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PHYTOECDYSTEROIDS OF PLANTS OF THE *Silene* GENUS. XIX. THE STRUCTURE OF SILENEOSIDE G

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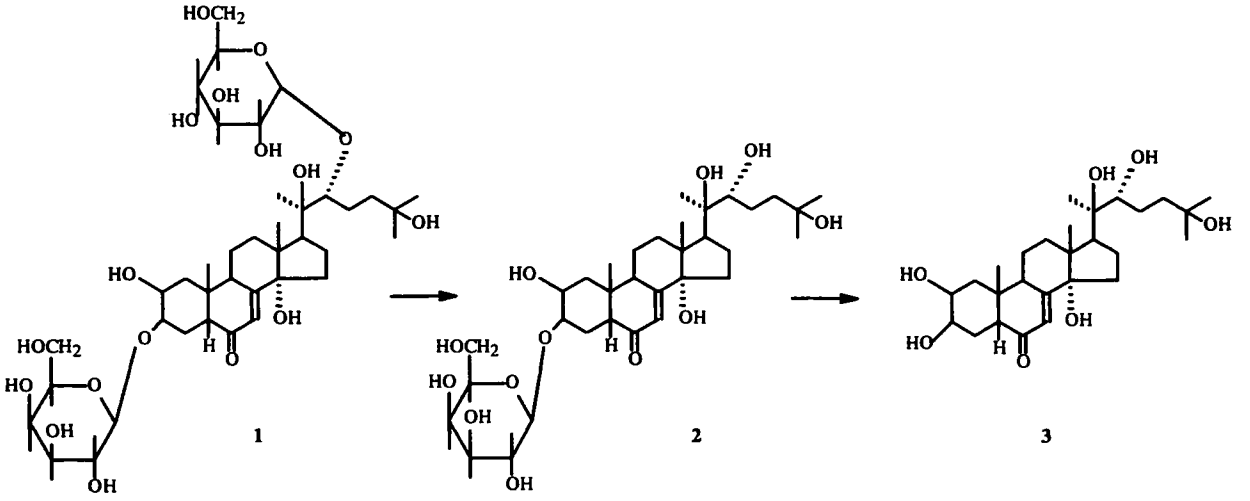
The phytoecdysteroid sileneoside G has been isolated from the roots of Silene brahuica *Boiss., and its structure has been established on the basis of chemical transformations and spectral characteristics as ecdysterone 3-O-α-D-galactopyranoside 22-O-α-D-glucopyranoside. Enzymatic hydrolysis yielded ecdysterone and sileneoside D.*

We have previously reported the isolation of a number of phytoecdysteroids from the roots of *Silene brahuica* Boiss. [1]. Continuing the study of this plant, we have isolated a new phytoecdysteroid — sileneoside G (**1**), composition $C_{39}H_{64}O_{17}$.

The UV spectrum of sileneoside G had an absorption maximum at 246 nm (log ε 4.01). The IR spectrum showed a broad absorption band at 3372 cm^{-1} (OH group) and also a maximum at 1653 cm^{-1} corresponding to the absorption of a keto group conjugated with a double bond. There was no peak of the molecular ion in the mass spectrum of ecdysteroid (**1**); it contained a peak with *m/z* 588 and the peak of a dehydration ion with *m/z* 570, and also peaks of fragments with *m/z* 363, 345, 327, 309, 99, 81, and 69, which are characteristic for ecdysterone [2, 3], and the peaks of ions with *m/z* 163 and 145, corresponding to a fragmented hexose. This permitted the assumption that sileneoside G was a glycoside of ecdysterone.

The ^{1}H and ^{13}C NMR spectra contained the signals of two anomeric protons at 5.62 and 5.69 ppm and of two anomeric carbon atoms at 103.68 and 104.04 ppm.



The acid hydrolysis of (**1**) led to *D*-glucose and *D*-galactose as the carbohydrate components.

In combination, all these facts showed that sileneoside G contained one residue of each monosaccharide and was a bioside (Table 1).

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TABLE 1. Chemical Shifts of the ^{13}C NMR Signals of Sileneoside G (1) and Ecdysterone (3) (C_5D_5N, δ, ppm, 0 — TMS)

C atom	Compound		C atom	Compound	
	1	3		1	3
1	39.36	38.8	15	31.71	32.0
2	68.05	68.0	16	21.42	21.6
3	79.12	68.0	17	49.85	50.1
4	31.61	32.3	18	18.05	17.6
5	51.41	51.1	19	24.24	24.3
6	203.01	203.2	20	77.81	76.9
7	121.46	121.6	21	22.28	21.5
8	166.54	165.7	22	90.96	77.5
9	34.22	34.5	23	27.11	27.2
10	38.50	35.5	24	41.75	42.4
11	21.01	21.0	25	69.72	69.7
12	31.97	31.6	26	29.73	29.9
13	48.05	48.1	27	29.73	29.9
14	84.15	84.2			
		3-O-α-*D*-galactose			
1	103.68		4	71.15	
2	70.93		5	72.44	
3	71.75		6	62.59	
		22-O-α-*D*-glucose			
1	104.04		4	71.19	
2	73.44		5	78.30	
3	79.10		6	62.74	

In the products of the enzymatic hydrolysis of (1), achieved with the gastric juice of the snail *Helix plectotropis*, we detected sileneoside D (2) [4] as a progenin. Consequently, the *D*-galactose residue was present in the steroid nucleus and was linked to the hydroxy group at C-3. The presence of ecdysterone (3) as the genin was shown by its identification in the products of enzymatic hydrolysis of sileneoside D (2) performed with the total enzymes from sweet almond [5].

The definitive position of the *D*-glucose residue was found in the following way. In the spectrum of sileneoside G (1) the signal of the C-22 carbon atom was present at 90.96 ppm and, in comparison with the corresponding signal in the spectrum of ecdysterone (3) at 77.5 ppm, was shifted downfield by 13.46 ppm. As we have shown previously [4], in sileneoside D (2) the *D*-galactose residue is attached to the hydroxy group at C-3 of ecdysterone by an α-glycosidic bond. In the PMR spectrum the signal of the anomeric proton of *D*-glucose resonated in the form of a doublet at 5.69 ppm with the SSCC $^3J = 4.03$ Hz, which showed the α-configuration of the glycosidic bond and the $^4C_1$-conformation of the monosaccharide in the pyranose form.

Thus, sileneoside G (1) is ecdysterone 3-O-α-*D*-galactopyranoside 22-O-α-*D*-glucopyranoside.

EXPERIMENTAL

For eluting columns we used the following systems: 1) chloroform—methanol—water (65:35:6); 2) the same components (70:23:3); 3) chloroform—methanol (4:1). The paper chromatography of the sugars was conducted on FN-11 paper using system 4) butan-1-ol—pyridine—water (4:6:3) with aniline phthalate as the revealing agent.

^{1}H and ^{13}C NMR spectra were recorded in C_5D_5N on a UNITY-400 Plus intrument (Varian).

For other information, see [6].

Isolation of the Ecdysteroids. The ecdysteroids obtained from the butanol fraction and subjected to preliminary purification were rechromatographed on a column of silica gel. Elution with system 1 led to the isolation of 53 mg (0.0010%) of sileneoside G (**1**), with the composition $C_{39}H_{64}O_{17}$, mp 225—227°C (from methanol—acetone), $[\alpha]_D^{20}$+121±2° (*c* 0.10, methanol). UV spectrum (C_2H_5OH, λ_{max}, nm): 245 (log ε 4.01). IR spectrum (KBr, ν, cm^{-1}): 3372 (OH), 1653 (7-ene-6-keto grouping).

Mass spectrum, *m/z* (%): 588 (M^+-162-3H_2O; 2.5), 570 (2), 426 (38), 363 (35), 345 (40), 327 (41), 311 (35), 309, (38), 300 (35), 145 (50), 143 (52), 99 (100), 81 (97), 69 (98).

PMR spectrum (δ, ppm, TMS): 0.97 (3H, s, CH_3-19), 1.24 (3H, s, CH_3-18), 1.40 and 1.47 (6H, s, CH_3-26 and CH_3-27), 1.67 (3H, s, CH_3-21), 2.79 (1H, dd, J=13.4 and 3.6 Hz, H-5), 2.96 (1H, t, J=8.5 Hz, H-17), 3.50 (1H, t, J=7.9 Hz, H-9), 3.78 (1H, d, J=9.1 Hz, H-22), 4.10 (2H, m, H-2 and H-3), 6.02 (1H, s, H-7), 5.62 (1H at C-1', d, J=3.9 Hz), 5.69 (1H at C-1", d, ^{3}J=4.03 Hz).

Acid Hydrolysis. Sileneoside G (5 mg) was hydrolyzed in 5 ml of 0.05% sulfuric acid at 100°C for 2 h. *D*-Glucose and *D*-galactose were detected in the hydrolysate by chromatography in system 4.

Enzymatic Hydrolysis of Sileneoside G (1). The enzyme from the snail *Helix plectotropis* was added to a solution of 15 mg of sileneoside G in 3 ml of water, and the mixture was kept at 36°C for 10 days. Then another 7 ml of water was added and the products were extracted with butanol. After evaporation of the solvent, the mixture of substances was chromatographed on a column of silica gel. Elution with system 2 yielded sileneoside D (**2**), mp 240—241°C (from methanol—acetone) identified by comparison with an authentic specimen from its R_f value on TLC (system 2) and by its IR spectrum.

Enzymatic Hydrolysis of Sileneoside D (2). A solution of 5 mg of sileneoside G (**2**) obtained as described above was treated with 2 ml of an aqueous solution of almond enzymes. After being kept at 36°C for 30 days, the reaction mixture was diluted with ethyl acetate and the solvent was evaporated off. The residue was chromatographed on a column of silica gel. Elution with system (**3**) gave ecdysterone (**3**), identical with an authentic specimen according to TLC (system 3).

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