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PHYTOECDYSTEROIDS OF *Silene viridiflora*

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The known ecdysteroids polipodin B, 2-deoxyecdysterone, ecdysterone, 26-hydroxypolipodin B, integristerone A, and sileneosides A and D were observed in the aerial part of Silene viridiflora *L. (Caryophyllaceae). 26-Hydroxypolipodin B was observed in this species for the first time.*

Key words: *Silene viridiflora*, ecdysteroids, PMR, IR, mass spectra.

Worldwide, about 700 representatives of the *Silene* genus are known. Of these, 206 occur in the CIS; 84, in Middle Asia [1, 2]. Phytoecdysteroids were first found in *S. praemixta* [3]. Investigation of 110 *Silene* species isolated and established the structure of 81 ecdysteroids [4, 5].

A large number of ecdysteroid-containing species has been noted in the *Silene* genus [6-10]. Many ecdysteroids were isolated for the first time from representatives of this genus. The ecdysteroid composition varies substantially between species. Qualitative and quantitative changes in the ecdysteroid composition were observed during plant development. The chemical composition of the synthesized compounds depended on the habitat [6].

We studied the chemical composition of ecdysteroids from *Silene viridiflora* introduced into the Siberian Botanical Garden and compared their qualitative and quantitative content with plants grown under different conditions in Tomsk and Tashkent (Table 1). This species has been grown in the Siberian Botanical Garden since 1992 as a source of phytoecdysteroids [11, 12]. At present, the most promising species for biomass and quantitative and qualitative content of ecdysteroids have been selected from 22 specimens, the seeds of which were obtained from botanical gardens in various countries (Austria, Germany, Spain, Poland, Romania, France, and Switzerland).

Specimens for which the seeds were obtained from Romania and Spain were the most promising for the theoretical yield of ecdysterone (Table 1). We compared the composition and quantitative content of ecdysteroids from plants introduced into Western Siberia and the Republic of Uzbekistan, the seeds of which were obtained from botanical gardens of Romania and the Republic of Uzbekistan. An evaluation of the contribution of organs to the phytomass and the ecdysterone in the whole plant in various phenophases [9] showed that the fraction of the total ecdysterone (mg, taking into account the phytomass of organs) in the leaves was greater than the total amount of them in other organs in all phenophases, although their content is greater in reproductive organs.

Analysis of the ecdysterone content in *S. viridiflora* of various ages indicated that the ecdysterone content was greatest in plants of the second year in all phenophases (Table 2).

It was found that specimens of the aerial part of *S. viridiflora* grown in different places synthesized different amounts of ecdysteroids. Furthermore, they had different compositions. This indicates that there is a dependence on habitat (Table 3).

The composition and ratio of ecdysteroids were specific for each specimen. This was probably due to morphologic and ecologic-geographic characteristics of the plants. However, the ecdysteroid content was greatest during budding regardless of the habitat [13, 14].

Several principal ecdysteroids were found in *S. viridiflora* plants using TLC and vanillin-H_2SO_4 [15] and column chromatography [14].

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TABLE 1. Ecdysterone Content in Promising *Silene viridiflora* Samples of Various Origin (2nd Year, Flowering)

Seed origin	Weight of phytomass from one dry plant, g	Ecdysterone content, % of dry phytomass			
		L	F	R	Ecdysterone content in phytomass of one dry plant, mg
Spain (Palermo)	80.5	0.7	1.5	0.3	5.5
Germany (Bonn)	70.5	0.8	1.4	0.2	4.9
Austria (Graz)	35.0	0.4	1.2	0.3	3.9
Poland (Bidgosz)*	48.0	0.6	1.6	0.2	5.0
Romania (Cluj-Napoca)*	66.0	1.1	1.9	0.5	7.2

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*Plants grown from seeds of the Siberian botanical garden. L = leaves; F = flowers; R = roots. Ecdysterone content in stem was 0.2% of dry phytomass.

TABLE 2. Ecdysterone Content in Aerial Parts of *Silene viridiflora** of Various Age as a Function of Development Phase (% of Dry Mass)

Plant age, year	Sprouting	Budding	Flowering	Fruitage	End of growth
1	0.5				0.2
2	0.9	1.1	1.0	0.7	0.4
3	0.5	0.6	0.6	0.2	0.1
4	0.4	0.4	0.4	0.2	0.1
5	0.2	0.3	0.2	0.1	0.1

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*Plant grown in the Siberian Botanical Garden.

TABLE 3. Ecdysteroid Content in Aerial Parts of *Silene viridiflora* Grown under Various Conditions

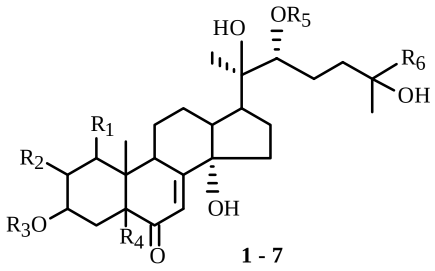
Content	Sprouting	Budding and flowering		Fruitage	End of growth
	1	1	2	1	1
Polipodin B (**1**)	0.15	0.25	0.25	0.20	0.10
2-Deoxyecdysterone (**2**)	-	-	0.20	-	-
Ecdysterone (**3**)	0.20	0.35	0.35	0.30	0.19
26-Hydroxypolipodin B (**4**)	-	-	0.04	-	-
Sileneoside D (**5**)	0.09	0.17	0.10	0.15	0.07
Integristerone A (**6**)	0.10	0.18	0.20	0.19	0.07
Sileneoside A (**7**)	-	-	0.08	-	

Plants grown on the experimental plot of ICPS, AS RUz (1), at the Siberian Botanical Garden (Tomsk) (2). Quantitative data correspond to yield upon isolating ecdysteroids (%).

The isolated pure ecdysteroids were identified using IR, mass, and PMR spectroscopies and comparison with authentic standards (see Experimental). Investigation of the ecdysteroid content of the Siberian specimen of *S. viridiflora* isolated **4** in addition to known compounds synthesized by plants introduced into Middle Asia [14].

The IR spectrum of **4** exhibits a broad absorption band at 3443-3368 cm^{-1} due to hydroxyl stretchings. Bands at 1687 cm^{-1} correspond to absorption of ketones conjugated to a double bond.

The mass spectrum of **4** has the molecular-ion peak at *m/z* 512. Comparison of the molecular ions of **4** (M^+ 512) and **1** (polipodin B, M^+ 496) showed that the molecular weight of the former is 16 amu larger [16]. This is consistent with the presence in **4** of an additional hydroxyl. Comparison of the PMR spectra of **4** and **1** [10, 17] has shown that the spectrum of **4** lacks the 6H signal for CH_3-26 and CH_3-27 at 1.36 ppm. Instead of it, two signals appear at 1.21 ppm (3H, s, CH_3-27) and 3.51 ppm (2H, s, CH_2 bound to OH). The appearance of this signal as a doublet proves the existence of 26-hydroxypolipodine B as a mixture of stereoisomers at C-27 in CH_3OH solution [17]. Our results are consistent with those previously published [17]. The results as a whole identify **4** as 26-hydroxypolipodin B or 5,20,26-trihydroxyecdysone [17], which was isolated previously from *S. nutans*.



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1: $R_1 = R_3 = R_5 = R_6 = H$, $R_2 = R_4 = OH$;
2: $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = H$;
3: $R_1 = R_3 = R_4 = R_5 = R_6 = H$, $R_2 = OH$;
4: $R_1 = R_3 = R_5 = H$, $R_2 = R_4 = R_6 = OH$;
5: $R_1 = R_4 = R_5 = R_6 = H$, $R_2 = OH$, $R_3 = \alpha$-D-Gal;
6: $R_1 = R_2 = OH$, $R_3 = R_4 = R_5 = R_6 = H$;
7: $R_1 = R_3 = R_4 = R_6 = H$, $R_2 = OH$, $R_5 = \alpha$-D-Gal

EXPERIMENTAL

PMR spectra were recorded on a Bruker WM-250 MHz instrument in $CDCl_3$ at 27°C with TMS standard; IR spectra, on a Perkin—Elmer System 2000 FTIR in KBr disks; mass spectra, on a JEOL JMS-700 instrument using ammonia as a reactive gas.

For chromatography, the solvent systems were: $CHCl_3$—CH_3OH (15:1, 1; 9:1, 2; 4:1, 3) and $CHCl_3$—CH_3OH—H_2O (4:1:0.1, 4).

Isolation of Ecdysteroids. Air-dried ground aerial organs (8.5 kg) of *S. viridiflora* grown in the Siberian Botanical Garden of Tomsk State University were extracted with CH_3OH (35 L × 6). The extract was condensed to 2.0 L and diluted with an equal amount of H_2O. The resulting precipitate was filtered off. The CH_3OH was evaporated. The aqueous solution was extracted with $CHCl_3$ (8 L), ethylacetate (4 L), and butanol (3 L). Solvents were evaporated in vacuum to afford ethylacetate (106 g) and butanol (450 g) fractions.

Separation of Ecdysteroids from Aerial Organs. The butanol fraction (150 g) was chromatographed over a silica-gel column.

Elution by system 1 isolated **2**, 5.67 g (0.2%, here and henceforth the yield is given per air-dried mass of raw material), $C_{27}H_{44}O_6$. After crystallization from CH_3OH—H_2O, mp 254-256°C, $[\alpha]_D^{23}$ +82.02 ± 2° (*c* 0.5, CH_3OH).

Based on the physical constants given above, **2** is identified as 2-deoxyecdysterone [18].

Elution of the column by system 2 isolated **1**, 7.08 g (0.25%), $C_{27}H_{44}O_8$, mp 252-254°C (acetone), $[\alpha]_D^{20}$ +94.2 ± 2° (*c* 0.5, CH_3OH).

Our results and comparison with an authentic specimen (TLC) identified **1** as polipodin B [16]. Elution by the same system isolated **3**, 9.92 g (0.35%), $C_{27}H_{44}O_7$, mp 241-242°C (acetone), $[\alpha]_D^{20}$ +58.9 ± 2° (*c* 0.3; CH_3OH).

These data and comparison of TLCs led us to the conclusion that **3** is ecdysterone [16].

Using system 3, we isolated **6**, 5.7 g (0.2%), $C_{27}H_{44}O_8$, mp 246-248°C (ethylacetate—methanol), $[\alpha]_D^{20}$ +36.2 ± 2° (*c* 0.33, CH_3OH).

Therefore, it can be assumed that **6** is integristerone A [16]. This is confirmed by the agreement of the mass spectra and PMR spectra for the studied compound and those for integristerone A and their TLCs.

Elution of the column by system 4 isolated **5**, 2.83 g (0.1%), $C_{33}H_{54}O_{12}$, mp 240-242°C (CH_3OH—H_2O), $[\alpha]_D^{20}$ +91.2 ± 2° (*c* 1.01, CH_3OH).

IR spectrum (KBr, ν, cm^{-1}): 3380-3430 (OH), 1648 (7-en-6-ketone).

Mass spectrum, *m/z* (%): 624 (0.5) $[M - H_2O]^+$, 606 (0.8), 588 (5), 570 (1), 514 (0.8), 507 (0.7), 490 (0.9), 473 (0.6), 462 (1), 444 (1), 426 (11), 411 (2), 408 (3), 393 (1), 375 (1), 363 (5), 345 (55), 327 (13), 309 (10), 300 (11), 284 (11), 145 (10), 143 (12), 135 (11), 99 (100), 81 (55), 69 (53).

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 0.86 (3H, s, CH_3-19), 1.09 (3H, s, CH_3-18), 1.25 (6H, s, CH_3-26/CH_3-27), 1.48 (3H, s, CH_3-21), 3.37 (1H, br.m, H-9), 3.75 (1H, br.m, H-22), 3.96 (2H, br.m, H-2,3), 5.48 (1H, d, ^3J = 3.9, H-1′), 6.10 (1H, br.s, H-7).

This compound was identified as sileneoside D based on the physicochemical constants and spectral data given above and the TLC data [19].

Further elution of the column by the same system isolated **7**, 2.27 g (0.08%), $C_{33}H_{54}O_{12}$, mp 254-256°C (CH_3OH—H_2O), $[\alpha]_D^{22}$ +93.1 ± 2° (*c* 1.03, CH_3OH).

IR spectrum (KBr, ν, cm^{-1}): 3370-3440 (OH), 1645 (7-en-6-ketone).

Mass spectrum, *m/z* (%): 624 (0.3) $[M - H_2O]^+$, 606 (0.8), 588 (0.5), 570 (0.2), 507 (2.5), 489 (11), 473 (2), 463 (2.2), 455 (3.8), 445 (5), 444 (4), 427 (34), 426 (35), 409 (12), 408 (11), 393 (3.8), 363 (3.7), 358 (5), 352 (17), 345 (100), 344 (94), 327 (67), 309 (22), 300 (34), 163 (20), 145 (22), 143 (33), 125 (22), 99 (66), 81 (44), 69 (42).

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 0.90 (3H, s, CH_3-19), 1.09 (3H, s, CH_3-18), 1.24 and 1.30 (6H, s, CH_3-26/CH_3-27), 1.49 (3H, s, CH_3-21), 3.59 (1H, br.m, H-22), 3.42 (1H, br.m, H-9), 4.0-4.20 (2H, br.m, H-2,3), 5.50 (1H, d, ^3J = 3.4, H-1′), 6.11 (1H, br.s, H-7).

Compound **7** was identified as sileneoside A by comparison with the TLC and IR spectrum of an authentic sample [20].

Isolation of 26-Hydroxypolipodin B (4). A fraction containing a mixture of two ecdysteroids (1.8 g) was rechromatographed over a column. Elution by system 3 produced successively **3** (0.6 g) and **4** (1 g, 0.035%), $C_{27}H_{44}O_9$, mp 236-238°C (ethylacetate—acetone), $[\alpha]_D^{23}$ +34.0 ± 2° (*c* 0.3, CH_3OH).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.92 (3H, s, CH_3-18), 0.99 (3H, s, CH_3-19), 1.21 (3H, s, CH_3-27), 1.29 (3H, s, CH_3-21), 2.37 (1H, m, H-17), 3.18 (1H, m, H-22), 3.51 (2H, s, CH_2-26), 4.17 (2H, m, H-2, H-3), 6.05 (1H, d, ^3J = 2.5, H-7).

Compound **4** with *m/z* 512 $[M]^+$ was identified by physicochemical constants and spectral data as 26-hydroxypolipodin B or 5,20,26-trihydroxyecdysone [17].

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