Alkaloid Profile of Bulgarian Species from Genus *Senecio* L.

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Twenty-seven pyrrolizidine alkaloids were identified by a detailed phytochemical study and GC/MS analysis on twelve Bulgarian species from genus *Senecio*. Twenty of them are new for the corresponding species. Other six structures reimain tentatively identified.

Key words: Genus *Senecio*, Pyrrolizidine Alkaloids, GC/MC Analysis

The capillary gas chromatography with mass spectrometry (GC/MS) is convenient and very often used method for analysis of a complex mixture containing pyrrolizidine alkaloids (PAs) (Witte *et al.*, 1993).

As a part of our systematic investigations on the species from the genus *Senecio* widespread in Bulgaria we studied the alkaloid profile of the well studied specis: *Senecio jacobaea* L., *S. erucifolius* L. (Witte *et al.*, 1992), *S. vernalis* Waldst & Kit., *S. vulgaris* L. (Borstel *et al.*, 1989), *S. nemorensis* L. (Smith and Culvenor, 1981), so Balkan endemit and chiefly characteristic for south-east Europe: *S. sylvaticus* L. (Roeder *et al.*, 1986), *S. pančičii* Degen, *S. carpathicus* (Herbich) Nyman, *S. subalpinus* Koch (Klasek *et al.*, 1968), *S. viscosus* L. (Smith and Culvenor, 1981), *S. papposus* (Reichenb.) Less. (Jovčeva *et al.*, 1978) and *S. rupestris* Waldst & Kit.

The GC/MS analysis was performed with GC Hewlett Packard 6890 plus MS detector Hewlett Packard 5973. Column: HP 5-MS (30 m × 0.25 mm *i.d.* df = 0.25 μm); conditions: injector 260 °C, temp. programme 100 °(2 min) – 280 °C, 5 °C/min, isothermal at 280 ° for 20 min; split ratio 1:50; carrier gas: He const. flow 0.8 ml/min, sample 1 mg crude alkaloid mixture (CAM). The ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ on a Bruker DRX 250 spectrometer with TMS as internal standard. Optical rotation was performed with a Perkin-Elmer 241 polarimeter.

Fresh plant material from each species was extracted with MeOH. The combined MeOH extracts after evaporation to dryness was acidified with 5% HCl. The N-oxides were reduced with Zn dust (24 h), made alkaline with 25% NH_4OH to pH 9 and extracted with $CHCl_3$ to afford CAM. Pure alkaloids were obtained after column chromatography of the CAMs and preparative TLC on mix fractions as was described in paper (Christov *et al.*, 2002).

The above ground parts from the studied species were collected during blooming from different regions on Bulgaria. Voucher specimens from all of them were deposited at the Herabarium of the Institute of Botany, Bulgarian Academy of Sciences SOM.

The alkaloids from the phytochemical studies have been identified by ^{1}H, in some case ^{13}C NMR 21-hydroxyintegerrimine, $[\alpha]_D - 5°$ ($c = 0.0075$ in $CHCl_3$) 7-angeloylheliotridine and comparisons with data in the literature (Logie *et al.*, 1994). The latter studies were supplemented by the results of the GC/MS analysis. In this case the alkaloids were identified by MS, comparisons with the data in literature (Borstel *et al.*, 1989; Jovčeva *et al.*, 1978; Witte *et al.*, 1992, 1993), and with already identified alkaloids, a model mixture from *S. macedonicus* (Christov *et al.*, 2002), as well as by Kovatch relative retention indices (RR_I). The latter was calculated by model mixture from normal hydrocarbons (C_{16}, $C_{18} \ldots C_{32}$) which was used as reference mixture (Kovatch 1958; Witte *et al.*, 1993). Twenty-seven PAs and caffeine have been identified as a result of these investigation (Table I). To our knowledge twenty of them are new for the corresponding species. The presence of caffeine in the species from genus *Senecio* is not typical. Other six remained tentatively identified. As in a previous investigation (Jovčeva *et al.*, 1978) procerine has been detected in *S. papposus.* Nemorensine and bulgarsenine isomers has the same MS like nemorensine resp. bulgarsenine and differ only by RR_I (Table I), most probable they are diastereomers of the corresponding alkaloids. The MS 70 eV of hydroxymethylnemorensine has a M^+ at

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Table I. The alkaloid profile of Bulgarian species from genus *Senecio*.

Species			**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
Alkaloids	RR_I	M^+ *m/z*	% (Fr. W)											
7-Angeloylretronecine	1820	237	+											
7-Angeloylheliotridine	1918	237												⊕*
7-Angeloylplatynecine	1856	239	+*											
9-Angeloylplatynecine	1896	239	+*											
Triangularine	2434	335	+											
Neotriangularine	2449	335	+*											
Sarracine	2460	337	+											
Neosarracine	2475	337	0.02*											
8-Episarracine	2492	337	+*											
8-Epineosarracine	2507	337	+*											
Senecivernine	2330	335			+*	+	+*				0.06	0.02		
Senecionine	2342	335			0.01	0.02	⊕	0.03	0.01	⊕	0.08	⊕		
Seneciphylline	2360	333		⊕		0.02	0.01	0.15	⊕	⊕	0.09	⊕		
Integerrimine	2402	335			0.02	+	+*	+*	+	+	+	+		
Retrocine	2585	351							+	+*	+	+		
21-Hydroxyintegerrimine	2684	351			⊕*				+					
Senecionine-12-Ac	2505	377				+*								
Seneciphylline-12-Ac	2531	375					⊕*	⊕*						
Spartioidine-12-Ac	2580	375						+*						
Platyphylline	2388	337											+	
Neoplatyphylline	2464	337					+*					+	+	
Senkirkine	2540	365							+*			0.08		0.02
Neosenkirkine	2617	365												+*
Nemorensine	2322	337											⊕	
Retroisosenine	2342	–											0.02	
Bulgarsenine	2428	337											0.09	
Doronenine	2475	335											+	
Caffeine	1842	194		+*										
Procerine	1974	255												+
Nemorensine-isomer*	2380	337											TN	
Nemorensine-isomer*	2394	337											TN	
Bulgarsenine-isomer*	2443	337											TN	
Bulgarsenine-isomer*	2482	337											TN	
Hydroxymethylnemorensine	2585	353											TN	

Species QAM *g*/Fr. W. *g*: **A**, *S. sylvaticus* 0.156/100; **B**, *S. carpathicus* 0.038/32; **C**, *S. rupestris* 0.290/600; **D**, *S. jacobaea* 0.088/178; **E**, *S. subalpinus* 0.180/600; **F**, *S. pančičii* 0.660/100; **G**, *S. erucifolius* 0.057/100; **H**, *S. viscosus* 0.053/138; **I**, *S. vulgaris* 0.710/180; **J**, *S. vernalis* 0.157/122; **K**, *S. nemorensis* 1.220/1000; **L**, *S. papposus* 0.030/50; ⊕ Alkaloids identified by spectral methods ^{1}H and ^{13}C NMR; + Alkaloids identified by GC/MS analysis and RR_I; ⊕*, +* Alkaloids new for the corresponding species; TN, Tentatively identified new natural products; * % (Fr. W), % in fresh weight plant material according to the results of GC analysis over 0.01%.

m/z 353(18) and is characteristic for nemorensine fragments at *m/z* 82(100), 95(25), 122(66), 138(24), 210(27), 238(4), 322(54). M^+ and the intensive fragment at *m/z* 322, M^+-CH_2OH define this alkaloid as hydroxymethylnemorensine. According to the fragmentation pattern most probable positions are at C-12 or C-15. To our knowledge the last five alkaloids (Table I) are new natural products.

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