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PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS *Silene*

XVI. 5α-SILENEOSIDE E FROM *Silene brahuica*

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From the epigeal part of Silene brahuica *we have isolated a new ecdysteroid — 5α-sileneoside E. From spectral characteristics its structure has been established as 5α-2-deoxy-α-ecdysone 3-O-β-D-glucopyranoside.*

We have previously reported the presence in *Silene brahuica* Boiss (fam. Caryophyllaceae) of 2-deoxy-α-ecdysone (**1**), 2-deoxyecdysterone (**2**), sileneoside E (**3**), ecdysterone (**4**), brahuisterone (**5**) [1] and sileneoside F (**6**) [2]. By rechromatography on a silica gel column of the mother solutions obtained in the isolation of sileneoside E (**3**) we have now isolated a phytoecdysteroid (**7**) with the composition $C_{33}H_{54}O_{10}$.

The IR spectrum of the ecdysteroid (**7**) showed a broad absorption band at 3380-3430 cm^{-1} corresponding to the stretching vibrations of hydroxy groups. A band at 1650 cm^{-1} corresponded to the absorption of a keto group conjugated with a double bond.

The peak of the molecular ion was absent from the mass spectrum of the ecdysteroid (**7**). The presence of peaks with *m/z* 592, 574, and 556, corresponding to the products of the successive dehydration of the molecular ion, in combination with fragments having *m/z* 284, 285, 99, and 81, which are characteristic for a 2-hydroxyecdysteroid [3, 4], and also the presence of peaks of ions having *m/z* 163 and 143, which are characteristic for a fragmented hexose, permitted the assumption that the ecdysteroid under investigation was a glycoside of a 2-deoxyecdysteroid. Furthermore, in the mass spectrum of ecdysteroid (**7**) we observed peaks with *m/z* 476 and 446, formed by the cleavage of the C-20-C-22 and C-17-C-20 bonds. The latter fact showed that the sugar residue was bound to the steroid nucleus.

It was established by GLC [5] that the ecdysteroid contained one molecule of *D*-glucose.

In the PMR spectrum of compound (**7**) the signals of the H-3 and H-22 protons were masked by those of the carbohydrate residue and it did not appear possible to determine the chemical shifts of the former with sufficient accuracy. Nevertheles, as can be seen from Table 1, the chemical shifts of the protons of the CH_3-18, -21, and -26/27 methyl groups of compounds (**7**) and compounds (**3**) and (**8**) were identical with one another. This may be evidence in favor of the formation of the glycosidic bond at C-3 of the (**7**) molecule. The anomeric proton resonated at 4.97 ppm with J = 7.5 Hz, which showed the β-configuration of the glycosidic center.

It can be seen from the Table that the chemical shift of CH_3-19 in compound (**7**) experiences a substantial diamagnetic displacement in comparison with that of 2-deoxy-α-ecdysone (**1**) ($\Delta\delta$ = 0.26 ppm). We observed a smaller (0.07 ppm) but symbatic displacement of the CH_3-19 signal in a comparison of the characteristics of the PMR spectra of sileneoside E (**3**) and compound (**7**). It has been shown previously that in the PMR spectra of 3β-hydroxy-5β-ergosta-7,22-dien-6-one and 3β-hydroxy-5α-ergosta-7,22-dien-6-one the CH_3-19 signals appear at 1.01 and 0.89 ppm, respectively [8]. It follows from this comparison of the characteristics of two pairs of epimeric compounds that on passing from a 5β- to a 5α- epimer there are diamagnetic displacements of the signals of the protons of the CH_3-19 group by 0.12 and 0.13 ppm. An analogous shift by 0.15 ppm has also been observed in the passage from 5β-2-deoxy-α-ecdysone to its 5α- epimer [6]. On the basis of these facts it may be assumed that rings *A* and *B* of the steroid part of glycoside (**7**) are *trans*-linked to one another, and H-5 has the α-orientation. This conclusion is confirmed by the fact that the products of the enzymatic hydrolysis of glycoside (**7**) by the

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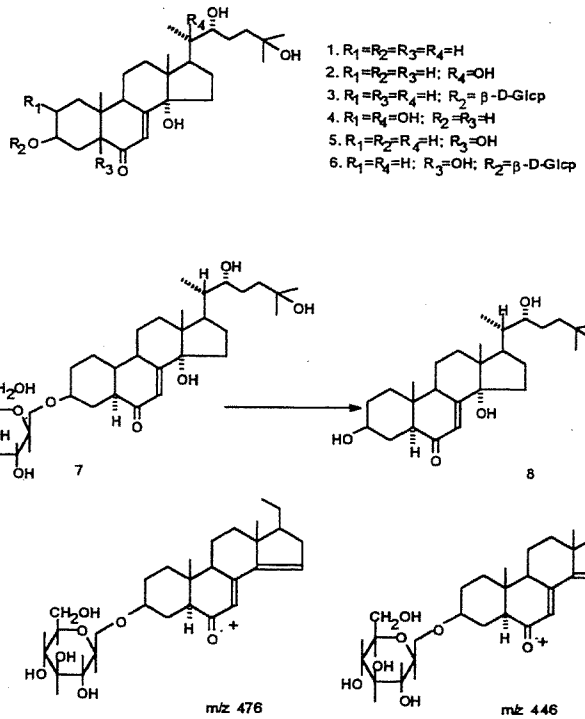
TABLE 1. Chemical Shifts of the Protons of 2-Deoxy-α-ecdysone (**1**), Sileneoside E (**3**), 5α-Sileneoside E (**7**), and 5α-2-Deoxy-α-ecdysone (**8**) (δ, ppm, Py-d_5, 0 — TMS)

Compound	Positions of the protons								
	H-3	H-9	H-22	H-7	H-1′	CH-18	CH-19	CH-21	CH-26/27
1	4.14, br.m $W_{1/2}\cong10$ Hz	3.52, m	4.08, br.d $^3J\cong9.2$ Hz	6.22, br.d $J\cong2$ Hz	–	0.75, s	1.07, d	1.31, d $^3J=6.6$ Hz	1.40, s
3	3.86, br.m $W_{1/2}\cong10$ Hz	3.48, br.m	4.1*	6.19, br.d J=2 Hz	4.90, d $^3J=7.5$ Hz	0.72, s	0.88, s	1.29, d $^3J=6.5$ Hz	1.40, s
7	*	3.44, br.m	*	6.1, br.s	4.98, d $^3J=7.5$ Hz	0.71, s	0.81, s	1.28, d J=6.5 Hz	1.40, s
8	4.04, br.m	–	4.04, br.m	6.17, d J=3 Hz	–	0.72, s	0.92, s	1.27, d J=6.5 Hz	1.40, s

*Masked by the signals of the protons of the carbohydrate residue. Symbols: s) singlet; d) doublet; br.d) broadened doublet; br.m.) broadened multiplet.

gastric juice of the snail *Helix plectotropis* were found to contain an aglycon identified as 5α-2-deoxy-α-ecdysone (**8**). Authentic 5α-2-deoxy-α-ecdysone was obtained by the alkaline isomerization of 2-deoxy-α-ecdysone [6].



Thus, glycoside (7) is the 5α- isomer of sileneoside E, i.e. 5α-2-deoxy-α-ecdysone 3-O-β-*D*-glucopyranoside.

EXPERIMENTAL

For the isolation of the ecdysteroids, the instruments, the chromatographic methods, and the GLC conditions, see [9].

Mass spectra were taken on a MKh-1310 instrument at an ionizing voltage of 50 V, and a temperature of 100-140°C, and PMR spectra on a BS-567 A instrument (100 MHz, Tesla) in C_5D_5N with TMS as standard.

Isolation of 5α-Sileneoside E (7). The mother solutions obtained in the isolation and recrystallization of sileneoside E (**3**) were combined and chromatographed on a column of silica gel. Elution was conducted with the chloroform—methanol—water (4:1:0.1) system. This led to the isolation of 13 mg of the amorphous ecdysteroid (**7**), $C_{33}H_{54}O_{10}$; ν_{max} (KBr), cm^{-1}: 3380-3430 (OH), 1650 (Δ^7-keto group).

Mass spectrum, *m/z* (%): 592 ($M^+ - H_2O$; 3), 574 (7), 556 (1.4), 476 (2), 448 (6), 446 (7), 430 (10), 412 (60), 395 (16), 394 (16), 379 (8), 343 (8), 314 (11), 311 (12), 285 (50), 284 (100), 269 (15), 163 (9), 145 (12), 143 (8), 99 (80), 81 (80), 69 (50).

Enzymatic Hydrolysis of 5α-Sileneoside E (7). A solution of 8 mg of the ecdysteroid (**7**) in 1 ml of water was treated with 1 ml of the enzyme complex from the snail *Helix plectotropis*, and the reaction mixture was left at 36-37°C for 8 days. Then it was treated with 5 ml of water and extracted with ethyl acetate. After evaporation of the solvent and recrystallization of the residue from methanol—water, we obtained 3 mg of a crystalline substance — 5α-2-deoxy-α-ecdysone (**8**), mp 224-226°C. On TLC in the chloroform—methanol (9:1) system, substance (**8**) proved to be identical with the 5α-2-deoxy-α-ecdysone obtained previously by the alkaline isomerization of 2-deoxy-α-ecdysone (**1**) [6].

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