PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS *Silene*.

V. SILENEOSIDE B — AN ECDYSTERONE DIGALACTOSIDE FROM *Silene brahuica*

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The phytoecdysteroid sileneoside B has been isolated from the roots of *Silene brahuica* Boiss.; it is ecdysterone 3,22-di-O-α-D-galactopyranoside.

We have isolated three new phytoecdysteroids containing sugar residues from the roots of *Silene brahuica* Boiss. (family *Caryophyllaceae*) — sileneosides A, B, and C. The structures of sileneosides A and C have been considered previously [1, 2]. In the present paper we discuss a proof of the structure of sileneoside B (I).

The mass spectrum of the ecdysteroid (I) contains fragments with m/z 363, 345, 327, 99, 81, and 69, which are characteristic for ecdysterone [3, 4], and also the peaks of ions m/z 163 and 145 which are characteristic of a fragmented hexose. The facts permit the assumption that sileneoside B is an ecdysterone glycoside.

It has been established by the GLC method [5] that glycoside (I) contains two D-galactose residues. The presence of ecdysterone (II) as the genin was shown by its identification in the products of enzymatic hydrolysis of sileneoside B (I) performed by the combined enzymes obtained from sweet almond [6]. In addition to ecdysterone (II), sileneoside A (III), i.e., ecdysterone 22-O-α-D-galactoside [1], was detected in the reaction mixture. Consequently, one of the two D-galactose molecules is present in the side chain and is attached to the hydroxy group of C-22.

In dry acetone solution, sileneoside B (I) gave a diacetonide (VII). The mass spectrum of compound (VII) does not contain the peak of the molecular ion, but the presence of a dehydration fragment with m/z 866 $(M - H_2O)^+$ confirms the presence of two D-galactose residues. The mass spectrum of the diacetonide (VII) also has the peak of an ion with m/z 547 formed as the result of the cleavage of C-20—C-22 bond which is characteristic for ecdysterone [3] and the splitting out of one molecule of water. This fragment gives grounds for considering that the second D-galactose residue is attached to the tetracyclic nucleus of the ecdysterone molecule.

The acetylation of sileneoside B (I) with acetic anhydride in pyridine led to a nonaacetate (V) and a decaacetate (VI).

The mass spectra of (V) and (VI) each have a peak with m/z 735 ($C_{37}H_{51}O_{15}$), likewise formed by the cleavage of the C-20—C-22 bond. The presence of the fragment with m/z 735 is an additional indication that the second D-galactose residue is attached to the steroid nucleus.

In the ^{13}C NMR spectrum of ecdysterone, the signals of the carbinol carbon atoms C-2 and C-3 are located at 68.0 ppm [7]. In the spectrum of sileneoside B (I), the same carbon atoms give signals at 67.9 and 78.9 ppm. Such a considerable downfield change in the chemical shift of one of them (Δδ 10.9 ppm) is undoubtedly due to the effect of glycosylation [8].

The establishment of the position of attachment of the second D-galactose residue reduced to choosing between the hydroxy groups at C-2 and C-3.

In the PMR spectrum of the diglycoside (I) (Table 1) there is a broadened multiplet with an intensity of two proton units at 3.96 ppm relating to the protons at C-2, and C-3 [1]. The spectrum of the decaacetate (VI) retains in this region of the spectrum at 4.02 ppm only a one-proton multiplet ($W_{1/2}$ = 8 Hz). This signal is obviously due to the proton geminal

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TABLE 1. Chemical Shifts of the Protons of Sileneoside B (I), Sileneoside A (III), their Acetyl Derivatives (IV) and (VI), and Ecdysterone (II) (δ, ppm, O = HMDS)

Compound	Positions of the protons					
	H-2	H-3	H-7	H-9	H-22	H-1'
I	3,96	3,96	6,03	3 34	3,61	5,40 d ^3J=3,9 Hz
II	3,9—4,2	3,9—4,2	6,07	3,43	3,70	—
III	4,0—4,2	4,0—4,2	6,11	3,42	3,59	—
IV	5,0—5,3	5,0—5,3	6,01	3,38	3 63	—
VI	5,08 $W_{1/2}$=21 Hz	4,02 $W_{1/2}$=8 Hz	6,00	3,42	3,59	~5,8

Compound	Positions of the protons					
	H-1"	CH_3-18	CH_3-19	CH_3-21	CH_3-26/27	OAc
I	5,45d ^3J=3,9 Hz	1,09	0,86	1,51	1,26 1,31	—
II	—	1,07	0,94	1,44	1,25 1,25	—
III	5,50 d ^3J=3,4 Hz	1,09	0,90	1,49	1,24 1,30	—
IV	~5,7	0,93	0,93	1,43	1,36 1,47	1,87—1,90 (6 CH_3); 2,07
VI	~5,8	0,92	0,89	1,42	1,37 1,48	1,80; 1,88—1,92 (6 CH_3); 1,98; 2,05; 2,07

Note: The spectra were taken on a JNM-4H-100/100 MHz instrument in C_5D_5N. The signals of the protons of the methyl groups are singlets; in all cases the H-7 proton appears in the form of a broadened singlet and the other signals (with the exception of H-1' and H-1") as braodened multiplets.

to a galactosyl residue, while the other component of the multiplet, corresponding to a hydrogen atom at a free hydroxy group, has undergone a paramagnetic shift as the result of acetylation. As also in the case of sileneoside A hepta-acetate (IV), the signal under consideration must be present in the 5.0-5.3 ppm interval [1]. And, in actual fact, in this region of the spectrum of the deca-acetate (VI) it is not difficult to find a broadened multiplet at 5.08 ppm ($W_{1/2}$ = 21 Hz).

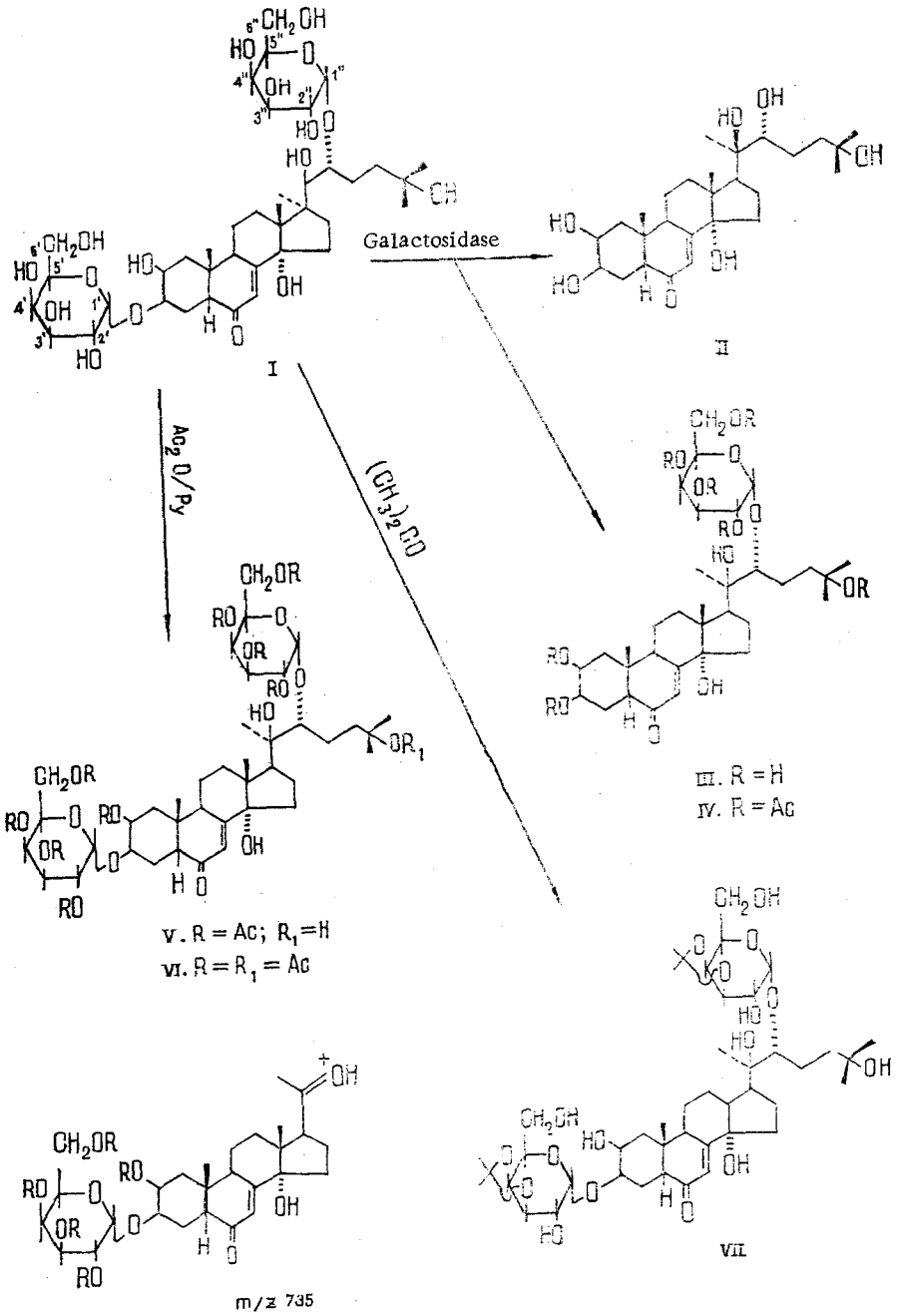
A study of the multifrequency resonance spectra of the acetate (VI) showed that the multiplicity of the signal under consideration at 5.08 ppm became simpler in stages on the successive superposition of additional radiofrequency fields with ν = 402 and 164 Hz, and on simultaneous irradiation with them it was converted into a singlet. Changes of just this type also took place when the signal at 4.02 ppm was observed with the superposition of additional radiofrequency fields with ν = 508 and 179 Hz.

All these facts permitted the unambiguous assignment of the signal at 4.02 ppm with a half-width of 8 Hz to a proton geminal to a D-galactose residue, and the multiplet at 5.08 ppm with a half-width of 21 Hz to a proton geminal to an acetyl group.

On the basis of the cis linkage of rings A/B in ecdysterone (II) one must apparently assume that in the deca-acetate (VI) the signal at 4.02 ppm with the smaller half-width (8 Hz) corresponds to an equatorial hydrogen atom at C-3. Consequently, the second D-galactose residue in sileneoside B (I) is attached through the hydroxy group at C-3.

It has been shown previously that in sileneoside A (III) [1], a D-galactose residue is attached through the hydroxy group at C-22 of ecdysterone by an α-glycosidic bond. The anomeric sugar protons H-1' (at C-3) and H-1" (at C-22) in sileneoside B have close indices: δ 5.40 ppm, J = 3.9 Hz, and δ 5.45 ppm, J = 3.9 Hz, respectively. These facts indicate the α configuration of the glycosidic center at C-3 as well.

The calculated molecular rotation difference between sileneoside B (I) and sileneoside A (III) ($[M]_{D(I)}$ +890°, $[M]_{D(III)}$ +597°, $\Delta[M]_D$ +293°) confirmed that the D-galactose residue was attached through the C-3 hydroxy group by an α-glycosidic bond [9].



Thus, sileneoside B is ecdysterone 3,22-di-O-α-D-galactopyranoside.

EXPERIMENTAL

For the isolation of the ecdysterones and for instruments, chromatographic methods, and GLC conditions, see [1, 2]. PMR spectra were taken in C_5D_5N on a JNM-48-100/100 MHz instrument (δ, 0 — HMDS), and ^{13}C NMR spectra on a Varian CFT-20 instrument in C_5D_5N (0 — TMS).

Sileneoside B (I). $C_{39}H_{64}O_{17}$, mp 236-238°C (from a mixture of methanol and ethyl acetate); $[\alpha]_D^{22}$ + 110.7 ± 2° (c 0.51; methanol). $\lambda_{max}^{C_2H_5OH}$: 246 nm (log ε 4.11) ν_{max}^{KBr} (cm^{-1}): 3370–3420 (OH), 1660 (Δ^7-6-keto grouping); CD (c 0.10 methanol): Δε = —6.3 (250 nm); Δε = +2.8 (330 nm).

Mass spectra, m/z (%): 642 (M^+ —162; 0.5), 624 (1), 606 (3), 588 (17), 570 (3), 490 (3), 462 (10), 444 (12), 429 (17), 426 (41), 411 (19), 409 (20), 363 (11), 345 (45), 327 (46), 309 (27), 300 (27), 163 (10), 161 (10), 145 (11), 143 (10), 99 (100), 81 (36), 69 (35).

Enzymatic Hydrolysis of Sileneoside B (I). To 71 mg of the diglycoside (I) was added 2 ml of an aqueous solution of the enzymes obtained from 670 g of sweet almonds [6]. After being kept at 27°C for 22 days, the reaction mixture was diluted with 20 ml of water and ex-

tracted with butanol (3 × 10 ml). The solvent was evaporated off and the residue was chromatographed on a column of silica gel. Elution with the chloroform–methanol (4:1) system gave 10 mg of ecdysterone (II) with mp 240-241°C (from acetone), $[\alpha]_D^{22}$ +60.7 ± 2° (c 0.87; methanol).

Continuing the elution of the column with the same solvent system led to 7 mg of sileneoside A (III), mp 254-256°C (from methanol–water), $[\alpha]_D^{22}$ +92.4 ± 2° (c 0.69; methanol), identical with an authentic sample according to both TLC and IR spectra [1].

Preparation of the 2,2',2",3',3",4',4",6',6"-Nonaacetate (V) and the 2,2',2",3',3",4',4",6',6",25-Decaacetate (VI) of Sileneoside B. A solution of 144 mg of sileneoside B (I) in 7 ml of pyridine was acetylated with 7 ml of acetic anhydride at room temperature for 7 days. Then the reaction mixture was diluted with water and extracted with chloroform (5 × 100 ml). The solvent was evaporated off and the residue was chromatographed on a column of silica gel. Elution with the benzene–acetone (4:1) system yielded 94 mg of the decaacetate (VI), $C_{59}H_{84}O_{27}$, mp 142-144°C (from benzene–hexane), $[\alpha]_D^{22}$ +119.6 ± 2° (c 0.11; methanol). ν_{max}^{KBr} (cm^{-1}): 3530 (OH); 1760, 1230-1250 (ester grouping); 1675 (Δ^7-6-keto grouping).

Mass spectrum, m/z (%): 1206 ($M - H_2O$; 1), 1188 (0.2), 1149 (0.3), 1146 (0.5), 1128 (0.4), 1030 (0.2), 845 (0.9), 827 (0.6), 817 (0.5), 816 (0.5), 799 (1), 781 (0.8), 780 (0.9), 757 (0.4), 735 (7), 717 (5), 700 (3), 699 (3), 675 (1), 657 (0.5), 622 (0.6), 619 (1), 528 (0.4), 511 (0.9), 495 (0.7), 447 (0.8), 429 (0.8), 411 (0.7), 387 (2), 331 (100), 289 (6), 299 (6), 211 (5), 187 (4), 169 (36), 157 (7), 151 (18), 138 (18), 126 (18), 109 (34), 99 (12), 81 (12).

Further washing of the column with the same mixture of solvents led to 20 mg of the nonaacetate (V), $C_{57}H_{82}O_{26}$, mp 126-128°C (from benzene–hexane), $[\alpha]_D^{22}$ +124.0 ± 2° (c 0.79; methanol). ν_{max}^{KBr} (cm^{-1}): 3560 (OH); 1765, 1230-1260 (ester grouping); 1675 (Δ^7-6-keto grouping).

Mass spectrum, m/z (%): 1164 ($M^+ - H_2O$; 0.4), 1146 (0.4), 1128 (0.5), 1104 (0.1), 1086 (0.3), 1068 (0.2), 1047 (0.4), 1030 (0.3), 1002 (0.2), 941 (0.3), 798 (0.9), 780 (0.5), 735 (2), 717 (1), 701 (0.6), 700 (0.6), 673 (0.5), 619 (0.4), 600 (0.4), 528 (0.4), 331 (100), 287 (7), 229 (8), 99 (45), 81 (40).

Preparation of Sileneoside B 3',4':3",4"-diacetonide (VII) from (I). A solution of 128 mg of sileneoside B (I) in 20 ml of anhydrous acetone was treated with 5 mg of molybdophosphoric acid, and the reaction mixture was shaken at room temperature until the components had dissolved completely. After 4 days, the mixture was diluted with 50 ml of water and was neutralized with sodium bicarbonate. The neutral solution was extracted with ethyl acetate, and the extract was chromatographed on a column of silica gel. Elution of the column with the chloroform–methanol (10:1) system yielded 15 mg of the 3'4':3"4"-diacetonide (VII), $C_{45}N_{72}O_{17}$, mp 185-187°C (from benzene–hexane), $[\alpha]_D^{22}$ +78.7 ± 2° (c 0.46; methanol). ν_{max}^{KBr} (cm^{-1}): 3430 (OH); 1665 (Δ^7-6-keto grouping).

Mass spectrum, m/z (%): 866 ($M^+ - H_2O$; 0.3), 851 (1), 848 (0.9), 833 (0.9), 830 (0.4), 789 (0.4), 749 (0.3), 688 (1), 675 (1), 668 (1), 664 (2), 649 (3), 646 (4), 628 (13), 613 (3), 610 (3), 588 (2), 570 (2), 554 (3), 547 (15), 530 (8), 503 (3), 502 (6), 455 (2), 444 (3), 443 (2), 426 (10), 345 (24), 327 (25), 203 (58), 145 (56), 99 (100), 81 (57).

SUMMARY

A new ecdysteroid has been isolated from the roots of the plant *Silene brahuica* Boiss.; it is ecdysterone 3,22-di-O-α-D-galactopyranoside.

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X-RAY STRUCTURAL STUDY OF 3β-ACETOXY-(25R)-5α-SPIROSTAN-12-ONE

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An x-ray structural study has been made of a compound with the composition $C_{29}H_{44}O_5$. The bond lengths and valence angles are the usual ones for compounds of this type. All the six-membered rings (A, B, C, and F) have the chair conformation. The five-membered rings D and E have the forms of 14α- and 22α-envelopes, respectively. Rings A, B, C, and D are trans-linked, and D and E cis-linked.

Steroid glycosides are widespread in the vegetable kingdom and possess an extremely interesting spectrum of biological action: they inhibit the growth of malignant neoplasms [1], they exhibit fungicidal action on some pathogenic fungi [2]; they bind cholesterol in the form of insoluble complexes [3]; they possess antimicrobial activity [4]; and they are modifying agents of bilayer lipid membranes [5]. The results of a determination of the dependence of the antitumoral activity of steroid glycosides on their composition and structure have been given previously [1, 6].

In order to establish its molecular structure, an x-ray study has been made of 3β-acetoxy-(25R)-5α-spirostan-12-one, with the composition $C_{29}H_{44}O_5$. The compound was obtained as the result of the hydrolysis of the steroid glycosides from century-plant agave [7]. After crystallization, the aglycone was acetylated and the product was obtained in the form of single crystals.

Figure 1 gives sketches of the molecule with the bond lengths and the valence and torsional angles. All the six-membered rings have the chair conformation. The greatest deviation from the ideal conformation is observed for ring C. The five-membered ring D forms a 14α-envelope, and E adopts the form of a 22α-envelope. The molecule as a whole is nonplanar. Rings A, B, C, and D are trans-linked, and D and E are cis-linked (Fig. 2). The mean plane of the six-membered ring F is approximately perpendicular to the plane of the neighboring five-membered ring E. This mutual orientation of these rings is determined by the tetrahedral configuration of the C(22) atom common to them. The angle between the planes of the C(20), C(22) and O(2) and the C(23), C(22), and O(3) fragments is 93.3°. The acetate group does not lie in the mean plane of the linked ring A–E. It makes an angle of 73° with the C(2)–C(3)–C(4) plane.

The interatomic distances and valences angles in the structure studied agree with the values observed in other compounds [8-13]. The C–C distances, except for the bonds of C(3) with the neighboring carbon atoms are close to the value for an ordinary $C(sp^3)$–$C(sp^3)$ bond. The C(12) atom is in a state of sp^2 hybridization, and the distance from it to the C(11) and C(13) atoms correspond to the standard value for bonds of the $C(sp^2)$–$C(sp^3)$ type. The C(3)–C(2) and C(3)–C(4) distances are shortened in comparison with the length of a corresponding ordinary bond. At the same time, the C(3)–O(1) bond is somewhat lengthened. Close values of the lengths of the bonds of the C(3) atom with the neighboring C and O atoms have been found in methyl 3β-acetoxy-17α-methyl-18-nor-5α-androstan-17β-carboxylate [13]. The C(12)–O(4) interatomic distance is close to the value for a double bond. The distances between the intracyclic oxygen atoms O(2) and O(3) and the carbon atoms correspond to ordinary bonds.

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