PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS *Silene*.

VII. SILENEOSIDE D — ECDYSTERONE 3-O-α-D-GALACTOPYRANOSIDE FROM *Silene brahuica*

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A phytoecdysteroid, sileneoside D, has been isolated from the roots of *Silene brahuica* Boiss. and it has been shown to be ecdysterone 3-O-α-D-galactopyranoside.

We have previously described the structures of sileneosides A, B, and C [1, a-c], isolated from *Silene brahuica* Boiss. (family Caryophyllaceae). In this communication we consider the structure of a new ecdysteroid of glycosidic nature — sileneoside D (I) — also isolated from this plant.

It was shown by GLC [2] that glycoside (I) contained one D-galactose residue. In the products of the enzymatic cleavage of sileneoside D (I) performed by enzymes obtained from almond [3], ecdysterone (II) was identified as the aglycone.

The acetylation of the ecdysterone galactoside (I) with acetic anhydride in pyridine gave a heptaacetate (III) (M^+ 936).

In the PMR spectrum of the heptaacetate (III), the signal of the H-22 proton and the signals of the 26/27-methyl groups had undergone paramagnetic shifts (Table 1) due to the acetylation of the corresponding hydroxy groups of the side chain. These results, together with the peaks of ions with m/z 622 [4] and 735 observed in the mass spectrum of the acetate (III) gave grounds for considering that the sugar was attached to the steroid moiety of the molecule.

In the ^{13}C NMR spectra of ecdysterone (II) [5] and of sileneoside D (I), the signals of the carbinol carbon atoms were characterized by the following values of the chemical shifts (ppm; the figures for sileneoside D are given in parentheses): 68.0 (68.0) C-2; 68.0 (79.0) C-3; 84.2 (84.1) C-14; 76.9 (76.8) C-20; 77.5 (77.5) C-22; and 69.7 (69.6) C-25. Thus, in the ^{13}C NMR spectrum of galactoside (I) only the signal relating to the C-2 or C-3 carbon atom had undergone a paramagnetic shift by +11.0 ppm due to glycosylation [1 c, 6].

The site of attachment of the D-galactose residue was established in the following way. In the PMR spectrum of glycoside (I) the H-2 and H-3 protons form a common 2-proton multiplet at 3.96 ppm [1c]. In the spectrum of the heptaacetate (III), in place of this multiplet a broadened one-proton singlet was observed at 4.09 ppm with $W_{1/2}$ = 8 Hz and a one-proton multiplet at 5.13 ppm with $W_{1/2}$ = 21 Hz. The signal at 4.09 ppm must be assigned to a proton geminal to a galactose residue. When a saturating frequency was superimposed on this signal (4.09 ppm), the one-proton multiplet with $W_{1/2}$ = 21 Hz at 5.13 ppm simplified to a quartet with ^3J = 11.8 and 4.2 Hz. Consequently, the multiplet at 5.13 ppm corresponded to a proton located geminally to an acetate residue in the C-2—C-3 diol group.

On the basis of the half-width of this signal ($W_{1/2}$ = 21 Hz), it had to be assigned to an axial hydrogen atom at C-2.

Thus, the D-galactose residue was attached to the hydroxy group at C-3.

It is known that in the ^{13}C NMR spectra of D-galactofuranose there is, in comparison with the pyranose form, a downfield shift of the signals of the carbon atoms, particularly C-4 [7, 8]. In the ^{13}C NMR spectrum of sileneoside D the signals of the carbon atoms of the sugar residue had the following values of their chemical shifts (ppm): 103.5 (C-1'); 70.9

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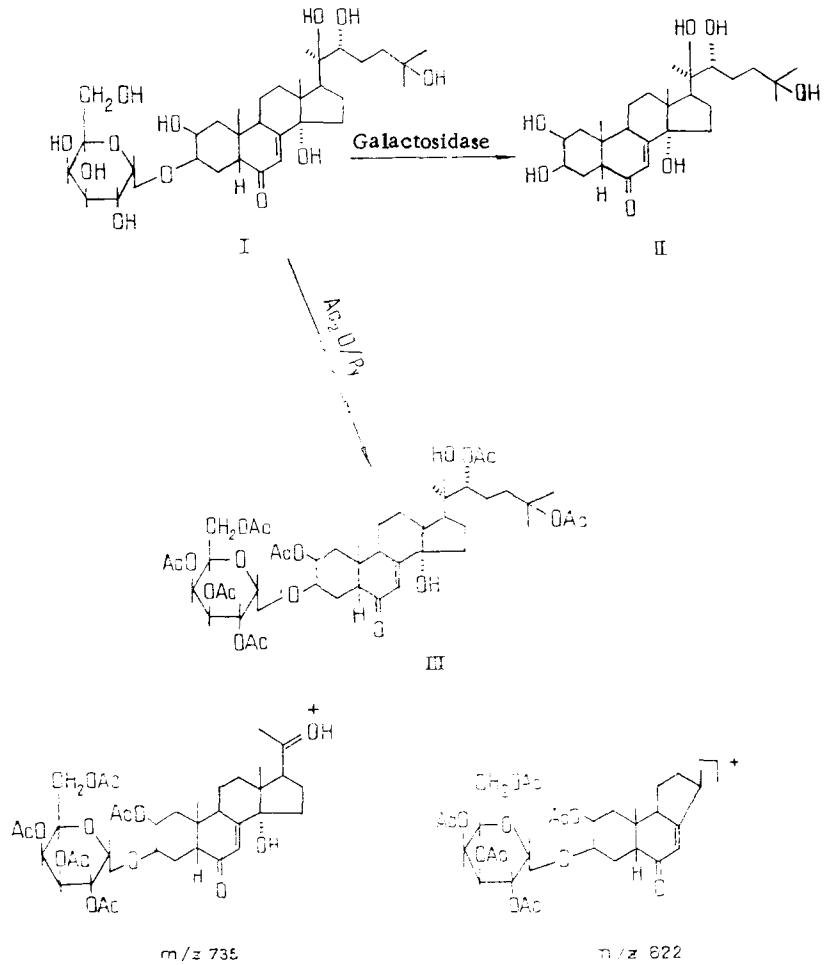
(C-2'); 71.6 (C-3'); 70.9 (C-4'); 73.3 (C-5') and 62.5 (C-6'). These figures indicate the pyranose form of the D-galactose residue [8].

The anomeric proton, H-1', of silenoside D (I) resonated at 5.48 ppm with J = 3.9 Hz (Table 1). These indices [9], and also the molecular rotation differences [10] between sileneoside D (I) and ecdysterone (II) shows the α configuration of the glycosidic center.

Thus, sileneoside D is ecdysterone 3-O-α-D-galactopyranoside.



We have previously isolated from *Silene brahuica* ecdysterone 3,22-di-O-α-D-galactopyranoside — sileneoside B [1c], and one of its progenins, ecdysterone 22-O-α-D-galactopyranoside — sileneoside A [1a].

The sileneoside D described in the present paper is a structural isomer of silenoside A. It can also be considered as the second natural progenin of sileneoside B.

EXPERIMENTAL

PMR spectra were taken on a JNM-100 instrument (C_5D_5N, ppm, 0 — HMDS), and ^{13}C NMR spectra on a CFT-20 instrument (Varian) (C_5D_5N, 0 — TMS). For other information, see [1a].

Sileneoside D (I). The mother liquors that had accumulated in the isolation of sileneoside A [1a] from 10 kg of *Silene brahuica* were rechromatographed repeatedly on a column of silica gel in the chloroform—methanol (4:1) system. After recrystallization from methanol—acetone, 136 mg (yield 0.0013% calculated on the air-dry raw material) of sileneoside D (I) was obtained with $C_{33}H_{54}O_{12}$, mp 240-242°C, $[\alpha]_D^{20}$ +91.2 ± 2° (c 1.01; methanol). $\lambda_{max}^{C_2H_5OH}$: 247 nm (log δ 415). ν_{max}^{KBr} (cm^{-1}): 3380-3430 (OH), 1648 (Δ^7-6-keto group). CD (c 0.10; methanol): $\Delta\varepsilon$ = 5.5 (250 nm), $\Delta\varepsilon$ = +2.2 (327 nm).

Mass spectrum, m/z (%): 624 ($M^+ - H_2O$ 0.5); 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 (1.0), 363 (5), 345 (55), 327 (13), 309 (10), 300 (11), 284 (11), 145 (10), 143 (12), 135 (11), 99 (100), 81 (55), 69 (53).

TABLE 1. Chemical Shifts of the Protons of Sileneoside D (I), Its Acetyl Derivative (III), and Ecdysterone (II) (d, ppm, 0 — HMDS)

Compound	Positions of the protons				
	H-2	H-3	H-7	H-9	H-22
I	3,96	3,96	6,10	3,37	3,75
II	3,9—4,2	3,9—4,2	6,07	3,43	3,70
III	5,13 $W_{1/2}$ =21 Hz	4,09 $W_{1/2}$ =8 Hz	6,07	3,49	5,29 br.d ^3J=10,2 and 1 Hz

Compound	Positions of the protons					
	H-1'	CH_3-18	CH_3-19	CH_3-21	CH_3-26/27	OAc
I	5,48 d ^3J=3,9 Hz	1,09	0,86	1,48	1,25 1,25	—
II	—	1,07	0,94	1,44	1,25 1,25	—
III	5,63 d ^3J=3,6 Hz	1,01	0,89	1,49	1,32 1,33	2,09; 2,01; 1,98; 1,95; 1,92; 1,85; 1,82

The protons of the methyl groups appeared in the form of a singlet; in all cases the signal of H-7 protons has the nature of a broadened singlet while the other signals (with the exception of that of H-1') were broadened multiplets.

Enzymatic Hydrolysis of Sileneoside D (I). A solution of 30 mg of sileneoside D (I) was treated with 7 ml of an aqueous solution of the enzyme obtained from 0.5 kg of sweet almonds [3]. After being kept at 26°C for 24 days, the reaction mixture was diluted with 16 ml of water and extracted with butanol (3 × 10 ml). The solvent was evaporated off and the residue was chromatographed on a column of silica gel. Elution with chloroform—methanol (9:1) yielded 17 mg of ecdysterone (II) with mp 239-240°C (from acetone), $[\alpha]_D^{22}$ +59.8 ± 2° (c 0.47; methanol), identical with an authentic sample according to TLC and IR spectrum, as well.

Sileneoside D 2,2'3',4',6',22,25-Heptaacetate (III). A solution of 134 mg of sileneoside D (I) in 6 ml of pyridine was acetylated with 6 ml of acetic anhydride at room temperature for 7 days. Then the reaction mixture was diluted with water, and the resulting precipitate (139 mg) was filtered off and chromatographed on a column of silica gel. Elution with benzene—acetone (4:1) gave 85 mg of the heptaacetate (III), $C_{47}H_{68}O_{19}$ with mp 188-190°C (from chloroform-methanol), $[\alpha]_D^{22}$ +119.5 ± 2° (c 0.91; methanol), ν_{max}^{KBr} (cm^{-1}): 3538 (OH), 1758, 1225-1260 (ester grouping); 1660 (Δ^7-6-keto grouping).

Mass spectrum, m/z (%): 936 (M^+; 0.01), 848 (1), 840 (0.7), 816 (1), 798 (3), 788 (2), 780 (3), 735 (3), 717 (3), 673 (1), 662 (0.7), 622 (3), 516 (0.7), 498 (0.7), 489 (0.5), 470 (0.8), 451 (0.7), 427 (1), 409 (2), 392 (1), 331 (100), 289 (2), 256 (4), 169 (50), 99 (5), 81 (10).

SUMMARY

A new ecdysterone has been isolated from the roots of the *Silene brahuica* Boiss. and has been shown to be ecdysterone 3-O-α-D-galactopyranoside.

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A NEW STEROID SAPONIN FROM THE LEAVES OF *Yucca aloifolia*

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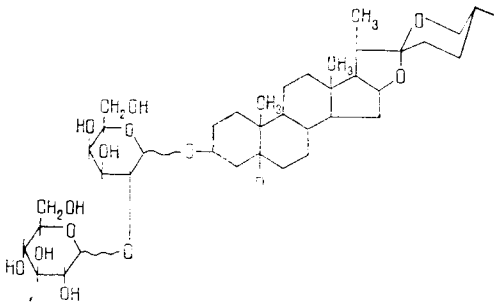
A new steroid saponin has been isolated from the air-dry leaves of *Yucca aloifolia* L., and it has been shown to be (25R)-5β-spirostan-3β-ol O-D-glucopyranosyl-(1 → 2)-D-galactopyranoside. The substance melts at 302-303°C $[\alpha]_D^{20}$ −27.2, (c 1.0; $CHCl_3$).

The genus *Yucca* is known for its content of steroid saponins [1]. From the leaves of *Y. aloifolia* L. (aloe yucca) we have isolated the steroid saponins smilagenin, tigogenin, hecogenin, chlorogenin, and gitogenin [2].

In the present paper we give the results of a study of the steroid saponins of the leaves of the aloe yucca collected in the Sukhumi Botanical Garden in the summers of 1980-1982.

In an ethanolic extract of the leaves by TLC analysis we established the presence of five glycosides, which we called, in order of increasing polarity, glycosides A, B, C, D, and E. A methanolic extract of the leaves was fractionated into less polar and more polar saponins. From the less polar materials, by chromatography on a column of silica gel, we isolated glycoside A. According to its physicochemical properties, the aglycone of the compound was smilogenin [3, 4]. The carbohydrate fraction from complete acid hydrolysis was found to contain glucose and galactose. After the reduction of the hydrolysate followed by acetylation, sorbitol and dulcitol acetates were identified in a ratio of 1:1. The nature of the bond between the glucose and galactose residues was established with the aid of Hakomori methylation [5] of the compound under investigation. As a result of the methanolysis of the methylated saponin followed by GLC, completely methylated methyl glucopyranoside, which obviously represents the terminal monosaccharide residue, and methyl 3,4,6-tri-O-methylgalactopyranoside, representing the monosaccharide residue attached to the aglycone, were identified in a ratio of 1:1. The methylated saponin was subjected to acid hydrolysis followed by reduction and acetylation. 1,5-Di-O-acetyl-2,3,4,6-tetra-O-methylsorbitol and 1,2,5-tri-O-acetyl-3,4,6-tri-O-methyldulcitol [6] were identified by chromato-mass spectrometry, which confirmed the presence of a (1 → 2) glycosidic bond between the glucose and galactose residues in the initial glycoside.



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