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20-HYDROXYECDYSONE FROM *Stemmacantha uniflora* SUBSP. *satzyperovii*

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The dynamics of 20-hydroxyecdysone content in vegetative and generative organs of Stemmacantha uniflora *subsp.* satzyperovii *(Soskov) Dittrich, which is distributed over Primorskii Krai, were investigated. A high content of 20-hydroxyecdysone during the plant vegetative period is characteristic of growing organs. The amount is maximal for young leaves during development of racemes (7.8 μg/mg) and for ripe achenes (11.15 μg/mg).*

Key words: *Stemmacantha uniflora* subsp. *satzyperovii*, Asteraceae, 20-hydroxyecdysone, HPLC.

Phytoecdysteroids are physiologically active substances that possess anabolic, adaptogenic, immunostimulating, and hypoglycemic action [1-4]. The predominant component of the ecdysteroid-containing plant fractions is 20-hydroxyecdysone (**1**), the main sources of which are rhizomes from plants of the Asteraceae family, *Stemmacantha carthamoides* (Iljin) Dittrich [synonyms: *Leuzea carthamoides* (Willd.) DC. and *Rhaponticum carthamoides* (Willd.) Iljin]. The plant is used in Chinese, Tibetan, and Mongolian medicine.

The distribution and seasonal dynamics of the content of **1** were studied in the vegetative and generative organs of species of the genus *Stemmacantha* Cass. [5-7]. Far-east representatives of this taxon have been little studied. We previously observed **1** in *Stemmacantha uniflora* subsp. *satzyperovii* (Soskov) Dittrich [*Rhaponticum satzyperovii* Soskov, *Rh. uniflorum* subsp. *satzyperovii* (Soskov) Worosch.] [8].

Our goal was to investigate the dynamics of the content of **1** in various parts of the Far-eastern plant *St. uniflora* subsp. *satzyperovii*.

St. uniflora subsp. *satzyperovii* is a perennial herbaceous plant with a thick soft down, a tough stem up to 1 m high, and lyre-shaped slightly separated leaves at the root. This subspecies is encountered rarely in Primorskii Krai. It grows on cliffs and rocky slopes overgrown with the bush and is often confined to limestone. The plant forms a rosette of leaves at the root in the first year and a generative runner in the second year.

Table 1 lists the content of **1** in the plant organs at various phenophases. A high content is observed during budding in the leaves and forming racemes and during massive flowering in the raceme and acheme involucre and in the apical part of the generative runner (stem and upper stem leaves).

During fruiting, the content of **1** is typically maximal in the achemes. The content of **1** in the vegetative organs of the aerial part of the plant decreases during this period. By the end of vegetation (desiccation of the aerial part), the content of **1** in the rhizomes and generative runners continues to decrease.

Thus, the amount of **1** is maximal in young leaves during raceme development (7.80 μg/mg) and in ripe achemes (11.15 μg/mg) during fruiting.

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TABLE 1. Content of 20-Hydroxyecdysone in Various Plant Parts of *Stemmacantha uniflora* subsp. *satzyperovii* as a Function of Growth

Phenophase (collection date and plant characteristics)	Plant organ	Content of **1**, μg/mg
Budding (May 14, development of generative runner, racemes 1-2 cm in diameter)	Rhizome	0.073±0.003
	Upper leaves	7.80±0.39
	Middle leaves	6.23±0.31
	Stem (upper part)	2.48±0.12
	Raceme	7.34±0.36
Massive flowering (June 18, height of generative runner up to 100 cm, leaves at the root up to 40 cm long, upper leaves 4-10 cm long, raceme 3.5-6 cm in diameter)	Rhizome	0.81±0.04
	Upper leaves	7.56±0.38
	Middle leaves	5.27±0.26
	Leaves at the root	3.87±0.19
	Stem (upper part)	7.43±0.37
	Stem (middle part)	1.73±0.08
	Stem (lower part)	2.36±0.12
	Petioles of lower leaves	2.59±0.13
	Petioles of middle leaves	2.51±0.12
	Raceme	5.53±0.27
	Flowers	1.94±0.09
	Involucre	6.62±0.33
	Pappus	0.62±0.03
	Achemes	4.77±0.24
Fruiting (July 18, achemes 3-5 mm long)	Upper leaves	1.80±0.09
	Middle leaves	2.00±0.10
	Leaves at the root	1.57±0.08
	Stem (upper part)	2.57±0.13
	Stem (middle part)	0.33±0.02
	Stem (lower part)	0.65±0.03
	Petioles of lower leaves	0.59±0.03
	Petioles of middle leaves	1.03±0.05
	Raceme	4.06±0.21
	Involucre	2.57±0.13
	Pappus	0.34±0.02
	Achemes	11.15±0.56
Acheme falling (August 16, start of dying off of upper part of generative runner)	Rhizome	0.59±0.03
	Middle leaves	2.61±0.13
	Stem (upper part)	0.69±0.03
	Stem (middle part)	0.31±0.01
	Petioles of lower leaves	1.06±0.05
	Raceme	4.98±0.25
	Involucre	0.57±0.03
	Pappus	0.54±0.03
	Achemes	5.19±0.15
Desiccation of aerial part (September 17, upper part of stem and upper dry leaves)	Rhizome	1.75±0.09
	Leaves at the root	2.66±0.13
	Stem (upper part)	0.64±0.03
	Stem (middle part)	0.59±0.03
	Stem (lower part)	1.35±0.07
	Petioles of lower leaves	0.69±0.03
	Raceme	1.98±0.09
End of vegetation (October 15, full dying off of aerial part, calathides without achemes)	Rhizome	0.71±0.03
	Old leaves	0.49±0.02
	Stem (middle part)	0.031±0.001
	Stem (lower part)	0.50±0.02
	Petioles of lower leaves	0.35±0.02

EXPERIMENTAL

UV spectra were recorded using a SPD-M6A (Shimadzu, Japan) diode-array detector; IR spectra, on a Spectrum-1000 FT-IR Fourier spectrometer (Perkin—Elmer, USA) in KBr disks; PMR spectra, in $CDCl_3$ solution on an AMX spectrometer (Bruker, Germany) at working frequency 500.12 MHz relative to TMS as an internal standard ($\delta = 0$). Mass spectra were recorded on an Agilent 1100 Series LC/MSD GC-MS (Hewlett Packard, USA) using chemical ionization at atmospheric pressure. The range of recorded masses was 150-1000 Da (positive-ion mode) at 70 V fragmentation voltage, 4 kV ionization-chamber voltage, 6 L/min drying-gas (N_2) flow rate, and 50 kgs/cm^2 diffuser-gas pressure.

Plant material was collected in 1998-2001 near Novonezhino village of Shkotovo region of Primorskii Krai. The content of **1** was determined in rhizomes and racemes and its separate parts: flowers, involucre, pappus, and achemes.

Isolation of 1. A weighed portion of air-dried plant material (5.0 g) was extracted three times with ethanol (70%, once with 100 mL, twice with 50 mL) on a water bath at 70-80°C. The combined aqueous ethanol solutions were evaporated in vacuum at 35-40°C. The remainder was worked up with hexane (3×40 mL) and diethylether (2×20 mL) to remove nonpolar impurities. Then the solution was lyophilized at -18°C in vacuum to afford a dry solid (55 mg) that was dissolved in water (1 mL). Preparative HPLC using a Zorbax ODS column (9.4 × 250 mm, 5 μm, DuPont, USA) and $CH_3CN:H_2O$ (20:80) as eluent isolated the fraction containing **1**. The elution rate was 6 mL/min; column temperature, 25°C. Detection was performed at λ 248 nm. The fraction containing **1** was analyzed by HPLC over a Zorbax ODS column (4.6 × 250 mm, 5 μm, DuPont, USA) using a Shim-Pack FLC-ODS guard column (4.6 × 50 mm, 3 μm, Shimadzu, Japan) and $CH_3CN:H_2O$ (20:80) as eluent at column temperature 55°C. The elution rate was 2 mL/min. Then the fraction was evaporated in vacuum at 35-40°C to remove CH_3CN. The remainder was lyophilized in vacuum at -18°C to afford chromatographically pure product (26 mg).

20-Hydroxyecdysone. IR spectrum (ν, cm^{-1}): 3420, 1723, 1650, 2968, 1381, 1446, 1289, 1144, 1056, 879. UV spectrum (CH_3CN, λ_{max}, nm): 248 (log ε 4.23). PMR spectrum (δ, ppm): 1.06 (3H, s, Me-19), 1.21 (3H, s, Me-18), 1.36 (6H, s, Me-26, Me-27), 1.58 (3H, s, Me-21), 3.00 (1H, t, H-17), 3.01 (1H, t, H-5), 3.58 (1H, t, H-9), 3.87 (2H, m, H-22), 4.17 (2H, m, H-2), 4.21 (2H, m, H-3), 6.25 (1H, s, H-7). Mass spectrum (*m*/*z*, %): 481 (100) $[M + H]^+$, 463 (30), 445 (45), 427 (18), 409 (5), 391 (3).

Quantitative Determination of 20-Hydroxyecdysone. Air-dried plant material (about 200 mg, accurately weighed, residual moisture <8.2%) was extracted with ethanol (70%, 10-15 mL) at 0°C for 30 d. The resulting extracts were filtered. A portion (0.9 mL) was selected and treated with water (12 mL). Solid-phase extraction was carried out over Supelclean C_{18} columns (Supelco, USA) using ethanol:water (3:2) as eluent. The amount of **1** was determined by HPLC using the conditions described above and a 20-hydroxyecdysone standard (Sigma, USA).

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