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PHYTOECDYSTEROIDS OF PLANTS OF THE *Silene* GENUS AND THE DYNAMICS OF THEIR ACCUMULATION

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The ecdysteroids of four species of the genus Silene *L. have been studied. Six ecdysteroids have been isolated and identified, and the dynamics of the accumulation of the ecdysteroids in relation to the phases of development of the plants have been determined.*

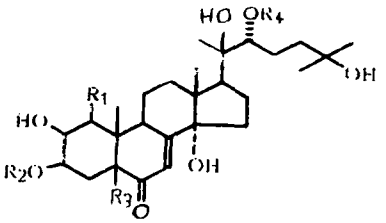
Ecdysteroids occupy a special position among the various steroid metabolites produced by plants. They possess anabolic, adaptogenic, tonic, hypoglycemic, and hepatoprotective effects and exert a positive action in the treatment of myocarditises [1].

Plants of the genus *Silene* (fam. Caryophyllaceae) appear promising in the search for ecdysone-containing plants among representatives of the domestic flora. The Caryophyllaceae family is distinguished from other families in which various ecdysteroids have been detected [2]. Up to the present, among representatives of the *Silene* genus ecdysteroids have been found in 20 species.

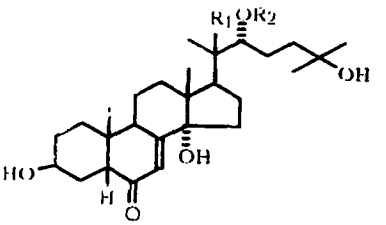
We have studied four species of *Silene* introduced into sierozem-oasis soil in an experimental plot of the Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan: *S. supina, S. viridiflora, S. nutans,* and *S. tatarica*. Seeds of these plants were obtained from the Republic of Uzbekistan Botanical Garden.

The air-dry epigeal parts of the plants gathered in all phenophases in 1992-1995 were analyzed.

By thin-layer chromatography using vanillin/sulfuric acid [3] and by column chromatography [4] we have shown in plants of all four species the presence of a number of main ecdysteroids with various structures: ecdysterone (**1**), polypodin (B) (**2**), 2-deoxyecdysterone 22-O-benzoate (**3**), integristerone A (**4**), sileneoside A (**5**), and sileneoside D (**6**).



1. $R_1=R_2=R_3=R_4=H$
2. $R_1=R_2=R_4=H$; $R_3=OH$
4. $R_2=R_3=R_4=H$; $R_1=OH$
5. $R_1=R_2=R_3=H$; $R_4=\alpha$-D-Gal
6. $R_1=R_3=R_4=H$; $R_2=\alpha$-D-Gal
7. $R_1=R_2=R_3=H$; $R_4=COC_6H_5$



3. $R_1=OH$; $R_2=COC_6H_5$
8. $R_1=OH$; $R_2=H$

Ecdysteroids (**1**), (**2**), (**4**), and (**6**) were isolated from *S. viridiflora*, (**1**) and (**5**) from *S. supina*, and (**1**) and (**3**) from *S. nutans* and *S. tatarica* (Fig. 1).

A study of the dynamics of the accumulation of the ecdysteroids showed that in the process of development of the plants their amount increased, reaching a maximum in the phase of mass budding and flowering, and it then fell to the end of veget-

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TABLE 1. Chemical Shifts of the Signals of the Protons of Ecdysterone (1), Ecdysterone 22-O-Benzoate (7), 2-Deoxyecdysterone (8), and 2-Deoxyecdysterone 22-O-Benzoate (3) (C_5D_5N, δ, ppm, 0 — TMS)

Compound	Positions of the protons									
	H-2	H-3	H-7	H-9	H-22	CH_3-18	CH_3-19	CH_3-21	CH_3-26; 27	arom. protons
Ecdysterone (1)	1.0	4.3	6.21	3.57	3.84	1.19	1.06	1.55	1.34	–
Ecdysterone 22-O-benzoate (7)	1.0	4.3	6.22	3.60	5.76	1.21	1.10	1.78	1.32	7.40(3H); 8.27(2H)
2-Deoxyecdysterone (8)	–	4.11	6.22	3.52	3.85	1.22	1.05	1.58	1.38	–
2-Deoxyecdysterone 22-O-benzoate (3)	–	4.14	6.25	3.58	5.77	1.23	1.07	1.81	1.32	7.53(3H); 8.28(2H)

*The signals of the protons of the methyl groups were singlets; in compound (4), H-7 appeared in the form of a doublet with J = 1 Hz, and in other cases as a broadened singlet; the H-2, H-3, H-9, and H-22 signals were multiplets.

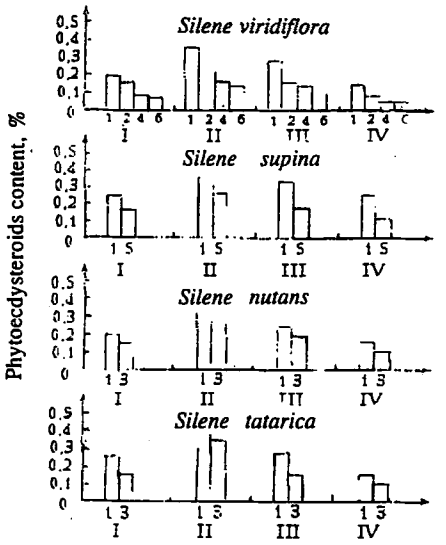

Fig 1. Dynamics of the accumulation of ecdysteroids according to the phases of development of the plants: I) beginning of vegetation; II) phase of mass budding and flowering; III) fruit-bearing phase; IV) end of vegetation.

ation (see Fig. 1). A similar dynamics of the accumulation of ecdysteroids has been described by Yu. D. Kholodova et al. for the epigeal part of *Serratula xeranthemoides* Bieb. [5].

It must be mentioned that the highest level of ecdysteroids in the form of ecdysterone (0.35-0.4%) was found in the budding and flowering phase in the epigeal parts of all the plant species studied. In this phase, 2-deoxyecdysterone 22-benzoate (**3**) was detected in amounts of 0.27 and 0.28%, respectively, in the plants *S. tatarica* and *S. nutans*. Sileneoside A (**5**), in an amount of 0.25%, was found only in *S. supina*.

The individual steroids isolated were identified on the basis of IR, PMR, and mass spectroscopies, and also by comparison with standard specimens (see the Experimental part).

In an investigation of the epigeal part of *S. nutans*, in addition to ecdysterone (**1**), we isolated the ecdysteroid (**3**).

Absorption in the IR spectrum at 1705 and 1285 cm^{-1}, in combination with bands characterizing a benzene ring (1610, 1587, 730 cm^{-1}), permitted the assumption that ecdysteroid (**3**) contained an ester group of aromatic nature. This was also indicated by the signals of five aromatic protons at 7.53 (3H) and 8.28 (2H) in the PMR spectrum and, in addition, by intensive peaks of ions in the mass spectrum with *m/z* 122, 105, and 77, which are characteristic of benzoic acid.

After the alkaline saponification of ecdysteroid (**3**), 2-deoxyecdysterone (**8**) was identified in the neutral fraction and benzoic acid in the acid fraction of the hydrolysate.

A comparison of the PMR spectra of 2-deoxyecdysterone (**8**) and of the ecdysteroid (**3**) showed that the signals of the 21-methyl groups had undergone a considerable downfield displacement (Table 1). This fact permitted the assumption that in compound (**3**) the benzoic acid esterified the hydroxy group at C-22.

A comparison of the chemical shifts of the protons of 2-deoxyecdysterone (**8**) and of the ecdysteroid (**3**) showed than, in addition to the signal of the 21-methyl group, the signal of the proton at C-22 had shifted. The downfield shifts of the signals of the C-22 and CH_3-21 protons showed that the benzoic acid residue was attached to the hydroxy group at C-22. An analogous downfield shift has been observed in the case of ecdysterone 22-O-benzoate (**7**) [6] (see Table 1).

Thus, ecdysteroid (**3**) was 2-deoxyecdysterone 22-O-benzoate.

The facts presented enabled the ecdysteroid (**3**) to be identified as 2-deoxyecdysterone 22-O-benzoate, which has been isolated previously from *S. tatarica*. This is the first time that it has been isolated from the plant *S. nutans*.

EXPERIMENTAL

For column and thin-layer chromatographies we used mixtures of chloroform and methanol in various ratios: 1) 9:1; 2) 4:1; 3) 4:1:0.1 [sic]. For other information, see [4].

Isolation of the Phytoecdysteroids from *S. viridiflora* L. The dried and comminuted epigeal part of *S. viridiflora* (3 kg) was extracted five times with 15 liters of ethanol. The extract was concentrated and was then diluted with water, and the resulting precipitate was removed. The ethanol was evaporated off in vacuum, and the aqueous residue was treated with hexane. The ecdysteroids were extracted from the purified aqueous fraction with butanol.

The dry residue obtained after the butanol had been distilled off was chromatographed on a column of silica gel. Elution of the column with system 1 led to the isolation of polypodin B (**2**), $C_{27}H_{44}O_8$, mp 252-254°C (from acetone), $[\alpha]_D$ +94.2 ± 2° (*c* 0.50; methanol).

From its spectral characteristics and also by a direct comparison with an authentic specimen by TLC, compound (**2**) was identified as polypodin B [4, 8, 9].

Subsequent elution of the column with system 2 yielded ecdysterone (**1**), $C_{27}H_{44}O_7$, mp 241-242 (from acetone), $[\alpha]_D^{20}$ +63.2 ± 2° (*c* 0.30; methanol) and integristerone A (**4**), $C_{27}H_{44}O_8$, mp 246-248°C (from a mixture of ethyl acetate and methanol), $[\alpha]_D^{20}$ +36 ± 2° (*c* 0.43; methanol) [4, 8].

Using system 3, we isolated seleneoside D (**6**), $C_{33}H_{54}O_{12}$, mp 240-242°C (methanol–acetone), $[\alpha]_D^{20}$ +91.0 ± 2° (*c* 0.56; methanol), identified by its physicochemical constants and spectral characteristics, and also by direct TLC comparison with an authentic specimen of sileneoside D [4, 10].

Isolation of Phytoecdysteroids from *S. supina* M.B. The air-dry comminuted epigeal organs (2.5 kg) of the plant were extracted in 10 liters of ethanol. The combined ethanolic extracts were concentrated to a syrupy mass. This was diluted with water, and the resulting precipitate, which contained no ecdysteroids, was filtered off. The excess of ethanol was distilled off in vacuum, and the aqueous residue was treated with chloroform to eliminate hydrophobic impurities. The ecdysteroids were exhaustively extracted from the purified solution with butanol. After evaporation of the butanol in vacuum, the total extractive substances were chromatographed on a column of silica gel, and were eluted first with system 1 and then with system 2. This led to the isolation of ecdysterone (**1**) [4, 8]. On further elution, with system 3, seleneoside A (**5**) was obtained.

Seleneoside A (5), $C_{33}H_{54}O_{12}$, mp 254-256°C (from methanol–water), $[\alpha]_D^{20}$ +93 ± 2° (*c* 0.50; methanol). IR spectrum (KBr, ν, cm^{-1}): 3370-3440 (OH), 1645 (Δ^7-6-keto grouping).

Mass spectrum, *m/z* (%): 624 ($M^+ + H_2O$; 0.3), 606(0.8), 588(0.5), 570(0.5), 507(3), 489(15), 473(5), 455(4), 445(6), 444(5), 427(34), 426(35), 409(12), 408(11), 393(4), 363(4), 358(7), 352(20), 345(100), 344(95), 327(67), 309(22), 300(34), 163(20), 145(22), 143(33), 125(22), 99(67), 81(44), 69(42).

PMR spectrum (C_5D_5N, δ, ppm, 0 — HMDS): 0.90 (s, CH_3-19), 1.09 (s, CH_3-18), 1.24/1.30 (s, CH_3-20/27), 1.49 (s, CH_3-21), 3.59 (br.s, H-22), 3.42 (br.s, H-9), 4.0-4.2 (br.s, H-2.3), 5.50 (d, J=3.4 Hz, H-1), 6.11 (br.s, H-7).

The facts presented, and also a direct comparison with an authentic specimen (TLC) permitted ecdysteroid (**5**) to be identified as sileneoside A [8].

Isolation of Ecdysteroids from *S. nutans* L. The comminuted epigeal parts of the plant (2.200 kg) were exhaustively extracted with ethanol (5 × 10 liters). The extract was concentrated, the residue was diluted with water, and the aqueous residue [sic] was treated with chloroform. Ecdysteroids were extracted from the purified aqueous fraction first with ethyl acetate and then with butanol.

The dry residue obtained after the distillation of the ethyl acetate was chromatographed on a column of silica gel. When the column was eluted with system 1, 2-deoxyecdysterone 22-O-benzoate (**3**) was isolated. Ecdysterone (**1**) [4, 8] was isolated by eluting the column with system 2.

The total substances remaining after the distillation of the butanol were chromatographed on a column of silica gel. Elution first with system 1 and then with system 2 gave an additional quantity of ecdysterone (**1**).

2-Deoxyecdysterone 22-O-Benzoate (3). $C_{34}H_{48}O_6$, IR spectrum (KBr, cm^{-1}): 3400-3500 (OH), 1650 (Δ^7-6-keto grouping), 1705, 1285 (ester), 1610, 1587, 730 (benzene ring).

Mass spectrum, *m/z* (%): 568 (M: 0.9), 550(1), 535(2), 532(2), 517(2), 484(2), 474(2), 472(5), 470(2), 428(52), 410(80), 397(29), 395(32), 347(99), 329(79), 234(73), 122(100), 99(85), 81(77).

For the PMR spectrum — see Table 1.

Alkaline Hydrolysis of 2-Deoxyecdysterone 22-O-Benzoate (3). A solution of 15 ml of ecdysteroid (**3**) in 4 ml of methanol was treated with 30 mg of potassium bicarbonate in 2 ml of water. The reaction mixture was left in a thermostat at 37-40°C for four days. Then it was diluted with water (15 ml), neutralized, and extracted with ethyl acetate. The ethyl acetate was distilled off to dryness, and the residue was recrystallized from methanol–water (1:1). This gave 7 mg of a substance with mp 253-255°C, identical with an authentic specimen of 2-deoxyecdysterone (**8**) [11].

The aqueous solution, after acidification with dilute (1:1) hydrochloric acid and extraction with ethyl acetate, yielded benzoic acid with mp 122°C.

Isolation of Ecdysteroids from *S. tatarica* (L) Pers. The air-dry epigeal part of the plant (1.5 kg) was extracted with 7 liters of ethanol. The extract was concentrated and diluted with water, and the resulting precipitate was separated off. The ecdysteroids were extracted from the purified aqueous fraction with ethyl acetate.

The dry residue obtained after the distillation of the ethyl acetate was chromatographed on a column of silica gel. Elution of the column with ethyl acetate led to the isolation of known ecdysteroids — ecdysterone (**1**) [4, 8] and 2-deoxyecdysterone 22-O-benzoate (**3**).

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