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Rhaponticum integrifolium AS A PRODUCER OF ECDYSTEROIDS

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From the point of view of the search for ecdysteroid-containing plants among representatives of the domestic flora, species of *Silene* and *Rhaponticum* have proved to be promising. It is known that the species *Rhaponticum carthamoides* Willd. is a producer of ecdysteroids possessing the activity of an insect molting hormone. The present communication is part of biological investigations directed to the introduction into cultivation of *Rh. integrifolium* C. Winkl. (fam. Asteraceae), which contains a considerable amount of ecdysteroids.

Under domestic conditions, *Rh. integrifolium* is found on detrital slopes in the middle band of the mountains of Central Asia: in particular, in the Surkhandar'inskaya oblast, in the Fergana range, and in the Pamiro-Alai. It is a perennial herbaceous plant with a height of 40—150 cm. The leaves are leathery, smooth-edged, elongated, elliptical, cauline, ovate, and tapered from both ends. The flowerhead is globular, 4.5—6 cm. The achenes are obovate, glabrous, clear, truncated at the apex, with a coronet-like peristome.

The plant was propagated by sowing seeds in October—November. In the first year of life it formed a rosette of radical leaves. Flowering and fruit-bearing began in the second year of life. Perennial specimens began to vegetate in the last ten days of March, budding was observed at the end of April—beginning of May, flowering in the middle of May, and ripening of the fruit in June. Intensive growth of the plant took place in April—May, its height in this period reaching 100—120 cm. The weight of 1000 seeds was 19.8 g.

The epigeal organs and seeds gathered in the first ten days of June in 1997—1998 were used as the material for analysis. The quantitative determination of ecdysteroids in the plant under study was carried out by the following method. By means of thin-layer chromatography, we found in extracts of the plant, using vanillin/sulfuric acid [1] and column chromatography [2], a series of main ecdysteroids with various structures: viticosterone E (**1**), 24(28)-dehydromakisterone A (**2**), ecdysterone (**3**), and integristerone (**4**). This is the first time that viticosterone E has been detected in this plant species.

A considerable amount of ecdysterone was present in the epigeal organs during the budding period. After budding, the level gradually fell. The results of analysis showed that ecdysterone accumulated in the largest amount in the seeds.

Ecdysteroids were isolated in the following way from the epigeal organs of *Rh. integrifolium* introduced into the sierozem soil of an experimental plot of the Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan. The dried and comminuted raw material was extracted five times with 10 liters of ethanol. The extract was concentrated and diluted with water, and the precipitate that deposited was removed. The ethanol was evaporated off in vacuum, and the aqueous residue was treated with chloroform. The ecdysteroids were extracted from the purified aqueous fraction with isopropanol—chloroform (1:1). The dry residue obtained after the solvent had been distilled off was chromatographed on a column of silica gel. On elution by system 1, 72 mg (0.0036%; here and below the yields have been calculated on the air-dry raw material) of viticosterone E (**1**) [3], $C_{29}H_{46}O_8$, was isolated; after crystallization from acetone it had mp 196—198°C, $[\alpha]_D^{22}$ +59.8±2° (*c* 0.18; methanol). [4].

The further elution of the column with system 1 gave 63 mg (0.0031%) of 24(28)-dehydromakisterone A (**2**), $C_{29}H_{44}O_7$, mp 245—246°C (methanol), $[\alpha]_D^{22}$ +62.0±2° (*c* 0.25; methanol).

On using system (2) we isolated 2.5 g (0.25%) of ecdysterone (**3**), $C_{27}H_{44}O_7$, mp 241—242°C (from acetone), $[\alpha]_D^{22}$ +63.2±2° (*c* 0.20; methanol).

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Elution of the column with system 3 gave 53 mg (0.0026%) of integristerone A (**4**), $C_{27}H_{44}O_8$, mp 246—248°C (from ethyl acetate—methanol) $[\alpha]_D^{22}$ +36±2° (*c* 0.32; methanol).

Ecdysteroids were isolated from the seeds of *Rh. integrifolium* in the following way: 0.5 kg of ground seeds were treated twice with chloroform for defatting and the elimination of other substances. Then they were extracted with ethanol five times, as described above. The dry residue obtained after distillation of the solvent was chromatographed on a column of silica gel. Washing the column with system 1 led to the isolation of 216 mg (0.04%) of 2-deoxyecdysterone (**5**) [5], $C_{27}H_{44}O_6$, mp 254—255°C (from methanol—water), $[\alpha]_D^{20}$ +82.0±2° (*c* 0.25; methanol).

By washing the column with system 1 we obtained 124 mg (0.02%) of 24(28)-dehydromakisterone A (**2**). The further washing of the column with system (**2**) led to the isolation of 500 mg (0.1%) of polypodin B (**6**), $C_{27}H_{44}O_8$, mp 252—254°C (from acetone), $[\alpha]_D^{20}$ +93.2±2°(*c* 0.23; methanol).

On continuing elution of the column with the same system we isolated ecdysterone (**3**). Washing the column with system 3 yielded 166 mg (0.03%) of integristerone A (**4**). No additional ecdysterone was isolated when the seeds were extracted with ethanol. The individual ecdysteroids isolated were identified from the results of IR, PMR, and mass spectroscopy, and also by direct comparison with authentic specimens.

The ecological and geographical condition of the growth of *Rh. integrifolium* do not influence the composition of the ecdysteroids, since the plant in its natural growth sites (Fergana range) also contains a set of compounds.

Thus, the results can be used to optimize the conditions for growing the plant in cultivation, and the recommended raw material can be cultivated successfully and used for obtaining the ecologically friendly tonic drug ékdisten.

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