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PHYTOECDYSTEROIDS OF THE PLANTS OF THE GENUS *Silene*
XV. SILENOSIDE F — BRAHUISTERONE 3-O-β-D-GLUCOPYRANOSIDE FROM *Silene brahuica*

M. Kh. Dzhukharova, Z. Saatov, N. D. Abdullaev, and N. K. Abubakirov

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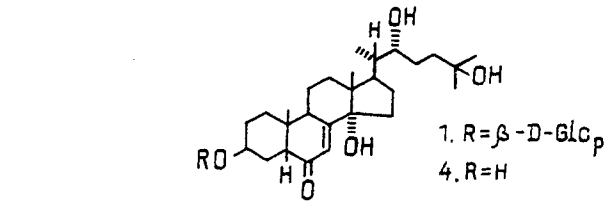
A new brahuisterone glycoside — silenoside F — has been isolated from the epigeal part of Silene brahuica *Bois. (fam. Caryophyllaceae). Its structure has been established by an analysis of spectral characteristics: 3β,5,14α,22R,25-pentahydroxy-5β-cholest-6-one 3-O-β-D-glucopyranoside.*

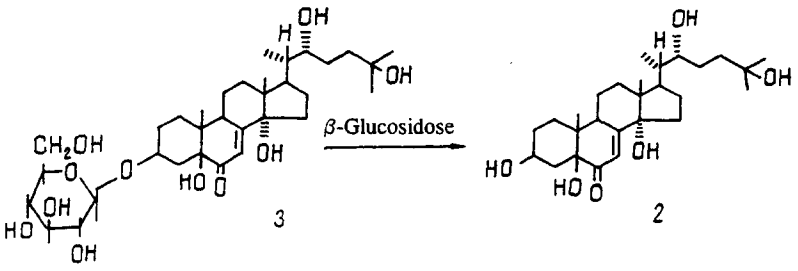
We have previously reported the isolation from *Silene brahuica* Bois. (fam. Caryophyllaceae) of two new compounds: silenoside E (2-deoxy-α-ecdysone 3-O-β-*D*-glucopyranoside (**1**)) and a phytoecdysteroid — brahuisterone (3β,5,14α-22R,25-pentahydroxy-5β-hydroxy-5β-cholest-6-one (**2**)) [1, 2].

We have isolated from the same source another new glycoside, which we have called sileneoside F (**3**). According to GLC results [3], it contained one *D*-glucose residue. In the products of the enzymatic cleavage of compound (**3**) in the presence of the gastric juice of the snail *Helix plectrotropis* we detected the presence of an aglycone which was identified as brahuisterone (**2**) [2]. The peak of an ion with *m/z* 446 observed in the mass spectrum (cleavage between C-17 and C-20) and the formation of fragments with *m/z* 428, 410, and 392 permitted the assumption that the carbohydrate residue was attached to the steroid nucleus [4, 5].

To determine the position of the carbohydrate residue we made a comparative analysis of the characteristics of the ^{13}C NMR spectra of sileneoside E (**1**), sileneoside F (**3**), and 2-deoxy-α-ecdysone (**4**) (Table 1). The results of the assignment of the signals in the spectrum of (**3**) showed that, as compared with those of compounds (**1**) and (**4**), the main changes in the values of the chemical shifts were observed for the carbon atom in rings *A* and *B*.

We must first mention the paramagnetic shift of the C-3 signal by 7.3 ppm (in comparison with C-3 of compound [**4**]) as the result of the glycosylation of the hydroxy group. The CH_3-19 signal had undergone a diamagnetic shift by 7.0 ppm under the action of the β-OH group at C-5. The latter was shown unambiguously by a 27.1 ppm shift of the C-5 resonance line on passing from compound (**4**) to sileneoside F (**3**) [6, 7].





Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 89 14 75. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 734-737, November-December, 1994. Original article submitted May 16, 1994.

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TABLE 1.Chemical Shifts of the ^{13}C Signals of Sileneoside E(**1**), Sileneoside F (**3**), and 2-Deoxy-α-ecdysone (**4**) (δ, ppm, C_5D_5N TMS)*

C-atom	1	3	4	C-atom	1	3	4
1	29.5 (a)	25.5	29.3 (a)	18	15.8	15.7	15.8
2	27.5	27.1	28.7 (a)	19	24.0	17.1	24.1
3	72.5	71.5 (a)	64.2	20	43.0	42.8	42.7
4	29.6 (a)	32.4	32.7	21	13.6	13.5	13.4
5	51.5	78.4	51.3	22	74.0	73.9	74.0
6	203.9	202.2	203.8	23(b)	25.6	25.5	25.2
7	121.3	120.2	121.1	24	42.5	42.3	42.0
8	165.9	166.4	166.4	25	69.7	69.6	70.0
9	34.5	36.7	34.4	26 (c)	30.2	30.0	29.9
10	36.7	42.8	36.8	27 (c)	30.0	29.9	29.6
11	21.5	21.7	21.4				
12	31.7	31.4	31.5	1'	103.3	101.3	
13	48.1	47.0	47.9	2'	75.2 (d)	75.2	
14	84.0	83.7	84.1	3'	78.6	78.3	
15	31.7	31.7	31.5	4'	71.8 (d)	71.7 (a)	
16(b)	26.7	26.5	26.5	5'	78.2	78.3	
17	48.3	48.1	48.1	6'	62.9	62.6	

*The assignment of some of the resonance signals with close values of the chemical shifts from carbon atoms monotypical with respect to substitution may be interchanged when they are shown by the same letters in the columns.

TABLE 2. Chemical Shifts of the Protons of Sileonoside E (**1**), Brahuisterone (**2**), and Sileneoside F (**3**) (δ, ppm, C_5D_5N, TMS)

Com-pound	Positions of the protons and groups								
	H-3	H-7	H-9	H-22	H-1'	CH_3-18	CH_3-19	CH_3-21	CH_3-26/27
1	3.86 br.m W1/2= 10Hz	6.19 br.m J=21 Hz	3.48 m	~4.1*	4.90 d ^3J=7.5 Hz	0.72 s	0.88 s	1.29 d ^3J=6.5Hz	1.40 s
2	4.06 br.m	6.20	3.53	4.06	—	0.74 s	1.14 s	1.29 d ^3J=6.4 Hz	1.40 s
3	~4.1*	6.24 br.s	3.53 m		4.90 d ^3J=7.5Hz	0.74 s	1.14 s	1.30 d ^3J=6.5Hz	1.40

*In the spectra of (**1**) and (**3**) the signals of protons 2'—6' of the carbohydrate residue resonated between 3.7 and 4.5 ppm.

It is known that, depending on the position of the glycosylated hydroxy group, the neighboring carbon atoms undergo a upfield shift by 1-6 ppm on the introduction of a carbohydrate residue [8, 9]. However, on passing from compound (**4**) to (**3**) no such chemical shift of C-4 (32.7 and 32.4 ppm, respectively) is observed. This fact finds its explanation if we take into

account the mutual compensation of the contributions with opposite signs of the hydroxy group at C-5 and the carbohydrate residue at C-3.

The assignment of the triplet signals at 25.5 and 27.1 ppm in the ^{13}C NMR spectrum of compound (**3**) under the conditions of incomplete suppression of C—H interactions was made in the light of the γ-contribution of the OH group at C-5 to C-1 and the effect of glycosylation at C-2. The paramagnetic shift of the C-10 resonance line under the action of the γ-contributions of the OH at C-5 amounted to 6.0 ppm, which is comparable to the change in the chemical shift of the analogous carbon atom on passing from ecdysterone to polypodine B [7]. The C-1′ anomeric carbon atom of compound (**3**) resonated at 101.3 ppm. The other glucopyranose carbon atoms were characterized by standard values of the chemical shifts [10, 11].

It must be mentioned that, previously, in [1], an inaccurcy was committed in the assignment of the C-3 signal in the ^{13}C NMR spectrum of sileneoside E (**1**). In the present communication (Table 1) we introduce a correction according to which the signal at 72.5 ppm has been assigned to this carbon atom.

In the PMR spectrum of compound (**3**), the anomeric proton resonated at 4.90 ppm with ^3J = 7.5 Hz, which showed the β-configuration of the glycosidic center. Table 2 gives the details of the PMR spectra of compounds (**1—3**). An analysis of these characteristics and, in particular, those relating to H-3 and CH_3-18, -19, -21, and -26/27 shows the stability of the values of the chemical shifts of the methyl groups, apart from CH_3-19, on passing from compound (**1**) to (**2**) and (**3**). The absence from the PMR spectrum of compound (**3**) of a signal characteristic for H-5 in combination with all the characteristics of the ^{13}C NMR spectrum showed the correctness of the conclusion that the aglycon of sileneoside F is in fact brahuisterone.

Thus, all the facts given above enable the structure of compound (**3**) to be established as 3β,5,14α,22R,25-pentahydroxy-5β-cholest-7-en-6-one 3-O-β-*D*-glucopyranoside.

EXPERIMENTAL

The PMR and ^{13}C NMR spectra were taken on a Varian XL-200 instrument (C_5D_5N, 0 — TMS). For other details, see [2].

Sileneoside F (3). The concentrated fractions obtained in the isolation of sileneoside E [1], brahuisterone [2], and 2-deoxyecdysterone 20,22-monoacetonide [12] were chromatographed on a column of silica gel in the chloroform—methanol—water (4:1:0.1) system. The ecdysteroid (**3**), with the composition $C_{33}H_{55}O_{11}$, amorphous, was isolated. UV spectrum C_2H_5OH, λ_{max}, nm) 243 (log ε 4.09). IR spectrum (KBr, ν, cm^{-1}): 3300-3500 (OH), 1680 (Δ^7-6-keto group). Mass spectrum, 608($M^+ - H_2O$; 0.4), 590 (3), 572 (3), 492 (2), 447 (23), 446 (23), 429 (98), 428 (100), 426 (24), 418 (26), 411 (50), 410 (98), 395 (16), 392 (17), 377 (42), 348 (56), 330 (26), 99 (57), 81 (55).

Enzymatic Hydrolysis of Sileneoside F (3). A solution of 42 mg of sileneoside F (**3**) in 3 ml of water was treated with 2 ml of commercial enzyme from the snail *Helix plectrotropis*, and the mixture was left at 38°C for 5 days. Another 10 ml of water was added to the reaction mixture and it was extracted with ethyl acetate. The solvent was evaporated off. The residue was chromatographed on a column of silica gel. Elution by the chloroform—methanol (15:1) system yielded 13 mg of brahuisterone (**2**) with mp 186-187°C (chloroform—methanol), the ecdysteroid (**2**) being identified from the spectral indices and by a direct PLC comparison (chloroform—methanol (9:1)), with an authentic sample of brahuisterone [2].

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