PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS *Silene*.

IV. SILENEOSIDE C — A GALACTOSIDE OF INTERGRISTERONE A FROM *Silene brahuica*

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UDC 547.926:591.147

The phytoecdysteroid sileneoside C has been isolated from the roots of *Silene brahuica* Boiss. It has been shown that sileneoside C is intergristerone A 22-O-α-D-galactoside.

We have previously reported the isolation from the roots of *Silene brahucia* Boiss. of three new phytoecdysteroids (compounds B, D, and E) and the determination of the structure of sileneoside A (substance B). Products D and E which we have called, respectively, sileneosides B and C, are also of glycosidic nature. In this paper we give a proof of the structure of sileneoside C (I).

Sileneoside C has absorption characteristics for ecdysteroids in the UV spectrum at 245 nm (log ε 4.01) and in the IR spectrum at 1658 cm^{-1}. The CD curve of compound (I) shows negative and positive Cotton effects, with $\Delta\varepsilon = -3.81$ (245 nm) and $\Delta\varepsilon = +1.36$ (338 nm). The nature of the CD curve shows the cis linkage of rings A/B [2].

The closeness of the values of the chemical shifts of the methyl groups of the PMR spectrum of compound (I) to the corresponding indices for integristerone A (II) (Table 1) permitted the assumption that the new ecdysteroid was glycoside of integristerone A. In actual fact, integristerone A (II) [4] was found in the products of the enzymatic hydrolysis of silenoside C with a total enzyme obtained from sweet almond [3]. It was shown by the GLC method [5] that glycoside (I) contained one D-galactose residue.

The acetylation of sileneoside C (I) with acetic anhydride in pyridine gave the heptaacetate (V) and the octaacetate (VI). The mass spectra of the acetates (V) and (VI) each had a peak with m/z 505 ($C_{27}H_{37}O_9$), formed through the cleavage of the C-20—C-22 bond. The presence of this fragment shows that the sugar residue was not attached to the steroid part of the molecule but was present in the side chain.

As can be seen from Table 1, the characteristics of the PMR spectra of acetates (V) and (VI) are extremely close, with the exception of the shifts of the C-6 and C-7 methyl groups. In the case of seleneoside C (I) and its heptaacetate (V), the signals of these CH_3 groups appear at 1.24/1.29 and 1.31/1.31 ppm, respectively. In the case of the octaacetate (VI), the same signals are shifted downfield (1.41/1.47 ppm), which is obviously connected with the presence of an acetyl group at C-25. Thus, the possibility of the attachment of the sugar residue at the C-25 hydroxyl is excluded.

In the acetates (III) and (IV) formed by the acetylation of integristerone A, the C-22 proton gives a signal shifted downfield, with resonance in the 5.0-5.2 ppm region. The analogous index for sileneoside C is 3.58 ppm. It changed little in the acetates of this glycoside (V) and (VI) — 3.64 and 3.62 ppm, respectively. Consequently, there are grounds for assuming that in glycoside (I) the sugar residue is attached precisely through the C-22 hydroxyl.

In the ^{31}C NMR spectrum of sileneoside C, the signal of the C-22 carbon atom appears at 90.76 ppm and is shifted downfield by 13.40 ppm in comparison with the corresponding signal (77.36 ppm) in the spectrum of integristerone A (II).

The facts given unambiguously show that attachment of the D-galactose residue through the hydroxy group at C-22.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 211-214, March-April, 1982. Original article submitted June 30, 1981.

0009-3130/82/1802-0193$07.50 © 1983 Plenum Publishing Corporation

TABLE 1. Chemical Shifts of the Protons of Sileneoside C (I) and of Integristerone A (II) and Their Derivatives (δ, ppm, O — HMDS)

Compound	Positions of the protons											
	H-1	H-2	H-3	H-7	H-9	H-22	H-1′	CH_3-18	CH_3-19	CH_3-21	CH_3-26/27	OAc
I	4,13	4,13	4,13	6,06	3,41	3,58	5,46 d, ^3J=3,3Hz	1,11	1,26	1,48	1,24 1,29	—
II	4,15	4,15	4,15	6,14	3,42	3,71	—	1,07	1,26	1,43	1,24 1,24	—
III	5,65	5,10—5,50		6,03	3,43	5,0—5,2	—	0,97	0,97	1,44	1,23 1,23	1,84; 1,88;, 1,92; 2,03
IV	5,62	5,10—5,50		6,02	3,45	5,0—5,2	—	0,97	0,97	1,44	1,30 1,30	1,81 (6H); 1,90 (6H); 2,02
V	5,30—5,75			6,01	3,42	3,64	5,80	0,91	1,03	1,39	1,31 1,31	1,86 (6H); 1,91 (9H) 2,03; 2,08
VI	5,30—5,75			5,99	3,41	3,62	5,81	0,91	1,01	1,38*	1,41* 1,47	1,85; 1,87 (6H);, 1,91 (9H); 2,03; 2,08

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 group have a singlet nature; 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′) as broadened multiplets. In the case of compounds (III) and (IV), the signals from H-22 overlap with the multiplets from H-2 and H-3. The values denoted by asterisks may be interchanged.

The spin-spin coupling constant (^3J = 3.3 Hz) and the magnitude of the chemical shift (δ 5.46 ppm) of the signal of the anomeric proton shows the α configuration of the glycosidic center [6]. This is also confirmed by a molecular rotation difference calculation [7].

Consequently, sileneoside (I) is integristerone A 22-O-D-galactoside (see Scheme).

EXPERIMENTAL

For methods of isolation, instruments, and conditions of chromatography, see [1].

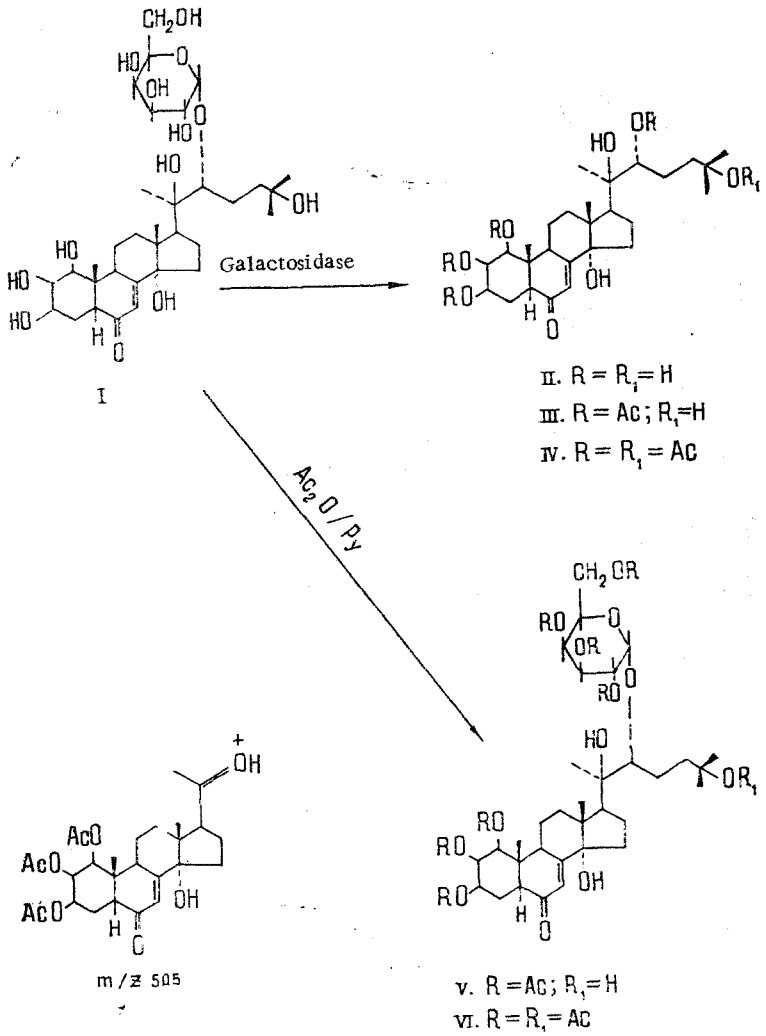
As in the determination of the structure of sileneoside A, sugars were identified in the form of the trimethylsilyl ethers of methyl glycosides by the GLC method on a Chrom-5 instrument using a column (3.7 m × 3 mm) containing Chromaton N-AW impregnated with 5% of SE-30 silicone phase. The thermostat temperature was 190°C and the rate of flow of the carrier gas, helium, was 44 ml/min.

Primes denote the carbon atoms of the sugar moiety.

Sileneoside C (I). $C_{33}H_{54}O_{13}$, mp 232-234°C (from methanol—ethyl acetate), $[\alpha]_D^{22}$ +48.0 ± 2° (c 1.02; methanol; $\lambda_{max}^{C_2H_5OH}$: 245 nm (log ε 4.01). ν_{max}^{KBr} (cm^{-1}): 3370, 3340 (OH); 1658 (Δ⁷-6-keto grouping). CD (c 0.12; methanol): Δε = −3.81 (245 nm); Δε = +1.36 (338 nm).

Mass spectrum: m/z (%): 460 (M^+ −180-H_2O 0.6), 422(4), 427(4), 424(2), 368(2), 361(4), 360(4), 343(8), 325(4), 316(4), 308(2), 301(2), 163(3), 145(4), 143(6), 125(5), 99(100), 81 (98).

Enzymatic Hydrolysis of Sileneoside C (I). To 50 mg of sileneoside C (I) was added 10 ml of an aqueous solution of the enzymes obtained from 0.5 kg of sweet almond [3]. After the reaction mixture had been kept at 26°C for 24 days, 20 ml of water was added to the vessel and the resulting mixture was extracted with butanol (3 × 15 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 integristerone A (II) with mp 242-244°C (from ethyl acetate—methanol), $[\alpha]_D^{22}$ +34.0 ± 2° (c 0.97; methanol), identical with an authentic sample also from its TLC behavior and its IR spectrum.



I

II. R = R1 = H
III. R = Ac; R1 = H
IV. R = R1 = Ac

V. R = Ac; R1 = H
VI. R = R1 = Ac

The acetylation of (II) as described previously [4] yielded integristerone A 1,2,3,22-tetraacetate (III), mp 170-172°C, and integristerone A 1,2,3,22,25-pentaacetate (IV), mp 150-152°C.

Sileneoside C 1,2,2',3,3',4',6'-Heptaacetate (V) and 1,2,2',3,3',4',6',25-Octaacetate (VI). A solution of 100 mg of sileneoside C (I) in 3 ml of pyridine was acetylated with 3 ml of acetic anhydride at room temperature for 7 days. Then the reaction mixture was diluted with water and the precipitate that deposited (110 mg) was filtered off and chromatographed on a column of silica gel. Elution with the benzene–acetone (5:1) system gave 35 mg of the octaacetate (VI), $C_{49}H_{70}O_{21}$, mp 140-142°C (benzene–hexane), $[\alpha]_D^{22}$ +52.4 ± 2° (c 0.69; methanol). $\lambda_{max}^{C_2H_5OH}$: 243 nm (log ε 4.10). ν_{max}^{KBr} (cm^{-1}): 3560 (OH); 1755, 1220-1270 (ester grouping); 1678 (Δ^7-6-keto grouping).

Mass spectrum: m/z (%): 976 ($M^+ - H_2O$: 0.03); 958 (0.04), 934(0.2), 916(0.6), 898(0.2), 874(0.2), 856(0.1), 838(0.1), 835(0.5), 643(0.2), 629(0.2), 615(2), 597(7), 587 (3.4), 569(7), 528(0.6), 505(14), 487(11), 461(8), 445(8.2), 443(4), 442(1), 427(7.8), 403(2.5), 392 (10), 385(5.7), 367(2.5), 343(11), 331(100), 327(2.5), 326(4), 325(12), 169(48), 152(31), 139(25), 109(25), 99(8), 81(9).

Further elution of the column with the same mixture of solvents led to the isolation of 32 mg of the heptaacetate (V), $C_{47}H_{68}O_{20}$, mp 160-162°C (benzene–hexane), $[\alpha]_D^{23}$ +97.5 ± 2° (c, 0.41; methanol). $\lambda_{max}^{C_2H_5OH}$; 243 nm (log ε 4.15), ν_{max}^{KBr} (cm^{-1}): 3565 (OH); 1755, 1235 (ester grouping); 1672 (Δ^7-6-keto grouping).

Mass spectrum: m/z (%): 934 ($M^+ - H_2O$; 0.03), 916(0.1), 898(0.2), 892(0.4), 874(0.4), 856(0.6), 838(0.4), 835(0.6), 817(0.3), 796(0.4), 772(0.5), 754(0.9), 736(0.2), 712(0.2), 681(0.8), 656(0.2), 652(0.4), 615(0.5), 597(1), 587(1), 569(8), 551(4), 532(3), 527(3), 505(6), 487(10),