (2002) Honey from plants containing pyrrolizidine alkaloids: a potential threat to health. J Agric Food Chem 50:2719–2730
35. Beales KA, Betteridge K, Colegate SM, Edgar JA (2004) Solid phase extraction and LCMS analysis of pyrrolizidine alkaloids in honeys. J Agric Food Chem 52:6664–6672
36. Betteridge K, Cao Y, Colegate SM (2005) An improved method for extraction and LCMS analysis of pyrrolizidine alkaloids and their N-oxides in honey: application to *Echium vulgare* honeys. J Agric Food Chem 53:1894–1902
37. Boppré M, Colegate SM, Edgar JA (2005) Pyrrolizidine alkaloids of *Echium vulgare* honey found in pure pollen. J Agric Food Chem 53:594–600
38. Bundesamt für Risiokobewertung (2007) Salatmischung mit Pyrrolizidinalkaloid-haltigem Greiskraut verunreinigt; Stellungnahme Nr.028/2007 des BfR vom 10, Jan 2007
39. Kugelman M, Liu WC, Axelrod M, McBride TJ, Rao KV (1976) Indidcine-N-oxide: the antitumor principle of *Heliotropium indicum*. Lloydia 39:125–128
40. Kovach JS, Ames MM, Powis G, Moertel CG, Hahn RG, Creagan ET (1976) Toxicity and pharmacokinetics of a pyrrolizidine alkaloid, indicine N-oxide. Cancer Res 39:4540–4544
41. Miser JS, Smithson WA, Krivit W, Hughes C, Davis D, Krailo M, Hammond D (1991) Phase II trial of indicine N-oxide in relapsed pediatric solid tumors. Investig N Drugs 9:339–342
42. Bull LB, Culvenor CCJ, Dick AT (1968) The pyrrolizidine alkaloids. North Holland Publishing, Amsterdam
43. Mattocks AR (1986) Chemistry and toxicology of pyrrolizidine alkaloids. Academic Express, New York/London
44. Rizk AFM (1991) Naturally occuring pyrrolizidine alkaloids. CRC Press, Boca Raton//Ann Arbor//Boston
45. Nowacki E, Byerrum RU (1962) A Study on thr biosynthesis of the crotalaria alkaloids. Life Sci 1:157–161
46. Hughes CA, Letcher R, Warren FL (1964) The senecio alkaloids. Part XVI. The biosynthesis of the "Necine" bases from carbon-14 precursors. J Chem Soc 4974–4978
47. Bottomley W, Geissman TA (1964) Pyrrolizidine alkaloids. The biosynthesis of retronecine. Phytochemistry 3:357–360
48. Bale NM, Crout DHG (1975) Determination of the relative rates of incorporation of arginine and ornithine into retronecine during pyrrolizidine alkaloid biosynthesis. Phytochemistry 14:2617–2622
49. Birecka H, Birecki M, Frohlich MW (1987) Evidence for arginine as the precursor of necines in *Heliotropium*. Plant Physiol 84:42–46
50. Birecka H, Birecki M, Cohen EJ, Bitonti AJ, McCann PP (1988) Ornithine decarboxylase, polyamines, and pyrrolizidine alkaloids in *Senecio* and *Crotalaria*. Plant Physiol 86:224–230
51. Khan HA, Robins DJ (1981) Pyrrolizidine alkaloid biosynthesis; Incorporation of ^{13}C-labelled putrescines into retronecine. J Chem Soc, Chem Comm 146–147
52. Khan HA, Robins DJ (1985) Pyrrolizidine alkaloid biosynthesis. synthesis of ^{13}C-labelled putrescines and their incorporation into retronecines. J Chem Soc, Perkin Trans I 101–105
53. Khan HA, Robins DJ (1981) Pyrrolizidine alkaloids: evidence for *N*-(4-Aminobutyl)-1,4-diaminobutane (Homospermidine) as an intermediate in teronecine biosynthesis. J Chem Soc, Chem Comm 554–556
54. Grue-Sorensen G, Spenser ID (1981) Biosynthesis of retronecine. J Am Chem Soc 103:3208–3210
55. Rana J, Robins DJ (1983) Pyrrolizidine alkaloid biosynthesis. Intact incorporation of [1,9-$^{13}C_2$]homosper-midine into retronecine. J Chem Res (S) 146–147
56. Ober D, Hartmann T (1999) Homospermidine synthase, the first pathway-specific enzyme of pyrrolizidine alkaloid biosynthesis, evolved from deoxyhypusine synthase. Proc Natl Acad Sci USA 96:14777–14782
57. Ober D, Hartmann T (2000) Phylogenetic origin of a secondary pathway: the case of pyrrolizidine alkaloids. Plant Mol Biol 44:445–450

58. Graser G, Hartmann T (2000) Biosynthesis of spermidine, a direct precursor of pyrrolizidine alkaloids in root cultures of *Senecio vulgaris* L. Planta 211:239–245
59. Robins DJ (1989) Biosynthesis of pyrrolizidine alkaloids. Chem Soc Rev 18:375–408
60. Kelly HA, Kunec EK, Rodgers M, Robins DJ (1989) Biosynthesis of the seco-pyrrolizidine base otonecine. J Chem Res (S) 358–359
61. Kunec EK, Robins DJ (1989) Pyrrolizidine alkaloid biosynthesis. Synthesis of ^{3}H-labelled trachelanthamidine and isoretronecanol and their incorporation into three pyrrolizidine bases (Necines). J Chem Soc Perkin Trans I 1:1437–1441
62. Hagan DB, Robins DJ (1990) Biosynthesis of the pyrrolizidine alkaloids cynaustraline and cynaustine in *Cynoglossum australe*. J Chem Res (S) 9:292–293
63. Crout DHG, Benn MH, Imaseki H, Geissman TA (1966) Pyrrolizidine alkaloids. The biosynthesis of seneciphyllic acid. Phytochemistry 5:1–21
64. Crout DHG (1966) Pyrrolizidine alkaloids. The biosynthesis of echimidinic acid. J Chem Soc (C) 21:1968–1972
65. Crout DHG (1967) Pyrrolizidine alkaloids. Biosynthesis of the angelate component of heliosupine. J Chem Soc (C) 1233–1234
66. Crout DHG, Davies NM, Smith EH, Whitehouse D (1970) Biosynthesis of the C_{10} necic acids of the pyrrolizidine alkaloids. Chem Comm 11:635–636
67. Crout DHG, Davies NM, Smith EH, Whitehouse D (1972) Pyrrolizidine alkaloids. The biosynthesis senecic acid. J Chem Soc, Perkin Trans I 671–680
68. Robins DJ, Bale NM, Crout DHG (1974) Pyrrolizidine alkaloids. Biosynthesis of monovrotalic acid, the necic acid component of monocrotaline. J Chem Soc, Perkin Trans I 2082–2086
69. Crout DHG, Whitehouse D (1977) Absolute configuration of 2,3-dihydroxy-3-methypentanoic acid, an intermediate in the biosynthesis of isoleucine, and its identity with the esterifying acid of the pyrrolizidine alkaloid strigosine. J Chem Soc, Perkin Trans I 544–549
70. McGaw BA, Wooley JG (1979) The biosynthesis of angelic acid in *Cynoglossum officinale*. Phytochemistry 18:1647–1649
71. Denholm AA, Robins DJ (1991) Biosynthesis of the 3-hydroxy-3-methylglutarate portion of the pyrrolizi-dine alklaoid monocrotaline. J Chem Soc, Chem Comm 19–21
72. Cahill R, Crout DHG, Mitchell MB, Müller US (1980) Isoleucine biosynthesis and metaboilsm: stereo-chemistry of the formation of L-isoleucine and of its conversion into senecic and isatinecic acid. J Chem Soc, Chem Comm 419–421
73. Cahill R, Crout DHG, Gregorio MVM, Mitchell MB, Müller US (1983) Pyrrolizidine alkaloid biosynthesis: stereochemistry of the formation of isoleucine in senecio species and of its conversion into necic acid. J Chem Soc, Perkin Trans I 173–180
74. Devlin JA, Robins DJ (1984) Pyrrolizidine alkaloids. Biosynthesis of trichodesmic acid. J Chem Soc, Perkin Trans I 1329–1332
75. Stirling IR, Freer IKA, Robins DJ (1997) Pyrrolizidine alkaloid biosynthesis. Incorporation of 2-amino-butanoic acid labeled with ^{13}C or ^{2}H into the necic acid portion of rosmarinine and senecionine. J Chem Soc, Perkin Trans I 677–680
76. Mattocks AR (1968) Toxicity of pyrrolizidine alkaloids. Nat (Lond) 217:723–728
77. Culvenor CCJ, Downing DT, Edgar JA, Jago MV (1969) Pyrrolizidine alkaloids as alkylating and antimitotic agents. NY Acad Sci 163:837–847
78. Jago MV, Edgar JA, Smith LW, Culvenor CCJ (1970) Metabolic conversion of heliotrine based pyrrolizidine alkaloids to dehydroheliotridine. Mol Pharmacol 6:402–406
79. Mattocks AR, White INH (1971) Pyrrolic metabolites from non-toxic pyrrolizidine alkaloids. Nat New Biol 231:114–115
80. Culvenor CCJ, Edgar JA, Smith LW, Jago MV, Peterson JE (1971) Active metabolites in the chronic hepatotoxicity of pyrrolizidine alkaloids, including otonecine esters. Nat New Biol 229:255–256
81. Mattocks AR (1972) Acute hepatotoxicity and pyrrolic metabolites in rats dosed with pyrrolizidine alkaloids. Chem Biol Interact 5:227–242

82. Mattocks AR, White INH (1971) The conversion of pyrrolizidine alkaloids to dihydropyrrolizine derivatives by rat-liver microsomes in vitro. Chem Biol Interact 3:383–396
83. Powis G, Ames MM, Kovach JS (1979) Metabolic conversion of indicine-N-oxide to indicine in rabbits and humans. Cancer Res 39:3564–3570
84. Chou MW, Wang YP, Yan J, Yang YC, Berger RD, Williams LD, Doerge DR, Fu PP (2003) Riddelliine N-oxide is a phytochemical and mammalian metabolite with genotoxic activity that is comparable to the parent pyrrolizidine alkaloid riddelliine. Toxicol Lett 145:239–247
85. Wang Y-P, Yan J, Fu PP, Chou MW (2005) Metabolic activation of the tumorigenic pyrrolizidine alkaloid, retrorsine, leading to DNA adduct formation in vivo. Int J Environ Res Public Health 2:74–79
86. Wang Y-P, Yan J, Beger RD, Fu PP, Chou MW (2005) Metabolic activation of the tumorigenic pyrrolizidine alkaloid, monocrotaline, leading to DNA adduct formation in vivo. Cancer Lett 226:27–35
87. Wang Y-P, Yan J, Fu PP, Chou MW (2005) Human liver microsomal reduction of pyrrolizidine alkaloid N-oxides to form the corresponding carcinogenic parent alkaloid. Toxicol Lett 155:411–420
88. Lin G, Cui Y, Hawes EM (1998) Microsomal formation of a pyrrolic alcohol glutathione conjugate of Clivorine firm evidence for the formation of a pyrrolic metabolite of an otonecine-type pyrrolizidine alkaloids. Drug Metab Dispos 26:181–184
89. Lin G, Cui Y, Hawes EM (2000) Characterization of rat liver microsomal metabolites of clivorine, an hepatotoxic otonecine-type pyrrolizidine alkaloid. Drug Metab Dispos 28:1475–1483
90. Culvenor CCJ, Edgar JA, Smith LW, Tweeddale HJ (1970) Dihydropyrrolizines. III. Preparation and reactions of derivatives related to pyrrolizidine alkaloids. Aust J Chem 23:1853–1867
91. Curtain CC, Edgar JA (1976) The binding of dehydroheliotridine to DNA and the effect of it and other compounds on repair synthesis in main and satellite band DNA. Chem Biol Interact 13:243–256. Hincks JR, Kim HY, Segal HJ, Molyneux RJ, Stermitz FR, Coulombe RA (1991) DNA cross-linking in mammalian cells by pyrrolizidine alkaloids: structure-activity relationships. Toxicol Appl Pharmacol 111:90–98
92. Kim H-Y, Stermitz FR, Coulombe RA (1995) Pyrrolizidine alkaloid-induced DNA-protein cross-links. Carcinogenesis 16:2691–2697
93. Kim H-Y, Stermitz FR, Li JK, Coulombe RA (1999) Comparative DNA cross-linking by activated pyrrolizidine alkaloids. Food Chem Toxicol 37:619–625
94. Pereira TN, Webb RL, Reilly PE, Seawright AA, Prakash AS (1998) Dehydromonocrotaline generates sequence-selective N-7 guanine alkylation and heat and alkali stable multiple fragment DNA crosslinks. Nucleic Acids Res 26:5441–5447
95. Coulombe RA, Drew GL, Stermitz FR (1999) Pyrrolizidine alkaloids crosslink DNA with actin. Toxicol Appl Pharmacol 154:198–202
96. Yan J, Nichols J, Yang Y-C, Fu PP (2002) Detection of riddelliine-derived DNA adducts in blood of rats fed riddelliine. Int J Mol Sci 3:1019–1026
97. Fu PP, Xia Q, Lin G, Chou MW (2002) Genotoxic pyrrolizidine alkaloids mechanisms leading to DNA adduct formation and tumorigenicity. Int J Mol Sci 3:948–964
98. Fu PP, Xia Q, Lin G (2004) Pyrrolizidine alkaloids – genotoxicity, metabolism enzymes, metabolic activation, and mechanism. Drug Metab Revs 36:1–55
99. Xia Q, Chou MW, Edgar JA, Doerge DR, Fu PP (2006) Formation of DHP-derived DNA adducts from metabolic activation of the prototype heliotridine-type pyrrolizidine alkaloid, lasiocarpine. Cancer Lett 231:138–145
100. Cheeke PR, Gorman GR (1974) Influence of dietary protein and sulphur amino-acid levels on the toxicity of *Senecio jacobaea* (tansy ragwort) to rats. Nutr Rep Int 9:197–207
101. Nigra L, Huxtable RJ (1992) Hepatic glutathione concentrations and the release of pyrrolic metabolites of the pyrrolizidine alkaloid, monocrotaline, from the isolated perfused liver. Toxicon 30:1195–1202

102. Reed RL, Miranda CL, Kedzierski B, Henderson MC, Buhler DR (1992) Microsomal formation of a pyrrolic alcohol glutathione conjugate of the pyrrolizidine alkaloid senecionine. Xenobiotica 1:1321–1327
103. Peterson JE, Jago MV (1980) Comparison of the toxic effects of dehydroheliotridine and heliotrine in pregnant rats and their embryos. J Pathol 131:339–355
104. Robertson KA (1982) Alkylation of N^2 in deoxyguanosine by dehydroretronecine, a carcinogenic metabolite of the pyrrolizidine alkaloid monocrotaline. Cancer Res 42:8–14
105. Peterson JE, Samuel A, Jago MV (1972) Pathological effects ofdehydroheliotridine, a metabolite of heliotridine-based pyrrolizidinealkaloids in the young rat. J Pathol 107:107–189
106. Prakash AR, Pereira TN, Reilly PEB, Seawright AA (1999) Pyrrolizidine alkaloids in human diet. Mutation Res 443:53–67
107. Allen JR, Hsu I-C, Carstens LA (1975) Dehydroretronecine induced rhabdomyosarcomas in rats. Cancer Res 35:997–1002
108. Shumaker RC, Robertson KA, Hsu IC, Allen JR (1976) Neoplastic transformation in tissues of rats exposed to monocrotaline or dehydroretronecine. J Natl Cancer Inst 56:787–789
109. Mattocks AR, Cabral JRP (1982) Carcinogenicity of some pyrrolizidine alkaloid metabolites and analogues. Cancer Lett 17:61–66
110. Peterson JE, Culvenor CCJ, Keeler RF, Tu AT (1983) Plant and fungal toxins. In: Handbook of natural toxins, vol 1. Marcel Dekker, New York, pp 637–681
111. Culvenor CCJ, Edgar JA, Jago MV, Outteridge A, Peterson JE, Smith LW (1976) Hepato- and pneumotoxicity of pyrrolizidine alkaloids and derivatives in relation to molecular structure. Chem Biol Interact 12:299–324
112. Williams DE, Reed RL, Kedziersk B, Dannan GA, Guengerich FP, Buhler DR (1989) Bioactivation and detoxication of the pyrrolizidine alkaloid senecionine by cytochrome P-450 enzymes in rat liver. Drug Metab Dispos 17:387–392
113. Miranda CL, Chung W, Reed RE, Zhao X, Henderson MC, Wang JL, Williams DE, Buhler DR (1991) Flavin-containing monooxygenase: a major detoxifying nzyme for the pyrrolizidine alkaloid senecionine in guinea pig tissues. Biochem Biophys Res Commun 178:546–552
114. McLean EK (1970) The toxic actions of pyrrolizidine (Senecio) alkaloids. Pharmacol Rev 22:429–483
115. Huxtable RJ (1989) Human health implications of pyrrolizidine alkaloids and herbs containing them. In: Cheeke PR (ed) Toxicants of plant origin, vol 1. CRC Press, Boca Raton, pp 42–86
116. Stegelmeier BL, Edgar JA, Steven M, Colegate SM, Gardner DR, Schoch TK, Coulombe RA jr (1999) Pyrrolizidine alkaloids plants, metabolism and toxicity. J Nat Toxins 8:5–116
117. Rasenack R, Müller C, Kleinschmidt M, Rasenack J, Wiedenfeld H (2003) Veno-occlusive disease in a foetus caused by pyrrolizidine alkaloids of food origin. Fetal Diagn Ther 18:223–225
118. Yee SB, Kinser S, Hill DA, Barton CC, Hotchkiss JA, Harkema JR, Ganey PE, Roth RA (2000) Synergistic hepatotoxicity from coexposure to bacterial endotoxin and the pyrrolizidine alkaloid monocrotaline. Toxicol Appl Pharmacol 166:173–185
119. Newberne M, Rogers AE (1973) Nutrition, monocrotaline and aflatoxin B_1 in liver carcinogenesis. Plant Food Man 1:23–31
120. White INH, Mattocks AR, Butler WH (1973) The conversion of thepyrrolizidine alkaloid retrorsine to pyrrolic derivatives in vivo andin vitro and its acute toxicity to various animal species. Chem Biol Interact 6:207–218
121. Tuchweber B, Kovacs K, Jago MV, Beaulieu T (1974) Effect of steroidal and nonsteroidal microsomal enzyme inducers on the hepatotoxicity of pyrrolizidine alkaloids in rats. Res Commun Chem Pathol Pharmacol 7:459–480
122. Lin JJ, Liu C, Svoboda DJ (1974) Long-term effects of aflatoxin B_1 and vocal hepatitis on marmoset liver: a preliminary report. Lab Invest 30:267–278