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PHYTOECDYSTEROIDS FROM *Serratula komarovii*

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Integristerone A and 2-deoxy-20-hydroxyecdysone were observed for the first time in the aerial and subterrean organs of the eastern Asian plant Serratula komarovii *Iljin. α-Ecdysone was not found in the plants. The dynamics of phytoecdysteroid content (integristerone A, 20-hydroxyecdysone, and 2-deoxy-20-hydroxyecdysone) in the vegetative and generative organs of this species were investigated.*

Key words: *Serratula komarovii*, Asteraceae, integristerone A, 20-hydroxyecdysone, 2-deoxy-20-hydroxyecdysone, α-ecdysone, HPLC.

Phytoecdysteroids are interesting as potential agents for stimulating natural protein synthesis without disrupting the endocrine system. The most active phytoecdysteroids can be used as preparations of choice because the use of anabolic steroids has several serous limitations in therapeutic practice and in sports, where they are classified as dopants [1-3]. We decided to research a representative of the *Serratula* L. genus because species of this genus are superproducers of 20-hydroxyecdysone (**1**), the predominant component of the phytoecdysteroid fractions, and because it contains several minor compounds [integristerone A (**2**), 2-deoxy-20-hydroxyecdysone (**3**), α-ecdysone (**4**), ponasterone A, inokosterone, etc.] [4-9].

Compound **2** was first isolated from flowers of *Rhaponticum integrifolium* C. Winkl. [10] and also observed in *S. xeranthemoides* Bieb. [11] and *S. coronata* L. [12]. Compound **3** was first found in *Blechnum minus* (R. Br.) Ettingsh. [13] and later isolated from *R. carthamoides* (Willd.) Iljin [14]. Compound **4** was observed in only *S. coronata* among the Asteraceae [15].

We previously found **1** in rhizomes of the eastern Asian species *S. komarovii* [16]. The content of **2**-**4** in *S. komarovii* has not been reported.

The goal of our work was to investigate the contents and dynamics of phytoecdysteroids in various organs of *S. komarovii* collected in 2002-2003 near Verkhneblagoveshchenskoe village of Blagoveshchensk region in Amur district.

S. komarovii is a perennial herbaceous plant with a straight nearly unbranched stem from 100-150 cm in height, with long petiolar, lyrate-pinnatipartite radical leaves, and single (rarely double) calathides up to 2-3 cm in diameter. It grows on stony slopes among shrubs, less frequently in pine forests. It occurs in the Far East primarily in Amur district, Khabarovsk and Primor′e territories, northeast China, and Mongolia.

Table 1 lists the contents of **1**-**3** in organs of *S. komarovii* at various development stages. Compound **4** was not observed in vegetative and generative organs of *S. komarovii* in all development stages.

This species typically has a sharp distribution gradient of **1**-**3** within a single plant and significant variations in the content of phytoecdysteroids in the same organs at different development times.

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TABLE 1. Content of 20-Hydroxyecdysone (**1**), Integristerone A (**2**), and 2-Deoxy-20-hydroxyecdysone (**3**) in Various Parts of *Serratula komarovii* as a Function of Growth Stage

Phenophase (collection date, plant characteristics)	Plant organ	Content, μg/mg		
		1	**2**	**3**
Start of vegetation (May 27; radical leaves formed, start of generative shoot development)	Rhizome	0.61±0.03	0.094±0.005	0.33±0.02
	Radical leaf	3.05±0.15	0.046±0.002	0.43±0.02
	Radical-leaf petiole	2.61±0.13	0.15±0.01	Tr.
Budding (June 25; generative shoot height 70 cm, inflorescences 4-7 mm in diameter)	Rhizome	0.68±0.03	0.030±0.002	0.25±0.01
	Radical leaf	1.19±0.06	0.074±0.004	-
	Radical-leaf petiole	0.84±0.04	0.023±0.001	Tr.
	Lower stem leaf	1.11±0.06	0.031±0.002	Tr.
	Middle stem leaf	1.67±0.08	0.042±0.002	-
	Upper stem leaf	1.44±0.07	-	0.23±0.01
	Stem (lower part)	0.54±0.03	-	Tr.
	Stem (middle part)	0.95±0.05	-	-
	Stem (upper part)	2.88±0.14	0.24±0.01	-
	Secondary stem	1.97±0.1	0.20±0.01	-
	Inflorescence	0.97±0.05	0.010±0.001	0.12±0.01
Flowering (July 26; generative shoot height up to 100 cm, inflorescences 2-2.5 cm in diameter)	Rhizome	0.75±0.04	0.071±0.004	0.36±0.02
	Radical leaf	0.20±0.01	-	-
	Radical-leaf petiole	0.24±0.01	Tr.	Tr.
	Young radical leaf	1.35±0.07	0.036±0.002	0.13±0.01
	Lower stem leaf	0.43±0.02	0.011±0.001	Tr.
	Middle stem leaf	0.84±0.04	0.10±0.005	Tr.
	Upper stem leaf	2.34±0.12	Tr.	-
	Stem (lower part)	0.11±0.01	-	0.13±0.01
	Stem (middle part)	0.052±0.003	Tr.	-
	Stem (upper part)	0.81±0.04	Tr.	-
	Flower	0.68±0.03	-	-
	Sheath	3.14±0.16	0.12±0.01	-
	Pappus	2.47±0.12	0.15±0.01	-
Fruiting, dying off of aerial part (August 21; ripe achenes, generative shoot starting to die off, dry radical and lower stem leaves)	Rhizome	1.53±0.08	0.065±0.003	0.47±0.02
	Radical leaf	0.14±0.01	Tr.	-
	Radical-leaf petiole	Tr.	-	Tr.
	Lower stem leaf	0.044±0.002	-	-
	Middle stem leaf	-	-	-
	Upper stem leaf	Tr.	Tr.	-
	Stem (lower part)	0.018±0.001	-	0.32±0.002
	Stem (middle part)	-	-	Tr.
	Stem (upper part)	0.014±0.001	Tr.	-
	Secondary stem	0.023±0.001	-	-
	Sheath	0.61±0.03	Tr.	-
	Pappus	0.42±0.02	-	0.23±0.01
	Seeds	4.68±0.23	-	0.80±0.04

Tr., trace (≤0.01 μg/mg). Dash, compounds not observed.

Compound **1** varied from 0.01 to 4.68 μg/mg in the aerial and subterrean part of *S. komarovii* depending on the organ and development stage. The maximum content was observed in actively developing parts of organs: during development of generative shoots, in leaves (3.05 μg/mg); during budding, in the upper stem (2.88 μg/mg); during flowering, in the sheath and

pappus (3.14 and 2.47 μg/mg, respectively); during fruiting, in seeds (4.68 μg/mg). The results indicate that the content of **1** gradually decreases in the aerial part of the plant and accumulates in rhizomes toward the end of the vegetative period. The general tendencies in the distribution of **1** that were found for *S. komarovii* agree with those for *S. coronata* growing in middle and eastern Europe [9].

A distinct trend in the distribution of **2** and **3** as a function of development stage was not found for these minor phytoecdysteroids in vegetative and generative organs of *S. komarovii* during the vegetative period. The maximum amount of **2** during budding was found in the upper stem (0.24 μg/mg); during flowering, in the sheath and pappus (0.12 and 0.15 μg/mg); during fruiting, in rhizomes (0.065 μg/mg). A high content of **3** (from 0.33 to 0.47 μg/mg) was observed in rhizomes at all development stages and in radical leaves (0.43 μg/mg) during budding. The maximum content of **3** (0.80 μg/mg) was found in seeds.

EXPERIMENTAL

UV spectra were recorded using an SPD-M6A diode-matrix detector (Shimadzu, Japan). Mass spectra were recorded on an Agilent 1100 Series LC/MSD (Hewlett Packard, USA) GCMS using chemical ionization at atmospheric pressure. The range of recorded masses was 150-1000 Da (positive-ion detection); fragmentor potential, 70 V; ionization chamber potential, 4 kV; carrier-gas (N_2) flow rate, 6 L/min; sputtering-gas (N_2) pressure, 50 kg·s/cm^2.

The contents of **1**-**4** were determined in rhizomes, stems of generative shoot, leaves (radical and stem), flower clusters, and flower organs: flowers, sheath, pappus, and seeds.

Phytoecdysteroids were identified using UV spectrophotometry and GCMS.

20-Hydroxyecdysone. UV spectrum (CH_3CN, λ_{max}, nm): 248 (log ε 4.23). Mass spectrum (*m*/*z*, %): 481 (100) $[M + H]^+$, 463 (30), 445 (45), 427 (18), 409 (5), 391 (3).

Integristerone A. UV spectrum (CH_3CN, λ_{max}, nm): 248 (log ε 4.09). Mass spectrum (*m*/*z*, %): 497 (7) $[M + H]^+$, 479 (32), 461 (100), 443 (53), 425 (4), 407 (1).

2-Deoxy-20-hydroxyecdysone. UV spectrum (CH_3CN, λ_{max}, nm): 248 (log ε 4.90). Mass spectrum (*m*/*z*, %): 465 (100) $[M + H]^+$, 447 (38), 429 (84), 411 (33), 393 (4).

Quantitative Determination of Phytoecdysteroids. Air-dried plant material (about 200 mg, accurate weight) was extracted with ethanol (70%, 5-10 mL) at 0°C for 30 days. The extracts were filtered. An aliquot (0.9 mL) was treated with water (12 mL). Solid-phase extraction was performed over a Supelclean C_{18} column (Supelco, USA) with elution by ethanol:water (3:2). The quantity of phytoecdysteroids was determined by HPLC on an LC-6A chromatograph (Shimadzu, Japan) over a Zorbax ODS column (4.6 × 250 mm, 5 μm, duPont, USA) and a Shim-Pack FLC-ODS precolumn (4.6 × 50 mm, 3 μm, Shimadzu, Japan) with elution by CH_3CN:H_2O (20:80) and column temperature 55°C. The flow rate was 2 mL/min. Signals were detected using an SPD-M6A (Shimadzu, Japan) diode-matrix UV detector. The retention times for the analytical conditions given above were integristerone A, 3.5 min; 20-hydroxyecdysone, 5.1 min; α-ecdysone, 11.81 min; and 2-deoxy-20-hydroxyecdysone, 15.89 min.

We used standards of 20-hydroxyecdysone prepared as previously described [17], 2-deoxy-20-hydroxyecdysone, α-ecdysone (Sigma, USA), and integristerone A obtained from the Institute of Petroleum Chemistry and Catalysis (Ufa).

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