SUMMARY

A new triterpenoid ketone of the cycloartane series — 3-dehydrocycloasgenin C — having the structure of 6α,16β,24,25-tetrahydroxy-24R-cycloartan-3-one — has been isolated from the roots of the plant *Astragalus tashkendicus* Bge. A passage to 3-dehydrocycloasgenin C from cycloasgenin C has been performed.

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BIOLOGICAL ACTIVITY OF PHYTOECDYSTEROIDS AND THEIR DERIVATIVES IN *in vitro* TESTS ON *Drosophila melanogaster* CELLS

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The specific biological activities of a number of ecdysteroids have been investigated in tests on the inhibition of the growth of a culture of *Drosophila melanogaster* cells and in experiments on the induction of the metamorphosis of imaginal disks. Some relationships between structure and biological action have been established.

We have investigated the biological activity of ecdysteroids isolated from plants growing in Central Asia, and also some ecdysteroid derivatives obtained chemically.

To determine activities we used tests based on the inhibiting action of the molting hormones on the growth of *Drosophila melanogaster* cells in a culture [1, 2] and also on the capacity of the ecdysteroids for inhibiting the metamorphosis of the imaginal disks — the lenticular rudiments of the organs of the adult fly [3].

With respect to the strength of their inhibiting action on the growth of cells in culture and their capacity for inducing the metamorphosis of the imaginal disks, the compounds that we investigated may be divided arbitrarily into three groups. To the first group must be assigned ecdysterone (I), polypodin B (II), and 24 (28)-dehydromakisterone A (III). All these compounds have a natural origin.

Ecdysterone (I) is the most common molting and pupation (metamorphosis) hormone; it has been detected in the majority of arthropods and is frequently found in plants. This compound can be considered as a standard.

Polypodin B (II) has been isolated only from plants; in its chemical structure it is close to ecdysterone (polypodin B is 5-hydroxyecdysterone). A high biological activity of polypodin B in tests on larvae of *Calliphora* flies and in experiments on the induction of evag-

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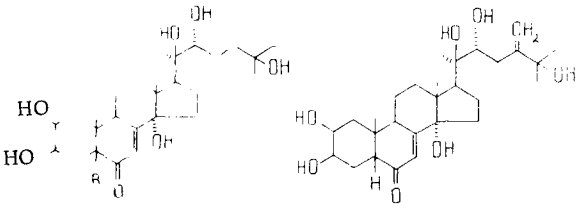
TABLE 1. Comparative Characteristics of the Biological Activities of the Ecdysteroids

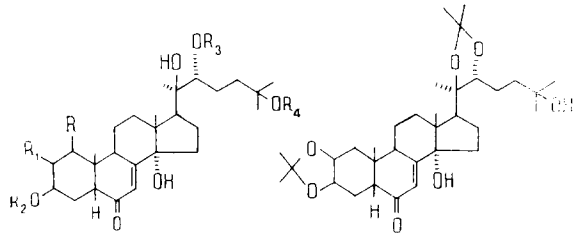
Ecdysteroid *	Suppression of cell growth, %	Induction of the metamorphosis of imaginal disks	Conclusion on activity
I. Ecdysterone [10]	95	Induces	Active
II. Polypodin B [11]	95	Induces [3]	Active
III. 24(28)-Dehydromakisterone [13]	95	Induces	Active
IV. 2-Deoxyecdysterone [7]	10–30	Induces †	Inactive
V. Integristerone A [12]	10—30	Does not induce	Inactive
VI. Sileneoside A [11]	5—10	Does not induce	Inative
VII. Ecdysterone 2,3,22,25- tetraacetate [10]	20—50	Does not induce	Inactive
VIII. Integristerone A 1.2.3.22-tetraacetate [12]	10—20	Does not induce	Inactive
XI. Ecdysterone 2,3:20,22-diacetonide [10]	20—50	Does not induce	Inactive

*Only those literature sources are shown in which the isolation of the ecdysteroids directly serving as the object of the present investigation from plant sources is described.
†Concentration greater than 0.1 μg/ml [4].



I R = H
II R = OH

III



IV R = R_1 = R_2 = R_3 = R_4 = H
V R_2 = R_3 = R_4 = H; R = R_1 = OH
VI. R = R_2 = R_4 = H; R_1 = OH; R_3 = α-D-Gal
VII R = H; R_1 = OAc; R_2 = R_3 = R_4 = Ac
VIII. R_4 = H; R = R_1 = OAc; R_2 = R_3 = Ac

IX

ination (metamorphosis) of the imaginal disks of *D. melanogaster* is known [4]. The effective inhibiting action of polypodin B on the growth of cells (Table 1) must therefore be regarded as completely normal.

24(28)-Dehydromakisterone A (III) is a rare ecdysteroid found only in plants. In contrast to ecdysterone (I), this compound has a methylene group at C-24. Its analog — makisterone A (24-methylecdysterone) — has been detected in the embryo of the bug *Oncopeltus fasciatus* [5] and has exhibited a high activity (at the level of ecdysterone in *in vivo* experiments [4]). In our experiments it was found that the replacement of the C-24 methyl group by a methylene group did not lead to a decrease in biological activity. In the strength of its action of the growth of cells and the degree of induction of metamorphosis of imaginal disks, 24(28)-dehydromakisterone A (III) was not inferior to ecdysterone.

The second group of ecdysteroids — 2-deoxyecdysterone (IV), integristerone A (V), and sileneoside A (VI) — are likewise substances of natural origin. In comparison with the compounds of the first group their inhibiting activity is weak and they do not promote the metamorphosis of imaginal disks.

2-Deoxyecdysterone (IV) has been detected both in plants [6, 7], and in animal organisms [8, 9]. The ecdysteroid (IV) suppresses the growth of cells by 10-30%, which agrees with observations made previously concerning its lower efficacy, in comparison with ecdysterone, in experiments on the induction of the metamoprhosis of imaginal disks [4]. It must be mentioned that in tests on larvae of *Calliphora* flies the activities of ecdysterone (I) and of 2-deoxyecdysterone (IV) were identical [8]. This is probably due to the fact that in the insect organism 2-deoxyecdysterone (IV) is rapidly converted into ecdysterone (I).

The weak activity in both tests of integristerone A (V) was unexpected. As compared with ecdysterone, this phytoecdysteroid contains an additional 1β-hydroxy group. The hypothesis has been expressed that the ecdysteroid molecule is bound to the receptor with the aid of the 2β- and 3β-hydroxy groups [4]. The fact that in the integristerone A (V) molecule the three hydroxy groups are adjacent to one another (1β, 2β, 3β) possibly leads to a competing interaction with the receptor of the 1β- and 2β-hydroxyls in place of the usual 2β- and 3β-hydroxyls. Such uncharacteristic attachment is obviously functionally inactive.

The reason for the low activity of sileneoside A (VI) probably resides in the presence of a D-galactose residue at carbon atom 22 of ecdysterone (sileneoside A = ecdysterone 22-O-α-D-galactopyranoside). It is known that a hydroxy group at C-22 largely determines the activity of ecdysone-like compounds, promoting their binding with the receptor [4]. In the case of sileneoside A (VI), the bulky sugar substituent blocks the position of binding of the side chain with the receptor, because of which its activity falls.

The third and last group of compounds consists of derivatives of ecdysterone and of integristerone A. These compounds are not found in nature and they are obtained by partial synthesis from native ecdysteroids. Their low activity in both tests can be explained by the assumption that the hydroxy functions interacting with the receptor have been replaced by acetic acid or acetone residues. The fluctuation of the results in experiments on the suppression of the growth of cell cultures with ecdysterone tetraacetate (VI) and its diacetonide (IX) are possibly explained by metabolic transformations leading partially to the formation of free ecdysterone.

It can be seen from Table 1 that ecdysteroids feebly suppressing the growth of cells also do not cause the metamorphosis of imaginal disks.

EXPERIMENTAL

The *D. melanogaster* 67j 25D cell culture, which had undergone subculturing for a long time [14] was used. The cells were cultivated on C-45 medium [15] with serum (Gibco). The initial concentration of the cells in the bottles (volume of medium 0.5 ml) was $1.0\text{-}1.5 \cdot 10^6$ cells/ml. After 7 days (without the addition of ecdysteroid), it had reached $6\text{-}8 \cdot 10^6$ cells in 1 ml. The imaginal disks were cultivated on C-58 medium, which consists of modified C-45 medium in which the glucose has been replaced by an equimolar amount of dulcitol, for 12 h. The ecdysteroids were used in a concentration of 0.1 μg/ml.

SUMMARY

The specific biological activities of a number of natural ecdysteroids and their derivatives have been investigated. It has been shown that substances weakly suppressing the growth of cell cultures do not initiate the metamorphosis of imaginal disks, either.

It has been found that the specific biological action of the ecdysteroids depends to a considerable degree on the accessibility of the hydroxy groups interacting with the receptor.

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