and extracted with ethyl acetate. After the solvent had been distilled off, the residue was recrystallized from methanol—ethyl acetate. This gave 7 mg of ecdysterone with mp 236-237°C, identified by TLC and also by mass spectrometry.

The aqueous solution, after acidification and extraction with ethyl acetate, gave 2 mg of benzoic acid with mp 120-121°C.

SUMMARY

A new ecdysteroid which has proved to be ecdysterone 20-O-benzoate has been isolated from whole Silene tatarica (L.) Pers. plants.

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PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS Silene.

XV. 2-DEOXY-α-ECDYSONE 22-O-BENZOATE FROM Silene wallichiana

Z. Saatov, M. B. Gorovits, and
and N. K. Abubakirov

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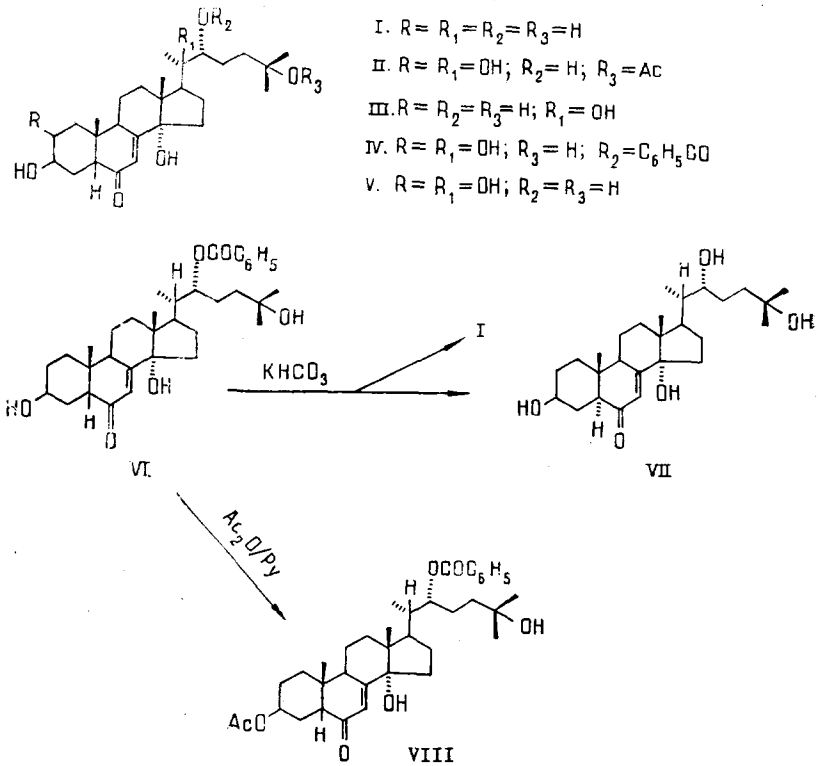
A new phytoecdysteroid, 2-deoxy-α-ecdysone 22-O-benzoate, has been isolated from the epigeal organs of Silene wallichiana Klotzch.

Continuing an investigation of the ecdysome-like substances of plants of the genus Silene (family Caryophyllaceae) [1], we have studied the phytoecdysteroids of the epigeal part



Institute of the Chemistry of Plant Substances, Uzbek SSR Academy of Sciences, Tashkent.
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TABLE 1. Chemical Shifts of the Protons of 2-Deoxy-α-ecdysone (I), of 2-Deoxy-α-ecdysone 22-O-Benzoate (VI), of 5α-2-Deoxy-α-ecdysone (VII), and of the Acetate (VIII)

Compound	Positions of the protons								
	3-H	22-H	7-H	18-CH_3	19-CH_3	21-CH_3	26/27-CH_3	aromatic protons	OAc
I	4,00	4,00	6,13	0,61	0,95	1,17	1,26	—	—
VI	3,96	5,47	6,08	0,63	0,90	1,13	1,20	7,40 (3H) 8,19 (2H)	—
VII	3,92	3,92	6,05 J=3 Hz	0,60	0,80	1,14	1,28	—	—
VIII	5,00	5,11	5,82 J=2 Hz	0,62	0,92	1,00	1,16	7,42 (3H) 8,00 (2H)	1,99

The spectrum of compound (VIII) was taken in $CDCl_3$ and those of the others in C_5D_5N. The signals of the protons of the methyl groups, apart from 21-CH_3, had a singlet nature. The 21-CH_3 group appeared in the spectrum of a doublet with J = 6 Hz. The signals of protons 3, 9, and 22 were multiplets. In compounds (VII) and (VIII), 7-H appeared in the form of a doublet while in the other cases it formed a broadened singlet.

of the plant S. wallichiana Klotsch. From this material, in addition to the known 2-deoxy-α-ecdysone (I), viticosterone E (II), 2-deoxyecdysterone (III), ecdysterone 22-O-benzoate (IV), and ecdysterone (V), we isolated the new ecdysteroid (VI).

The IR spectrum (1730, 1290, 1610, 710 cm^{-1}), and also the peaks of ions with m/z 122 ($C_7H_6O_2$), 105 (C_7H_5O), and 77 (C_6H_5) in the mass spectrum showed that the ecdysteroid (VI) contained a benzoic acid residue. The signals of five aromatic protons in the PMR spectrum at 7.40 ppm (3 H) and 8.19 ppm (2 H) showed the presence of a single benzoate group in this compound.

The mass spectrum of the substance under investigation showed the peaks of ions with m/z 332, 331, 285, 284, 234, 233, which enabled compound (VI) to be assigned to the 2-deoxyecdysteroids [2].

In a comparison of the PMR spectra of 2-deoxy-α-ecdysterone (I) and of the ecdysteroid (VI), an appreciable difference was found only in the position of the resonance lines of the proton at C-22. In the spectrum of the benzoate (VI), the signal of this proton was shifted downfield by 1.47 ppm. On this basis, it was possible to assume that ecdysteroid (VI) was 2-deoxy-α-ecdysone 22-O-benzoate. From the products of the alkaline hydrolysis of the benzoate (VI) the ecdysteroids (I) and (VII) were isolated, in addition to benzoic acid. Compound (I) was identified as 2-deoxy-α-ecydsone [3, 4].

The PMR spectrum of substance (VII) (Table 1) showed an appreciable downfield shift of the signal of the 19-methyl group as compared with that for ecdysteroid (I) ($\delta\Delta$ = 0.15 ppm). The values of the chemical shifts of the other signals had scarcely changed. This paramagnetic shift permitted the assumption [1, 5, 6] that ecdysteroid (VII) was 5α-2-deoxy-α-ecdysone. An authentic sample of this compound was obtained by the alkaline isomerization of ecdysteroid (I). Compound (VII) proved to be identical with this.

It is obvious that on the alkaline hydrolysis of the benzoate (VI) the isomerization of the 2-deoxy-α-ecdysone (I) took place simultaneously. According to our observations, in an alkaline medium the equilibrium for the 2-deoxyecdysteroids is substantially shifted in the direction of the 5α-isomer. In contrast to this, for the 2,3-dihydroxyecdysteroids and, in particular, for the ecdysteroid ester (IV), the isomerization process is practically imperceptible [9].

What has been said above permits the conclusion that ecdysteroid (VI) was 2-deoxy-β-ecdysone 22-O-benzoate.

In agreement with the suggested structure, acetylation of this compound gave the 3-monoacetate (VIII).

EXPERIMENTAL

Mixtures of chloroform and methanol, in various ratios – 1) (15:1); 2) (9:1); and 3) (4:1) – were used for column and thin layer chromatography.

PMR spectra were taken on a JNM-4H-100/100 MHz instrument ($CDCl_3$, C_5D_5N, δ, 0 – HMDS).

For further information, see [8].

Isolation of the Phytoecdysteroids. The epigeal part of Silene wallichiana Klotsch was gathered in June, 1984 (gorge of the R. Vabasai, Malguzar range, UzSSR). The dried and comminuted raw material (15 kg) was exhaustively extracted with methanol (100 liters). The extract was concentrated, the residue was diluted with water, and the precipitate that deposited was removed. The methanol was evaporated off and the aqueous residue was treated first with hexane and then with ethyl acetate. The solvents were distilled off in vacuum.

The ethyl acetate extract (45 g) was chromatographed on a column of alumina (1 kg) with elution by system 1. This led to the isolation of 12 g of 2-deoxy-α-ecdysone (I) (0.071%; the yields here and below are calculated on 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) [3, 4].

Further elution of the column with system 1 yielded 900 mg of viticosterone E (II) (0.006%), $C_{29}H_{46}O_8$, mp 194-196°C (from acetone), $[\alpha]_D^{20}$ +58.6 ± 2° (c 0.52; methanol) [7, 8].

Washing the column with system 2 gave 6 g of 2-deoxy-α-ecdysone (III) (0.040%), $C_{27}H_{44}O_6$, mp 254-256°C (from aqueous ethanol); $[\alpha]_D^{23}$ +82.0 ± 3° (c 1.3; methanol) [3, 4].

Subsequent washing of the column in the same system yielded 5 g of ecysterone 22-O-benzoate (IV) (0.033%), $C_{34}H_{48}O_8$, mp 202-205°C (from methanol–water) $[\alpha]_D^{2*}$ = +45.0 ± 2° (c 1.1; methanol) [9].

Elution of the column with system 3 gave 7 g of ecdysterone (V) (0.046%), $C_{27}H_{44}O_7$, mp 241-242°C (from acetone, $[\alpha]_D^{20}$ +59.6 ± 2° (c 0.4; methanol) [10].

2-Deoxy-α-ecdysone 22-O-Benzoate (VI). The mother liquor (230 mg) remaining after the recrystallization of the 2-deoxy-α-ecdysone (I) was chromatographed on a column of silica gel. Elution with system 1 gave 162 mg of the ecdysteroid (VI) (0.0010%), $C_{34}H_{48}O_6$, mp 182-185°C (from methanol–water), $[\alpha]_D^{20}$ +141.1 ± 2° (c 0.40; methanol); $\lambda_{max}^{C_2H_5OH}$: 234 nm (log ε 4.09), ν_{max}^{KBr}, cm^{-1}: 3400-3440 (OH); 1665 (Δ⁷-6-keto grouping); 1740, 1290 (ester); 1610, 710 (benzene ring). CD (c 0.10; methanol); Δε = +3.08 (221 nm); Δε = −8.9 (242 nm); Δε = +1.67 (332 nm).

Mass spectrum, m/z (%): 552 (M^+; 4), 534 (7), 524 (15), 519 (4), 506 (16), 476 (1.7), 430 (3), 412 (23), 402 (22), 397 (24), 394 (24), 384 (41), 379 (11), 369 (11), 361 (4), 351 (10), 332 (7), 331 (11), 303 (2), 285 (41), 284 (66), 234 (66), 233 (82), 122 (22), 105 (100), 99 (59), 81 (59), 77 (59), 69 (59), 51 (10).

Alkaline Hydrolysis of 2-Deoxy-α-ecdysone 22-O-Benzoate (VI). A solution of 20 mg of the ecdysteroid (VI) in 5 ml of methanol was treated with 50 mg of potassium bicarbonate in 3 ml of water. The reaction mixture was left in a thermostat in an atmosphere of nitrogen at 38°C for 2 days. It was then diluted with water and 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 11 g of 5α-2-deoxy-α-ecdysone (VII), $C_{27}H_{44}O_5$, mp 225-227°C (from methanol–water), $[\alpha]_D^{20}$0 ± 2° (c 0.40; methanol); $\lambda_{max}^{C_2H_5OH}$: 242 nm (log ε 4.10), ν_{max}^{KBr}, cm^{-1}: 3380-3430 (OH); 1670 (Δ⁷-6-keto grouping): CD (c 0.12; methanol): Δε = −6.00 (245 nm), Δε = +2.35 (330).

Mass spectrum, m/z (%): 430 (M^+–H_2O; 7), 424 (19), 412 (4), 397 (12), 379 (4), 361 (6), 343 (6), 332 (23), 314 (23), 299 (13), 284 (46), 234 (38), 233 (38), 99 (100), 81 (61).

The further washing of the column with the same solvent system led to 3 mg of 2-deoxy-α-ecdysone (I), mp 235-236°C (from aqueous ethanol); $[\alpha]_D^{23}$ 93.2 ± 2° (c 1.5; methanol), identified with an authentic sample [3, 4] with the aid of TLC and a mixed melting point.

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*As in Russian original – Publisher.

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

2-Deoxy-α-ecdysone 3-O-Acetate 22-O-Benzoate (VIII). A solution of 68 mg of the ecdysteroid (VI) in 1 ml of pyridine was acetylated with 1 ml of acetic anhydride at room temperature for 24 h. The excess of reagents was eliminated in vacuum. The residue was recrystallized from methanol–water. This gave 52 mg of the monoacetate (VIII), $C_{36}H_{50}O_7$, mp 255-257°C; $[\alpha]_D^{20}$ 85.0 ± 2° (c 0.80; methanol). ν_{max}^{KBr}, cm^{-1}: 3480-3540 (OH): 1710, 1740, 1285, 1255, 1230 (ester group); 1610, 1590, 725 (benzene ring); 1640 (Δ^7-6-keto grouping).

Mass spectrum, m/z (%): 594 (M^+; 9), 576 (30), 566 (46), 561 (18), 548 (30), 534 (4), 516 (6), 488 (16), 472 (14), 454 (46), 444 (23), 439 (23), 436 (46), 426 (62), 421 (17), 412 (14), 394 (19), 384 (18), 379 (19), 366 (46), 356 (18), 326 (100), 285 (23), 284 (22), 276 (82), 275 (81), 257 (19), 121 (46), 105 (82), 99 (46), 81 (46), 69 (43).

5α-2-Deoxy-α-ecydsone (VII) from (I). To 200 mg of the ecdysteroid (I) in 15 ml of methanol was added 100 mg of potassium bicarbonate in 2 ml of water. The reaction mixture was left at room temperature in an atmosphere of nitrogen for 24 h. Then it was neutralized with acetic acid, the methanol was evaporated off to half-volume, and this residue was diluted with water and extracted with ethyl acetate. The ethyl acetate extract was evaporated to dryness. The residue was chromatographed on a column of silica gel. Elution in system 1 gave 120 mg of 5α-2-deoxy-α-ecdysone, $C_{27}H_{44}O_5$, mp 224-226°C (from aqueous methanol), $[\alpha]_D^{20}$ 0 ± 2° (c 0.40; methanol). The substance obtained was identified by comparison with the ecdysteroid (VII) isolated on the alkaline isomerization of the benzoate (VI), and also from its R_f value in TLC and by its spectral characteristics.

SUMMARY

From the epigeal part of Silene wallichiana Klotsch (family Caryophyllaceae) have been isolated the known ecdysteroids 2-deoxy-α-ecdysone, viticosterone E, 2-deoxy-α-ecdysone, ecdysterone 22-O-benzoate, and ecdysterone, and also a new phytoecdysteroid which has proved to be 2-deoxy-α-ecdyzone 22-O-benzoate.

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