PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS Silene.

XVI. VITICOSTERONE E 22-O-BENZOATE FROM Silene wallichiana

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A new phytoecdysteroid, viticosterone E 22-O-benzoate (I), $C_{36}H_{50}O_9$, mp 147-149°C (methanol—water), $[\alpha]_D^{20}$ +63.2° (methanol), has been isolated from the epigeal organs of Silene wallichiana Klotzsch. The alkaline hydrolysis of (I) led to viticosterone E and benzoic acid. The acetylation of (I) gave the 2,3-diacetate (II), $C_{40}H_{54}O_{11}$, mp 152-153°C (methanol—water), $[\alpha]_D^{20}$ +65.5° (methanol). Details of the IR, PMR, and mass spectra of (I) and (II) are given.

Continuing a study of the ecdysteroids of Silene wallichiana Klotzsch [Oberna wallichiana (Klotzsch) Ikonn] (family Caryophyllaceae) [1], we have isolated a new ecdysteroid, (I), from the epigeal part of this plant.

It followed from the spectral characteristics of ecdysteroid (I) that its molecule contained acyl groupings. The presence of a benzoate group was shown by the 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, and also by absorption characterizing a benzene ring (1610, 1585, 730 cm^{-1}) in the IR spectrum. In the PMR spectrum, the signals of five aromatic protons at 8.18 ppm (3H) and 7.34 ppm (2H) showed

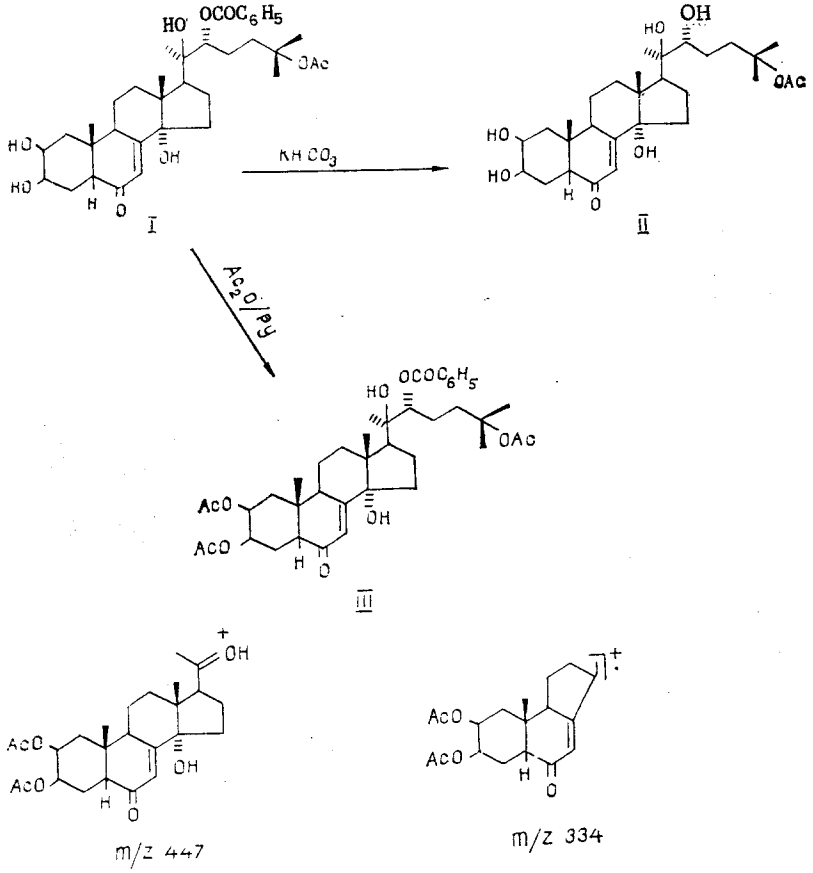

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TABLE 1. Chemical Shifts of the Protons of Compounds (I)-(III) (δ, ppm, 0 − HMDS)

Compound	Positions of the protons									
	2,3-H	9-H	22-H	7-H	18-CH_3	19-CH_3	21-CH_3	26/27-CH_3	aromatic protons	OAc
I	4,00—4,14	3,50	5,54	6,12	1,10	0,98	1,63	1,30; 1,33	7,34 (3H) 8,18 (2H)	1,84 (3H)
II	4,02—4,05	3,50	3,67	6,03	1,08	0,94	1,46	1,29; 1,35	—	1,79 (3H)
III	5,02—5,46	3,55	5,72	6,17	1,19	1,07	1,77	1,42; 1,44	7,40 (3H) 8,24 (2H)	1,95 2,01 2,04

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

that there was only one benzoate group. In addition, in the PMR spectrum at 1.84 ppm a three-proton singlet showing the presence of an acetyl group was observed.

On the alkaline saponification of ecdysteroid (I), viticosterone E (II) [2, 3] was identified in the neutral fraction, and benzoic acid was detected in acid fraction of the hydrolysate.

The acetylation of ecdysteroid (I) gave the acetate (III). The mass spectrum of compound (III) showed strong peaks of ions with m/z 447 ($C_{25}H_{35}O_7$) and 334 ($C_{19}H_{26}O_5$) [4], indicating that the benzoic acid residue was present in the side chain. In actual fact, when the PMR spectra of viticosterone E (III) and of ecdysteroid (I) were compared, the only appreciable difference was found in the positions of the resonance lines of the proton at C-22. In the spectrum of ecdysteroid (I), the signal of this proton was shifted downfield by 1.87 ppm (δ_I = 5.54, δ_{II} = 3.67, $\Delta\delta$ = 1.87 ppm) (Table 1). The facts given show that the hydroxy group at C-22 was esterified with benzoic acid. Consequently, ecdysteroid (I) was viticosterone E 22-O-benzoate.

EXPERIMENTAL

Chloroform—methanol mixtures (15:1) (1) and (100:1) (2) were used for column and thin-layer chromatography.

PMR spectra were taken on a BS-567 A instrument (100 MHz, Tesla) (C_5D_5N, δ, 0 − HMDS). For other information see [3].

Viticosterone E 22-O-Benzoate (I). By elution with system 1 in a column of silica gel, the mother liquors (325 mg), containing 2-deoxy-α-ecdysone-22-O-benzoate [1] gave 185 mg of ecdysteroid (I) (yield 0.0012% calculated on the air-dry raw material). $C_{36}H_{50}O_9$, mp 147-149°C (methanol—water), $[\alpha]_D^{20}$ +63.2 ± 2° (c 0.65; methanol). $\lambda_{max}^{CH_2H_5OH}$: 234 nm (log ε 4.11). ν_{max}^{KBr}, cm^{-1}: 3220-3300 (OH), 1660 (Δ^7-6-keto grouping); 1730, 1285 (ester groupings); 1610, 1585, 730 (benzene ring).

Mass spectrum, m/z (%): 566 (M^+ − CH_3COOH; 0.2), 548(0.6), 530(0.7), 514(0.3), 486(0.5), 468(2), 444(6), 426(40), 416(10), 408(23), 398(5), 398(5), 393(13), 383(4), 375(13), 365(12), 363(13), 329(12), 327(12), 301(23), 300(23), 250(60), 249(30), 185(12), 161(23), 122(100), 105(80), 99(16), 81(26), 77(43), 69(27), 51(27).

Alkaline Hydrolysis of Viticosterone E 22-O-Benzoate (I). A solution of 20 mg of ecdysteroid (I) in 5 ml of methanol was treated with 50 mg of potassium bicarbonate in 3 ml of water. The reaction mixture was left in the thermostat at 38°C for 2 days. Then it was diluted with water and was neutralized, and the methanol was evaporated off in vacuum. 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. Elution with system 1 gave 8 mg of viticosterone E (II), mp 195-196°C (acetone), identical with an authentic sample according to GLC and to IR and PMR spectroscopy.

The aqueous solution after acidification with dilute (1:1) hydrochloric acid and extraction with ethyl acetate yielded 2 mg of benzoic acid with mp 122°C.

Viticosterone E 22-O-Benzoate 2,3-Diacetate (III). A solution of 50 mg of ecdysteroid (I) in 2 ml of pyridine was acetylated with 2 ml of acetic anhydride at room temperature for 24 h. The excess of reagents was eliminated in vacuum. The residue was chromatographed on a column of silica gel. Washing with system 2 yielded 35 mg of the acetate (III), $C_{40}H_{54}O_{11}$, mp 152-153°C (from methanol–water) $]\alpha]_D^{20}$ +65.5 ± 2° (c 0.31; methanol); ν_{max}^{KBr}, cm^{-1}: 3470 (OH), 1725, 1740, 1260 (ester group); 1630, 1590, 720 (benzene ring); 1670 (Δ^7-6-keto grouping).

Mass spectrum, m/z (%): 650 ($M^+ - CH_3COOH$, 0.4), 632(1), 614(1.6), 572(0.4), 551(1.6), 534(13), 528(10), 510(18), 500(13), 492(29), 468(4), 447(32), 429(45), 385(45), 384(31), 345(27), 334(79), 327(64), 311(43), 309(27), 283(27), 232(64), 231(42), 122(86), 105(100), 99(27), 81(61), 69(64).

CONCLUSIONS

From the epigeal part of Silene wallichiana Klotzsch (family Caryophyllaceae) has been isolated a new phytoecdysteroid, viticosterone E 22-O-benzoate.

LITERATURE CITED

1. Z. Saatov, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 852 (1987).
2. H. Rimpler, Tetrahedron Lett., No. 5, 329 (1969).
3. Z. Saatov, M. B. Gorovits, N. D. Abdullaev, B. Z. Usmanov, and N. K. Abubakirov, Khim. Prir. Soedin., 738 (1981).
4. M. Koreeda, K. Nakanishi, S. Imai, T. Tsuchiya, and N. Wasada, Mass Spectrosc., 17, No. 2, 669 (1969).

ANALYSIS OF OLIGOSPIROSTANOSIDES IN A SUSPENSION CULTURE OF Dioscorea deltoidea CELLS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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A method has been developed for the quantitative determination of oligospirostanosides in a culture of Dioscorea deltoidea cells with the aid of HPLC on a LiChrosorb RP-18 column. Elution was performed with acetonitrile–water (50-75%) with detection at 207 nm.

In recent years, several highly productive strains of a cell culture of Dioscorea deltoidea synthesizing steroid glycosides of both the furostanol and spirostanol series have been obtained in the Institute of Plant Physiology [IFR] of the Academy of Sciences of the USSR [1-3]. In a suspension culture of yam cells of the strain IFR DM-0.5, after hydrolysis with exogenous enzymes, deltonin and dioscin – spiro analogs of the deltoside and protodioscin formed in the cells in vitro [4] – were detected. We have previously developed a spectrophotometric method permitting the determination of the amounts of glycosides of the furostanol series in a cell culture [2, 3]. In the present paper we describe a method for analyzing oligospirostanosides. The method was developed with the use of a cell culture of Dioscorea deltoidea, strain IFR DM-0.5.

To convert the oligofurostanosides into oligospirostanosides the cell mass was first subjected to enzymatic hydrolysis with preparations of cellulytic and pectolytic enzymes. It must be mentioned that in this procedure an additional liberation of forms of the glycoside strongly bound to the cell walls is possible [5].

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