



REFERENCE SUBSTANCES FOR HERBAL PRODUCTS

As one of the leading manufacturers internationally, PhytoLab offers over 1,500 extensively documented herbal reference substances of all classes of natural compounds. Our portfolio currently includes a total of 29 pyrrolizidine alkaloids, 27 pyrrolizidine alkaloid N-oxides and one necine base. Most of our pyrrolizidine alkaloids reference substances are certified as primary reference standards.

Well-known food contaminants

Pyrrolizidine alkaloids are common secondary plant metabolites that have been identified in more than 6000 plant species from at least twelve higher plant families. **It has been estimated that about 3 % of all flowering plants from all continents contain pyrrolizidine alkaloids.** More than 660 pyrrolizidine alkaloids and pyrrolizidine alkaloid N-oxides with varying toxic properties have been identified so far. Pyrrolizidine alkaloids are therefore probably the most widespread group of natural substances with toxicological relevance for humans and animals. Plants produce these compounds as a defence against herbivory by insects and mammals.

Occurrence

Due to their widespread occurrence, pyrrolizidine alkaloids are well-known contaminants of various foodstuffs such as leaf lettuces, cereals, spices, teas, herbal teas, and honey. Animal feedstuff can be affected as well. Horses are particularly vulnerable to PA toxicity. The most common source for the pyrrolizidine alkaloids are weeds from the families of the Asteraceae (e.g. genus *Senecio*, known as groundsel or ragwort), Boraginaceae (most genera) and Fabaceae (e.g. genus *Crotalaria*, known as rattlepods). About 95 % of all pyrrolizidine alkaloids occur in the three aforementioned families as well as in Orchidaceae and Apocynaceae. Honey can be contaminated with pyrrolizidine alkaloids if plants from these and other families are visited by the honey bees.

Currently available phyproof® anthocyanins & anthocyanidins

Reference Substance	Product #
7-Acetylintermedine and N-oxide	83759/83795
7-Acetyllycopsamine and N-oxide	84275/85874
Echimidine and N-oxide	86541/83590
Echinatine and N-oxide	86118/84093
Echiumine and N-oxide	83749/84164
Erucifoline and N-oxide	83432/83433
Europine and N-oxide	83237/83238
Heliosupine and N-oxide	86678/84092
Heliotrine and N-oxide	80403/83236
Indicine and N-oxide	83234/83235
Integerrimine and N-oxide	83968/83969
Intermedine and N-oxide	82424/83446
Spartioidine and N-oxide (NEW)	86055/86056
Trichodesmine and N-oxide	83438/84738
Junceine and N-oxide	85643/85644
Lasiocarpine and N-oxide	80412/83220
Lycopsamine and N-oxide	89726/83447
Monocrotaline and N-oxide	89251/82629
Retronecine	83901
Retrorsine and N-oxide	89775/82630
Riddelliine and N-oxide	84102/84103
Rinderine and N-oxide	84162/84163
Sceleratine and N-oxide	84073/84074
Senecionine and N-oxide	89789/82631
Seneciphylline and N-oxide	89275/82632
Senecivernine and N-oxide	83436/83437
Senkirkin	89274
Jacobine and N-oxide	83434/83435
Jaconine	83970
Usaramine and N-oxide	84274/84703

phyproof® Reference Substances

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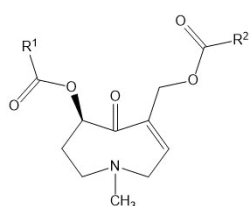
Structural properties

From a chemical point of view pyrrolizidine alkaloids are mono- or diesters of 1-hydroxymethyl pyrrolizidine (necine base) and aliphatic mono- or dicarboxylic acids (necic acids). **Otonecine-type, platynecine-type, and the C7-diastereomeric retronecine-type and heliotridine-type pyrrolizidine alkaloids** are distinguished, their structure depending on the substitution pattern of the necine base. Besides the linear mono- and diesters also cyclic diesters can occur, if esterification occurs with both carboxyl groups of a dicarboxylic acid. Necic acids are typically branched mono- or dicarboxylic acids with a chainlength of 5-10 carbon atoms carrying various substituents. Biosynthetically they are derived from amino acids.

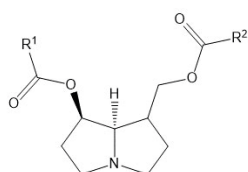
Toxicity

A prerequisite for the toxicity is an 1,2-unsaturated necine core structure that forms an ester with at least one branched C5-carboxylic acid. Pyrrolizidine alkaloids with these structural characteristics have been found to be genotoxic and carcinogenic in animal experiments. In larger quantities pyrrolizidine alkaloids can also cause **acute**

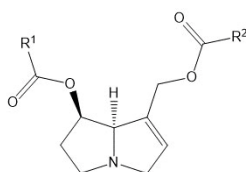
Structures of necine bases



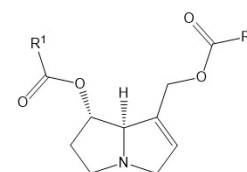
Otonecine type



Platynecine type



Retronecine type



Heliotridine type

and chronic liver damage. It was found that the toxicity of monoesters of the hydroxymethyl group of the necine base is enhanced if a second hydroxyl group is attached to C-7 of the necine core. The toxicity is further intensified if a diester involving the second hydroxyl group is formed. **The highest toxicity is attributed to cyclic diesters.** Except for otonecine-type pyrrolizidine alkaloids plants usually contain a mixture of pyrrolizidine alkaloids and their N-oxides. A comparable toxicity is attributed to both forms upon oral ingestion as the N-oxides are metabolized by reductases to the underlying alkaloid. Further metabolism of 1,2-unsaturated pyrrolizidine alkaloids by certain liver enzymes (cytochrome P450 monooxygenases) results in highly reactive pyrrole esters that can form DNA and protein adducts, that are ultimately responsible for the toxic activity.

phyproof® PA standards

For a reliable quantitative analysis of pyrrolizidine alkaloids and their N-oxides well characterized reference substances are essential. Because of large differences in response factors (e.g. in LC-MS analysis) as well as in toxicity, the quantitative analysis of individual pyrrolizidine alkaloids is preferred over a non-selective sum method. **Currently we offer a total of 57 pyrrolizidine alkaloids**, including pyrrolizidine alkaloid N-oxides and necine bases, all of them supplied together with a comprehensive certificate of analysis. For up-to-date information on prices and specifications please contact us or visit our webshop at phyproof.phytolab.com.

Regulation of PA in Food

Recently, maximum limits have been imposed by EU Directive (EU) 2023/915 for Honey, Herbal Infusions, Spices and Food Supplements (Regulation (EU) 2023/915 on maximum levels for certain contaminants in food). For Herbal medicinal products the EMA HMPC has set a maximum allowable dose of 1µg/day (adults) (EMA HMPC Public statement on the use of herbal medicinal products containing toxic, unsaturated pyrrolizidine alkaloids EMA/HMPC/893108/2011 Rev. 1). Only very recently the united States Pharmacopoeia has implemented a general monograph on adulterant PA. US Pharmacopoeia General Chapter <1567> PYRROLIZIDINE ALKALOIDS AS CONTAMINANTS, DOI: https://doi.org/10.31003/USPNF_M16216_02_01, Official from 1 December, 2023

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