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Pyrrolizidine Alkaloid containing Plants used in Mongolian Traditional Medicine: *Lappula myosotis* Moench.

Helmut Wiedenfeld*[1], Amarsanaa B.[2], Altanchimeg D.[2], and Narantuya S.[2]

[1]Pharmazeutisches Institut der Universität, An der Immenburg 4, D-53121 Bonn, Germany
[2] Institute of Chemistry and Chemical Technology, Mongolian Academy of Science, Ulaanbaatar-51, Mongolia

Summary

Lappula myosotis Moench. (Boraginaceae) is a plant growing wide-spread in the Mongolian Aimags Khubsgul, Khangai, Khentei, Mongol dahurica, Altai and Alasha Gobi [1]. From this plant four pyrrolizidine alkloids were isolated and their structures determined using spectroscopical methods: lycopsamine, intermedine and their acetylderivatives. This plant is used in the Mongolian traditional medicine externally but on account of its high level of alkaloids (~ 0.2%) the usage of *L. myosotis* may be hazardous for humans.

Keywords

Lappula myosotis; Pyrrolizidine alkaloids; Lycopsamine; Intermedine; Acetylderivatives

Introduction

Pyrrolizidine alaloids (PA) are hazardous for humans and domestic animals. Several reports of intoxications can be found in literature [2-8]. Ongoing with our studies on PA-containing medicinal plants from Mongolia [9-11] we investigated *Lappula myosotis* Moench. (Boraginaceae). This plant and the related species *L. intermedia* is used in Tibetian and Mongolian traditional medicine for the external treatment of broken bones as well as for wound-healing and articular swellings

[12,13]. As *Lappula* species are known to contain pyrrolizidine alkaloids (PA) [14-16] we collected during flowering season 2003 samples of *L. myosotis* at different habitats in Tuv aimag for the identification and estimation of its PA content.

In Austria the use of plants or preparations from them which contain toxic PA is forbidden on account of human and animal toxicity data [17]. In Germany the Federal Health Bureau in 1992 established guidelines for the defense of remedy risks leading to regulations for the sale of herbal products containing PA: The daily uptake of PA is restricted to a dose of 1µg for internal and 100 µg for external administration. In general, the use of such products is limited to six weeks per year [18].

For the evaluation os a possible human risk we investigated aerial plant material of *L. myosotis* for its PA content. From *L. glochidiata* as well as from *L. intermedia* echinatine and lasiocarpine were isolated [14,15]. Furthermore from Egyptian *L. spinocarpos* angeloylheliotridine, supinine, vridiflorine, amabiline, trachelanthamine, lycopsamine, intermedine and their acetylderivatives have been detected using GC-MS [16]. From the here investigated *L. myosotis* we succeeded in the isolation of four PA. The structures were determined using spectroscopical methods (MS, 2D-NMR) and found to be lycopsamine, intermedine, acetyllycopsamine and acetylintermedine.

Results and Discussion

The PA **1 - 4** were isolated from alcoholic plant extracts (Fig. 1). Important structural information could be received by the GC-MS spectra. An identical fragmentation pattern between m/z 138 and m/z 80 indicates for all compounds retronecine or its isomeric form heliotridine as the basic moiety. The molecular formulae could be deduced from the $[M]^+$-Peaks at 341 ($C_{17}H_{27}NO_6$ = **3**, **4**) and 299 ($C_{15}H_{25}NO_5$ = **1**, **2**).

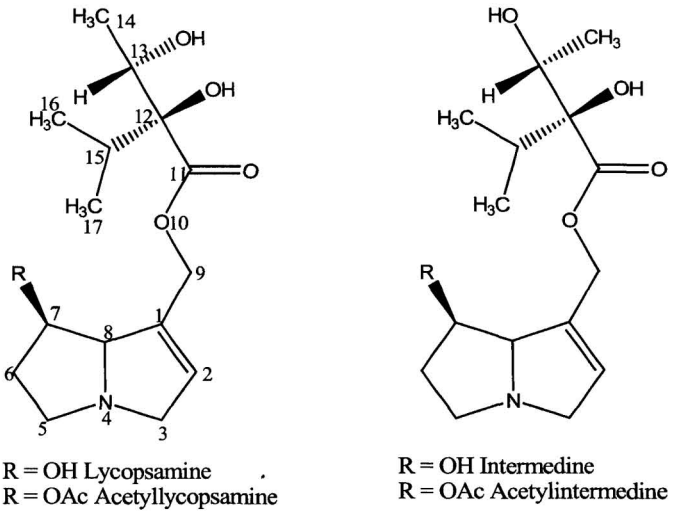

Figure 1: Structures of the PA

Further data were received by NMR. **1** – **4** are shown to be retronecine esters on account of their ^{13}C shift for C-6 (about 35-36 ppm; the C-7 *S*-configurated heliotridine shows values about 33 ppm). Further structural elucidation was done by interpretation of H,H- and C,H-correlated spectra. Important information is provided by the ^{13}C chemical shift of C-7 (**1/2**: ≈70; **3/4**: ≈71 ppm) and C-8 (**1/2**: ≈78; **3/4**: ≈75 ppm). These signals determine a retronecine-*O*-9 ester for **1** and **2** and a retronecine-*O*-9-*O*-7 diester for **3** and **4**, respectively [19-21]. The stereochemistry at C-12 can be deduced by interpretation of the shift-difference of the C-9H_2 AB-system [20,21]. Values from 0-0.2 ppm indicate *S*-configuration, higher values the opposite one (ΔHz = 0.1 ppm in **1** - **4**). The configuration at C-13 is shown by the H-13 and C-13 data as well as by the shift differences of the ^{13}C-NMR data for the methyl goups C-16/C-17 [21]. Thus, the values in **1/3** for C-13 of 3.9 and 71 ppm and in **2/4** of 4.1 and 69 ppm as well as the ΔHz C-16/C-17 values (1.5 ppm: **1/3**; 0.2 ppm **2/4**) give evidence for a 12*S* 13*S* configuration (= (-)-viridifloric acid) in **1/3** and a 12*S* 13*R* configuration (= (+)-trachelanthic acid) in **2/4**, respectively. These

data agree with those already described for lycopsamine **1**, intermedine **2** and their *O*-7-acetylderivatives **3**, **4** [22-24].

PA themselves are showing no or very low toxicity but these compounds can undergo a toxification process in the liver of humans or animals to highly toxic alkylating pyrrols.

On account of structure toxicity relationships the here described PA **3** and **4** should produce toxic side effects whereas **1** and **2** should show a moderate toxicity. The quantification of the PA content in the plants was carried out by gaschromatography and resulted in amounts about 0.2% (dried plants). On account of this PA level the medical use (external application) of *L. myosotis* exhibits a human risk when is it administered in higher doses than 50 mg daily (according to the German restriction of a limitation of 100 µg toxic PA daily and externally).

Experimental

Plant material

Upper earth material of *Lappula myosotis* Moench. was collected at several places in Tuv aimag during flowering season in August 2003. The material was identified by Dr. Ch. Sanchir, and a voucher specimen was deposited at the Institute of Botany, Mongolian Academy of Science (UBA). The plants were air-dried and pulverised.

Isolation of alkaloids

Extraction of plant material (aerial parts; 500 g) was carried out as described earlier [25]. The isolation of the PA was done using prep. TLC [silica gel F_{254}, CH_2Cl_2 -MeOH-NH_4OH (25%), 75:24:1].

General

NMR-spectra (Bruker AC-400) were measured in $CDCl_3$/ D_6-DMSO at 400 and 100 MHz, respectively. GC-MS (Hewlett-Packard, G1800C GCD system) : GC: 150° (5 min.) - 250°C, 10°/min.; HP-1, 25m x 0.32 mm; Inj.: 250°C, det.: 280°C; R_t:

1: 12.78 min., **2**: 12.86 min., **3**: 13.28 min., **4**: 13.37 min.; MS: 220°C; interface: 250°C; 2000 emV.

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