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PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS *Rhaponticum*
POLYPODIN B 22-O-BENZOATE FROM
Rhaponticum carthamoides

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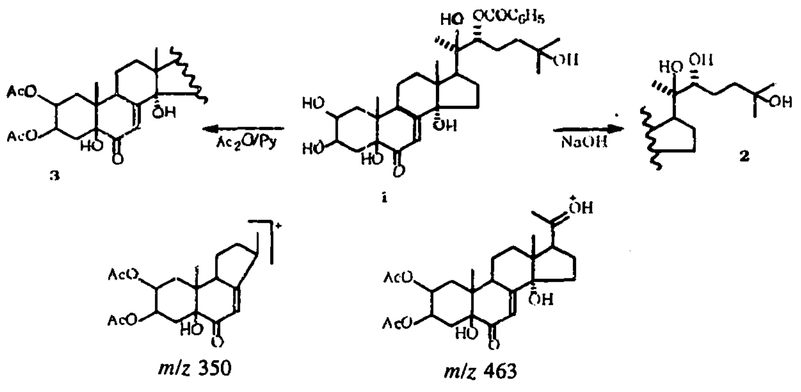
A known ecdysteroid — α-ecdysone — and a new phytoecdysteroid — polypodin B-22-O-benzoate — have been isolated from the seeds of Rhaponticum carthamoides *(Willa) Iljin. Alkaline hydrolysis of the latter gave polypodin B. Its structure has been established on the basis of chemical transformations and spectral characteristics.*

Continuing the study of the phytoecdysteroids of the seeds of the plant *Rhaponticum carthamoides* (fam. Compositae) [1], we have isolated the known ecdysteroid α-ecdysone. Together with this compound, we isolated a new ecdysteroid (1) with the composition $C_{34}H_{48}O_9$.

The IR spectrum of substance (1) contained absorption bands due to hydroxy groups (3432 cm^{-1}) and to an α,β-unsaturated keto grouping (1654 cm^{-1}). In addition, absorption bands were detected at 1710 and 1281 cm^{-1} (ester) and 1610, 1587, and 714 cm^{-1} (aromatic ring), permitting the assumption that there was an ester group of aromatic nature in ecdysteroid (1). This was also indicated by the signals of five aromatic protons at 7.35 ppm (3H) and 8.24 ppm (2H) in the PMR spectrum and also by intense peaks of ions with *m/z* 122 ($C_7H_6O_2$), 105 (C_7H_5O), and 77 (C_6H_5) characteristic of the fragmentation of benzoic cid.

After the alkaline saponification of ecdysteroid (1), polypodin B (2) was identified in the neutral fraction and benzoic acid in the acid part of the hydrolysate.



The peaks observed in the mass spectrum of ecdysteroid (1) of an ion with *m/z* 379 (C-20–C-22 cleavage) and of its derivatives with *m/z* 361, 343, and 325 permitted the assumption that the benzoic acid was not bound to the steroid nucleus but was attached to the side-chain [2-4]. The acetylation of ecdysteroid (1) led to the diacetate (3). The mass spectrum of (3) showed intense peaks of ions with *m/z* 463 and 350 [5], confirming that the benzene residue was present in the side-chain.

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A comparison of the chemical shifts of the protons in the PMR spectra of polypodin B and ecdysteroid (1) revealed that in the latter the signals of the protons at C-22 and 21-CH_3 were shifted downfield, which could be due to the addition of the benzoic acid residue to the hydroxy group at C-22.

Thus, ecdysteroid (1) is polypodin B-22-O-benzoate.

EXPERIMENTAL

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

Isolation of Phytoecdysteroids. The fractions enriched with compound (1) obtained in the isolation of 24(28)-dehydromakisterone A [1] were chromatographed on a column of silica gel in the chloroform–methanol (9:1) system, with the isolation of 25 mg of ecdysteroid (1). Further washing of the column with the same system gave 150 mg of α-ecdysone.

α-Ecdysone — $C_{27}H_{41}O_6$, mp 236-238°C (methanol–water), $[\alpha]_D^{20}$ +63.5 ± 2° (methanol). A comparison by TLC in the chloroform–methanol (9:1) system with an authentic specimen of α-ecdysone showed their identity. The IR, mass, and PMR spectra of the compound and of α-ecdysone agreed completely [6].

Polypodin-B 22-O-Benzoate (1). $C_{34}H_{48}O_9$, mp 196-198°C (from methanol–water).

IR spectrum (KBr, ν, cm^{-1}): 3432 (OH), 1654 (7-ene-6-keto grouping), 1710, 1281 (ester), 1610, 1587, 714 (benzene ring).

Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 442 (M^+-$C_6H_5COOH-2H_2O$; 47), 429 (99), 409 (57), 391 (29), 373 (29), 361 (100), 343 (47), 325 (47), 325 (47), 299 (57), 298 (56), 265 (45), 229 (57), 122 (95), 105 (71), 81 (23), 77 (71).

PMR (100 MHz C_5D_5N, δ, ppm HMDS): 1.00 (3H, s, CH_3-19), 1.07 (3H, s, CH_3-18), 1.17 (6H, s, CH_3-26 and CH_3-27), 1.65 (3H, s, CH_3-21), 3.50 (1H, m, H-9), 4.15 (2H, m, H-2 and H-3), 5.70 (1H, d, J=7.5 Hz; H-22), 6.17 (1H, br.s, H-7), 7.35 (3H, m), and 8.24 (2H, d, d, J=1; 7.5 Hz aromatic protons).

Alkaline Hydrolysis of Polypodin B-22-O-benzoate (1). A solution of 10 mg of ecdysteroid (2) in 3 ml of 0.5% methanolic caustic soda was left at room temperature for a day, and was then diluted with water (10 ml) and neutralized. The methanol was evaporated off, and the aqueous residue was extracted with ethyl acetate. The solvent was distilled off to dryness and the residue was chromatographed on a column of silica gel, with elution by the chloroform–methanol (9:1) system. This gave 6 mg of a substance with mp 252-254°C (acetone), identical with an authentic specimen of polypodin B [7]. Benzoic acid with mp 122°C was isolated from the aqueous solution after acidification with dilute (1:1) hydrochloric acid and extraction with ethyl acetate.

Polypodin B-2,3-Di-O-acetate-22-O-benzoate (3). A solution of 10 mg of ecdysteroid (1) in 2 ml of pyridine was acetylated with 2 ml of acetic anhydride at room temperature for a day. Then the reaction mixture was evaporated, and the dry residue was chromatographed on a column of silica gel. Elution with the chloroform–methanol (30:1) system gave 9 mg of the diacetate (3), $C_{38}H_{52}O_{11}$, mp 160-162°C (from aqueous methanol).

IR spectrum (KBr, ν, cm^{-1}): 3446 (OH), 1722, 1744, 1695, 1252, 1218 (ester groups), 1603, 716 (benzene ring), 1669 (Δ^7-6-keto grouping).

Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 532 (M^+-$C_6H_5COOH-2CH_3$; 23), 528 (23), 509 (74), 495 (53), 492 (27), 463 (50), 445 (39), 350 (44), 122 (45), 105 (100), 81 (69), 71 (64), 69 (64).

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