TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS.

XVIII. 3-DEHYDROCYCLOASGENIN C FROM *Astragalus taschkendicus*

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A new genin — 3-dehydrocycloasgenin C — has been isolated from the roots of the plant *Astragalus taschkendicus* Bge. (*Leguminosae*) and its structure has been established on the basis of chemical transformations and spectral characteristics as 6α,16β,24,25-tetrahydroxy-24R-cycloartan-3-one.

Continuing a study of the methylsteroids of *Astragalus taschkendicus* Bge. (*Leguminosae*) [1], we have isolated a new compound of genin nature which we have called 3-dehydrocycloasgenin C (I, scheme). The present paper is devoted to a proof of the structure of this substance.

The molecular formula of the genin (I), $C_{30}H_{50}O_5$, the presence in its PMR spectrum of two one-proton doublets at 0.266 and 0.56 ppm (Table 1), which are characteristic for a 1,1,2,2-tetrasubstituted cyclopropane ring, and also the signals of seven methyl groups, permitted this compound to be assigned to the triterpenoids of the cycloartane series [2]. In agreement with this, the IR spectrum showed an absorption band at 3055 cm^{-1} corresponding to a CH_2 group of a cyclopropane ring [3]. The IR spectrum also showed absorption at 3540-3310 cm^{-1} (OH) and 1750 cm^{-1} (C=O).

Cotton effects on the CD curve of 3-dehydrocycloasgenin C (I) were similar in magnitudes and signs [$\Delta\varepsilon$ = −0.04 (322 nm), $\Delta\varepsilon$ = +1.60 (287 nm)] to the corresponding indices of the 6α-hydroxy-3-ketocycloartanes [1, 4]. On this basis, it was possible to assume that compound (I) contained a keto function at C-3 and a 6α-secondary hydroxy group.

An additional confirmation of the presence of a 6α-hydroxy group in the molecule of the genin (I) was the presence in the PMR spectrum of a one-proton multiplet at 3.60 ppm. This magnitude practically coincides with the value of the chemical shift for 6β-H in the PMR spectrum of cycloasgenin C (III) [5]. As was to be expected, the signal of one of the methyl groups in the PMR spectrum of 3-dehydrocycloasgenin C (I) taken in deuteropyridine shifted downfield under the influence of the 6α-hydroxy function and was observed at 1.64 ppm [1, 4-6].

Taking into account the presence of a keto function at C-3, it followed from the molecular formula of compound (I), $C_{30}H_{50}O_5$, that the side chain had an acyclic structure.

The acetylation of 3-dehydrocycloasgenin C (I) with acetic anhydride in pyridine gave the triacetate (IV), the IR spectrum of which contained hydroxyl absorption. Consequently, the three remaining unidentified oxygen functions were represented by hydroxy groups.

In the mass spectrum of genin (I) there was the peak of an ion with m/z 327 arising by the cleavage of the C-17—C-20 bond and the elimination of one molecule of water. Thus, the side chain and the polycyclic moiety of the molecule each contained two hydroxyls.

In the PMR spectrum of the genin (I), protons located geminally to secondary hydroxy groups resonated at 3.60, 3.68, and 4.56 ppm. The correctness of this conclusion was confirmed by the paramagnetic shift of the signals under consideration in the spectrum of the triacetate (IV). This meant that the fourth hydroxy group must have a tertiary nature. The chemical shift of the weakest-field signal (4.56 ppm) in the PMR spectrum of genin (I) agreed well with that for the 16α-H atom in the spectrum of cycloasgenin C (III) [5]. We are therefore justified in assuming that the molecule of 3-dehydrocycloasgenin C (I) contains a 16β-hydroxy group.

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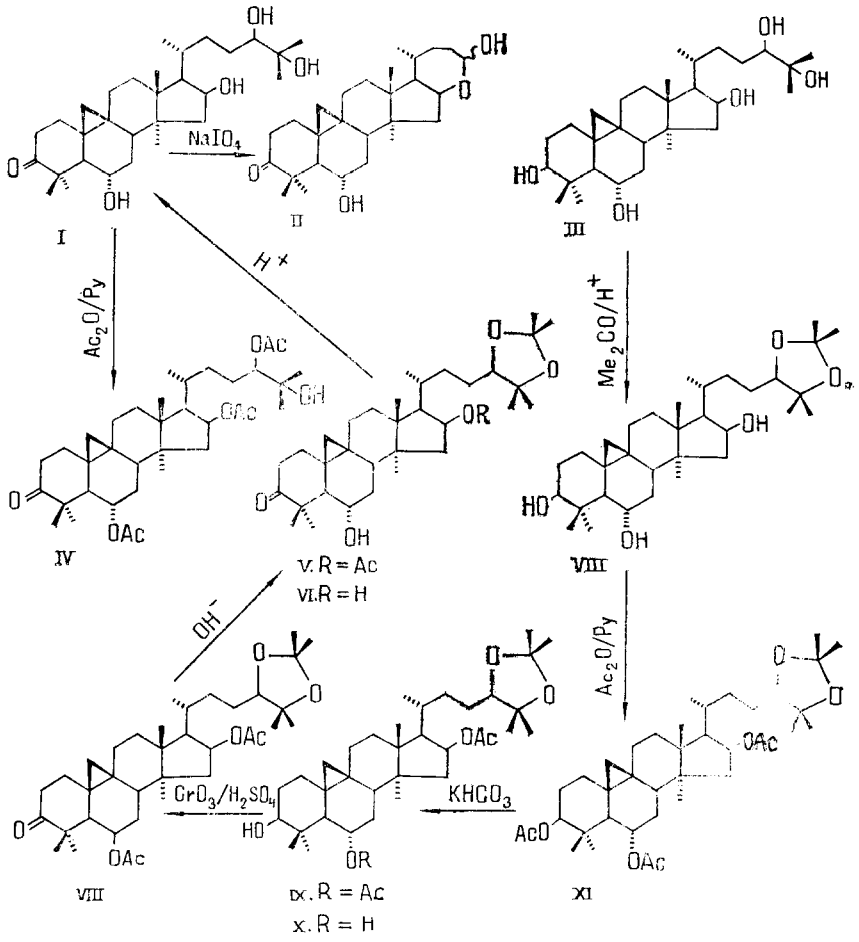
The periodate oxidation of genin (I) led to the nor compound (II) (M^+ 430). The formation of substance (II) showed that the hydroxy groups of the side chain formed an α-glycol system. The loss of 60 mass units on passing from (I) (M^+ 490) to (II) unambiguously determined the position of the glycol group at C-24—C-25.

In the PMR spectrum of the nor compound (II) it was possible clearly to trace the signal of the H-24 anomeric proton at 4.56 ppm. The latter indicated the semiacetal nature of substance (II). This was also shown by a diamagnetic shift of the H-16 signal observed in the form of a sextet at 4.15 ppm. The facts given determined substance (II) as 6α,24-dihydroxy-16β,24ξ-epoxy-25-norcycloartan-3-one.

It has been shown that in the cycloasgenin C (III) [5] and its 3-keto-11α-hydroxy derivative — cycloasgenin B [4] — isolated previously from the same plant, the C-24 chiral center has the R configuration. On this basis, we ascribed a similar stereochemistry to the C-24 atom of the genin (I) under consideration



Thus, 3-dehydrocycloasgenin C (I) is 6α,16β,24,25-tetrahydroxy-24R-cycloartan-3-one.

To confirm the proposed structure we effected the passage from cycloasgenin C (III) to 3-dehydrocycloasgenin C (I). For this purpose the pentaol (III) was converted into the 24,25-acetonide (VII) [5], from which the triacetate (XI) was obtained by acetylation [5]. Saponification of (XI) under mild conditions led to the formation of the diacetate (IX) and the monoacetate (X). It can be seen from a comparative analysis of the PMR spectra of compounds (XI), (X), and (IX) (Table 1), that substance (IX) was the 6,16-diacetate and (X) the 16-monoacetate.

The Jones oxidation [7] of the 6,16-diacetate (IX) gave the 3-keto derivative (VIII). The IR spectrum of the ketone (VIII) showed an absorption band at 1710 cm^{-1}, and in the PMR spectrum the H-3 signal was absent.

TABLE 1. Chemical Shifts of the Protons of 3-Dehydrocycloasgenin C (I), Cycloasgenin C (III) and their Derivatives (δ, ppm 0 — HMDS

Compound	Positions of the protons						
	H-3	H-6	H-16	2H-19	H-24	CH_3 group	OAc
I	—	[3.60 m*]	[4.56 m $W_{1/2}$=14Hz]	[0,26; 0.56 д. ^2J=4Hz]	[3,68q* ^3J=10and3Hz]	[0,86; 1,01 d ; 1,28; 1,34; 1,38 (2×CH_3); 1,64]	—
II	—	3,50 m $W_{1/2}$=16 Hz	4.15 sx ΣJ=23 Hz	0,38; 0,56 д, ^2J=4 Hz	4,56 m $W_{1/2}$=14 Hz	0,88 d; 0,90; 1,08; 1,18; 1,28	—
III	[~3,60m*]	[~3,60m*]	[4.54m]	[0.21; 0,48 d]	[~3.60 m*]	[0,91; 0,98d; 1.21; 1,27; 1,35; 1,36; 1,73]	—
IV	—	4,45m $W_{1/2}$=15 Hz	5,26 m $W_{1/2}$=1 6 Hz	0,20; 0,44d, ^2J=4 Hz	4,66q ^3J=10and 3Hz	0,86; 0,88 d; 1,02; 1 ,04; 1,08; 1,12 (2×CH_3)	1,96; 1,99; 2,02
V	—	3,48m*	5.20 m $W_{1/2}$=14 Hz	0,39; 0,59 d ^2J=4 Hz	3,48m*	0,88 d; 0 91; 1,02; 1,10; 1,16 (2×CH_3); 1,24; 1,29; 1.32	1,97
VI	—	3,47m*	4.41 sx $\Sigma J \approx 22$ Hz	0,38; 0.59 d ^2J=4 Hz	3,62q * ^3J=9and 4 Hz	0,88; 0.92 d; 1.04; 1.10; 1,16; 1,18; 1,26; 1,29; 1,34	—
VIII	—	4,66 sx	5.20 m	0,46; 0,70 d ^2J=4 Hz	3,54 q	0,89; ~0,90 d; 1,02; 1,08; 1,10 (2×CH_3); 1,17; 1 24; 1.32	1,96; 1,97
IX	3,26q	4.68sx	5.20 sx	0,30; 0,52 d ^2J=4 Hz	3,52 q	0,86; ~0,87 d; 0,89; 0,93; 1,03; 1,07; 1,18; 1,27; 1,32	1,96; 1,98
X	3,22 m	3,48 m	5,20 m $W_{1/2}$=14 Hz	0,31; 0,45 d ^2J=4 Hz	3,48m*	0,88 d; 0,89 (2×CH_3); 1,02; 1,08; 1,17; 1,18; 1,24; 1,32	1,96
XI	4,53m*	4,53m*	5,16 m	0,30; 0,55d	3,46 q	0,80; 0.88 d; 0,90; 0,94; 1.01; 1,07; 1,17; 1,24; 1.31	1,90; 1,96; 1.98

The spectra were taken in $CDCl_3$ or in C_5D_5N. The indices given in square brackets were obtained with the use of C_5D_5N. The signals marked with an asterisk in the horizontal rows are superposed upon one another. The signals of the methyl groups have a singlet nature with the exception of the CH_3 at C-20, which has a doublet nature; d — doublet; q — quartet; sx — sextet; m — multiplet.

Saponification of the 3-keto derivative (VIII) led to compounds (V) and (VI). The IR, mass, and PMR spectra of substance (V) showed the presence of one acetate group. A weak-field signal at 5.20 ppm, assigned to H-16, in the PMR spectrum of product (V) determined the position of the acetyl group at C-16.

According to its IR, mass, and PMR spectra, compound (VI) contained no acetyl groups. The treatment of product (VI) with a 0.5% methanolic solution of sulfuric acid led to a substance which was identical with 3-dehydrocycloasgenin C (I) from its physicochemical constants, spectral characteristics, and its R_f value on TLC.

The preparation of 3-dehydrocycloasgenin C (I) from cycloasgenin C (III) showed the position of the oxygen functions at C-3, C-6, C-6, C-16, C-24, C-25 and the stereochemistry of the chiral centers as C-6α, C-16β, and C-24R.

EXPERIMENTAL

For general observations see [1]. The following solvent systems were used: 1) chloroform–methanol (15:1); 2) chloroform–methanol (20:1); 3) chloroform–ethyl acetate (1:3); 4) benzene–chloroform–ethyl acetate (5:1:1); 5) benzene–ethyl acetate (2:1); and 6) benzene–ethyl acetate (1:1).

PMR spectra were taken on a Varian XL-100-15 instrument in deuterochloroform or deuteropyridine (δ, ppm, 0 – HMDS).

Isolation of 3-Dehydrocycloasgenin C (I). The intermediate fractions collected in the isolation of cycloasgenins A and B [1] were repeatedly chromatographed on a column with elution by system 1. This gave 70 mg (0.0039% of the air-dry raw material) of 3-dehydrocycloasgenin C (I).

3-Dehydrocycloasgenin C (I), $C_{30}H_{50}O_5$, mp 208-210°C (from ethyl acetate), $[\alpha]_D^{22}$ + 82.5 ± 2° (c 0.8; methanol). ν_{max}^{KBr}, cm^{-1}: 3540-3310 (OH), 3055 (CH_2 of a cyclopropane ring), 1705 (C=O at C-3). CD (c 0.1, methanol): Δε = −0.04 (322 nm), Δε = +1.60 (287 nm). Mass spectrum, m/z (%): M^+ 490 (5.1), 472 (10.3), 454 (7.7), 439 (12.8), 421 (7.7), 413 (7.7), 395 (12.8), 386 (6.4), 329 (15.4), 327 (35.9), 311 (12.8), 309 (17.9), 234 (30.8), 203 (48.7), 165 (69.2), 161 (66.6), 149 (100).

6α,24-Dihydroxy-16β,24ξ-epoxy-25-norcycloartan-3-one (II) from (I). To 30 mg of 3-dehydrocycloasgenin C (I) in 3 ml of methanol was added 95 mg of sodium periodate in 0.5 ml of water and the mixture was left at room temperature for 2.5 h. Then the excess of oxidant was decomposed by the addition of a few drops of ethylene glycol. After this, the reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with water, dried with anhydrous sodium sulfate, and evaporated. The residue was chromatographed on a column with elution by system 2. This gave 19 mg of the nor compound (II), $C_{27}H_{42}O_4$, mp 121-123°C (from system 2), $[\alpha]_D^{22}$ + 67.5 ± 2° (c 0.77; methanol). ν_{max}^{KBr}, cm^{-1}: 3480-3370 (OH), 3055 (CH_2 of a cyclopropane ring), 1708 (C=O at C-3). Mass spectrum, m/z (%): M^+ 430 (9.0), 415 (6.4), 412 (17.9), 397 (19.2), 379 (10.3), 369 (6.4), 361 (3.2), 341 (4.5), 329 (20.5), 327 (11.5), 311 (7.7), 309 (10.3), 256 (11.5), 246 (11.5), 231 (15.4), 213 (23.0), 203 (20.5), 149 (100).

3-Dehydrocycloasgenin C 6,16,24-Triacetate (IV) from (I). 3-Dehydrocycloasgenin C (I) (30 mg) was acetylated with 0.25 ml of acetic anhydride in 0.5 ml of pyridine at room temperature for 24 h. After the solvents had been distilled off, the residue was chromatographed on a column with elution by system 2. This gave 27 mg of the amorphous triacetate (IV), $C_{36}H_{56}O_8$, $[\alpha]_D^{21}$ + 90.9 ± 2° (c 0.88; methanol). ν_{max}^{KBr}, cm^{-1}: 3560-3435 (OH), 3060 (CH_2 of a cyclopropane ring); 1750-1705, 1250 (C=O at C-3 and ester groups). Mass spectrum, m/z (%): M^+ 616 (1.9), 598 (4.8), 556 (25.0), 538 (6.7), 514 (4.8), 496 (32.7), 481 (19.2), 478 (9.6), 470 (10.6), 463 (6.7), 454 (8.7), 436 (15.4), 421 (25.0), 403 (11.5), 369 (75.0), 149 (100).

Cycloasgenin C 24,25-Acetonide (VII) from (III). To 786 mg of cycloasgenin C (III) was added 35 ml of acetone containing 0.2% of sulfuric acid. The reaction mixture was stirred and was then left at room temperature for 2 days. After this it was poured into 150 ml of water and the reaction products were extracted with chloroform. The chloroform extract was washed with water, dried with anhydrous sodium sulfate, and evaporated. The residue was chromatographed on a column with elution by system 3. This gave 740 mg of compound (VII) with mp 188-189°C (from ethyl acetate), $[\alpha]_D^{22}$ + 60 ± 2° (c 0.9; methanol) [5].

Cycloasgenin C 3,6,16-Triacetate 24,25-Acetonide (XI) from (VII). Cycloasgenin C acetonide (VII) (740 mg) was acetylated with 8 ml of acetic anhydride in 16 ml of pyridine at room temperature for 4 days. After this, the reaction mixture was poured into ice water and the resulting precipitate was filtered off and washed with water. The product was purified by chromatography on a column with elution by system 4. This gave 880 mg of substance (XI) with mp 133-134°C (from methanol), $[\alpha]_D^{22}$ + 91.7 ± 2° (c 0.8; methanol) [5].

6,16-Diacetate (IX) and 16-Monoacetate (X) of Cycloasgenin C 24,25-Acetonide from (XI). To 880 mg of substance (XI) in 20 ml of methanol was added a solution of 200 mg of $KHCO_3$ in 20 ml of the same solvent, and the mixture was left at 60°C for 30 days. Then it was poured into 200 ml of water and extracted with chloroform. The chloroform extract was washed with water and dried with anhydrous sodium sulfate. The residue after evaporation of the solvent was chromatographed on a column with elution by system 5. This gave 450 mg of the 6,16-diacetate of cycloasgenin C 24,25-acetonide (IX), $C_{37}H_{60}O_7$, mp 97-99°C (from methanol), $[\alpha]_D^{22}$ + 125 ± 2° (c 0.96; methanol). ν_{max}^{KBr}, cm^{-1}: 3560-3480 (OH), 3040 (CH_2 of a cyclopropane ring), 1738, 1250 (ester group). Mass spectrum, m/z (%): M^+ 616 (2.0), 601 (57.1), 598 (7.1), 556 (65.3), 538 (100), 523 (8.2), 499 (20.4), 481 (24.5), 463 (14.3), 438 (26.5), 423 (24.5), 405 (30.6), 371 (12.2), 331 (14.3), 311 (26.5), 293 (26.5), 273 (48.9).

Washing the column with the same system yielded 130 mg of the 16-monoacetate of cycloasgenin C 24,25-acetonide (X), $C_{35}H_{58}O_6$, mp 211-213°C (from methanol), $[\alpha]_D^{22}$ + 72.3 ± 2° (c 0.83; methanol). ν_{max}^{KBr}, cm^{-1}: 3540-3495 (OH), 3047 (CH_2 of a cyclopropane ring), 1725, 1250 (ester group). Mass spectrum, m/z (%): M^+ 574 (0.36), 559 (37.0), 556 (11.1), 538 (11.1), 481 (9.3), 456 (25.9), 439 (12.9), 423 (29.6), 405 (22.2), 329 (81.5), 311 (100).

6α,16β,24,25-Tetrahydroxy-24R-cycloartan-3-one 6,16-Diacetate 24,25-Acetonide (VIII) from (IX). At −4°C, 1 ml of the Jones reagent [7] was added to 450 mg of the 6,16-diacetate 24,25-acetonide of cycloasgenin C (IX) in 50 ml of acetone, and the reaction mixture was stirred for 30 min. The excess of oxidant was destroyed by the addition of a few milliliters of methanol. Then the mixture was diluted with water and the reaction products were extracted with chloroform. The chloroform extract was washed with water and dried with anhydrous sodium sulfate. The residue from the evaporation of the chloroform was chromatographed on a column with elution by system 4. This yielded 420 mg of substance (VIII), $C_{37}H_{58}O_7$, mp 132-134°C (from methanol), $[\alpha]_D^{22}$ + 220 ± 2° (c 0.5; methanol). ν_{max}^{KBr}, cm^{-1}: 3045 (CH_2 of a cyclopropane ring); 1710 (C=O at C-3); 1740, 1250 (ester group). Mass spectrum, m/z (%): M^+ 614 (1.7), 599 (100), 554 (41.7), 539 (12.5), 497 (29.2), 479 (25.0), 436 (66.7), 421 (58.3), 369 (50.0), 351 (25.0), 331 (20.8), 309 (75.0), 295 (20.8), 273 (58.3).

6α,16β,24,25-Tetrahydroxy-24R-cycloartan-3-one 16-Monoacetate 24,25-Acetonide (V) and 6α,16β,24,25-Tetrahydroxy-24R-cycloartan-3-one 24,25-Acetonide (VI) from (VIII). A solution of 1 g of potassium hydroxide in 50 ml of methanol was added to 420 mg of compound (VIII) in 100 ml of the same solvent, and the mixture was boiled on the water bath for 80 h. Then it was diluted with 2 volumes of water and the methanol was evaporated off. The resulting precipitate was filtered off, washed with water, and dried. Then it was chromatographed on a column with elution by system 6. This gave 110 mg of substance (V), $C_{35}H_{56}O_6$, mp 253-255°C (from methanol), $[\alpha]_D^{22}$ + 166.7 ± 2° (c 0.42; chloroform). ν_{max}^{KBr}, cm^{-1}: 3540 (OH), 3065 (CH_2 of a cyclopropane ring), 1705 (C=O at C-3), 1720, 1280-1250 (ester group). Mass spectrum m/z (%): M^+ 572 (5.7), 557 (20.5), 554 (7.9), 512 (5.7), 497 (4.5), 479 (3.4), 468 (5.7), 454 (40.9), 436 (31.8), 421 (29.5), 327 (100), 309 (65.9).

On continuing the elution of the column with the same system, we obtained 150 mg of compound (VI), $C_{33}H_{54}O_5$, mp 199-201°C (from methanol), $[\alpha]_D^{22}$ + 88 ± 2° (c 0.5; methanol). ν_{max}^{KBr}, cm^{-1}: 3535-3400 (OH), 3060 (CH_2 of a cyclopropane ring); 1710 (C=O at C-3). Mass spectrum, m/z (%): M^+ 530 (16.6), 515 (100), 512 (27.7), 497 (13.3), 494 (11.1), 472 (33.3), 454 (72.2), 439 (55.6), 436 (38.9), 432 (22.2), 426 (27.7), 421 (50.0), 411 (22.2), 399 (77.7), 327 (83.3), 309 (88.9).

6α,16β,24,25-Tetrahydroxy-24R-cycloartan-3-one (I) — 3-Dehydrocycloasgenin C — from (VI). A solution of 80 mg of substance (VI) in 15 ml of methanol containing 0.5% of sulfuric acid was heated at 40°C for 3 h. After this, the reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with water, dried with anhydrous sodium sulfate and evaporated. The residue was chromatographed on a column with elution by system 1. This gave 45 mg of 6α,16β,24,25-tetrahydroxy-24R-cycloartan-3-one (I), $C_{30}H_{50}O_5$, mp 208-210°C (from ethyl acetate), $[\alpha]_D^{22}$ + 83 ± 2° (c 0.7; methanol). This compound was also shown to be identical with 3-dehydrocycloasgenin C from the characteristics of its IR, mass, and CD spectra and its R_f values on TLC (systems 1 and 2).

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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