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PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS Silene.

IX. ECDYSTERONE 22-O-BENZOATE FROM Silene scabrifolia

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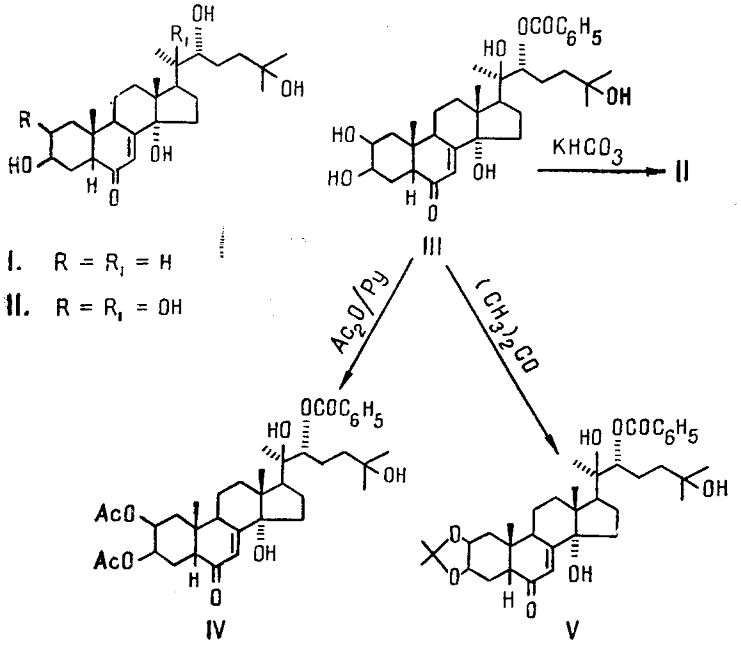
An ecdysteroid which has proved to be ecdysterone 22-O-benzoate has been isolated from the epigeal organs of Silene scabrifolia Kom.

We have included S. scabrifolia Kom. within the sphere of our investigations of the ecdysteroids of plants of the genus Silene (family Caryophyllaceae) [1, 2]. From the epigeal organs of this plant, in addition to the known 2-deoxy-α-ecdysone (I) and ecdysterone (II), we have isolated the new ecdysteroid (III).

Absorption in the UV spectrum at 1710 and 1285 cm^{-1}, in combination the the bands characteristic for the benzene ring (1610, 1587, 720 cm^{-1}) permitted the assumption that compound



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(III) contained an ester group of aromatic nature. This was shown by the signals of five aromatic protons at 7.39 (3 H) and 7.99 (2 H) in the PMR spectrum, and also by strong peaks of ions with m/z 122 ($C_7H_6O_2$), 105 (C_7H_5O), and 77 (C_6H_5), which are characteristic of benzoic acid.

When the ecdysteroid (III) was subjected to alkaline saponification, ecdysterone (II) was identified in the neutral fraction and benzoic acid in the acid fraction (see above).

In a dry acetone solution of the compound (III) that we had isolated, a monoacetonide (V) was formed. The mass spectrum of this derivative had a strong peak of an ion with m/z 403 ($C_{24}H_{35}O_5$) due to the cleavage of the C-20—C-22 bond, which is characteristic for ecdysterone acetonide [3, 4].

The fragment with m/z 403 showed that the benzoic acid was not attached to the steroid nucleus but to the side chain.

The acetylation of the benzoate (III) led to a diacetate (IV).

The PMR spectrum of the ecdysterone benzoate (III) had a one-proton multiplet at 5.06 ppm corresponding in the nature of the signal and the magnitude of the chemical shift to a proton geminal to an acyl residue at C-22. The chemical shift of the same signal in the PMR spectrum of the diacetate (IV) had scarcely changed.

The facts given, taken together, show the attachment of a benzoic acid residue to the hydroxy group at C-22.

Thus, ecdysteroid (III) is ecdysterone 22-O-benzoate. This is not the first time that acyl derivatives of ecdysteroids at the side chain have been detected in plants. In particular, viticosterone E [5-7], 22-acetylcyasterone [8], and α-ecadysone 22-sulfate [9] are known.

EXPERIMENTAL

For column chromatography and thin-layer chromatography we used mixtures of chloroform and ethanol in various ratios: 1) 50:1, 2) 50:2, 3) 9:1, 4) 4:1.

PMR specta were taken on a JNM-4H-100/100 MHz instrument ($CDCl_3$, δ, 0 — HMDS).

For other details, see [5].

Isolation of the Phytoecdysteroids. The epigeal part of the plant *Silene scabrifolia* Kom. was gathered in June, 1984 (gorge of the R. Uvabsai, Dzhizak Province, UzSSR). The dried and comminuted plant (15 kg) was exhaustively extracted with ethanol (120 liters). The extract was concentrated, the residue was diluted with water, and the precipitate that deposited was separated off. The ethanol was evaporated, and the aqueous residue was extracted with ethyl acetate and then with butanol. After evaporation of the solvent in vacuum, 170 g of ethyl acetate fraction and 190 g of butanol fraction were obtained.

The ethyl acetate fraction was chromatographed on a column of alumina (2 kg). System 2 eluted 30 g of 2-deoxy-α-ecdysone (I) (0.20%; the yields here and below are given as calculated to the air-dry raw material), $C_{27}H_{44}O_5$, mp 235-236°C (from aqueous ethanol); $[\alpha]_D^{23}$ +93.3 ± 2° (c 1.5; methanol).

The fraction obtained when the column was washed with system 3 was rechromatographed on SiO_2. Elution with the same system gave 25 g (0.16%) of ecdysterone benzoate (III). Subsequent elution of the column with system 4 gave 7 g (0.046%) of ecdysterone (II), $C_{27}H_{44}O_7$, mp 241-242°C (from acetone), $[\alpha]_D^{20}$ +59.6 ± 2° (c 0.4; methanol).

Ecysterone 22-O-Benzoate (III), $C_{34}H_{48}O_8$, mp 202-205°C (from methanol—water); $[\alpha]_D^{20}$ +45.0 ± 2° (c 1.1; methanol). $\lambda_{max}^{C_2H_5OH}$: 235 nm (log ε 4.36). ν_{max}^{KBr}, cm^{-1}: 3420-3470 (OH), 1600 (Δ^7-6-keto grouping); 1710, 1285 (ester group); 1610, 1587, 720 (benzene ring).

Mass spectrum, m/z (%): 584 (M^+; 0.02), 566 (0.1), 548 (0.2), 533 (0.4), 530 (0.3), 462 (0.8), 445 (7), 444 (23), 429 (23), 427 (39), 426 (84), 411 (56), 408 (23), 393 (24), 375 (24), 363 (39), 357 (24), 353 (25), 345 (24), 329 (24), 327 (23), 315 (24), 301 (56), 300 (54), 251 (39), 250 (84), 249 (80), 232 (24), 231 (24), 152 (80), 126 (81), 122 (81), 109 (56), 105(100), 99 (52), 81 (52), 77 (53), 69 (52), 51 (53), 43 (53).

PMR spectrum, ppm: 0.83 (3 H at C-18, s); 0.89 (3 H at C-19, s); 1.11 (6 H at C-26 and C-27, s); 1.33 (3 H at C-21, s); 3.94 (2 H at C-2 and C-3, m); 5.06 (H at C-22, m, $W_{1/2}$ = 8 Hz);

5.78 (H at C-7, broadened singlet); 7.39 (3 H, m) and 7.99 (2 H, m, aromatic protons).

Alkaline Hydrolysis of Ecdysterone 22-O-Benzoate (III). A solution of 50 mg of the ecdysteroid (III) in 10 ml of methanol was treated with 100 mg of potassium bicarbonate in 4 ml of water. The reaction mixture was left in a thermostat at 40°C for 5 days. Then it was diluted with water (20 ml), neutralized, and extracted with ethyl acetate. The solvent was distilled off to dryness and the residue was recrystallized from acetone, giving 10 mg of a substance with mp 240-242°C, $[\alpha]_D^{20}$ +59.0 ± 2° (c 0.4; methanol), identical with an authentic sample of ecdysterone.

The aqueous solution, after acidification with dilute (1:1) hydrochloric acid and extraction with ethyl acetate yielded 3 mg of benzoic acid with mp 122°C.

Ecdysterone 2,3-Di-O-acetate 22-O-Benzoate (IV). A solution of 383 mg of ecdysteroid (III) in 4 ml of pyridine was acetylated with 4 ml of acetic anhydride at room temperature for 20 h. Then the reaction mixture was diluted with water (30 ml) and the precipitate that deposited (395 mg) was filtered off and chromatographed on a column of silica gel. Elution with system 1 gave 200 mg of the diacetate (IV), $C_{38}H_{52}O_{10}$, mp 162-164°C (from methanol), $[\alpha]_D^{20}$ +82.3 ± 2° (c 0.85; methanol). ν_{max}^{KBr}, cm^{-1}: 3470-3520 (OH); 1725, 1745, 1260, 1220 (ester groups), 1610, 1587, 720 (benzene ring); 1665 (Δ^7-6-keto grouping).

Mass spectrum, m/z (%): 528 ($M^+ - C_6H_5COOH - H_2O$; 7), 510 (36), 495 (35), 477 (2), 470 (2), 449 (2), 437 (7), 399 (6), 393 (8), 385 (15), 334 (24), 311 (8), 232 (33), 152 (69), 126 (67), 122 (94), 105 (100), 99 (18), 81 (18), 77 (51), 51 (40), 43 (54).

PMR spectrum, ppm: 0.83 (3H at C-18, s); 1.00 (3 H at C-19, s); 1.14 (6 H at C-26 and C-27, s); 1,33 (3 H at C-21, s); 3.10 (H at C-9, m); 5.05 (2 H at C-2 and C-22, m); 5.28 (H at C-3, m); 5.86 (H at C-7, br.s); 7.48 (3 H, m) and 8.01 (2 H, m – aromatic protons).

Ecdysterone 2,3-Acetonide 22-O-Benzoate (V). A mixture of 180 mg of ecdysterone benzoate (III) and 20 ml of anhydrous acetone was treated with 5 mg of molybdophosphoric acid, and the mixture was shaken at room temperature until the compound (III) had dissolved completely. After a day, the reaction mixture was diluted with water (30 ml) and was neutralized with potassium bicarbonate. The neutral solution was extracted with ethyl acetate and the extract was chromatographed on a column in system 1 to give 100 mg of the 2,3-monoacetonide (V), $C_{37}H_{52}O_8$, mp 242-244°C (from chloroform), $[\alpha]_D^{20}$ +33.3 ± 2° (c 0.66; methanol). ν_{max}^{KBr}, cm^{-1}: 3455 (OH); 1725, 1710, 1265 (ester groups); 1610, 1585, 715 (benzene ring); 1645 (Δ^7-6-keto grouping.

Mass spectrum, m/z (%): 624 (M^+, 0.02), 609 (15), 591 (0.9), 573 (0.9), 502 (0.9), 487 (4), 466 (22), 451 (21), 403 (46), 385 (21), 327 (21), 290 (22), 267 (22), 163 (28), 139 (37), 126 (53), 122 (52), 105 (100), 99 (37), 81 (22), 77 (23), 59 (22), 43 (52).

PMR spectrum, ppm: 0.84 (3 H at C-18, s); 0.93 (3 H at C-19, s); 1.18 (6 H at C-26 and C-27, s); 1.32 (3 H at C-21, s); 4.20 (2 H at C-2 and C-3, m); 5.13 (H at C-22, m); 5.80 (H at C-7, br.m); 7.47 (3 H, m), and 8.00 (2 H, m – aromatic protons).

SUMMARY

A new ecdysteroid, ecdysterone 22-O-benzoate, has been isolated from the epigeal organs of Silene scabrifolia Kom.

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