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PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS Melandrium

I. POLYPODIN B 22-ACETATE FROM Melandrium turkestanicum

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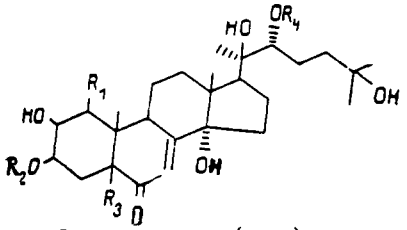
Known ecdysteroids – ecdysterone, ecdysterone 22-acetate, polypodin B, integristerone A, and sileneosides A and D – and a new phytoecdysteroid – polypodin B 22-acetate – have been found in the epigeal parts of the plant Melandrium turkestanicum (Rgl.) Vved. (family Caryophyllaceae).

Extending the range of plants of the family Caryophyllaceae containing ecdysteroids that have been studied [1], we have investigated Melandrium turkestanicum (Rgl.) Vved., not infrequently growing on the rocky slopes in the central zone of the mountains of the Pamir-Alai and the western Tien-Shan. From the epigeal organs of this plant we isolated known ecdysteroids: ecdysterone (I), ecdysterone 22-acetate (II), polypodin B (III), integristerone A (IV), and sileneosides A (V) and D (VI). In addition to these compounds, we isolated a new ecdysterone (VII), with the composition $C_{29}H_{46}O_9$.



I. $R_1=R_2=R_3=R_4=H$
II. $R_1=R_2=R_3=H$; $R_4=Ac$
III. $R_1=R_2=R_4=H$; $R_3=OH$
IV. $R_1=OH$; $R_2=R_3=R_4=H$
V. $R_1=R_2=R_3=H$; $R_4=\alpha$-*D*-Gal
VI. $R_1=R_3=R_4=H$; $R_2=\alpha$-*D*-Gal
VII. $R_1=R_2=H$; $R_3=OH$; $R_4=Ac$

The IR spectra of substance (VII) had absorption bands due to hydroxy groups (3400-3500 cm^{-1}) and to an α,β-saturated keto grouping (1685 cm^{-1}). In addition to them, absorption at 1735 and 1260 cm^{-1} and the presence of a three-proton singlet at 2.04 ppm in the PMR spectrum (Table 1) showed the presence of one acetyl group in this compound.

The enzymatic hydrolysis of the acetate (VII) by the combined enzymes isolated from bakers' yeast [2], and also alkaline saponification, led to polypodin B (III) [3, 4]. The peak of an ion with m/z 379 (C-20–C-22 cleavage) observed in the mass spectrum of ecdysterone (VII), and its derivatives with m/z 361, 343, and 325, permitted the assumption that the acetyl group was located in the side chain and not in the steroid nucleus [3, 5-7].

On comparing the PMR spectrum of polypodin B (III) and the acetate (VII), an appreciable difference was observed only in the position of the resonance lines of the proton at C-22,

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TABLE 1. Chemical Shifts of the Protons of Ecdysterone (I), Ecdysterone 22-Acetate (II), Polypodin B (III), and Polypodin B 22-Acetate (VII) (δ, ppm; 0 – TMS)

Compound	Positions of the protons								
	H-2,3	H-7	H-9	H-22	CH_3-18	CH_3-19	CH_3-21	CH_3-26/27	OAc
I	4,6—4,3	6,21	3,57	3,84	1,19	1,09	1,55	1,34	—
II	4,52	6,24	3,57	5,52	1,14	1,07	1,62	1,32	2,00
III	4,11	6,15	3,55	3,81	1,19	1,13	1,56	1,35	—
VII	4,10	6,25	3,62	5,49	1,16	1,16	1,65	1,35	2,04

The spectra were taken in C_5D_5N. The signals of the protons of the methyl groups were singlets; in all cases the H-7 proton appeared in the form of a broadened singlet and the other signals as broadened multiplets.

which were shifted downfield by 1.68 pm and apepared at 5.49 ppm. An analogous downfield shift was observed in the case of ecdysterone 22-acetate (Table 1).

On the basis of what has been said above, it may be concluded that ecdysteroid (VII) was polypodin B 22-acetate (2β,3β,5,14α,20R,22R,25-heptahydroxy-5β-cholest-7-ene 22-acetate).

EXPERIMENTAL

Thin-layer chromatography (TLC) was conducted on Silufol plates. For column chromatography we used KSK silica gel and silica gel L 100/160 (Czechoslovakia). The solvent systems used were chloroform–methanol: 1) (15:1); 2) (9:1); and 3) (4:1); and chloroform–methanol–water: 4) (4:1:0.1); and 5) (70:23:2). The ecdysteroids were detected on TLC by spraying with vanillin/sulfuric acid followed by heating at 110-120°C for 2-5 min [8].

Mass spectra were recorded on a MKh-1310 instrument at an ionizing voltage of 50 V and a temperature of 100-140°C; IR spectra on a UR-20 spectrometer in KBr; and PMR spectra on a BS-567A instrument (100 MHz, Tesla) in C_5D_5N (δ, 0 – TMS).

Isolation of the Main Phytoecdysteroids. The epigeal part of the plant Melandrium turkestanicum (Rgl.) Vved. was collected in May, 1986 (Takhta-Koracha pass, Zeravshan range). The dried and comminuted raw material (7 kg) was exhaustively extracted with methanol (35 liters). The extract was concentrated, the residue was diluted with water, and the resulting precipitate was taken off. The methanol was evaporated, and the aqueous residue was treated with chloroform. The ecdysteroids were extracted from the purified aqueous fraction first with ethyl acetate and then with butanol.

The dry residue (40 g) obtained after the distillation of the ethyl acetate was chromatographed on a column of silica gel. Elution was performed first with system 1, giving 210 g of a mixture of three substances (for their further treatment, see below), and then with system 2. This led to the isolation of 120 mg (0.0017%) of polypodin B (III) (here and below, the yields given are calculated on the air–dry material), $C_{27}H_{44}O_8$, mp 251-252°C (acetone), $[\alpha]_D^{20}$ +91.2 ± 2° (c 0.32; methanol) [3, 4].

When the column was eluted with system 3, 6.0 g (0.085%) of ecdysterone (I) was isolated; $C_{27}H_{44}O_7$, mp 239-241°C (from acetone), $[\alpha]_D^{20}$ +59.6 ± 2° (c 0.41; methanol) [4, 8].

The combined substances (124 g) remaining after the butanol had been distilled off were chromatographed on a column of silica gel. Elution with system 4 gave an additional 0.042% of ecdysterone (I).

The further washing of the column with the same system led to 350 mg (0.005%) of integristerone A ($C_{27}H_{44}O_8$, mp 245-246 (from ethyl acetate–methanol); $[\alpha]_D^{20}$ +35.6 ± 2° (c 0.32; methanol) [4, 9].

Elution of the column with system 5 led to the isolation of 200 mg (0.0028%) of sileneoside A (V), $C_{33}H_{54}O_{12}$, mp 253-255°C (from methanol–water); $[\alpha]_D^{20}$ +92.7 ± 2° (c 0.47; methanol) [4].

The mother solution remaining after the crystallization of the sileneoside A was concentrated and chromatographed on a column of silica gel with elution by system 5. This gave

120 mg (0.0017%) of sileneoside D (VI), $C_{33}H_{54}O_{12}$, mp 240-241°C (from methanol–acetone), $[\alpha]_D^{20}$ +90.1 ± 2° (c 0.35; methanol) [10].

Isolation of Ecdysterone 22-Acetate (II) and Polypodin B 22-Acetate (VII). The mixture of three acdysteroids (210 mg) obtained from the ethyl acetate fraction and subjected to preliminary purification was rechromatographed on a silica gel column. Elution with system 1 led to the successive isolation of 27 mg (0.0004%) of polypodin B 22-acetate (VII) and 50 mg (0.0001% of substance (II), $C_{29}H_{46}O_8$, mp 152-154°C (acetone), which was identified by its physicochemical constants and spectral characteristics as ecdysterone 22-acetate [8, 11].

Polypodin B 22-acetate (VII); $C_{29}H_{46}O_9$, mp 150-152°C (methanol–water), $[\alpha]_D^{20}$ +120 ± 2° (c 0.30; methanol). ν_{max}^{KBr} (cm^{-1}): 3400-3500 (OH), 1685 (Δ^7-6-keto grouping), 1735, 1260 (ester group).

Mass spectrum, m/z (%): 520 ($M^+ - H_2O$; 0.3), 502 (0.7), 484 (1), 460 (18), 442 (100), 427 (18), 424 (14), 409 (9), 391 (4.5), 379 (1), 361 (4), 343 (5.9), 325 (5), 266 (18), 265 (18), 203 (8), 185 (11), 153 (51), 152 (50), 99 (22), 81 (25), 69 (41).

Hydrolysis of Polypodin B 22-Acetate (VII) [giving (III)]. Enzymatic. A mixture of 5 mg of the ecdysteroid (VII) and 0.2 ml of alcohol was added to 10 ml of a freshly prepared aqueous extract from 1 g of bakers' yeast. After the reaction mixture had been kept at 36-38°C for 15 days, it was diluted with 20 ml of water and extracted with ethyl acetate (3 × 15 ml). The ethyl acetate fraction was evaporated to dryness. The residue as dissolved in chloroform–methanol (4:1) and the solution was filtered through a small layer of silica gel. After the solvent had been distilled off, the reaction product was recrystallized from acetone. This gave 2 mg of a compound with mp 251-252°C which was identified by direct comparison in TLC and from its mass spectrum as polypodin B (III) [3, 4].

With Alkali. The acetate (VII) (10 mg) was added to 5 ml of a 0.5% aqueous methanolic solution of $KHCO_3$. The reaction mixture was left at room temperature for 3 h and was then diluted with water and neutralized with acetic acid, and the reaction product was extracted with ethyl acetate. The solvent was distilled off to dryness, and the residue was recrystallized from acetone. This gave 5 mg of polypodin B (III) mp 251-252°C [3, 4].

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