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Pyrrolizidine Alkaloids from *Ligularia sibirica* Cass. and *Tephroseris integrifolia* L.

Helmut Wiedenfeld[1*], Narantuya S.[2], Dumaa M.[3] and Monhbaatar A.[3]

[1]Pharmazeutisches Institut der Universität, An der Immenburg 4, D-53121 Bonn, Germany
[2] National Medical University of Mongolia, Choidog St. 3, Ulaanbaatar 48/111, Mongolia
[3]Institute of Chemistry and Chemical Technology, Mongolian Academy of Science, Ulaanbaatar-51, Mongolia

Summary

Tussilagine, isotussilagine, neo-tussilagine and neo-isotussilagine were isolated from *Ligularia sibirica* whereas *Tephroseris integrifolia* was found to contain senkirkine, otosenine, hydroxysenkirkine and O7-angeloylheliotridine. The structures were determined using spectroscopical methods (GC-MS; NMR).
Keywords: Ligularia sibirica, Tephroseris integrifolia, pyrrolizidine alkaloids

Introduction

In our studies on medical as well as forage plants from Mongolia which contain toxic pyrrolizidine alkaloids (PA) [1,2] we investigated *Ligularia sibirica* Cass., Asteraceae, as well as *Tephroseris integrifolia* L., Asteraceae, (syn. *Senecio integrifolius*, *Senecio campestre*) which both occur in large amounts on pastures and meadows in Bulgan and Huvsgul Aimag in Mongolia. From here several reports about yak intoxications were given by farmers and breeders. Similarily, literature citations confirmed this problem [3,4]. On account of botanical aspects it could be expected that both species should contain PA. We therefore collected both plants at their natural habitats and investigated them for their PA content. From *Ligularia sibirica* only the non-toxic compounds tussilagine and its isomers could be isolated whereas *Tephroseris integrifolia* contains the toxic PA senkirkine, otosenine, hydroxysenkirkine and O7-angeloylheliotridine.

Experiments

Plant material. Ligularia sibirica Cass. was collected at several places on wet meadows between Erdenet and Bulgan during flowering period in August 2000 and 2001. *Tephroseris integrifolia* L. was collected near Tsagaan-nuur, Huvsgul Aimag, in August 2000.
The material was identified by Prof. Dr. E. Jaeger, Inst. F. Geobotanik, University of Halle, Germany and by Dr. E. Ganbold, Inst. of Botany, Mongolian Academy of Sciences, voucher specimen were deposited at the herbarium of Mongolian Academy of Sciences, Ulaanbaatar.
The plants were air-dried and pulverised.

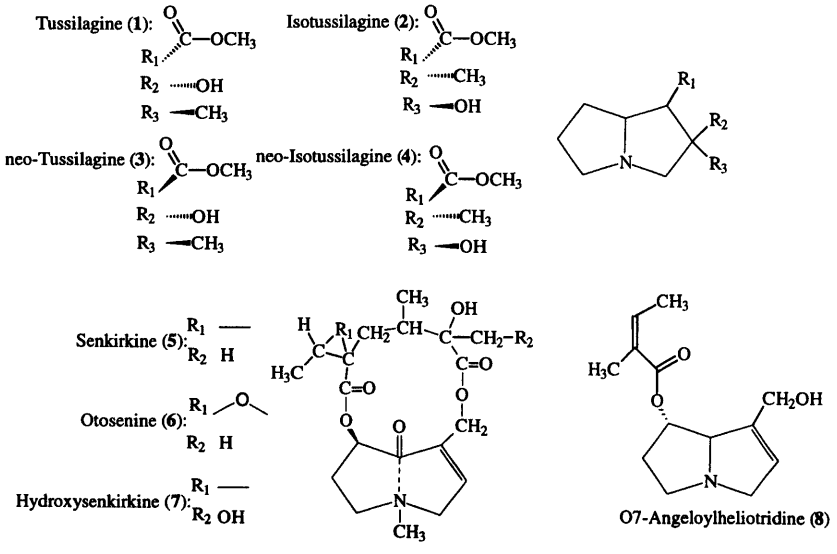
Isolation of alkaloids: Extraction of plant material (aerial parts; 500 g) was carried out as described earlier [5,6]. The isolation of the PA from *L. sibirica* was done using flash-chromatography (150x3 cm, silicagel 60, 0.04-0.063mm, Merck, Darmstadt, Germany) and elution with CH_2Cl_2-MeOH mixtures (500ml each from 80:20 to 50:50) and monitored by GC. A final purification by CC (30x1cm, silicagel 60, 0.063-0.200mm, Merck, Darmstadt, Germany; eluent: CH_2Cl_2-MeOH 78:22) yielded PA **1** to **4.** From *T. integrifolia* the PA **5** to **8** were isolated as oily compounds by 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**: 2.71 min., **2**: 3.10 min., **3**: 2.83 min., **4**: 3.23 min., **5**: 17.93 min., **6**: 17.87 min., **7**: 18.63 min., **8**: 9.43 min.; MS: 220°C; interface: 250°C; 2000 emV.

Results and Discussion

The PA were isolated from alcoholic plant extracts using CC-flash-chromatography and prep. TLC. Their structures were determined by GC-MS as well as NMR-spectroscopy.

The spectroscopical data of the PA from *Ligularia sibirica* are the same as those reported earlier for tussilagine and its isomers **1** – **4** [7,8]. The data of the PA from *Tephroseris integrifolia* are in accordance (within a range of 1.5 ppm = C-NMR and 0.2 ppm = H-NMR) with those reported for senkirkine **5** [9,10], otosenine **6** [11], hydroxysenkirkine **7** [12] and O7-angeloylheliotridine **8** [13].



From the Chinese species *Tephroseris integrifolia* var. *fauri* O7-angeloylheliotridine was isolated, too. But, in difference to the here investigated Mongolian *T. integrifolia,* only saturated otonecine derivatives (no double-bond in 1,2 position of the necine system) were found [14].

PA themselves show only low or no acute toxicity. But, in the liver of humans and animals these compounds are metabolised to highly toxic alkylating pyrrols in case their structure shows a double-bond in position 1,2 of the necine, a non-substituted α-position to the nitrogen atom and esterification of the OH-groups of the necine (monoesters are less-toxic than diesters) [15,16,17]. This structure toxicity relationship implicates that tussilagine and its isomers (**1** – **4**) are non-toxic compounds whereas PA **5** – **7** can produce toxic side effects. PA **8** should show a moderate toxicity.

The quantification of the PA content in *T. integrifolia* (GC) resulted in amounts of less than 1% (dry weight). Our observations in the above mentioned Mongolian Aimags confirmed that the breeders and farmers are mowing the pastures during the summer period for the production of winter food for their animals. We assume that this may be the main pathway for a PA uptake by the animals and that in this way *T. integrifolia* can take part in animal poisoning reported from Huvsgul Aimag.

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