

University of Szeged
Department of Pharmacognosy

**Investigation of plants containing unusual ecdysteroids
with ring in their side-chain**

Summary of Ph.D. Thesis

Attila Ványolós

Supervisor: Prof. Dr. Báthori Mária

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Introduction

Ecdysteroids are hormones of arthropods and many other invertebrates. The first ecdysteroid, ecdysone was isolated from silkworm pupae (*Bombyx mori*) by German scientists, Butenandt and Karlson, in 1954. Ecdysteroids play essential roles in all insects, as they regulate major transitional events during insect growth: molting, metamorphosis, reproduction and diapause. In the 1960s, phytoecdysteroids, the plant analogs of ecdysteroids, were identified in several plant species.

So far, less than 2% of the world's flora has been investigated for the presence of ecdysteroids, but more than 300 compounds have already been identified. The levels of ecdysteroids in plants are usually between 0.1 and 3% of the dry weight, which is 1000-fold higher than in insects.

Their roles in plants are still unclarified, but it is believed that ecdysteroids offer protection against non-adapted phytophagous invertebrates. Their ready availability in different plant species has allowed pharmacological studies, which have suggested that they have many positive pharmacological properties. Ecdysteroids are not endogenous participants in the mammalian metabolism, and are not toxic to mammals. They can be toxic to insects by disrupting hormonal processes upon ingestion, which has led to the development of safe insecticides. Nevertheless, the ecdysteroids themselves are not used in the control of pests because of their high polarity and their environmental instability. In contrast, bisacylhydrazines, functional analogs of ecdysteroids, are successful and selective pest control agents.

The most important action of ecdysteroids exerted on mammals is the stimulation of protein synthesis without the adverse androgenic, antigonadotropic and thymolytic side-effects described after the administration of vertebrate steroid hormones.

The mode of action of anabolic activity and the metabolism of ecdysteroids in mammals is not fully understood. Besides their anabolic action several other pharmacological effects of ecdysteroids have been reported: adaptogenic, wound healing, antidiabetic and multi-drug resistance reversal properties.

The basic chemical structure of ecdysteroids is a cyclopentano-perhydrophenanthrene (sterane) skeleton with a side-chain on C-17 (**Figure 1**). Characteristic structural element of ecdysteroids is a 7-en-6-one chromophore in ring B; the C/D ring junction is generally *trans*, whereas the A/B ring is normally *cis*. The number of hydroxy groups can be 2-8. The presence of a cyclic ether or a lactone ring (5- or 6-membered) at C-17 can occur.

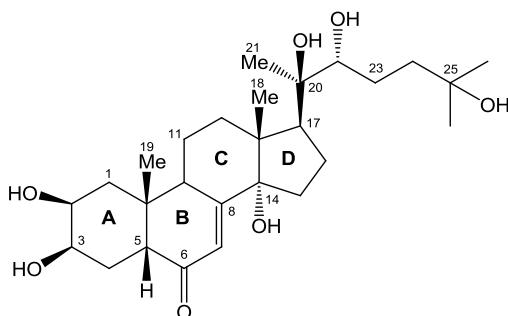


Figure 1. Structure of the most common ecdysteroid, 20-hydroxyecdysone.

Aims of the study

The extensive ecdysteroid research at the Department of Pharmacognosy dates back to the late 1970s. During this period a novel, rapid isolation process has been developed by the ecdysteroid research group, based on simplified, improved methodology, which has led to the isolation of many new phytoecdysteroids. The large-scale extraction of ecdysteroids is a key feature of ecdysteroid research, since the synthesis of ecdysteroids is not economically feasible. The investigation of ecdysteroids is a developing

area of biomedical chemistry, which involves plant screening, identification of the biologically active compounds and study of the practical application possibilities.

Our most important aims were as follows:

- To study the ecdysteroid profile of the aerial parts of *Ajuga reptans* var. *reptans*, the rhizome of *Polypodium vulgare* and the roots of *Serratula wolffii*, which involves the isolation and structure determination of new phytoecdysteroids?
- It was a main objective to find new compounds with unusual structures:
 - The isolation of ecdysteroids with the rare C₂₉-ecdysteroid skeleton
 - The identification of phytoecdysteroids which form a ring in the side-chain
- To isolate novel compounds with potentially high molting activity
- To provide a wide range of structurally different ecdysteroids for MDR studies, which would help reveal specific structure–activity relationships and identify compounds with potential MDR-reversal effects.

Materials and methods

Plant material

The herb *A. reptans* var. *reptans* was collected in August 2003 from harvested populations at the Experimental Station of the Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary. A voucher specimen (collection number A0308) has been deposited at the Department of Ecotoxicology, Plant Protection Institute, Budapest, Hungary. Rhizomes of *P. vulgare* L. were collected in October 2008 from three Hungarian locations: the environs of Veszprém, Egerbakta and Kőszeg. Roots of *S. wolffii* Andrae were collected from cultivated populations in August 2003 from Herencsény, Hungary. Voucher specimens with collection numbers

P71 (*P. vulgare*) and S94 (*S. wolffii*) have been deposited at the Department of Pharmacognosy, University of Szeged, Hungary.

Reagents and standard ecdysteroid samples

Solvents of HPLC grade were from Merck (Darmstadt, Germany). Solvents of analytical grade were from Reanal (Budapest, Hungary). Reference ecdysteroids were available from earlier isolation work and fully characterized in previous studies. Their identities and purities were verified by NMR, MS and HPLC.

Chromatographic methods used

The plant material was subjected to percolation carried out with methanol. The methanolic extract was prepurified using a multiple-step clean-up procedure based on simple, preparative-scale separation techniques. The prepurification process comprised three steps: fractionated precipitation, partition and solid-phase extraction. The isolation of different ecdysteroids from the prepurified plant extract needed the utilization of a combination of sophisticated preparative-scale chromatographic methods, which included NP-CC on alumina, RP-CC on octadecyl-silica, RPC on silica, and preparative HPLC. The entire isolation process was controlled by using NP-TLC.

Structure determination

The known ecdysteroids were identified by direct comparison of their physical and spectroscopic characteristics with those available in the literature. In addition to chromatography the structures of all compounds were characterized and determined by different spectroscopic methods, e.g. UV spectroscopy, NMR and MS. The structural elucidation of the compounds involved the evaluation of the MS and NMR spectral data in comparison with those for the well-known ecdysteroid, 20-hydroxyecdysone.

Results and discussion

Five ecdysteroids were isolated and characterized from *A. reptans*, three of them are new natural compounds (**Figure 2**). Thirteen compounds were isolated and characterized from the rhizome of *P. vulgare*, three of them were discovered for the first time in a natural source (**Table 1**). Three ecdysteroids were obtained and characterized from *S. wolffii*, two of these compounds are new natural substances (**Figure 3**).

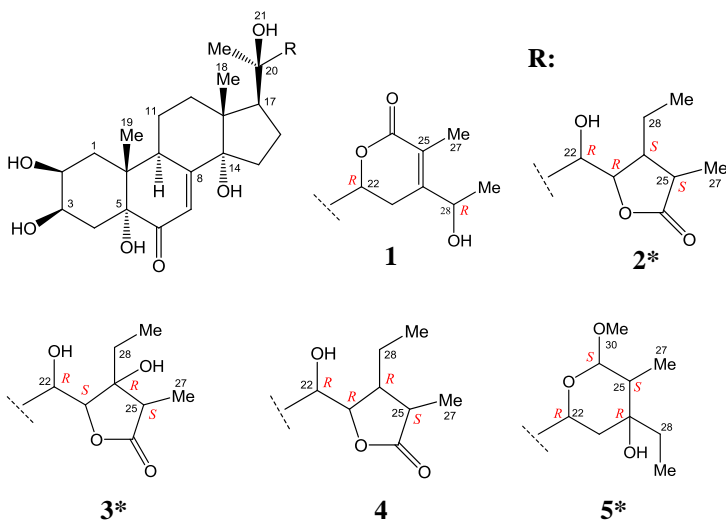


Figure 2. Structures of compounds isolated from *A. reptans*: 24-dehydroprecyasterone (**1**), reptanslactone A (**2**)*, reptanslactone B (**3**)*, breviflorasterone (**4**) and sendreisterone (**5**)*. New compounds are denoted by asterisks (*)

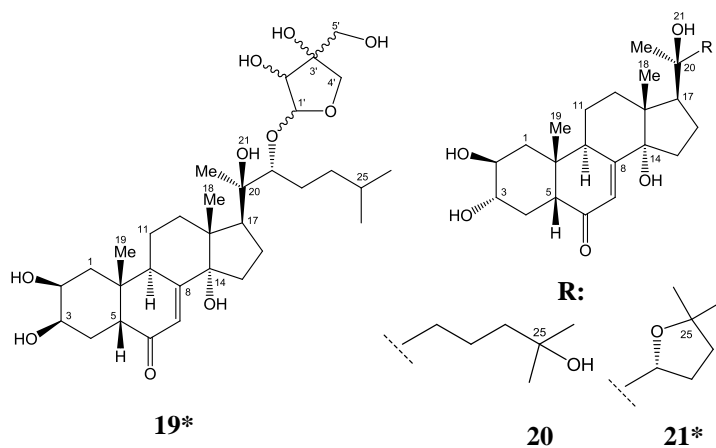


Figure 3. Structures of compounds isolated from *S. wolffii*: ponasterone A-22-apioside (**19**)*, 3-*epi*-22-deoxy-20-hydroxyecdysone (**20**) and 3-*epi*-shidasterone (**21**)*. New compounds are denoted by asterisks (*)

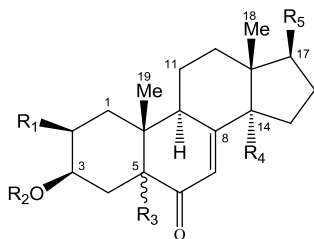
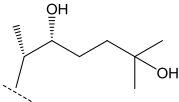


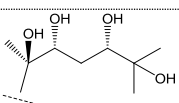
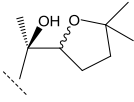
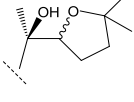
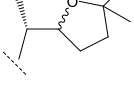
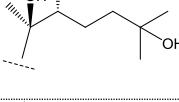
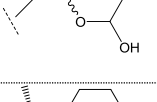
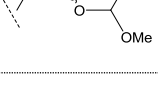


Table 1. Compounds isolated from *P. vulgare*. New compounds are denoted by asterisks (*)

Ecdysteroid	R_1	R_2	R_3	R_4	R_5
pterosterone (6)	OH	H	β H	OH	
rubrosterone (7)	OH	H	β H	OH	$=O$
5-hydroxyrubrosterone (8)	OH	H	β OH	OH	$=O$

5-hydroxyecdysone (9)*	OH	H	β OH	OH	
5-hidroxiposztsteron (10)	OH	H	β OH	OH	
poststerone (11)	OH	H	β H	OH	
abutasterone (12)	OH	H	β H	OH	
shidasterone (13)	OH	H	β H	OH	
5-hydroxyshidasterone (14)	OH	H	β OH	OH	
20-deoxyshidaszterone (15)*	OH	H	β H	OH	
polypodine B-2-glucoside (16)*	Glu	H	β OH	OH	
polypodosaponin (17)	H	Rha-Glu	α H	H	
26-methoxy-polypodosaponin (18)	H	Rha-Glu	α H	H	

Importance of the isolated ecdysteroids

Ecdysteroids with unusual structures

- Lactone-type compounds

Four of the isolated *Ajuga* ecdysteroids (**1-4**) possess a lactone ring, formed by the 26-carboxyl group and a 22- or 23-hydroxy group in the side-chain. Ecdysteroids containing a five- or six-membered lactone ring in their side-chain are rare, they were first considered specific to *Ajuga* species, but they have recently been found in other species (*Cyathula*, *Leuzea*, *Silene*), too.

- Compounds with ether or acetal ring in the side-chain

Ecdysteroids possessing an ether or acetal ring in their side-chain are characteristic of fungi, have also been found in species of the Lamiaceae family, in *Polypodium* spp. and were also isolated from *S. wolffii* by our research group. In three ecdysteroids, shidasterone (**13**) and shidasterone derivatives (**14-15**), the 22-hydroxy group forms an intramolecular ether linkage with the 25-hydroxy group. Shidasterone is fairly common in plants, whereas its derivatives are rare. The 20-deoxy derivative of shidasterone (**15**), which was previously synthesized from 20-hydroxyecdysone, is a new natural compound. Compounds **5**, **17** and **18** have unusual structures which are closely related to that of ajugacetalsterone A, isolated from *Ajuga nipponensis*, but the 26-hydroxy group at **5** and **18** is methylated.

- Ecdysteroids with hydroxyl groups on C-5

C-5 is the characteristic hydroxylation position in ecdysteroids from the common polypody: several of the isolated ecdysteroids possess a 5-hydroxy group. The common occurrence of basic ecdysteroid compounds together with their corresponding 5-hydroxy compounds proves that the final hydroxylation step occurs at C-5, in accordance with an earlier

hypothesis. Compound **9** is a new, 5-hydroxylated ecdysteroid which is present in the plant together with the parent compound, ecdysone.

- Epimer compounds

3-*Epi*-22-deoxy-20-hydroxyecdysone (**20**) and 3-*epi*-shidasterone (**21**) belong among the ecdysteroids that contain a 3 α -hydroxy group. The number of such reported 3-*epi*-ecdysteroids is 21, whereas there are around 350 known ecdysteroids. The presence of these 3-*epimers* in plants is rather unusual; they are mainly biosynthesized in insects. In contrast with the 3-*epi*-ecdysteroids, the other *epi*-ecdysteroids, such as 22-, 14- and 25-*epi*-ecdysteroids, occur only in plant species.

Biological activity of the isolated compounds

Our isolated ecdysteroids deserve attention for their unusual structures: all three species (*A. reptans*, *P. vulgare* and *S. wolffii*) biosynthesize compounds which possess a five- or six-membered ring in their side-chain. Such ecdysteroids with a cyclic ether, acetal or lactone ring are very useful for structure-activity relationship studies, and can reveal essential features of biological activity. The biological activities of some compounds with ring in the side-chain from *A. reptans* were determined via the oral aphid [*Acyrtosiphon pisum* (Harris)] test by our research group. Breviflorasterone (**4**) proved to be most active ($LC_{50} = 3.65$), while reptanslactone B (**3**) ($LC_{50} = 78.81$) and sendreisterone (**5**) ($LC_{50} = 95.14$) exhibited only low oral activity against aphid larvae. The activities of these compounds can be explained on the basis of the polarities and the characteristic structural elements of the side-chain. Compound **4** is more balanced in term of polarity as compared to **3**, which contains more hydroxy groups than **4**. Although compounds **4** and **5** have the same number of hydroxy groups, the latter contains a six-membered ring with several relatively bulky substituents (methoxy and ethyl), which probably decreases the activity, and the receptor-binding capacity.

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Scientific papers related to the thesis

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- II. **Ványolós A**, Simon A, Tóth G, Polgár L, Kele Z, Ilku A, Mátyus P, Báthori M. 2009. C-29 ecdysteroids from *Ajuga reptans* var. *reptans*. *J. Nat. Prod.* **72**: 929-32. If: **3.159**
- III. Simon A, **Ványolós A**, Béni Z, Dékány M, Tóth G, Báthori M. 2011. Ecdysteroids from *Polypodium vulgare* L. *Steroids* **76**: 1419-24. If: **3.106**
- IV. **Ványolós A**, Béni Z, Dékány M, Simon A, Báthori M. 2012. Novel ecdysteroids from *Serratula wolffii*. *ScientificWorldJournal* (in press). If: **1.524**

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