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Pyrrolizidine alkaloids from seven wild-growing *Senecio* species in Serbia and Montenegro

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Abstract: The genus *Senecio* (family Asteraceae) is one of the largest in the world. It comprises about 1100 species which are the rich source of pyrrolizidine alkaloids. Plants containing pyrrolizidine alkaloids are among the most important sources of human and animal exposure to plant toxins and carcinogens. The pyrrolizidine alkaloids of seven *Senecio* species (*S. erucifolius*, *S. othonnae*, *S. wagneri*, *S. subalpinus, S. carpathicus*, *S. paludosus* and *S. rupestris*) were studied. Fourteen alkaloids were isolated and their structures determined from spectroscopic data (^{1}H- and ^{13}C-NMR, IR and MS). Five of them were identified in *S. erucifolius*, four in *S. othonnae*, two in *S. wagneri*, four in *S. subalpinus*, two in *S. carpathicus*, three in *S. paludosus* and three in *S. rupestris.* Seven pyrrolizidine alkaloids were found for the first time in particular species. The results have chemotaxonomic importance. The cytotoxic activity and antimicrobial activity of some alkaloids were also studied.

Keywords: *Senecio*; pyrrolizidine alkaloids; antitumor and antimicrobial activity.

INTRODUCTION

The toxic pyrrolizidine alkaloids (PA) are a large group of related compounds that occur in plants, mainly in species of *Crotalaria* (Leguminosae), *Senecio* and related genera (Compositae), *Heliotropium*, *Trichodesma*, *Symphytum*, *Echium* and other genera of the Boraginaceae.

The worldwide distributed genus *Senecio* (family Asteraceae) is a rich source of pyrrolizidine alkaloids. It was shown that the alkaloid pattern differs between some subspecies.[1–4] Previous investigations of alkaloids from seven *Senecio* spe-

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cies (*S. erucifolius*, *S. othonnae*, *S. wagneri*, *S. subalpinus*, *S. carpathicus*, *S. paludosus* and *S. rupestris*) are presented in short in Table I.[5–27]

TABLE I. Alkaloids (**1**–**37**) isolated from species of genus *Senecio* (Columns: I – *S. erucifolius*; II – *S. othonnae*; III – *S. wagneri*; IV – *S. subalpinus*; V – *S. carpathicus*; VI – *S. paludosus*; VII – *S. rupestris*). The Arabic numbers in the columns correspond to the reference numbers of the papers

Alkaloid	I	II	III	IV	V	VI	VII
Senecionine (**1**)	5–11	12	–	5, 13, 14	–	–	5, 15, 16
Seneciphylline (**2**)	5, 6, 8, 9, 10, 11	17	–	5, 13, 14	5	18–20	–
O-Acetylseneciphylline (**3**)	10	–	–	–	–	–	–
Seneciphylline *N*-oxide (**4**)	21	–	–	–	–	–	–
O-Acetylseneciophylline *N*-oxide (**5**)	21	–	–	–	–	–	–
Senecivernine (**6**)	6, 10	–	–	5	–	–	5
Integerimmine (**7**)	5, 6, 9, 10	–	–	5, 13	–	–	5, 15, 16
21-Hydroxyintegerimmine (**8**)	5	–	–	–	–	–	5
Integerimmine *N*-oxide (**9**)	21	–	–	–	–	–	–
Senkirkine (**10**)	5, 9	–	5	–	–	–	–
O-Acetylsenkirkine (**11**)	9	–	–	–	–	–	–
Neosenkirkine (**12**)	–	–	5	–	–	–	–
Otosenine (**13**)	–	17, 22, 23	–	–	–	–	–
Doronine (**14**)	–	22, 23	–	–	–	–	–
Platyphylline (**15**)	–	12	–	–	–	–	–
Neoplatyphylline (**16**)	–	–	–	5	–	–	–
7-Angeloylplatynecine (**17**)	–	–	–	–	–	24	–
9-Angelylplatynecine (**18**)	–	–	–	–	–	25	–
Retronecine (**19**)	–	12	–	–	–	–	–
Onetine (**20**)	–	17, 22	–	–	–	–	–
Othonnine (**21**)	–	12	–	–	–	–	–
Retrorsine (**22**)	8	12	–	–	–	–	16
Dihydroretrorsine (**23**)	–	–	–	–	–	24	–
Erucifoline (**24**)	6, 10	–	–	–	–	–	–
O-Acetylerucifoline (**25**)	10	–	–	–	–	–	–
Erucifoline N-oxide (**26**)	21	–	–	–	–	–	–
Eruciflorine (**27**)	6	–	–	–	–	–	–
Procerine (**28**)	–	–	5	–	–	–	–
Racemonine (**29**)	–	–	–	–	–	26, 27	–
Racemocine (**30**)	–	–	–	–	–	26, 27	–
Racemodine (**31**)	–	–	–	–	–	24	–
Floridanine (**32**)	–	22, 23	–	–	–	–	–
Senecioracenine (**33**)	–	–	–	–		25	–
Sarracine (**34**)	–	–	–	–	–	25	–
Riddeline (**35**)	–	12	–	–	–	–	–
7-Angelylheliotridine (**36**)	–	–	5	–	–	–	–
Spartioidine (**37**)	6	–	–	–	–	–	–

Despite the fact that secondary metabolites (especially PAs) of *Senecio* species have been the subject of investigations for many years, interest in them remains. The investigations of the activities of plants PAs indicated their neurotoxic, mutagenic, carcinogenic, but also antitumor effects.[28,29] PAs are readily absorbed from the digestive tract and cause harmful effects only after undergoing activation in the liver to toxic metabolites. The effects include a variety of changes leading to permanent damage to genes and chromosomes, the ability of cells to divide, the development of cancer and even cell death. Some of them are strong toxins for humans and domestic animals.[30,31] The acute toxicity of PAs varies widely. The rat LD_{50} of most alkaloids known to be significant for human health are in the range of 34–300 mg/kg, although some approach 1000 mg/kg.

On the other hand, many *Senecio* species are used in traditional medicine in Asia and Africa, which makes them a very interesting for phytochemical investtigation.

In this study, the pyrrolizidine alkaloids from seven *Senecio* species (*S. erucifolius*, *S. othonnae*, *S. wagneri*, *S. subalpinus*, *S. carpathicus*, *S. paludosus* and *S. rupestris*) were isolated and their structure elucidated. Also, the cytotoxicity and antimicrobial activity of some of PAs were investigated.

EXPERIMENTAL

General

The IR spectra were measured in the form of KBr pellets on a Perkin-Elmer FT-IR spectrometer 1725X. The ^{1}H- (200 MHz) and ^{13}C-NMR (50 MHz) spectra were recorded on a Varian Gemini 2000 spectrometer. The mass spectra were obtained on a Finnigan MAT 8230, BE DCI (150 eV, iso-butane).

Silica gel, 0.008 mm (Merck, Darmstadt, Germany), was used for preparative column chromatography (CC) and silica gel F-254 (Merck, Darmstadt, Germany) for analytical and preparative thin layer chromatography (TLC).

Plant material

The studied species and collection data are listed in Table II.

TABLE II. Studied species and collection data

Species	Collection data
S. erucifolius	Deliblatska peščara, Serbia, July 2003
S. othonnae	Sinjajevina, Montenegro, August 2003
S. wagneri	Maja Rusalija, Montenegro, July 2004
S. subalpinus	Hajla, Montenegro, July 2003
S. carpathicus	Stara planina, Serbia, July 2004
S. paludosus	Suva planina, Serbia, July 2005
S. rupestris	Lisa, Montenegro, July 2005

Extraction and isolation

The dried and powdered plant material of each sample was extracted with methanol. After solvent removal, the residue was dissolved in 1.0 M sulfuric acid, washed with CH_2Cl_2, the pH adjusted to 9.0 with NH_4OH, extracted with CH_2Cl_2 and purified by silica gel CC and

prep. TLC to yield pure alkaloids. The elution was commenced with CH_2Cl_2:methanol:NH_4OH (9:1:0.1) and the polarity was gradually increased. The spots were detected under UV_{254}, by Dragendorff reagent or by spraying with 50 % H_2SO_4.

Investigation of antiproliferative and antimicrobial activity

Stock solutions of the isolated alkaloids were prepared in DMSO at a concentration of 10 mM and afterwards diluted with nutrient medium (RPMI 1640), supplemented with L-glutamine (3 mM), streptomycin (100 μg/ml), penicillin (100 IU/ml), 10 % heat inactivated (56 °C) fetal bovine serum and 25 mM Hepes, adjusted to pH 7.2 with bicarbonate solution and applied to the target cells (human cervix carcinoma HeLa cells, human myelogenous leukemia K562 cells, human melanoma Fem-X cells and normal human peripheral blood mononuclear cells) at various final concentrations ranging from 0 to 100 μM. HeLa and Fem-X cell survival was determined indirectly by measuring the total cellular protein by the Kenacid Blue R dye binding method.[32] Inhibition of the growth of PBMC and K562 cells was determined by the MTT test.[33]

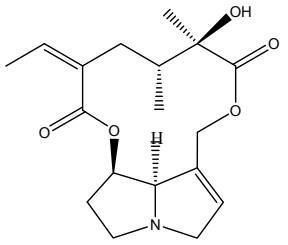
The antibacterial activity of alkaloids (otosenine, seneciphylline and a mixture of senecionine and seneciphylline) was tested against the microorganisms *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger*. The concentration of the tested samples was 1.0 mg/ml. The surface of an agar plate was inoculated by streaking the bacterial suspension (*ca.* 10^5 cfu/ml). After incubation at 37 °C for 24 h, the inhibitory effect was determined as the prevention of visible growth.

RESULTS AND DISCUSSION

The isolation procedure for alkaloids from the crude extract (of seven *Senecio* species: *S. erucifolius*, *S. othonnae*, *S. wagneri*, *S. subalpinus*, *S. carpathicus*, *S. paludosus* and *S. rupestris*) yielded fourteen alkaloids (**1**, **2**, **4**, **6**, **7**, **10**, **13**, **14**, **17**, **33**, **36**, **38**–**40**) (Table III, Scheme 1). The structural assignments of alkaloids were based on comparison of their spectral data with those published in literature.[34,35]

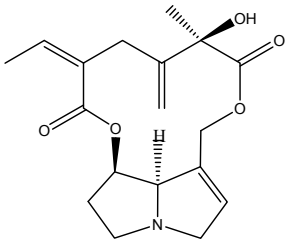
TABLE III. Alkaloids isolated from species of genus *Senecio* (Columns: I – *S. erucifolius*; II – – *S. othonnae*; III – *S. wagneri*; IV – *S. subalpinus*; V – *S. carpathicus*; VI – *S. paludosus*; VII – – *S. rupestris)*. Mark PA indicates alkaloids isolated for the first time from the *Senecio* species

Alkaloid	I	II	III	IV	V	VI	VII
Senecionine (**1**)	+	–	–	+	–	–	+
Seneciphylline (**2**)	+	–	–	+	+	–	–
Seneciphylline *N*-oxide (**4**)	+	–	–	PA	PA	–	–
Senecivernine (**6**)	–	–	–	–	–	–	+
Integerimmine (**7**)	–	–	–	–	–	–	+
Senkirkine (**10**)	–	–	+	–	–	PA	–
Otosenine (**13**)	PA	+	–	–	–	–	–
Doronine (**14**)	–	+	–	–	–	–	–
7-Angeloylplatynecine (**17**)	–	–	–	–	–	+	–
Senecioracenine (**33**)	–	PA	–	–	–	–	–
7-Angelylheliotridine (**36**)	–	–	+	–	–	–	–
7-Angelylretronecine (**38**)	–	–	–	–	–	PA	
Neopetasitenine (**39**)	–	PA	–	–	–	–	–
Senecionine *N*-oxide (**40**)	PA	–	–	PA	–	–	–



1

40 N-oxide of 1



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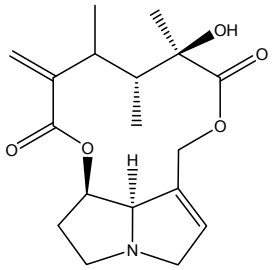
4 N-oxide of 2



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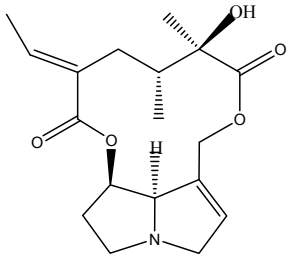

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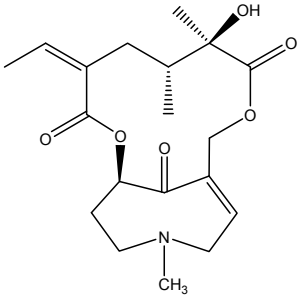

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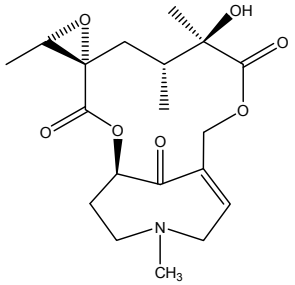

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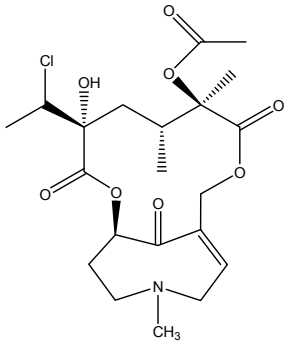

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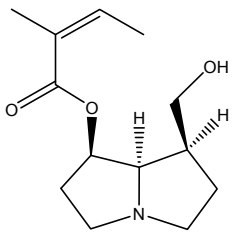
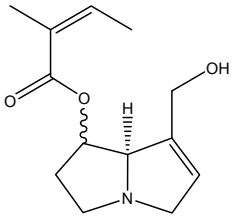
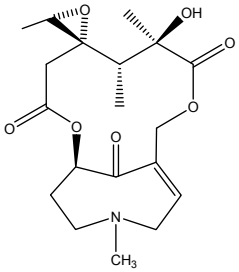


17



36 7α

38 7β



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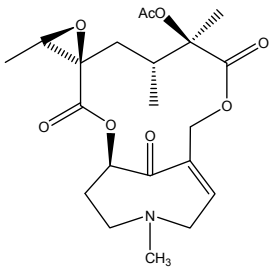

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Scheme 1.

Previous studies of *S. erucifolius* resulted in the isolation and identification of 17 PAs (**1**–**11**, **22**, **24**–**27** and **37**). In the present study, three of the previously identified alkaloids (senecionine, seneciphylline and seneciphylline *N*-oxide) and an additional two, namely otosenine (**13**) and senecionine *N*-oxide (**40**), are now reported and identified for the first time as alkaloids in *S. erucifolius.*

Four alkaloids were identified from *S. othonnae*, two of them (otosenine and doronine) were previously reported in this plant. Senecioracenine (**33**) and neopetasitenine (**39**) are alkaloids isolated for the first time from this species.

Four PAs, namely senkirkine, neosenkirkine, procerine and 7-angeloylheliotridine (**10**, **12**, **28** and **36**) were previously isolated from *S. wagneri.* This investtigation confirmed the presence of two alkaloids senkirkine (**10**) and 7-angeloylheliotridine (**36**).

Senecionine (**1**) and seneciphylline (**2**) identified in *S. subalpinus* are two of five previously isolated alkaloids. However, seneciphylline *N*-oxide (**4**) and senecionine *N*-oxide (**40**) are alkaloids new for this plant.

One (**2**) and five (**1**, **6**, **7**, **8** and **22**) alkaloids were previously isolated from *S. carpathicus* and *S. rupestris*, respectively. In this investigation, the presence of seneciphylline (**2**) as well as senecionine, senecivernine and integerimmine (**1**, **6** and **7**) respectively, were confirmed. Seneciphylline *N*-oxide (**4**) was isolated from *S. carpathicus* for the first time.

One (7-angeloylplatynecine) of nine previously reported alkaloids of *S. paludosus* was identified in the present study. An additional two alkaloids, namely senkirkine (**10**) and 7-angeloylretronecine (**38**), had not been previously isolated from this plant.

In order to anticipate antitumor action, the antiproliferative activity of some alkaloids (senkirkine and a mixture of senecionine and seneciphylline, which were isolated in sufficient quantities to allow bioassays) against malignant cell lines (human cervix carcinoma HeLa cells, human myelogenous leukemia K562 cells and human melanoma Fem-X cells) and normal human cells (PBMC) was tested. The investigated alkaloids (in concentrations from 0 to 100 μM) exhibited no cytotoxic effects against any of the tested human cancer cells.

The antimicrobial action of otosenine, seneciphylline and a mixture of senecionine and seneciphylline against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger* was also, investigated. Only the senecionine-seneciphylline mixture (2:1) exhibited a weak (on the limit of detection) inhibitory effect against *A. niger* at a concentration of 1 mg/ml.

CONCLUSIONS

Fourteen pyrrolizidine alkaloids were isolated and identified from seven *Senecio* species (*S. erucifolius*, *S. othonnae*, *S. wagneri*, *S. subalpinus*, *S. carpathicus*, *S. paludosus* and *S. rupestris*). Seven of them were identified for the first time in the particular species. The results are of chemotaxonomic importance.

Senkirkine and a mixture of senecionine and seneciphylline at the investigated concentrations did not exhibit antiproliferative effects against the tested human malignant cell lines. However, a mixture of senecionine and seneciphylline exhibited a weak antimicrobial effect against *Aspergillus niger*.

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ИЗВОД

ПИРОЛИЗИДИНСКИ АЛКАЛОИДИ ИЗ СЕДАМ САМОНИКЛИХ БИЉНИХ ВРСТА РОДА *Senecio* КОЈЕ РАСТУ У СРБИЈИ И ЦРНОЈ ГОРИ

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Један од најбројнијих родова на свету је род *Senecio* (фамилија Asteraceae). Обухвата око 1100 врста које представљају богат извор пиролизидинских алкалоида. Најзначајнија изложеност људи и животиња биљним токсинима и карциногенима потиче од биљака које садрже пиролизидинске алкалоиде. У овом раду изоловани су пиролизидински алкалоиди из седам врста рода *Senecio* (*S. erucifolius*, *S. othonnae*, *S. wagneri*, *S. subalpinus*, *S. carpathicus*, *S. paludosus* и *S. rupestris*), а њихове структуре одређене на бази спектроскопских података (^{1}H- и ^{13}C-NMR, IR и MS). Изоловано је укупно 14 алкалоида, пет из *S. erucifolius*, четири из *S. othonnae*, два из *S. wagneri*, четири из *S. subalpinus*, два из *S. carpathicus*, три из *S. paludosus* и три из *S. rupestris*. Седам пиролизидинских алкалоида је по први пут изоловано из неких појединачних врста. Добијени резултати имају хемотаксономски значај. Испитана је цитотоксичност и антимикробна активност изолованих алкалоида.

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