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PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS Silene. XVII. ECDYSTERONE 22,25-DI-O-BENZOATE FROM Silene scabrifolia

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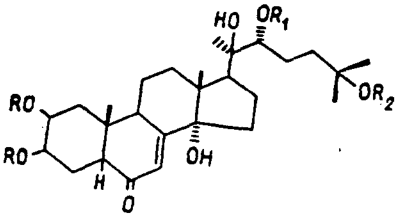
The new phytoecdysteroid ecdysterone 22,25-di-O-benzoate (IV) has been isolated from the epigeal organs of Silene scabrifolia Kom. The alkaline hydrolysis of (IV) gave ecdysterone 25-O-benzoate and ecdysterone (I). Details of the IR, UV, mass, PMR, and ^{13}C NMR spectra of compound (IV) are given.

Continuing a study of the phytoecdysteroids of the plant Silene scabrifolia, family Caryopyllaceae [1-3], from the mother liquors enriched with weakly polar derivatives we have isolated phytoecdysteroids (II), (III), and (IV).

Compound (II) was identified from its physicochemical constants and spectral characteristics as ecdysterone 2,3-monoacetonide 22-O-benzoate, which has been obtained by partial synthesis from ecdysterone 22-O-benzoate (VI) [1]. This is the first time that phytoecdysteroid (II) has been isolated from plant sources.

Ecdysteroid (III) proved to be identical with ecdysterone 2,3-monoacetonide, which has been isolated previously from Rhaponticum carthamoides (Willd) Iljin [4].

The spectral characteristics of ecdysteroid (IV) (absorption in the UV spectrum at 1720, 1295, 1610, 1590, and 730 cm^{-1} and intense peaks of ions with m/z 122 ($C_7H_6O_2$), 105 (C_7H_5O), and 77 (C_6H_5) in the mass spectrum) indicated the presence of a benzoate grouping. Signals in the PMR spectrum relating to ten aromatic protons at 7.39 ppm (6 H) and 8.25 ppm (4 H) showed the presence of two benzoic acid residues (Table 1).



I. $R = R_1 = R_2 = H$
II. $R = {>}CMe_2$; $R_1 = COC_6H_5$; $R_2 = H$
III. $R = {>}CMe_2$; $R_1 = R_2 = H$
IV. $R = H$; $R_1 = R_2 = COC_6H_5$
V. $R = R_1 = H$; $R_2 = COC_6H_5$
VI. $R = R_2 = H$; $R_1 = COC_6H_5$

The alkaline saponification of compound (IV) gave ecdysterone (I) and substance (V), which, according to its IR and mass spectra, still contained a benzoate grouping. The signals of five aromatic protons in the PMR spectrum of substance of (V) indicated the presence of only one benzoic acid residue.

It can be seen from Table 1 that, in comparison with ecdysterone, in ecdysteroid (V) the signals of the 26/27-methyl groups had undergone a considerable downfield shift. This fact permitted the assumption that in compound (V) the benzoic acid esterified the hydroxy group at C-25 and, consequently, it was ecdysterone 25-O-benzoate. It also follows from

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TABLE 1. Chemical Shifts of the Protons of Ecdysterone (I), Ecdysterone 22,25-Di-O-benzoate (IV), Ecdysterone 25-O-Benzoate (V), and Ecdysterone 22-O-Benzoate (VI) (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	Aromatic protons
I	4,0—4,3	6,21	3,57	3.84	1,19	1.06	1,55	1,34	—
IV	3,9—4,3	6,22	3,52	5.66	1,18	1,06	1,73	1,54	7,39(6H) 8.25(4H)
V	4,0—4,2	6,22	3,57	3,90	1,22	1.07	1,65	1,62	7,38(3H) 8,15(2H)
VI[1]	4,0—4,3	6,22	3,60	5.76	1.21	1,10	1.78	1.32	7,40(3H) 8,27(2H)

The signals of the methyl groups had a singlet nature; the H-7 signal appeared in the form of a broadened singlet; the H-2, H-3, H-9, and H-22 signals were multiplets.

TABLE 2. Chemical Shifts (ppm) of the Carbon Atoms of Ecdysterone (I) [7], Ecdysterone 22,25-Di-O-benzoate (IV), and Ecdysterone 22-O-Benzoate (VI)

C atom*	Compound		
	I	IV	VI
1	37.8	37.9	37,9
2	68,0	68,0	68,1
3	68.0	68,0	68,1
4	32.3	32,2	32,4
5	51.1	51,4	51,3
6	203,2	202.2	203.4
7	121,6	121,0	121,8
8	165.7	165,5	165,8
9	34.5	34,3	34.4
10	38,5	38,7	38,7
11	21.0	21,5	21,6
12	31,6	31,8	31,7
13	48,1	48,0	48,2
14	84,2	83,8	84,1
15	32,0	31,8	32,0
16	21,6	22.2	22,5
17	50.1	50,4	50,6
18	17,6	17,5	17.8
19	24,3	24.3	24,4
20	76,9	76,5	76.6
21	21,5	21,2	21,1
22	77,5	80,9	81,3
23	27,2	25,4	26.2
24	42,4	39,1	41,8
25	69.7	82,3	69,2
26	29,9	25,6	29,6
27	29,9	25,9	30.0
O—C=O	—	166,9	167,0
1′	—	131,7; 131,7	131,8
2′, 6′	—	129,8; 130,1	130.0
3′, 5′	—	128,8; 128,8	128,7
4′	—	133,0; 133,1	133,0

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*Primes indicate the carbon atoms of the benzene rings.

the facts given that the dibenzoate (VI) was based on ecdysterone, and one of the benzoate groups was attached to the hydroxyl at C-25.

A comparison of the chemical shifts of the protons of ecdysterone and the dibenzoate (IV) (see Table 1) showed that in the substance under investigation the signals of the protons at C-21 and of the 21-CH_3 group were shifted downfield, as well as the signals of the 26/27-methyl groups. The downfield shifts of the C-22 and 21-CH_3 protons can be explained by the presence of a benzoic acid residue attached to the hydroxy group at C-22 [1].

The results of a comparative study of the ^{13}C NMR spectra (Table 2) of ecdysterone and the ecdysteroids (IV) and (VI) confirmed the presence of benzoate groups at C-22 and C-25. It can be seen from Table 2 that in the case of the dibenzoate (IV), the signals of the C-22 and C-25 carbons were shifted downfield, while the values of the chemical shifts of all the other carbinol atoms of the two compounds were similar.

Thus, ecdysteroid (IV) was ecdysterone 22,25-di-O-benzoate.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were taken on a Tesla BS-567A spectrometer (100 MHz) in C_5D_5N (δ, 0 – TMS). For other information, see [1].

Ecdysterone 2,3-Monoacetonide 22-O-Benzoate (II). The enriched fractions obtained in the isolation of ecdysterone 22-O-benzoate [1], 5α-ecdysterone 22-O-benzoate [2], and ecdysterone 20,22-monoacetonide [3] were chromatographed on a column of silica gel in the chloroform—methanol (50:1) system. This permitted the isolation of 500 mg of the ecdysteroid (II), $C_{37}H_{50}O_8$, mp 258-260°C (from methanol—water), 242-244°C (from chloroform), $[\alpha]_D^{20}$ +34.2 ± 2° (c 0.60; methanol). The compound was identified from its IR, PMR, and mass spectra and also its R_f value on TLC (chloroform—methanol (25:1) system) as ecdysterone 2,3-monoacetonide 22-O-benzoate, which has been synthesized previously from ecdysterone 22-O-benzoate [1].

Ecdysterone 2,3-Monoacetonide (III). Subsequent washing of the column with the chloroform—ethanol (50:1) system led to the isolation of 200 mg of ecdysteroid (III), $C_{30}H_{48}O_6$, mp 242-244°C (from ethyl acetate), $[\alpha]_D^{20}$ +59.2 ± 2° (c 0.32; methanol). The properties of the substance obtained showed that it was identical with ecdysterone 2,3-monoacetonide isolated from *Rhaponticum carthomoides* [4].

Ecdysterone 22,25-Di-O-benzoate (IV). By continuing the elution of the column with the same system (chloroform—methanol (50:1)), 123 mg was isolated of the amorphous ecdysteroid (IV), $C_{41}H_{52}O_9$, $[\alpha]_D^{20}$ +80.1 ± 2° (c 0.22; methanol). $\lambda_{max}^{C_2H_5OH}$: 235 nm (log ε 4.10), ν_{max}^{KBr} cm^{-1}; 3460—3520 (OH), 1670 (Δ^7-6-keto grouping), 1720, 1295 (ester), 1610, 1590, 730 (benzene ring). Mass spectrum, m/z (δ): 548 (M^+—C_6H_5COOH—H_2O; 0.8) 530(1), 515(0.2), 512(0.2), 479(0.2), 467(0.2), 462(0.4), 461(0.4), 450(2.8), 444(5), 426(28), 408(30), 393(13), 375(30), 363(42), 357(28), 345(28), 329(13), 327(14), 301(30), 300(30), 250(50), 163(30), 122(98), 105(100), 99(17), 81(30), 77(54), 51(50).

Alkaline Hydrolysis of Ecdysterone 22,25-Di-O-benzoate (IV) to (V) and (I). A solution of 50 mg of the dibenzoate (IV) in 10 ml of methanol was treated with 100 mg of potassium bicarbonate in 4 ml of water. The reaction mixture was left at 39°C in a thermostat for three days, and then 25 ml of water was added and, after neutralization, extraction was carried out with ethyl acetate. The solvent was distilled off to dryness. The residue was chromatographed on a column of silica gel. Elution with the chloroform—methanol (25:1) system yielded 15 mg of amorphous ecdysterone 25-O-benzoate (V), $C_{34}H_{48}O_8$, $[\alpha]_D^{20}$ +60.9 ± 2° (c 0.36; methanol), ν_{max}^{KBr}, cm^{-1}: 3400—3500 (OH). 1665 (Δ^7-6-keto grouping), 1710, 1300 (ester), 1610, 1590, 725 (benzene ring).

Mass spectrum, m/z (%): 426 (M^+ — C_6H_5COOH — H_2O; 2.4), 411(0.8), 410(0.8), 393(0.6), 363(8), 345(28), 327(19), 311(4), 309(4), 301(6), 202(8), 185(10), 122(48), 105(100), 77(54).

When the column was washed with chloroform—methanol (9:1), 10 mg of compound (I) was isolated with mp 239-241°C (acetone), which was identified by its R_f value in TLC (chloroform—methanol (4:1) system) and spectral characteristics in comparison with an authentic sample of ecdysterone [5, 6].

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