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PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS Melandrium

II. MELANDRIOSIDE A – A GALACTOSIDE OF VITICOSTERONE E FROM Melandrium turkestanicum

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The isolation and determination of the structure of the new phytoecdysteroid melandroside A are described; it is viticosterone E 22-O-α-D-galactopyranoside.

We have shown that the epigeal organs of the plants Melandrium turkestanicum (Rgl) Vved (family Caryophyllaceae) are a source of various ecdysteroids, including sileneosides A and D [1].

Rechromatography on a column of silica gel of the mother liquors obtained in the isolation of these ecdysteroids led to the isolation of a new phytoecdysteroid (I), with the composition $C_{35}H_{56}O_{13}$, which we have called melandrioside A.

The IR spectrum of compound (I) contained, in addition to the absorption due to hydroxy groups (3400-3450 cm^{-1}) and to an α,β-unsaturated keto grouping (1670 cm^{-1}), absorption bands at 1720 and 1270 cm^{-1} showing the presence of an ester group. The presence of such a group was confirmed by a three-proton singlet in the PMR spectrum at 1.84 ppm.

The results of an analysis by the GLC method of the products of the methanolysis of the ecdysteroid (I) showed that this contained a D-galactose residue and was a monoside.

The enzymatic hydrolysis of melandrioside A by the total enzymes isolated from sweet almond led to the formation of a product (II) which was identified as viticosterone E [2, 3].

Attention is attracted by the small upfield shift of the H-22 signal in compound (I) as compared with that for viticosterone E (II) in the PMR spectra of these substances (Table 1). A similar shift has been observed for sileneoside A (III) in comparison with ecdysterone (V) (see Table 1). If it is also borne in mind that in the mass spectrum of (I) there is the characteristic peak of an ion with m/z 363, it may be assumed that the D-galactose residue in melandrioside A is located at C-22 (see scheme on top of following page).

The spin–spin coupling constant (^3J = 3.2 Hz) and the chemical shift (δ 5.46 ppm) of the signal of the anomeric proton showed the α-configuration of the glycosidic center [4].

To confirm the structure of glycoside (I), we saponified the heptaacetate of sileneoside A (IV), which has been described previously [5], with 0.5 $KHCO_3$ in methanol. As was to be expected, under these conditions the acetyl group at C-25 remained unaffected, and the reaction product coincided in its physicochemical constants and spectral characteristics with melandrioside A (I).

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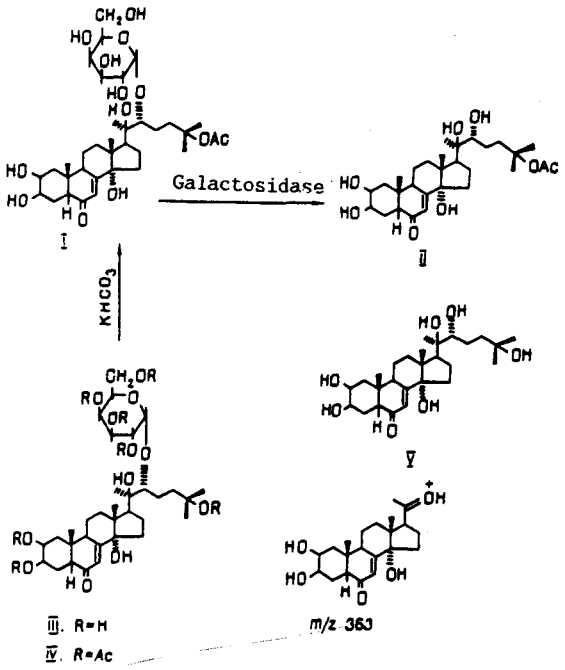

Thus, melandrioside A is viticosterone E 22-O-α-D-galactopyranoside.

EXPERMENTAL

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-567A instrument (100 MHz, Tesla) in C_5D_5N (δ, 0 – HMDS). For the conditions of performing TLC, see [6]; for general observations, see [1].

Isolation of Melandrioside A (I). The mother solutions obtained after the separation of sileneosides A and D were combined and chromatographed on a column of silica gel with elution by chloroform–ethanol (4:1). This yielded 52 mg (0.00071%) of melandrioside A, $C_{35}H_{56}O_{13}$, mp 204-206°C (methanol–ethyl acetate), $[\alpha]_D^{20}$ +92.3 ± 2° (c 0.17; methanol); ν_{max}^{KBr} (cm^{-1}): 3400-3450 (OH), 1670 (Δ^7-6-keto grouping); 1720, 1270 (ester group). Mass spectrum, m/z (%): 588 ($M^+ - CH_3COOH-2H_2O$; 0.7), 573 (0.4), 570 (0.8), 468 (1.5), 444 (18), 426 (80), 411 (26), 408 (60), 393 (27), 363 (18), 345 (100), 328 (98), 327 (99), 301 (40), 300 (40), 145 (30), 145 (32), 125 (32), 99 (32), 81 (32), 69 (32).

Methanolysis of Melandrioside A (I). A solution of 3.0 mg of glycoside (I) in 3 ml of absolute methanol containing 5% of hydrogen chloride was boiled for 16 h. Then an equal volume of water was added to the reaction mixture, the acid was neutralized with silver car-

TABLE 1. Chemical Shifts of the Protons of Compounds (I)-(III) and (V) (δ, ppm, 0 – HMDS)

Compound	Positions of the protons									
	H-2,3	H-7	H-9	H-22	H-1'	CH_3-18	CH_3-19	CH_3-21	CH_3-26/27	OAc
I	3,99–4,10	6,04	3,47	3,58	5,46 d ^3J=3,2 Hz	1,07	0,91	1,51	1,40;1,47	1,84(3H)
II [3]	4,02–4,05	6,03	3,50	3,67	—	1,08	0,94	1,46	1,29;1,35	1,79(3H)
III [5]	4,0–4,2	6,11	3,42	3,59	5,50 d ^3J=3,4 Hz	1,09	0,90	1,49	1,24;1,39	
V	3,9–4,2	6,07	3,43	3,70	—	1,07	0,94	1,44	1,25;1,25	

The spectra were taken in C_5D_5N. In all cases, the signals of the protons of the methyl groups were singlets; the H-7 proton appeared in the form of a broadened singlet, and the other signals (with the exception of H-1') as broadened multiplets.

bonate, and the precipitate was filtered off. The filtrate was evaporated to dryness. D-Galactose was detected by the GLC method [6]. For quantitative determination, the methanolysis of melandrioside A (I) was performed with the use of D-glucose as internal standard. According to the results of quantitative determination, melandrioside A contained one D-galactose residue.

Enzymatic Hydrolysis of Melandrioside A (I). To 15 ml of glycoside (I) was added 5 ml of an aqueous solution of the total enzymes obtained from 0.5 kg of sweet almond [7]. After the reaction mixture had been kept at 27°C for 24 days, it was treated with 15 ml of water and was then extracted with ethyl acetate (3 × 10 ml). The solvent was evaporated off. The residue was chromatographed on a column of silica gel. Elution with the chloroform–methanol (9:1) system yielded 7 mg of the ecdysteroid (II) with mp 195-197°C (from acetone), $[\alpha]_D^{20}$ [59.0 ± 2° (c 0.72; methanol), shown to be identical with an authentic sample of viticosterone E by comparison on TLC and from its IR spectrum [2, 3].

Melandrioside A (I) from Sileneoside A Heptaacetate (IV). A solution of the acetate (IV) [5] in 5 ml of methanol was treated with 25 mg of potassium bicarbonate in 3 ml of water. The reaction mixture was left at room temperature for two days. Then it was diluted with water and neutralized, and the methanol was evaporated off. The aqueous solution was extracted with ethyl acetate. The solvent was distilled off to dryness, and the residue was chromatographed on a column of silica gel. Elution with chloroform–methanol (15:1) system gave 4 mg of the glycoside (I) with mp 202-204°C (methanol–ethyl acetate), which was shown to be identical with native melandrioside A from its mobility in TLC and its IR and mass spectra.

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