# ECDYSTEROIDS OF FLOWERING PLANTS (ANGIOSPERMAE)*

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A review is given of the ecdysone-like substances found in recent years in flowering plants. The structures of new compounds are presented. The role of molting hormones in the ecological interrelationships between plants and insects is discussed.

The unusual fact that insect molting hormones — ecdysones — are produced in considerable amounts by some plants raises a number of interesting problems. The questions arise: why are the molting hormones needed by plants; what is their role in the ecological interrelationships between plants and insects; is it impossible to use ecdysone-like substances for practical purposes?

Insect hormones in objects of vegetable origin were discovered in 1966 by Nakanishi et al. [1]. In studying the components of an extract of the leaves of *Podocarpus nakaii* — a plant popular in Eastern medicine — they isolated four related compounds — ponasterones A, B, C, and D. What surprised the authors was that ponasterone A had a structure close to that of α-ecdysone (I) — the hormone of the mulberry silkworm *Bombyx mori*. Biological tests confirmed the similarity of their effects.

Almost simultaneously, the Australian chemists Galbraith and Horn [2] isolated an actual molt hormone — ecdysterone (II) — from *Podocarpus elata*. The number of ecdysone-like compounds found in plants began to rise rapidly. Supplementing the zooecdysones, the idea of phytoecdysones came into being.

Even the first workers showed that ecdysones are fairly widespread in the vegetable kingdom. Thus, of 1056 species (738 genera, 186 families) of the flora of Japan studied molting hormone activity was possessed by plant extracts belonging to 77 families (Chilo test) [3].

These results apparently require correction. To establish the presence of phytoecdysones, use is made mainly of biological tests based on the capacity of ecdysone-like substances for causing sclerotization (hardening and darkening) of the cuticle of the larva as it changes into the pupa. The test on the bluebottle fly *Calliphora erythrocephala* is most frequently used. For this purpose, an ethanolic extract of the material under investigation is introduced by means of a microsyringe into the lower part of the body of the larva isolated by ligating and the process of pupation is observed. In quantitative evaluation, the so-called calliphora unit (CU) is used, which corresponds to that amount of molting hormone that must be administered in order to achieve a 50% pupation of the larvae taken in the investigation. On an average, 1 CU corresponds to 0.01 μg (10 ng) of crystalline α-ecdysone, but depending on the strain of fly it may range from 7.5 to 20 ng.

Molting activity is determined by approximately the same method on the larvae of other insects: *Bombyx mori* (mulberry silkworm) [5], *Musca domestica* (housefly) [6, 7], *Samia synthis* (castor-bean silkworm) [8], *Sarcophaga peregrina* (gray flesh fly) [9], etc. The test on the rice pest *Chilo suppressalis* (stem moth) differs somewhat from the others [10]. The test is very simple in the technique of its application but is less sensitive than biotests on *Calliphora* and *Musca* and is apparently less objective. A method of determining ecdysone-activity *in vitro* on the imaginal disks of the legs of the fly *Drosophila melanogaster* has also been suggested [11].

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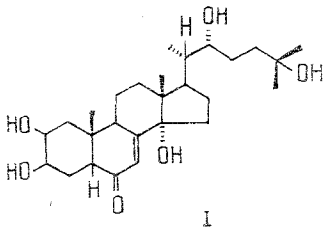
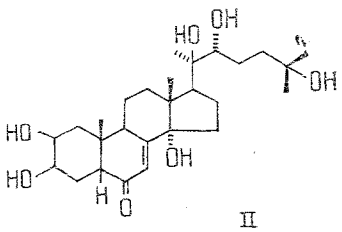



I




II

The main hormones of insect larvae: α-ecdysone (I) and ecdysterone (II).

The activities of the ecdysones in different insects are different and therefore the biotests do not correlate with one another [12, p. 15]. In particular, the housefly is 10 times more sensitive to the main molting hormones than large blubottle flies. Both in the test on *Calliphora* and in the test on *Musca* the activities of α-ecdysone (I) and ecdysterone (II) are almost identical, while in the test on *Sarcophaga ecdysterone* is twice as, and in the test on *Samia*, conversely, half as, active as α-ecdysone.

Because of differences in chemical structure, the activities of the ecdysone-like compounds in one and the same biotest do not agree. For example, in the test on the housefly ponasterone A is twice as active as ponasterone B and five times as active as ponasterone C [5, 6, 13].

In the testing of plant extracts it is also necessary to bear in mind the fact that they may contain compounds with an anti-ecdysone action. The presence of such compounds will mask the true ecdysone content. Thus, in *Ajuga decumbens* in addition to ecdysterone (II) and cyasterone (V), which possess pronounced molting activity, ajugalactone (V) inhibiting this action (Chilo test) has been detected [14]. It is not excluded that a substance possessing a high ecdysone activity by one biotest may prove to be anti-ecdysonic by another.

All this makes the screening of plants for the presence of ecdysones with the aid of biotests alone unreliable. In the best case, biotests can be used only for the preliminary, purely qualitative, evaluation of plant materials.

In order not to confuse the concrete compound α-ecdysone (I) with other substances related to it, recently in place of the name ecdysones the use of the term ecdysteroids (zooecdysteroids and phytoecdysteroids, as the case may be) has been recommended [15]. It is in harmony with other collective names such as corticosteroids, cardiosteroids, etc. At the same time, ecdysteroid is not a suitable term for a molting hormone. In the first place, it is not a biological action which is being put forward but a common feature of chemical composition. Such a compound may or may not possess molting activity. The most important elements determining whether a compound is an ecdysteroid are obviously the following:

the substance must be a steroid;

it must contain a ketonic grouping conjugated with a $\Delta^7$ bond in the C-6 position. The absence of a double bond at C-7 (cheilanthones A and B) or its shift to the C-8 position must be considered as an exception, since such compounds do not as a rule possess molting activity;

the overwhelming majority of ecdysteroids have hydroxy groups at C-3 and C-14 positions, while in individual compounds [3-dehydroecdysterones, silenosterone (XV)] the hydroxyl at C-3 has been oxidized to a ketone group and the hydroxyl at C-14 has been dehydrated to a double bond (stachysterone B, podecdysone B); and

there must be a side chain of the cholesterol type at C-17. Only two compounds — rubrosterone and poststerone — do not have a long side chain but in view of the combination of other elements their structures are assigned to the ecdysteroids.

On the basis of features of their chemical structure, spectrophotometric methods have been developed for determining ecdysteroids after their preliminary separation on a thin layer of silica gel or alumina. One of such methods is based on the Chugaev reaction (zinc chloride in acetic acid + acetyl chloride), which gives colored complexes with steroids capable of undergoing dehydration [16]. Another method is based on the fact that the UV spectra of the ecdysteroids have an absorption maximum at 242 nm (log ε 4.07) due to the presence of the $\Delta^7$-6-ketone grouping in the molecule. The intensity of this extremum permits it to be used as an analytical band [17].

Methods have been developed for determining phytoecdysteroids by gas-liquid chromatography [18-20], but the most promising method is liquid chromatography. This method has been used for the preparative separation of the ponasterones in the leaves of *Podocarpus nakaii* [21] and for the ecdysterone and inokosterone in the roots of *Achyranthes fauriei* [22, 23]. High-resolution liquid chromatography has been used to isolate molting hormones and their metabolites in biological materials [24, 27].

Instrumental methods are suitable mainly for qualitative analysis of the ecdysteroids in those plants where their presence has already been established. As applied to new materials, however, in accordance with the traditional chemistry of plant substances, only those facts that are confirmed by the isolation and physicochemical characterization of individual compounds serve as a positive evaluation.

Taking this condition into account, phytoecdysteroids have so far been found in about 90 species of plants belonging to 41 genera and 20 families [28].

DIVISION **POLYPODIOPHYTA**

Family. Osmundaceae
Osmunda
Family. Gleicheniaceae
Gleichenia

Family. Polypodiaceae
Criosinus
Cheilanthes
Cyclosorus
Lemmaphyllum
Neocheiropteris
Pleopeltis
Polypodium
Pteris

Family Aspidiaceae
Athyrium
Dryopteris
Matteuccia
Onoclea

Family. Aspidiaceae
Thelypteris
Family. Blechnaceae
Blechnum
Strutiopteris
Woodwardia
Family. Pteridiaceae
Pteridium

DIVISION **PINOPHYTA (GYMNOSPERMAE)**

Family. Podocarpaceae
Dacrudium
Podocarpus

Family. Taxaceae
Taxus

DIVISION **MAGNOLYPHYTA (ANGIOSPERMAE)**

Family. Commelinaceae
Cyanotis
Family. Liliaceae
Paris
Trillium
Family. Moraceae
Morus
Family. Amaranthaceae
Achyranthes
Bosea
Cyathula
Gomphrena
Family. Aisoaceae
Trianthema
Sesuvium
Family. Caryophyllaceae
Lychnis
Silene

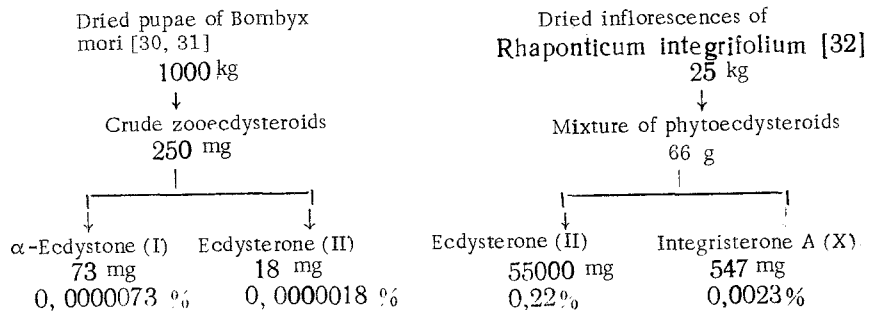
Family. Ranunculaceae
Helleborus
Family. Stachyuraceae
Stachyurus
Family. Convolvulaceae
Ipomoea
Family. Verbenaceae
Vitex
Family. Labiatae
Ajuga
Family. Compositae
Rhaponticum
Serratula

Apparently, even at an early stage the question of the existence of an interconnection between taxonomic hierarchies and the distribution of phytoecdysteroids in plants was discussed. All that can be stated definitely is that ecdysone-like compounds have been found in the main divisions of higher plants — Polypodiophyta, Pinophyta, and Magnoliphyta. Compounds with molting activity have not been found in higher fungi and algae, although it is true that they have not been studied intensively [29].

The advantage of plant raw material as a source of ecdysteroids over animal organisms is not a matter of doubt.

In the first place, the amount of zooecdysteroids in their sources is usually very low — from hundredths of a milligram to a few milligrams per 1 kg of live weight. Thus, in the first experiments from 500 kg of silkworm pupae only 25 mg of α-ecdysone was isolated. Consequently, the method was improved — from one tonne of dry pupae it was possible to obtain 250 mg of unpurified total zooecdysteroid [30, 31]. In plants, however, the concentration of phytoecdysteroids may reach 1% and more [32], i.e., from a tonne of dried plant material it is possible to obtain up to 10 kg of phytoecdysteroids.

Comparative Amounts of Ecdysteroids in Insects and Plants



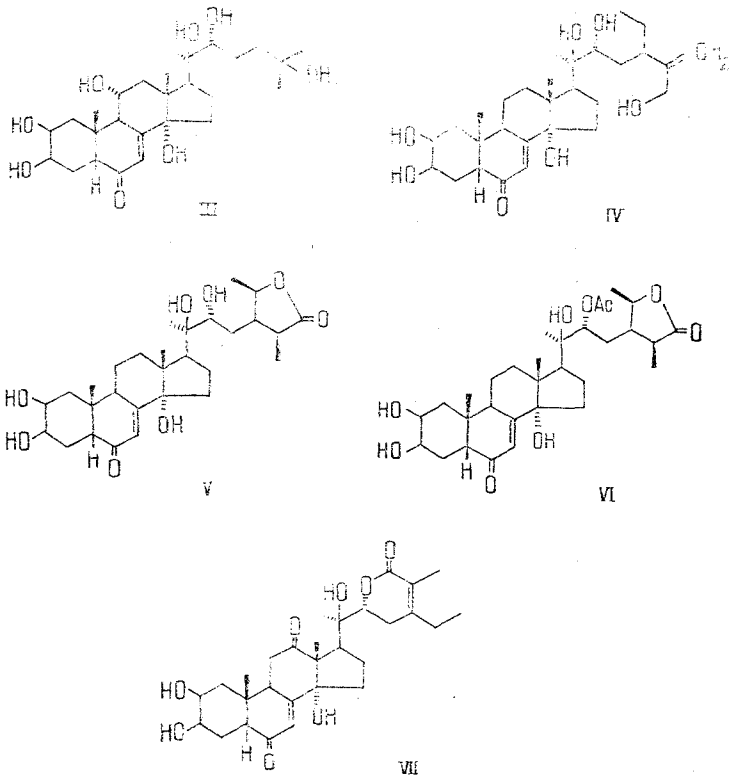

In the second place, in the structural respect, phytoecdysteroids are more diverse than zooecdysteroids. The functions of the molting hormones in insects are fulfilled by the α-ecdystone (I) and ecdysterone (II). The other compounds found in their organisms (26-hydroxyecdysone, 26-hydroxyecdysterone, 2-deoxy-α-ecdysone (XIII), 2-deoxyecdysterone (XIV), 3-dehydroecdysterone, 26-hydroxy-25-deoxyecdysterone, and 24-methylecdysterone) are perhaps only metabolites of the main substances. Almost all these substances are also found in plants, but in addition to them among the ecdystone-like compounds of plant origin acetates, methyl ethers, cinnamates, and compounds with lactone groupings on the side chains have also been described. It is true that not all phytoecdysteroids possess molting activity.

We have studied the phytoecdysteroids of flowering plants (Magnoliphyta=Angiospermae). Our choice was due to the fact that it is precisely flowering plants which form the bulk of the continental green kingdom. With respect to the number of species, flowering plants considerably exceed all the other groups of higher plants taken together. Our work appeared all the more necessary since the opinion has previously been expressed that ecdysone-like substances are present only on the Polypodiophyta [3].

Of the materials that we have studied, there was more or less complete information only about the phytoecdysteroids of plants of the genus *Ajuga* of the Japanese flora. Characteristic for the compounds isolated from this genus of plants is the presence of a 5- or 6-membered lactone ring in the side chain. They make up the group of phytoecdysteroids containing 29 carbon atoms in the molecule and are characteristic only of the vegetable kingdom If, as shown below, the precursor of ecdysterone (II) in plants is cholesterol (XXI), the biosynthesis of the ecdysteroids of *Ajuga* possibly takes place through sitosterol (XXII) or stigmasterol (XXIII), which also contain 29 carbon atoms in the molecule.

We have investigated for their ecdysteroid content the epigeal and hypogeal organs of the plant *A. turkestanica* growing in Central Asia. Substances typical for the genus *Ajuga* were found: ecdysterone (II), cyasterone (V) [33], ajugalactone (VII) [34], and ajugasterone B (IV) [35]. New among the ecdysterones of the 29-C series is 22-acetylcyasterone (VI), isolated in appreciable amounts from the leaves of the plant [36].

Another previously unknown compound — turkesterone (III) — accumulates mainly in the roots of the plant [37, 38]. The side chain of the ecdysteroid is identical in structure with that of ecdysterone. The steroid part of the molecule, however, contains an additional hydroxy group the position of which was established by spectral methods. Turkesterone could also be called 11α-hydroxyecdysterone (III).

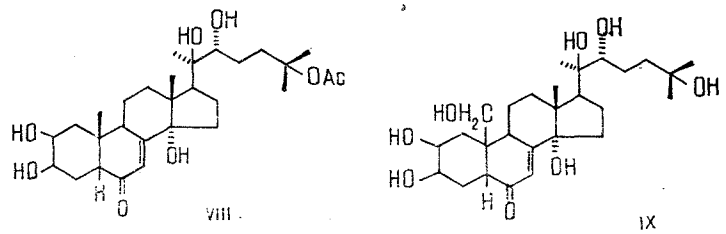


Phytoecdysteroids of *Ajuga turkestanica* (in addition to ecdysterone (II)) turkesterone (III), ajugasterone B (IV), cyasterone (V), 22-acetylcyasterone (VI), and ajugalactone (VII).

A large part of our investigations was devoted to ecdysteroids of plants of the family Compositae. The plants of this family occupy a very high position on the evolutionary ladder of the vegetable kingdom, and the question of whether they are capable of synthesizing ecdysteroids is of no little importance for investigating chemotaxonomic links.

A good source of phytoecdysteroids has proved to be plants of the genera *Serratula* and *Rhaponticum*. Attention was first drawn to the presence of considerable amounts of ecdysterone in the flower heads of *Serratula inermis* by Ya. K. Yatsyuk and G. M. Segal' [39]. We have investigated several species of plants of the genus *Serratula*. In the flowerheads of *S. sogdiana*, in addition to known compounds — ecdysterone (II) and viticosterone E (VIII) [40, 41] — we detected a new substance, sogdisterone (IX) [42].

A characteristic feature of the sogdisterone molecule is the presence of a hydroxy group at C-19. A decisive point in establishing this fact was that in the PMR spectrum of sogdisterone (IX) the signal corresponding to the C-19 angular methyl group was absent. As is well known, among steroids of plant origin some cardiac aglycones (strophanthidol, ouabagenin) have a hydroxy group at C-19. There are no differences in the structure of the side chain between sogdisterone and ecdysterone. The structure of sogdisterone corresponds to 19-hydroxyecdysterone (IX).



Phytoecdysteroids of *Serratula sogdiana* (in addition to ecdysterone (II)): vitocosterone E (VIII) and sogdisterone (IX).

TABLE 1. Flowering Plants from which Ecdysteroids have been Isolated

| Family, genus, species | Plant organ* | Ecdysteroids | Amount, % of the weight of the dry material |
|---|---|---|---|
| **LILIACEAE** | | | |
| Paris quadrifolia L. [80] | p | Ecdysterone | 0,020 |
| | | Polypodine B | 0,010 |
| **CARYOPHYLLACEAE** | | | |
| Silene brachuica Boiss. | f, l | Ecdysterone | 0,030 |
| | r | " | 0,094 |
| | f, l | Integristerone A | 0,020 |
| | r | " | 0,045 |
| | f, l | Polypodine B | 0,002 |
| | " | Viticosterone E | 0,0012 |
| | r | Sileneoside A† | 0,020 |
| | " | Sileneoside B† | 0,0045 |
| | " | Sileneoside C† | 0,0032 |
| S. latifolia (Mill.) Rendle et Britt. | f, l | Ecdysterone | TLC |
| | " | 2-Deoxyecdysterone | TLC |
| | " | 2-Deoxy-α-ecdysone | TLC |
| S. longicalycina Kom. | f, l | Ecdysterone | TLC |
| S. praemixta M. Pop. [52, 53] | f, l | Ecdysterone | 0,650 |
| | " | 2-Deoxy-α-ecdysone | 0,120 |
| | " | 2-Deoxyecdysterone | 0,082 |
| | " | Silenosterone† | 0,003 |
| | " | Premixisterone† | 0,002 |
| S. wallichiana Klatzsch. | " | Ecdysterone | TLC |
| **LABIATAE** | | | |
| Ajuga chia Schreb. | p | Ecdysterone | 0,010 |
| A. turkestanica Regel. [33—38] | l | " | 0,020 |
| | r | " | 0,045 |
| | l | Cyasterone | 0,025 |
| | r | " | 0,010 |
| | l | Ajugalactone | 0,001 |
| | r | " | 0,001 |
| | l | Ajugasterone B | 0,002 |
| | r | " | 0,003 |
| | l | 22-Acetylcyasterone† | 0,050 |
| | r | Turkesterone† | 0,052 |
| **COMPOSITAE** | | | |
| Rhaponticum carthamoides (Willd.) Iljin ssp. orientale (Serg.) Soskov. [17, 48] | f | Ecdysterone | 0,276 |
| | l | Ecdysterone | 0,568 |
| | r | " | 0,142 |
| | " | Integristerone A | 0,010 |
| | " | Integristerone B | 0,0002 |
| | " | 24(28)-Dehydromakisterone A | TLC |
| Rh. integrifolium C. Winkl. [32, 45—47] | f | Ecdysterone | 0,230 |
| | " | Integristerone A† | 0,013 |
| | " | Integristerone B† | 0,0003 |
| | " | 24(28)-Dehydromakisterone A† | 0,0002 |
| Rh. luratum C. Winkl. ex Iljin | f | Ecdysterone | TLC |
| Rh. nanum Lipsky | p | Ecdysterone | 0,007 |
| | | Integristerone A | 0,0025 |
| Serratula algida Iljin | f | Ecdysterone | TLC |
| S. centauroides L. | l | " | TLC |
| | " | Viticosterone E | TLC |
| S. coronata L. | l, f | Ecdysterone | 0,071 |
| | " | α-Ecdysone | 0,003 |
| | " | Viticosterone E | 0,0008 |
| S. procumbens L. | l, f | Ecdysterone | TLC |
| | | Viticosterone E | TLC |
| S. quinquefolia MB | l | Ecdysterone | TLC |
| S. sogdiana Bunge [40—42] | f | " | 0,520 |
| | l | " | 0,170 |
| | f | Viticosterone E | 0,0014 |
| | l | Viticosterone E | 0,027 |
| | f | Sogdisterone† | 0,003 |
| S. xeranthemoides Bieb. (=S. erucifolia L.) [49] | f | Ecdysterone | 0,260 |
| | " | Integristerone A | 0,150 |

*Conventional abbreviations: p — whole plant; f — flowers; l — leaves; r — roots.

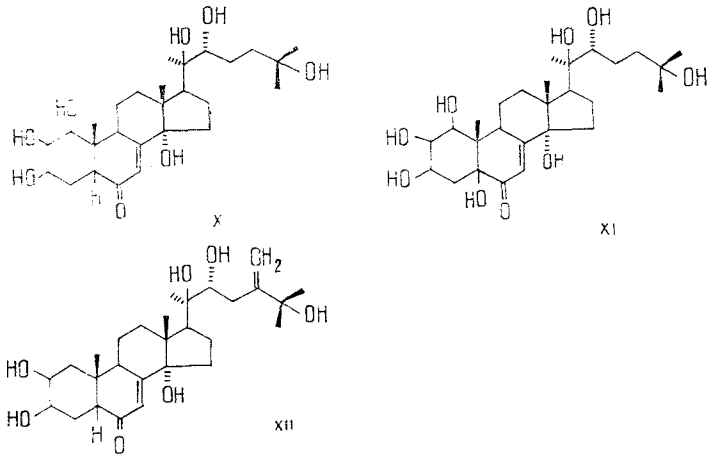
†Ecdysteroids described for the first time.

Viticosterone E (VIII) has been detected previously in the leaves of *Vitex megapotamica* (family Verbenaceae) [43]. Its structure was established on the basis of spectral characteristics. The individual functional groups may also have a different configuration, and we therefore found it necessary to perform the partial synthesis of viticosterone E (VIII) from ecdysterone (II). The synthesis, performed by two methods, confirmed that viticocosterone E. (VIII) is the 25-monoacetate of ecdysterone [41].

A mass-spectrometric study of the nature of the fragmentation of the side chains of the acetates and acetonides of ecdysterone and of viticosterone E revealed some distinguishing features having analytical value [44]. The results obtained were subsequently used to establish the structures of other phytoecdysteroids.

Ecdysone (I) has been found among other ecdysteroids in *S. coronata* — a rare example of the detection of a true molting hormone in plants.

Some interesting compounds have been isolated from *Rhaponticum integrifolium*. In this material, as also in other flowering plants, there is a considerable amount of ecdysterone (II) [45]. In addition to this, another three phytoecdysteroids have been found: integristerone A (X) [46], integristerone B (XI) [32], and 24(28)-dehydromakisterone A (XII) [47].




Phytoecdysteroids of *Rhaponticum carthamoides* and *Ph. integrifolium* (in addition to ecdysterone (II)): integristerone A (X), integristerone B (XI), and 24(28)-dehydromakisterone A (XII).

The first two compounds are the most interesting. Both phytoecdysteroids possess a considerable hydrophilicity and are characterized by the presence of a large number of hydroxy groups in the steroid part of the molecule. Integristerone A contains a total of six, and integristerone B a total of seven hydroxy groups. A decisive role in the establishment of the structures of both compounds was played by the possibility of obtaining isomeric acetonides, readily distinguished from one another at the 1,2 and 2,3 hydroxyls.

There is the same composition of phytoecdysteroids in the legendary plant of Eastern Siberia — *Rh. carthamoides*, *Leuzea sofloridea*, or maral root [17, 48]. In their adaptogenic tonic, and stimulating action, preparations of the plant resemble ginseng. An extract from the roots of *Rh. carthamoides* has been admitted into the official pharmacopeia but so far it is not known to what the medicinal action of the plant so popular in folk medicine is due.

The small *Rh. nanum* with matchstick-like roots growing in the alpine and subalpine zones of the Western Tien-Shan contains ecdysterone (II) and integristerone A (X).

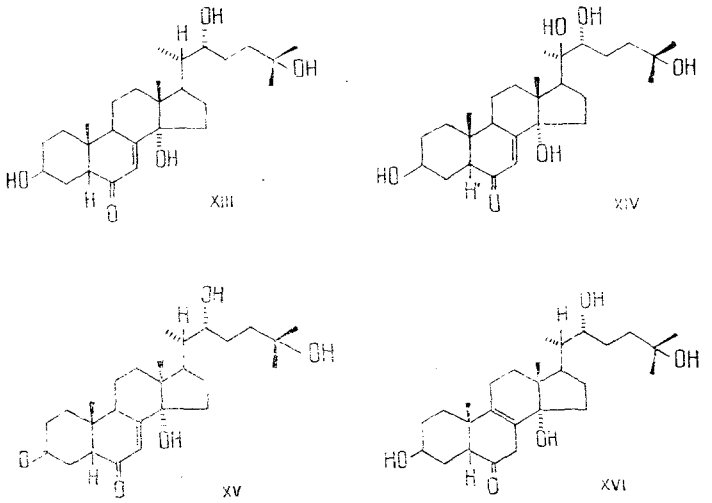
Integristerone A is apparently one of the commonest phytoecdysterones in the plant world. In addition to the plants mentioned, we have detected it in *Serratula xeranthemoides* [49] and *Silene brachuica*.

There are great prospects for finding ecdysteroids in plants of the family Caryophyllaceae. This is one of the large families among flowering plants and its representatives can be found in all the continents of the terrestrial globe as far as Antarctica.

We have studied several species of *Silene* and have found ecdysone-like substances in all of them.

Characteristic for plants of this genus is the presence in them of a considerable amount of 2-deoxyecdysteroids — compounds containing no hydroxy group at C-2 of the steroid nucleus. In particular, such compounds include the 2-deoxy-α-ecdysone (XIII), a hormone found in *Bombyx mori* and extremely important for the sexual development and maturation of the larvae [50]. In the vegetable kingdom, this compound and the related 2-deoxvecdysterone (XIV) are characteristic only of *Blechnum minus* — a plant belonging to the Polypodiophyta [51]. The amount of 2-deoxyecdysteroids in *Silene* is 10 times greater than that in *Blechnum*. Incidentally, we may note that in biotesting on *Chilo suppressalis* plants of the genus *Silene* showed a negative result, and of the Caryophyllaceae only the genus *Lychnis* gave a positive result [3].

In the flowers and leaves of *S. praemixte* — in addition to ecdysterone (II), 2-deoxy-α-ecdysone (XIII) and 2-deoxyecdysterone (XIV) — two compounds of low polarity have been found [52, 53]. We have called them silenosterone (XV) and premixisterone (XVI). The first of these compounds contains a keto group in position 3 and can therefore be called 2-deoxy-3-dehydro-α-ecdysone.



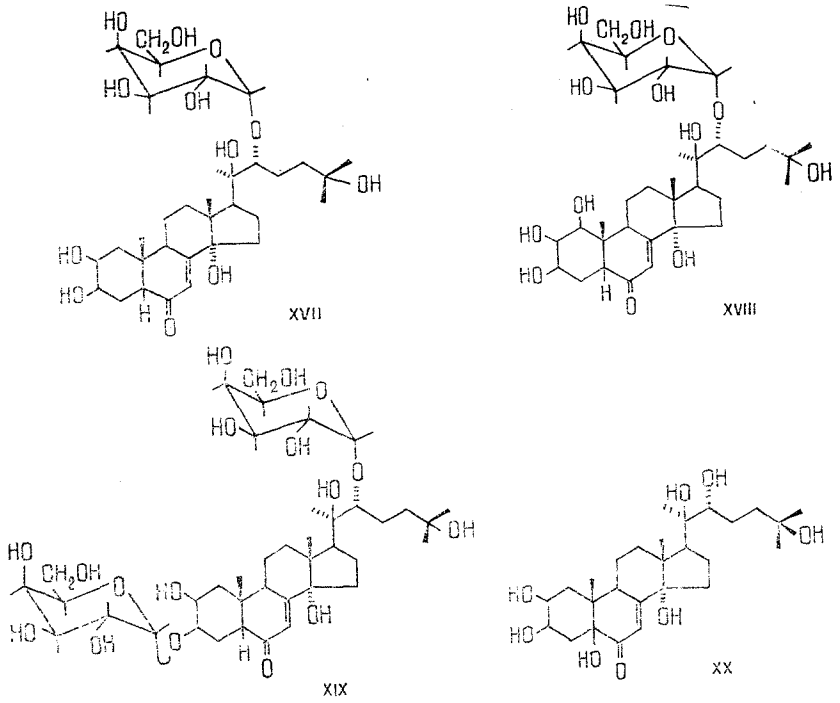

Phytoecdysteroids of *Silene praemixta* (in addition to ecdysterone (II)): 2-deoxy-α-ecdysone (XIII), 2-deoxyecdysterone (XIV), silenosterone (XV), and premixisterone (XVI).

Ecdysteroids with a keto group at C-3 have not been isolated in the native state. 3-Dehydro-α-ecdysone and 3-dehydroecdysterone have been described only as metabolites of the main molting hormones — ecdysterone and α-ecdysone — in insect homogenates [54, 55]. It is not excluded that in the plant organism, as well, silenosterone (XV) and premixasterone (XVI) play the role of metabolites of the main phytoecdysteroids, all the more since they are found in comparatively small amounts.

A collection of phytoecdysteroids with numbers of hydroxy groups from three to seven or eight has enabled us to study the nature of the fragmentation of such substances under the action of electron impact in more detail [56]. In an analysis of the mass spectra of natural ecdysteroids and their acetates several common features have been elucidated which facilitate the determination of the structures of new compounds.

The composition of the ecdysteroids of *Silene brachuica* is unusual. Zoo- and phytoecdysones do not usually form glycosides in nature. Only one glycosidic compound is known — ponasteroside A (ponasterone A 3β-D-glucoside) from *Pteridium aquilinium* [57]. Consequently, it proved unexpected when it was possible to isolate directly from the roots of *S. brachuica* three compounds of glycosidic nature, which were called sileneosides A, B, and C (not to be confused with the triterpene glycoside silenoside isolated from *S. latifolia* [58]). Sileneoside A (XVII) has the structure of ecdysterone 22-α-D-galactoside, and sileneoside B (XVIII) is integristerone A 22-α-D-galactoside. Attention is attracted by the α configuration of the glycosidic center formed by the D-galactose, contrary to Klyne's rule [59]. Also unusual is the position of the sugar residue at the C-22 hydroxyl. The overwhelming

majority of steroid glycosides have the sugar residues attached to the C-3 hydroxyl. Sileneoside C (XIX) apparently has the structure of ecdysterone 3,22-bis-α-D-galactopyranoside. The presence in flowers of glycosidic compounds of the ecdysteroids is indicative of their active role in metabolic processes.




Phytoecdysteroids of *Silene brachuica* (in addition to ecdysterone (II)): integristerone A (X), and viticosterone E (VIII): sileneoside A (XVII) sileneoside B (XVIII), sileneoside C (XIX), and polypodine B (XX).

At the present time, including those compounds which we have described for the first time, the chemical structures of approximately 60 ecdysteroids produced by the plant organism have been established. It must be assumed that this is not the limit. In essence, the investigation of the vegetable kingdom for phytoecdysteroids is only just beginning and on more careful investigation they may be detected in the most unexpected materials.

The information given in Table 1 on the amounts of individual substances must be considered only as indicative. Depending on the site of growth and the phase of development of the plants, both the total amount of phytoecdysteroids and the ratios of the individual components may vary.

Why are molting hormones needed by plants?

It is obvious that science will not quickly answer this question, just like the question of why plants need alkaloids, isoprenoids, antibiotics, coumarins, tannins, and other organic compounds of comparative low molecular weight. Once they were unsuccessfully called secondary metabolites, emphasizing not so much their significance as the dependence of their origin on primary substances (proteins, nucleic acids, lipids, polysaccharides). In the past, this type of compounds was not infrequently identified with the final product of the metabolism and they were considered as waste materials. The position has apparently changed somewhat. Now few dispute the importance of their role in biological processes. Consequently, the terms "low-molecular-weight bioregulators" is gaining ever greater popularity, although this name, as well, in our opinion, by no means completely reflects their true purpose.

So far as concerns the ecdysteroids attention is attracted by their high physiological activity in *in vivo* and *in vitro* experiments in very diverse directions. The most attractive opinion appears to be that of a beneficial role of the ecdysteroids in the growth and development of plants, i.e., in a function similar to their role in the life of arthropods. In this connection, the capacity of the ecdysteroids for exerting a favorable influence on the nitrogen metabolism is extremely important. In other words they stimulate the synthesis

of proteins in the plant organism and activate cell mitoses. It is not fortuitous that the phytoecdysteroids accumulate with great frequency in the reproductive organs of plants — in the flowers and fruit. For example, 2% of ecdysterone (II) has been detected in the inflorescences of *Serratula inermis* [39].

More vulnerable is the hypothesis of the protective function of the phytoecdysteroids. An artificial administration of an excess of molting hormones leads to a serious disorder of the mechanism of metamorphosis, which permits them to be equated with insecticides. However, here it must be borne in mind that the ecdysteroids are not toxic and even on direct contact with insects are relatively harmless. In order to show their insecticidal action, one must ensure that a large amount reaches the organism. Caterpillars of the silkworm, however, absorb an incredible amount of mulberry leaves containing ecdysterone and inokosterone [60], without any harm but, rather, with benefit to themselves. Very frequently in the field it is possible to see a plant rich in ecdysteroids that is at the same time literally plastered with a multitude of insects. In any case, the ecdysone-like substances are not repellants, i.e., substances frightening insects away. In experiments with labeled compounds on the plants *Podocarpus elata* [61-63], *P. macrophyllus* [64, 65], and *Polypodium vulgare* [66], it has been shown that the direct source of the ecdysteroids in the plants is cholesterol (XXI). In the process of biosynthesis detachment and subsequent addition of the side chain does not take place: cholesterol labeled with $^{14}C$ at C-4 of the steroid skeleton is converted into ecdysterone (II) to the same degree as the analogous compound labeled at the C-26 carbon of the side chain. The mechanisms of the biosynthesis of the ecdysteroids in plants and insects, at least in the last stage, are either similar or identical — $^{14}C$-4-labeled cholesterol is converted by the larvae of *Bombyx mori* into a mixture of α-ecdysone and ecdysterone [67, 68].

The capacity for biosynthesizing steroids appeared in the earliest stages of life on the earth — considerable amounts of sterols are produced by the blue-green algae [69], the rise of which dates back almost 2.5-3 thousand million years. The blue-green algae that appeared on the historic arena in the pre-Cambrian period were the oldest autotrophic organisms capable of independently assimilating carbon dioxide and liberating oxygen. This was done with the aid of sunlight, and therefore we are justified in considering steroid compounds as one of the first products of photosynthetic activity.

Insects appeared comparatively later. This took place at the end of the Paleozoic era, approximately 250 million years ago. An enormous interval of time separates us from that ancient era. But the butterflies, cockroaches and, dragon-flies living then differed but little from the insects alive today. Consequently, for their normal vital activity they already needed ecdysteroids.

There is no doubt of the fact that the physiological mechanism of the molting of insects was borrowed from marine organisms. Insects are not the only class of arthropods requiring molting hormones for growth and development. Ecdysterone, 2-deoxyecdysterone, and compounds similar to them have been found in the chitinous shells of crayfish and crabs. Apparently, the biological processes of molting regulating by ecdysones in various classes of arthropods, if they are not identical, at least have much in common. It is sufficient to inject a crab with the universal molting hormone ecdysterone for accelerated molting to take place [70].

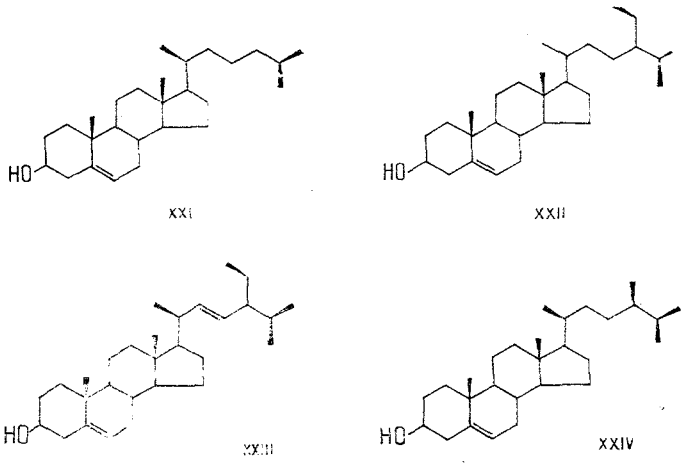
It will be nothing unusual if later investigations confirm that the biosynthesis of the zooecdysteroids in arthropods leading an aquatic form of existence also takes place via cholesterol. If this is the case, the precursors of the modern insects that found terrestrial life copied an existing mechanism which, of course, was improved and adapted to the conditions of existence in a different environment in the course of evolutionary development. This relates not only to molting and pupation. In the insect organism, the zooecdysteroids participate in the endocrine regulation of a multiplicity of other processes. They are necessary for the normal functioning of the sexual apparatus, especially of the ovaries, affect the central nervous system, accelerate the regeneration of individual organs, and ensure the regularity and completeness of the biorhythms, in particular, the diapause.

It is insufficient merely to copy the mechanism of hormonal regulation; it is not less important that a material carrier of this function should be available. For a better understanding of ecological links it is not superfluous to remember that the insect organism is not capable of synthesizing steroids from simple acyclic compounds and requires that they

are supplied with the food [71-74]. This is an important condition of the hypothesis that we have developed.

The main demands on plant food of insects and vertebrates are apparently the same — the food must contain proteins, carbohydrates, certain vitamins, and some mineral salts. In addition to products ensuring normal existence, other substances pass from plants into the animal organism and, in the first place, such low-molecular-weight compounds as alkaloids, terpenes, organic acids, various types of glycosides, etc. In principle, any plant possesses a sufficient calorie content, but the qualitative compositions of the secondary metabolites of different species of plants are different. Consequently, it is doubtful whether the products of the secondary metabolism possess any appreciable food value whatever. The animal organism has the right to deal with them according to its own judgment: to eliminate them through the excretory system or to use them for any other purposes not connected with the constant renewal of the primary substances.

Insects have adapted cholesterol (XXI) and, possibly, other sterols similar in structure — β-sitosterol (XXII), stigmasterol (XXIII), campesterol (XXIV), and others — for the synthesis of molting hormones. The enzyme systems in the insect organism are capable *in vivo* of shortening phytosterols with "superfluous" carbon atoms to cholesterol by dealkylation at C-24 [75, 76].




Possible precursors of ecdysteroids in plants: cholesterol (XXI), β-sitosterol (XXII), stigmasterol (XXIII), campesterol (XXIV).

Of course, we are not justified in excluding the direct route of the immediate utilization of phytoecdysteroids, as well. By the time of the emergence of arthropods on dry land ferns and gymnosperms were already growing in abundance on it. Did they not serve as the first insect molting hormones? The vigorous growth in the Lower Cretaceous series of flowering plants intensified mutual adaptation and at the same time the dependence of the two large kingdoms of life upon one another. Insects attracted by the color and scent of flowers visited flowering plants particularly willingly, and the latter, thanks to cross-pollination, rapidly developed. In the first place, those evolutionary branches and insects that were most adapted for mutual existence obtained the stimulus for development. Mutual adaptation took place not only in the direction of the adaptability of morphological and anatomical characteristics but also along the line of the suitability and desirability of individual metabolic products taking part in the symbiosis of the organism. Symbiosis benefited only from the fact that the products of the vital activity of one partner promoted the growth and development of the other. Many plant sterols, including ecdysterols, could prove to be mutually beneficial and mutually acceptable substances of this type.

In the process of natural selection — the main motive force of evolution — a mutant capable of producing a metabolite with more useful properties obtained definite advantages in the matter of survival and gradually displaced a related species in nature. Not all inhabitants of the green kingdom of antiquity have become established and some whole groups of plants have disappeared, finding themselves in an evolutionary dead end.

The correctness of the hypothesis of the direct use of phytoecdysteroids can be illustrated by the following example [77].

As is well known, insects feed on one or a few closely similar plants (monophagous), a a large group of plants belonging to one family (oligophagous), or an even wider group of plants including several closely related families (polyphagous). There are practically no insects that feed on all plants.

The ordinary mulberry silkworm *Bombyx mori* is monophagous. It is reared exclusively on the leaves of the mulberry *Morus alba*, which belongs to the family of Moraceae. The same family includes osage orange (*Maclura aurantica*), the Kazinoki paper mulberry (*Broussonetia kazinoki*), various species of *Cudrania* used as hedges (*Cudrania triloba*, *C. javenesis*), and the common fig (*Ficus carica*). The leaves of these plants were tested as food for silkworms. Their caterpillars willingly ate them and grew for some time but sooner or later they died. In any case, they did not once reach an age leading to pupation. At the same time, attempts to rear silkworm caterpillars on the leaves of the tall nettle (*Urtica procera*) and the Chinese elm (*Ulmus parvifolis*) — plants belonging to two families close to the Moraceae, the Urticaceae and the Ulmaceae — also proved unsatisfactory.

What is the reason for the death of the caterpillars? Is it not in the fact that only *Morus alba* produces ecdysteroids?

The sterol compositions of the plants mentioned above have not yet been adequately studied. However, it is not a matter of doubt that the plants contain cholesterol or at least, β-sitasterol and stigmasterol. Indeed, sterols are indispensable components of all plant cells. It is obvious that for silkworm larvae it is not sufficient that they can transform other steroid compounds into α-ecdysone and ecdysterone. The amount of molting hormones taken indirectly with their food is also important for their normal development. The demand for phytoecdysteroids rises in the stage of the transformation of the caterpillars into the pupae. Is this not why in modern sericulture, where a tendency to pass to an artificially composed food is being felt, it is impossible to succeed without adding a certain amount of mulberry leaves?

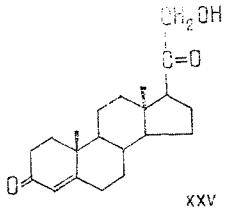
The nature of the nontrophic, i.e., effected outside the food chain ("that to which it serves as food") links between plants and insects may be more complicated than in the example given above. We may admit error or, in any case, consider too narrowly when in attempting to predict the role of individual metabolites we start, as it were, from the points of view of the plant itself, alone. By recognizing the characteristic of a direct link, we are not justified in denying the existence of a feedback — from insect to plants. Evolution has been based on a complex interrelationship between animals and plants, the two branches of life having developed in dialectic unity. The specific causal links are deeply buried and difficult to elucidate on superficial observation. The study of the role of various classes of natural substances acquire sense only at the level of the population and biocenosis, i.e., communities of organisms. However narrow the range with which we may deal in the understanding of vital phenomena, from the molecular level to the level of the whole organism, we must keep within our field of view the life of nature as a whole, in all forms of its manifestation and in all its diversity.

To the three main types of adaptation in ecology — structural, physiological, and behavioral — we are obviously right to add another not unimportant type — biochemical.

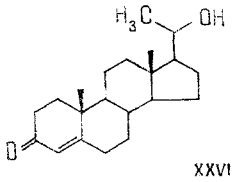
In conclusion, returning to the question originally propounded — "why do plants need molting hormones?" — we may convince ourselves that it should be followed by another, namely "in the force of what causes did insects begin to use steroid compounds (concretely, ecdysteroids) belonging to the vegetable kingdom, as molting hormones?"

Such a change does not appear strange if we recall the following example from chemical evolution.

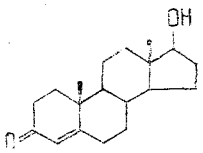
The fairly common water beetle *Dysticus marginalis*, when protecting itself from fish and frogs, secretes a milky liquid containing more than 10% of cortexone (XXV) [78]. The protective secretion of the water beetle *Illybius fenestratus* contains pregnelolone (XXVI), testosterone (XXVII), estrone (XXVIII), and estradiol (XXIX) [79]. Thus, the paradoxical question arises unhidden: why did water beetles prefer to use as protective pheromones those steroid compounds which in higher animals fulfil the function of adrenal hormones and sex hormones?



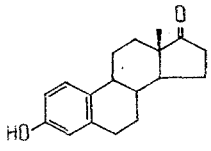

XXV



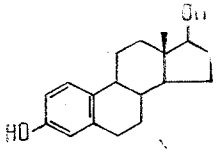

XXVI




XXVII




XXVIII




XXIX

The adrenal hormones and sex hormones of higher animals in the protective secretions of the water beetles *Dysticus marginalis* and *Illybius fenestratus*: cortexone (XXV), 4-hydroxypregn-20-en-3-one (XXVI), testosterone (XXVII), estrone (XXVIII), and estradiol (XXIX).

What is the use to man of molting hormones?

On the practical level, the discovery of plants containing ecdysteroids in considerable amount can be used in various directions:

chemically individual molting hormones permit a more far-reaching examination of the mechanism of the hormonal regulation of vitally important functions of insects;

on the basis of a real source of raw materials it is perhaps possible successfully to suggest biological insecticides harmless for the environment;

the idea of using molting hormones directly for active intervention on the metamorphosis of the silkworm with the aim of increasing its productivity is attractive; and

new preparations with an anabolic action for use in medicine and animal husbrandry can perhaps be created.

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# ASPARAGUS POLYSACCHARIDES.

## I. ISOLATION AND CHARACTERIZATION OF POLYSACCHARIDES OF *A. neglectus*: GLUCOMANNANS OF THE ROOTS

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Water-soluble polysaccharides and pectin substances have been isolated from various organs of *A. neglectus* and their quantitative amounts and monosacchardie compositions have been determined. Native acetylated glucomannans (A, B, and C) containing 13, 15, and 20% of acetyl groups and being homogeneous according to the results of gel chromatography have been isolated from the roots. On the basis of the results of periodate oxidation, methylation, and IR spectroscopy it has been established that the neutral polysaccharides of the roots of *A. neglectus* is a mixture of three glucomannans consisting of β-1→4 linear-bound D-gluco- and D-mannopyranose residues.

In Central Asia, the genus *Asparagus* is represented by 13 species [1]. There are reports on the study of the carbohydrates of *Asparagus* [2-6], but the water-soluble polysaccharides (WSPSs) and pectin substances (PecSs) of this genus have been little studied.

We have investigated the amounts of polysaccharides and their monosaccharide compositions in various organs of *A. neglectus* Kar. et Kir collected on May 17, 1977 (Temirlik, KirgSSR) and previously treated with chloroform and ethanol to eliminate low-molecular-weight substances.

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