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STUDY OF ECDISTEROIDS FROM SERRATULA CORONATA L. HERB

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Serratula coronata is a plant-producer of ecdysteroids, which is of interest for the introduction into pharmaceutical practice and the development of adaptogenes. As a result of chromatographic separation, five main ecdysteroids were isolated from the aboveground part of Serratula coronata L., introduced in the Botanical Garden of VILAR and identified on the basis of NMR spectroscopy data. The presence of four previously identified compounds in Serratula coronata L. was confirmed: 20-hydroxyecdysone (1), ayugasterone C (2), α-ecdysone (3) and taxisterone (4). For the first time, a new compound – 20-hydroxyecdysone 20,22-propylidene (5) was isolated.

Keywords: Serratula coronate L., phytoecdysteroids, 20-hydroxyecdysone, Ajugasterone C, α-ecdysone, taxisterone, 20-hydroxyecdysone 20,22-propylidene, NMR spectroscopy

Ecdysteroids are triterpene compounds consisted of four condensed rings having 27 or 28–29 carbon atoms. Ecdysteroids are widespread in the flora and fauna and have a wide spectrum of pharmacological activity. The source for their synthesis is cholesterol or other plant sterols [1]. More than 510 ecdysteroids are known, most of which are phytoecdysteroids [2] isolated from plants. Ecdysteroids have a wide range of pharmacological activity, exhibit an anabolic effect [3], which is used not only in the pharmaceutical industry, but also in the agricultural industry [4], they reduce cholesterol, relieve the symptoms of osteoporosis, improve skin regeneration, exhibit hepatoprotective, anti-inflammatory, adaptogenic effects [5–9]. The most common phytoecdysone is 20-hydroxyecdysone (ecdysterone, β-ecdysone) [10,11], which is a white crystalline powder that is poorly soluble in water [12].

To this date, two ecdysteroid-containing medicines are produced in Russia: Ecdisten® (containing 20-hydroxyecdysone [13], made of Rhaponticum carthamoides) and a liquid extract of Leuzea carthamoides roots [13]. Low toxicity and high biological activity are the main criteria of prospectivity for the use of medicines based on phytoecdysteroids. Thus, the LD50 for 20-hydroxyecdysone in case of abdominal and oral administration to mice is 6 g/kg [14] and 9 g/kg [15], respectively. The limiting factor in the production of these medicines is the low content of target substances in medicinal plant raw materials and a poor raw material base. As alternative objects containing ecdysteroids, a plant of the Asteraceae family, Serratula coronata L. (synonyms – S. wolfii Andrae, S. manshurica Kitag), is of particular interest [9]. Thus, in the herb of this plant, the total content of ecdysteroids can exceed 2%, which makes it promising for use as a source for obtaining these compounds [16].

Various researchers have found more than 50 phytoecdysteroids [17] and a wide variety of flavonoids (3-methylquercetin, 3-methylquercetin-3′-O-β-D-glucuronopyranoside, 3-methylquercetin-4′-O-β-D-glucuronopyranoside, 3-methylcampferol, apigenin, isocampferide, quercetin, quercetin-3′-O-β-D-glucuronopyranoside, quercetin-4′-β-D-glucoside, kaempferol, luteolin, luteolin-4′-β-D-glucoside, rutin), phenolcarboxylic acids (caffeic, ferulic, chlorogenic, neochlorogenic acids), higher fatty acids and their derivatives (linoleic, linolenic, palmitic and their methyl esters), sesquiterpenoids (caryophyllene, caryophyllene oxide, germacrene D), cyclitols ((-)-inositol) [18–23].

The effective doses of the main phytoecdysteroids isolated from Serratula coronata are presented in Table. 1. The content of the studied substances is subject to significant fluctuations in different parts of the plant, and also depends on the phase of its development [24,25]. In addition, scientists have shown that the Serratula coronata growing in different ecological and geographical conditions has a different qualitative and quantitative composition of ecdysteroids, which, in turn, indicates the presence of different chemotaxonomic races of this plant in nature [24].

In this regard, the **purpose** of this work was to isolate and identify the main (dominant) phytoecdysteroids of the Serratula coronata herb growing in the experimental field of the Botanical Garden of the All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), in order to detect differences in the chemical composition of this plant population in comparison with the information previously described in the literature.

MATERIALS AND METHODS

The object of the study was the dried aboveground part (herb) of the Serratula coronata (2018), introduced in the VILAR Botanical Garden

Table 1

EFFECTIVE DOSES OF THE MAIN PHYTOECDYSTEROIDS ISOLATED FROM SERRATULA CORONATA [26,27]

Ecdysteroids	ED50	Reference
20-hydroxyecdysone	$7{,}5 \cdot 10^{-9}$M	[27]
ecdysone	$1{,}1 \cdot 10^{-6}$M	[27]
polipodin B	$1{,}0 \cdot 10^{-9}$M	[27]
inocosteron	–	
Ajugasterone C	$6{,}2 \cdot 10^{-8}$M	[26]
22-O- acetyl 20-hydroxyecdysone	–	
taxisterone	$9{,}5 \cdot 10^{-8}$M	[26]
3-эпи-20-гидроксиэкдизон	$1{,}6 \cdot 10^{-7}$M	[26]

(Moscow). Raw materials were harvested in the 3–5-th year of the growing season in the phase of the beginning of flowering, the length of the shoots was 45–65 cm and the herb was dried in natural conditions at temperature of 28–34°C and at relative humidity of 52–65% for 10 days. The dried raw materials were ground to the size of particles passing through a 3 mm mesh screen (Kraft, Russia).

Extracts were prepared by three-time dynamic maceration of raw materials with 70% ethyl alcohol (vol.) at a temperature of (50±2) °C for 60 minutes. Raw materials/ extraction solvent ratio was 1:10 (by weight). The obtained water-alcohol extracts were combined and evaporated in the vacuum-rotary evaporator Heidolph Basis Hei-VAP ML (Germany) at a temperature of (50±2) °C until the distillation residue was obtained, which was quantitatively transferred to a separating funnel for sequential triple extraction with chloroform and n-butanol in a ratio of 1:1. The chloroform and n-butanol extracts were combined and evaporated in a rotary evaporator under vacuum at a temperature of 50±2°C until the solvents were completely removed. Thus, the target chloroform (non-polar) and n-butanol (polar) fractions were extracted.

To extract the individual compounds from the chloroform fraction, column chromatography was used, the column diameter was 1.0 cm and the height of the sorbent layer was 10 cm, on a silica gel made by Woelm (Germany) with a particle size of 80 microns. The "cyclohexane – isopropanol" system from 95:5 to 50:50 (vol.) was used as the mobile phase. The assessment of the phytoecdysteroid content in each fraction was analyzed by TLC on Sorbfil ПТСХ-АФ-УФ 20x20 plates in the "chloroform – methanol – water" system at the ratio of 26: 14: 3. The target fractions were combined and evaporated using a vacuum-rotary evaporator and re-chromatographed in the "chloroform-methanol" system from 98: 2 to 80:20 (vol.).

To remove the accompanying phenolic compounds, the n-butanol fraction was chromatographed on the Woelm (Germany) neutral aluminum oxide – II degree of activity (according to Brockman). The column diameter is 7 cm, the height of the sorbent layer is 30 cm and the eluent is "chloroform-methanol" with an increase in the gradient of the latter from 2 to 50%. Eluates from the column were analyzed by TLC on Sorbfil ПТСХ-АФ-УФ 20x20 plates in the systems "chloroform-methanol" at ratio of 90:10 and "chloroform-methanol-water" at ratio of 26:14: 3 (vol.). The plates were viewed in UV light at 254 nm and developed with a 25% solution of phosphoric-molybdenum acid. Fractions containing 20-hydroxyecdysone and other minor ecdysteroids were combined and evaporated using a rotary evaporator under vacuum to dry. The extracted fractions were re-chromatographed on silica gel made by Woelm (Germany) with a particle size of 80 microns. The column diameter is 1.5 cm, the height of the sorbent layer is 20 cm, the "chloroform – methanol" systems from 98:2 to 70:30 (vol.) were used as the mobile phase. The assessment of the phytoecdysteroid content in each fraction was analyzed by TLC on Sorbfil ПТСХ-АФ-УФ 20x20 plates in the "chloroform-methanol-water" system at the ratio of 26: 14: 3 (vol.). The target fractions containing individual compounds were combined and evaporated using a vacuum-rotary evaporator.

To determine the chemical structure of the isolated substances, ^{1}H – and ^{13}C-NMR spectra were taken in CD_3OD and DMSO with the Gemini 200 NMR spectrometer (Varian, USA).

RESULTS AND DISCUSSION

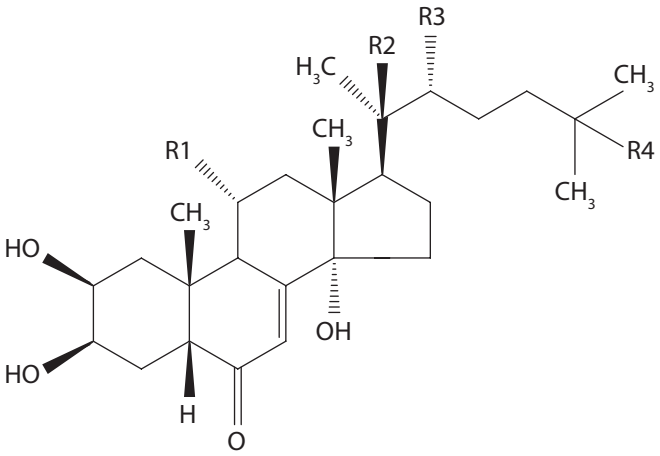
As a result of the study, five phytoecdysteroids were isolated from the Serratula coronata herb – four from the polar (n-butanol) fraction and one from the non-polar (chloroform) fraction. Using NMR spectroscopy (Table. 2) it was found that

Table 2

DATA OF ^{13}C NMR SPECTRA OF COMPOUNDS 1–4 (125 MHZ)

Number of a carbon atom	1		2		3		4	
	CD_3OD	DMSO-D^6	CD_3OD	DMSO-D^6	CD_3OD	DMSO-D^6	CD_3OD	DMSO-D^6
1	37.41	36.61	37.39	36.57	37.35	36.61	40.19	40.76
2	68.73	66.76	68.71	66.70	68.69	66.74	69.22	66.87
3	68.54	66.58	68.51	66.54	68.49	66.56	68.85	66.65
4	32.84	31.51	32.86	31.48	32.84	31.51	33.57	31.97
5	51.80	50.07	51.78	50.02	53.35	51.44	53.06	51.04
6	206.44	202.66	206.51	202.64	206.41	202.57	206.93	203.07
7	122.15	120.44	122.01	120.31	121.99	120.33	123.01	120.82
8	167.96	165.21	167.59	164.79	168.06	165.32	165.99	163.30
9	35.13	33.19	35.30	33.26	35.07	33.11	43.23	41.21
10	39.28	37.61	39.25	37.57	39.23	37.61	39.38	38.15
11	21.52	20.07	21.60	20.08	21.58	20.10	69.79	67.34
12	32.53	30.84	32.06	30.27	32.38	30.61	44.08	42.45
13	48.58	46.85	48.12	46.41	48.11	46.29	*	46.74
14	85.25	82.99	85.09	82.66	85.49	83.27	85.16	82.81
15	31.79	30.29	32.06	30.53	31.59	30.11	32.13	30.29
16	21.52	20.25	27.00	25.63	21.98	20.63	21.81	20.79
17	50.51	48.69	48.82	47.03	51.77	50.06	50.45	48.38
18	18.05	17.10	16.19	15.22	18.12	17.14	19.16	17.97
19	24.42	23.83	24.45	23.81	24.47	23.83	24.90	24.05
20	77.92	75.71	43.43	41.74	75.95	73.17	78.05	75.38
21	21.07	20.94	13.32	12.84	26.47	26.45	21.26	20.21
22	78.44	76.22	75.26	72.33	45.87	44.91	78.24	75.49
23	27.36	26.09	25.38	24.07	20.08	18.72	30.77	29.09
24	42.41	41.36	42.25	41.19	45.47	44.49	37.94	36.10
25	71.29	68.71	71.40	68.74	71.38	68.78	29.50	27.44
26	28.98	28.98	29.15	29.05	29.35	29.22	23.69	22.94
27	29.69	29.91	29.57	29.87	29.11	29.56	23.03	22.21

** Signal under solvent*



	R1	R2	R3	R4
1	H	OH	OH	OH
2	H	H	OH	OH
3	H	OH	H	OH
4	OH	OH	OH	H

FIG. 1. *Structural formulas of isolated phytoecdysteroids (1-4)*

the isolated phytoecdysteroids are 20-hydroxyecdysone (1), Ajugasterone C (2), α-ecdysone (3) and taxisterone (4) (Fig. 1). The obtained NMR spectra of the isolated substances correspond to the available literature data [28,29]. In Tables 2 and 3, along with the values of chemical shifts in methanol, the values of chemical shifts in dimethyl sulfoxide (DMSO) are given. In DMSO, the relaxation time of carbon atoms is significantly lower than in other solvents, due to its higher viscosity, which significantly reduces the time to provide ^{13}C NMR spectra. To a greater extent, this applies to quaternary carbon and carbon of the carbonyl group, the relaxation time of which can exceed 10 seconds.

A new, previously unidentified ecdysteroid (5) $C_{31}H_{50}O_7$ with molar mass of 534.74 Da was isolated from the low-polar (chloroform) fraction of the Serratula coronata herb.

In the ^{13}C NMR spectra (CD_3OD, DMSO-D^6) of substance 5, 31 signals appear. The chemical shifts of 25 of them almost completely coincide with the similar signals in the spectra of 20-hydroxyecdysone (Table. 2 and 3). The results indicate that substance 5 is a derivative of 20-hydroxyecdysone. The two signals in substance 5 and 20-hydroxyecdysone, the chemical shift of which is significantly different, apply to the carbons at position 20 and 22. A signal the chemical shift of which in substance 5 has a value of 109.92 ppm (CD_3OD), according to the literature data [20,31], is manifested in the spectrum of 20-hydroxyecdysone acetonides. The calculated 13C-NMR spectrum for the but-2-dioxyylidene fragment $CH_3C(O)_2CH_2CH_3$ gives the following values: 23,3 (1), 110,5 (2), 32,2 (3) and 8.3 (4) ppm, which are close to the values of carbon atoms in substance 5. It should be noted that in the ^1H-NMR spectrum of substance 5, the fourth methyl group of the aliphatic chain provides a signal in the form of a triplet at 0.86 ppm (DMSO-D^6), which also confirms the presence of a butylidene fragment in the test substance.

When analyzing the resulting data, it can be assumed that the isolated new phytoecdysteroid corresponds to 20,22-(but-2-ylidene) 20-hydroxyecdysone (Fig. 2).

20-hydroxyecdysone (1) is dominant in terms of the content of the isolated ecdysteroids that is 78%, other ecdysteroids are, respectively:

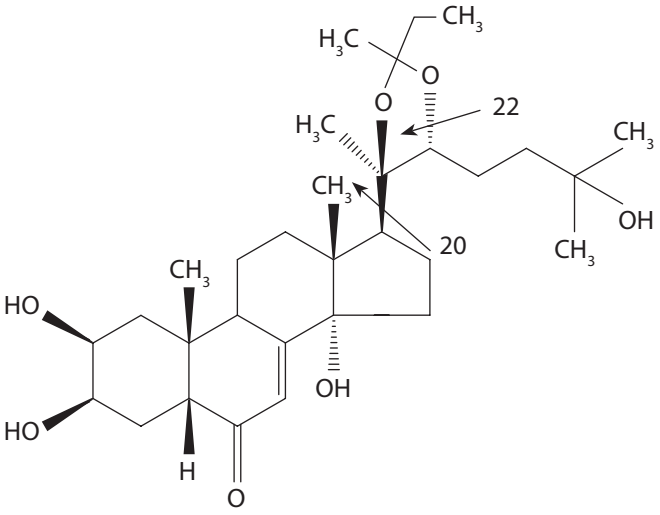


FIG. 2. *Structural formula of 20,22-(but-2-ylidene) 20-hydroxyecdysone*

Table 3

DATA OF ^{13}C NMR –SPECTRA OF COMPOUND 5 (125 MHZ)

Number of a carbon atom	5	
	CD_3OD	DMSO-D^6
1	37.43	36.57
2	68.73	66.72
3	68.53	66.54
4	32.86	31.48
5	51.78	50.02
6	206.39	202.57
7	122.12	120.50
8	167.61	164.57
9	35.19	33.15
10	39.23	37.53
11	21.54	20.01
12	32.39	30.58
13	*	46.67
14	85.50	83.61
15	31.75	30.15
16	22.47	20.92
17	50.69	48.80
18	17.66	16.54
19	24.43	23.80
20	85.32	82.95
21	22.91	21.98
22	83.03	81.14
23	24.74	23.14
24	42.23	40.99
25	71.11	68.40
26	29.07	28.91
27	29.44	29.64
28 O-**C**-O	109.92	107.78
29 OC**CH_3**	24.11	23.63
30 C**CH_2**CH_3	36.12	34.50
31 CCH_2**CH_3**	9.42	8.98

** Signal under solvent*

Ajugasterone C (2) – 11%, α-ecdysone (3) – 6%, taxisterone (4) – 1.2% of the total amount of isolated compounds by weight.

CONCLUSIONS

The results of the phytochemical study of the Serratula coronata herb growing on the territory of the Botanical Garden of the All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR) confirm the available literature data on the qualitative composition of ecdysteroids and the dominant share of 20-hydroxyecdysone among these compounds.

Along with this, the chemical structure of a new phytoecdysone of the Serratula coronata herb – 20-hydroxyecdysone 20,22-propylidene – was first isolated and identified.

The study was carried out within the framework of the implementation of the VILAR research plan on the subject No. 0576-2019-0010 "Search for active fractions of natural compounds, development of methods for their production from plant raw materials, standardization methods and creation of modern dosage forms based on them"

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