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Pyrrolizidine Alkaloids in *Senecio nemorensis* L. from Mongolia

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Summary

We isolated four Pyrrolizidine Alkaloids (PA) from *Senecio nemorensis* L. growing in Mongolia. Their structures were elucidated by spectroscopic methods to be 7-Senecioyl-9-sarracinoyl-retronecine, Retroisosenine, Doriasenine and Bulgarsenine. The alkaloidal pattern is very similar to that of the European *Senecio nemorensis* L., ssp. *nemorensis* (Rchb.) Celak. The medicinal use of this plant or of preparations from it may be hazardous to human health because of the high PA level in the plant (≈ 0.1%) and the fact that three of the PA are known to have toxic side-effects.

Keywords: Senecio nemorensis, Mongolia, Pyrrolizidine alkaloids; 7-Senecioyl-9-sarracinoyl-retronecine, Retroisosenine, Doriasenine, Bulgarsenine.

Introduction

Senecio nemorensis L., ssp. *Fuchsii* (Gmel.) Celak (Asteraceae) is a plant common in Europe and Asia. Its extracts are used as a haemostyptic agent in gynaecology [1-6]. In traditional medicine, the drug is said to have anti-diabetic properties when administered in form of a herbal tea.

Since it belongs to the Senecio tribe, the plant contains Pyrrolizidine Alkaloids (PA). This is important because those PA that have a double-bond in position 1-2 of their five-member ring system (necine) and occur as diesters (open-chain or macrocycle) show a toxic potential.

We identified several different PA from genetically identical plant material by means of positive and negative CI-MS [7]. The main PA show the structures of the non-toxic Platyphylline, Sarracine and Fuchsisenecionine. Toxic ones like Senecionine and Triangularine were found as minor constituents.

Because of the generally low PA content (less than 0.01%) and the fact that only traces of toxic PA occurred, the plant can be declared a non-toxic Senecio species.

In contrast, the subspecies *nemorensis* (*Senecio nemorensis* L., ssp. *nemorensis* (Rchb.) Celak) not only contains mainly toxic PA (Doronenine, Retroisosenine) and only one non-toxic PA (Bulgarsenine), but also shows an alkaloidal content 10 times higher than that of *fuchsii* [7].

It may also be of interest that 7-Senecioylretronecine, 7-Senecioyl-9-sarracinoylretronecine, Bulgarsenine and Sencalenine [8] as well as from *S. doria* Doriasenine [9] were isolated from the botanically similar *Senecio* species *cacaliaster*.

Until now, this very atypical mixture of macrocyclic and open-chain mono/diester PA was found only in species of the so-called nemorensis-group. One may safely assume that this PA pattern can be used as a chemotaxonomical marker for members of this plant group.

In context with our studies on Senecio species, which are used in the traditional medicine of Mongolia, we investigated *S. nemorensis*. It should be noted that in Mongolia, there is only a description of a single species, named *Senecio nemorensis* L. (syn. *S. octoglossus* DC.), which is not subdivided into further subspecies [10].

It was our objective to explain the structures of the PA in order to evaluate a possible hazardous effect on human health. Furthermore, we wanted to find out whether the Mongolian *S. nemorensis* is related to our European subspecies *nemorensis* or *fuchsii*.

Results and Discussion

TLC analysis of a methanolic extract from *S. nemorensis* (Mongolia) showed the existence of eight PA. Four of them are major components whereas the others occur only as traces.
We successfully isolated the four main constituents. This was done by CC-flash-chromatography, followed by purification of the resulting fractions using normal pressure CC.

We elucidated the structures by spectroscopical methods and established them to be 7-Senecioyl-9-sarracinoylretronecine (**1**), Retroisosenine (**2**), Doriasenine (**3**) and Bulgarsenine (**4**) (figure 1).
The total amount of PA in the dried plant material is about 0.1%.

These results prove that *S. nemorensis* from Mongolia shows an alkaloidal content similar to the one found in the European *Senecio nemorensis*, ssp. *nemorensis*.

The structures of the isolated PA are further evidence of the similarity of the species. In both species, Bulgarsenine and Retroisosenine are the main PA. Furthermore, the PA Doriasenine (**3**) is structurally very similar to the Doronenine (**5**) found in *S. nemorensis*, ssp. *nemorensis*. We may assume that the open-chain Diester PA Doriasenine, as well as the 7-Senecioyl-9-sarracinoylretronecine, are precursors of the macrocyclic Doronenine on account of the biosynthetic pathway of the PA, which starts from building the five-member bicyclus (necine), followed by esterification with simple amino acids and final closure of the macrocycle.

There is substantial evidence that *Senecio nemorensis* L.(Mongolia) is closely related to (or maybe even identical with) our European *Senecio nemorensis* L., ssp. *nemorensis* (Rchb.) Celak.
In addition, the structures of the PA isolated from this species clearly shows that the medicinal administration of plant material may be hazardous to human health. Extracts and other preparations have to be scrutinised with respect to their PA content.

Experiments

The plant was collected in August 1999 in the Terelj mountains of central Aimag of Mongolia at an altitude of 1800 m. The species was identified by Dr. Ch. Sanchir, and a voucher specimen was deposited at the Institute of Botany, Mongolian Academy of Science (UBA).

The dried and pulverised plant material was extracted with MeOH, followed by a liquid-liquid purification as described earlier [11].

The crude alkaloidal extract was applied on a flash-column (150x3 cm, silicagel 60, 0.04-0.063mm, Merck, Darmstadt, Germany), eluted with CH_2Cl_2-MeOH mixtures (500ml each from 80:20 to 50:50) and monitored by TLC (silicagel glass plates 20x20 cm, 0.25 mm; CH_2Cl_2:MeOH:NH_4OH (25%) 85:14:1; Detection: Dann-Mattocks [12,13]) as well as by GC [14]. We collected four fractions containing PA **1**-**4**. Final separation and purification was done using CC (30x1cm, silicagel 60, 0.063-0.200mm, Merck, Darmstadt, Germany), eluting with a CH_2Cl_2-mixture 70:30 yielding the four PA as oily compounds.

Structure elucidation was performed through MS- as well as 2D-NMR (homoeo- as well as heteronuclear) spectroscopy. The NMR analysis in particular gave clear proof of the structures shown in fig. 1 (**1**-**4**). In order to distinguish between the similar structures of the acidic parts of **2** and **4** on the one hand and between those of **1** and **3** on the other, was done as follows:

The macrocyclic diesterification is proven by the AB-shifting of C-9H_2 (4.54 and 4,15 ppm in **4**; 5.08 and 4,16 in **2**). The signals of the 2 quaternary carbons (C-12: 87.1 and C-15: 81.7 ppm) as well as the 2 methylen carbons (C-16: 47.1 and C-14: 46.0 ppm) are important for the verification of the structure of **2**, whereas in **4** the important ones are C-14 (35.6 ppm), C-15 (156.2 ppm) and C-16 (116.9 ppm). All spectroscopic data are in accordance with those already reported [15,16]. In contrast to **2** and **4**, the signals for C-9H_2 in **1** and **3** occur at 4.62 and 4.71 ppm, respectively, indicating the open-chain diester. In both cases the esterification at position 9 shows the identical structure of a sarracinic acid (C-12: 158, C-13: 138, C-15: 62 ppm). **1** and **3** only differ in the CH_2OH-group at position 20 (**1**: C-20: 26.9, C-21: 20.8; **3**: C-20: 66.8, C-21: 15.9 ppm (= Z-configuration)). In **1** and **3**, the spectroscopic data are in accordance with those already reported, too [9,17].

Acknowledgements

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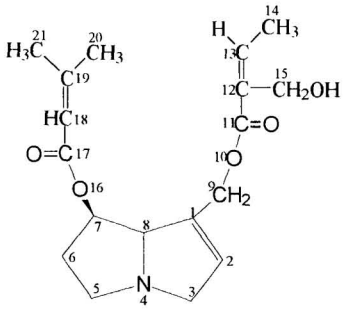
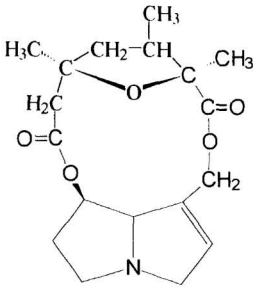
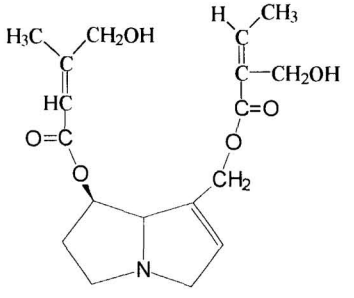


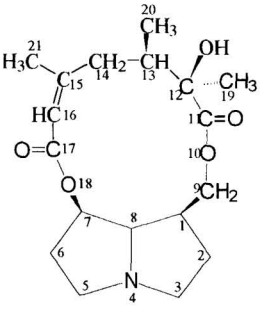
7-Senecioyl-9-sarracinoylretronecine (1)



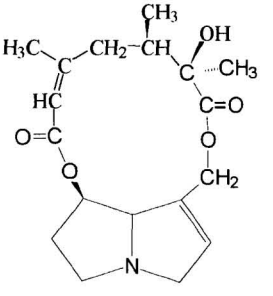
Retroisosenine (2)



Doriasenine (3)



Bulgarsenine (4)



Doronenine (5)

Figure 1: Structures of the Pyrrolizidine alkaloids

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