Ecdysteroids as Varying Chemical Constitutents of *Silene* Species Growing in Hungary

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Abstract

48 *Silene* species (Caryophyllaceae family) were tested by TLC/densitometry for ecdysteroids. It turned out that 50 % of the species contained 20-hydroxyecdysone (β-ecdysone) justifying that this compound is of widespread occurrence in this genus. *Silene italica, S. nutans, S. otites* contain, beside 20-hydroxyecdysone, integristeron A, both in varying quantities. *Silene otiotes* produced the highest ecdysteroid concentration in June and July, when *S. nutans* contained the least ecdysteroids. If the organs were compared with each other, in the case of both species, there is a decrease in the yield in the order inflorescence, leaf, root and stem.

INTRODUCTION

Ecdysteroids are steroid derivatives with a cholest-7-en-6-one skeleton, derived biosynthetically from cholesterol. They are highly hydroxylated (3-8 OH) and generally substituted with a sterin side-chain on carbon 17. The B/C- and C/D-ring junctions are always *trans*. The A/B-ring junction is normally *cis* (5ß) and rarely *trans.* Most ecdysteroids possess a hydroxyl group in the 14α-position. The 14α-hydroxy-7-en-6-one chromophore results in a characteristic ultra-violet absorption with a λ_{max} at 245 nm.

Ecdysteroids are widely distributed natural compounds occurring in both plants and invertebrates. They regulate a series of important physiological functions, mostly in insects and other arthropods and in vertebrates. The biological function of ecdysteroids is to regulate metamorphosis in insects (Karlson et al., 1965; Hoffmeister, 1966). There are several important pharmacological effects on vertebrates also. They are mildly anabolic without the androgenic activity associated with vertebrate steroids or their analogues. A number of preparations and tonics with reported roborant, adaptogenic and antidepressive effects are commercially available. A number of reports suggest that ecdysteroids may be effective in the control of diabetes. The ecdysteroid derivatives exhibit a hepatoprotective action. Some phytoecdysteroids have been reported to be potential cancer chemoprotective agents. To study their physiological effects and to use them in various medicines, plants are useful sources for ecdysteroid preparations (Bergmasco and Horn, 1983; Sláma. and Lafont, 1995).

Such plants, belonging to the Caryophyllaceae family, are convenient sources of 20-hydroxyecdysone (Báthori et al. 1987). We studied the occurrence of ecdysteroids in the *Silene* genus of the Caryophyllaceae family and the species examined and those found positive for ecdysteroids are listed in Table 1 (48 *Silene* species examined /24 containing ecdysteroids).

Here we present a survey of some species of *Silene* for ecdysteroids. Our aims were:

- gathering new data on the distribution of ecdysteroids both in closely related taxa, like in the genus *Silene* of the Caryophyllaceae.
- Studying the quantitative variation of 20-hydroxyecdysone, the most frequently occurring compound during the development of plants and also its distribution among organs. As models *Silene otites* (L.) Wibel, *Silene nutans* L. and *Silene italica* L. *ssp. nemoralis* have been chosen.

EXPERIMENTAL

Standard and Standard Solution

Standard 20-hydroxyecdysone, 2-deoxy-20-hydroxyecdysone, 2-deoxyecdysone and integristerone A were obtained through our earlier experimental work. Their purity was 99.8 % (by HPLC). Spectral data such as ^{1}H-NMR, ^{13}C-NMR, MS, IR, UV and melting points were the same as had been reported in the literature. A standard solution was prepared by dissolving 0.5 mg of 20-hydroxyecdysone in 10 ml of methanol.

Plant Material

Plant material used was collected partly from their natural habitat and partly from the experimental field of the Institute of Ecology and Botany of the Hungarian Academy of Sciences (Vácrátót, Hungary). The plant populations for comparative studies were grown from seeds obtained from botanical garden seed exchanges.

Chromatography

TLC was performed on 20x20 cm silica gel 60F_{254} plates of Merck, Darmstadt, Germany. Two different solvent systems were used for the validation of the mobile phase:

A: toluene - acetone - ethanol - (96 %) ammonia 50: 70: 16: 4,5 (v/v/v/v)

B: chloroform - methanol - benzene 25: 5: 3 (v/v/v)

The solvent system B was used to validate the method for the quantification of 20-hydroxyecdysone, 2-deoxy-20-hydroxyecdysone and 2-deoxyecdysone.

Calibration

The calibration was performed in the range of 0.9-6.75 μg of 20-hydroxyecdysone standard. The applied load was: 2, 5, 7, 10, 13, 15 μl from the standard solution, in duplicate. Calibration curves for 20-hydroxyecdysone were constructed by plotting peak area (y axis) against the respective amounts of samples applied to the plate (x axis).

Quantification

Quantification was done by using 6 loads of the standard (2, 5, 7, 10, 13,15 μl) and triplicates from different sample solutions, on the same plate. The chromatograms were scanned by a densitometer (Shimadzu CS-9301PC) using absorbance/reflectance mode (Báthori et al., 1999; Janicsák and Máthé, 1996).

RESULTS

Ecdysteroid content of the plants was screened by thin-layer chromatography (TLC). The plant sources were extracted with methanol and the analytical procedures were performed from the crude extract without any preliminary clean-up procedure. For the TLC analyses, at least two different solvent systems were used (Solvent Systems A and B). In Table 1 the results of the preliminary tests are listed. The '+' marks mean that the sample was found to contain 20-hydroxyecdysone by TLC. It turned out that 50 % of the *Silene* taxa contained 20-hydroxyecdysone. The species listed in Table 1. grow well in our experimental field and seemingly can be easily cultivated in Hungary.

The 20-hydroxyecdysone content of *Silene* species, such as *Silene otites* (L.) Wibel, *Silene nutans* L. and *Silene italica* L. *ssp. nemoralis* was determined by TLC/densitometry at 254 nm. The determinations were performed from both fresh and dry above ground parts of the plants, collected in April.

The 20-hydroxyecdysone content was always higher in the dried plants than in the fresh ones. The 2-deoxy-20-hydroxyecdysone and 2-deoxyecdysone content of *Silene otites* was much lower in the dried plant sample than in the fresh one. We observed this reverse phenomenon also for integristerone A content of *Silene nutans*. Integristerone A was present in the fresh plant in higher concentration than in the dried sample (Figure1.).

The organ dependent variation in the 20-hydroxyecdysone content of *Silene otites* and *Silene nutans* was also studied. Flowers showed the highest ecdysteroid content in

both species. High levels were also found in leaves with lower amounts in stems. Reproductive organs represent a relatively small mass in the case of *S. nutans* and leaves give the main part of plant mass and also the highest 20-hydroxyecdysone content. With *S. otites*, the inflorescence (flowers) contains the highest amounts of ecdysteroids and gives the greater part of the herb.

The seasonal variation in the 20-hydroxyecdysone content of *Silene otites* and *Silene nutans* herbs was investigated by TLC/densitometry. The average concentration of 20-hydroxyecdysone in plant samples was studied in five phonological phases: in the rosette, stem formation, budding, blossoming and fruit ripening stages. Maximal ecdysteroid content of *Silene otites* was observed during intense growth: at budding and flowering. In the samples of *Silene nutans*, leaves show the highest content of 20-hydroxyecdysone at the beginning of the vegetation period (Figure 2).

DISCUSSION

Our results definitely suggest that the occurrence of ecdysteroids in the Caryophyllaceae family is restricted to certain genera. *Silene*, *Lychnis* and *Melandrium* of the subfamily Silenoideae were proved to contain ecdysteroids, while in the species belonging to other genera, ecdysteroids were not found. Therefore the ecdysteroids can be considered as specific chemotaxonomic signals of the genera *Silene*, *Lychnis* and *Melandrium* (Báthori et al., 1987*).* In all, some 40 % of the *Silene* species proved to be ecdysteroid positive.

Comparing our results on *Silene* species with those of others, it turns out that the *Silene* genus can be characterised by the presence of ecdysteroids. Results of our quantitative determination show that *Silene otites*, *Silene nutans* and *Silene italica ssp. nemoralis* have produced the highest amount of ecdysteroids (1-2 %).

The change in the 20-hydroxyecdysone content during processing is a consequence of the oxidation of apolar ecdysteroids with 4 and 5 hydroxyl groups (2-deoxyecdysone, 2-deoxy-20-hydroxyecdysone) to 20-hydroxyecdysone. Similar oxidation takes place in the case of integristerone A. The integristerone A content decreases during the drying process. Integristerone A is transformed to polar, highly hydroxylated ecdysteroids.

The 20-hydroxyecdysone content of *Silene otites* herb dynamically changes during the vegetation period. Its concentration in the herb of *S. otites* is the highest at the rosette stage; it decreases till the stage of stem formation and then there is a strong increase at the stage of budding and blossoming. In the period of fruit ripening, the content of 20-hydroxyecdysone decreases to a small degree.

Analyses of the 20-hydroxyecdysone content of the plant extract have been done traditionally by radioimmune assay (RIA), biological tests, spectrophotometry and HPLC. RIA and biological tests have not proved to be specific enough; cross reactions with other steroids often interfere. Spectrophotometry requires pre-purification, otherwise several odd compounds may interfere with the UV absorption spectra. HPLC also requires sophisticated pre-separation clean-up. Our TLC procedure is simple and suitable for routine examination of 20-hydroxyecdysone in plant samples. In addition, its reliability is comparable to that of HPLC.

Literature Cited

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Tables

Table 1. Ecdysteroid occurrence in *Silene* species (Bátori et al., 1994)

Species	Present/ absent	Species	Present/ absent
S. altaica Pers	-	*S. gallica* L.	+
S. apetala Willd.	-	*S. inflata* Sm	+
S. armeria L.	+	*S. linicola* C.C. Gmelin	-
S. bellidifolia Juss.	-	*S longiflora* Ehrh.	+
S. bergiana Lindman	-	*S. maritime* (With.) A. & Löve	+
S. bupleorides L.	-	*S. micropetala* Lag.	-
S. catholica L.	-	*S. multicaulis* Guss.	-
S. chlorantha (Willd.) Ehrh.	+	*S. multiflora* (Waldst. & Kit.) Pers..	-
S. chlorifolia cordifolia All.	-	*S. nemoralis* Waldst. & Kit.	-
S. ciliata Pourret	-	*S. noctiflora* L.	+
S. coelia-rosa (L.) Godron	+	*S. nutans* L.	+
S. colorata Poiret	-	*S. otites* (L.) Wibel.	+
S. compacta Fischer	-	*S. pendula* L.	+
S. conica L.	-	*S. requienii* Otth.	-
S. cretica L.	-	*S. rupestris* L.	+
S. cucubalus Wibel.	+	*S. saxifraga* L.	-
S. densiflora D Urv.	-	*S. schafta* S. G. Gmel	+
S. dichotoma Ehrh.	-	*S. schumeckeri* Wettst.	-
S. dioica (L.) Clairv.	-	*S. sendtneri* Boiss.	+
S. echinata Otth	+	*S. tatarica* (L.) Pers.	+
S. fabarioides Hausskn.	+	*S. vallesia* L.	+
S. flavescens Waldst. & Kit.	+	*S. viridiflora L.*	+
S. frivaldszkyana Hampe.	+	*S. vulgaris* (Moench) Garcke	+
S. fruticulosa L.	-	*S. zawadzkii* Herbich	+

Figures

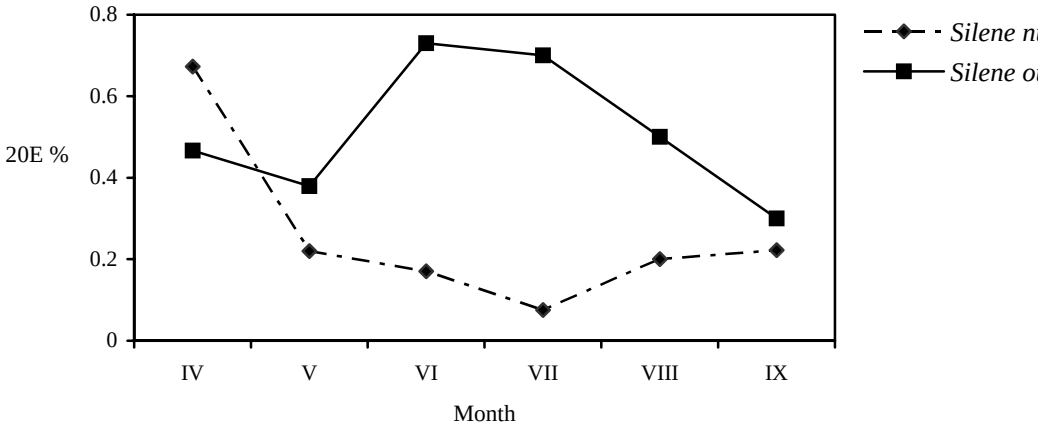

Fig.1. 20-hydroxyecdysone (20E) contents of dried herbs.

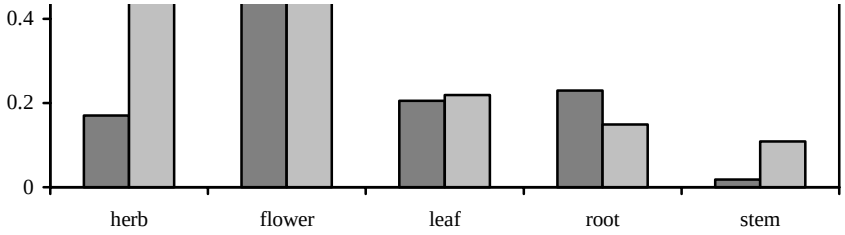

Fig.2. 20-Hydroxyecdysone (20E) contents of dried various organs.