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PHYTOECDYSTEROIDS OF PLANTS OF THE *Silene* GENUS. 2-DEHYDROXYECDYSTERONE-3-O-BENZOATE FROM *Silene wallichiana*

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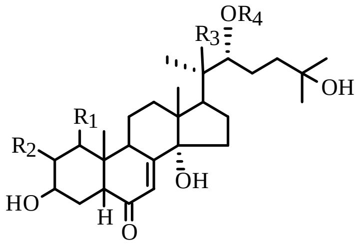
The known ecdysteroids 2-dehydroxyecdysone, 2-dehydroxyecdysterone, ecdysterone-22-O-benzoate, ecdysterone, integristerone A, and a new ecdysteroid identified as 2-dehydroxyecdysterone-3-O-benzoate are isolated from roots of Silene wallichiana *Klotzsch.*

Key words: ecdysteroids, 2-dehydroxyecdysone, 2-dehydroxyecdysterone, ecdysterone-22-O-benzoate, ecdysterone, integristerone A, 2-dehydroxyecdysterone-3-O-benzoate.

In continuation of studies of ecdysteroids from *Silene wallichiana* Klotzsch [*Oberna wallichiana* (Klotzsch) Ikonn, Caryophyllaceae] [1], we investigated phytoecdysteroids of the plant roots. In addition to the known 2-dehydroxyecdysone (**1**), 2-dehydroxyecdysterone (dehydroxycrustecdysone) (**2**), ecdysterone-22-O-benzoate (**3**), ecdysterone (**4**), and integristerone A (**5**), a new ecdysteroid (**6**) was isolated.



1. $R_1=R_2=R_3=R_4=H$
2. $R_1=R_2=R_4=H$; $R_3=OH$
3. $R_1=H$; $R_2=R_3=OH$; $R_4=C_6H_5CO$
4. $R_1=R_4=H$; $R_2=R_3=OH$
5. $R_1=R_2=R_3=OH$; $R_4=H$

IR spectra (1733, 1290, 1610, 720 cm^{-1}) and peaks for ions with *m/z* 122 ($C_7H_6O_2$), 105 (C_7H_5O), and 77 (C_6H_5) in the mass spectrum indicate that compound **6** contains a benzoic acid moiety. The PMR spectrum contains signals for five aromatic protons at 8.12 (2H) and 7.37 ppm (3H) that are consistent with the presence of one benzoate group.

In addition to the peaks indicated above, the mass spectrum of substance **6** exhibits peaks with *m/z* 347, 329, 303, 234, and 233, which indicate that it is a 2-dehydroxyecdysteroid [2].

Comparison of the PMR spectra of 2-dehydroxyecdysterone (**2**) and ecdysteroid **6** shows a significant difference only for the position of the proton on C-3. In the spectrum of **6**, this proton shifts to weak field by 1.23 ppm. Therefore, the benzoate group is located namely on this C atom (Table 1).

The mass spectrum of **6** has a peak with *m/z* 338, which forms via cleavage of rings *C*/*D* [3]. The presence of these fragments indicates that the benzoic acid is bonded to the steroid part of the molecule.

Alkaline hydrolysis of ecdysteroid **6** produces compounds **2** and **7** in addition to benzoic acid. Compound **2** was identified as 2-dehydroxyecdysterone [4].

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TABLE 1. Proton Chemical Shifts* of 2-Dehydroxyecdysterone (**2**), 2-Dehydroxyecdysterone-3-O-benzoate (**6**), and 5α-2-dehydroxyecdysterone (**7**) (δ, ppm, 0 = HMDS)

Compound	H-3	H-7	H-9	H-22	CH_3-18	CH_3-19	CH_3-21	CH_3-26/27	Aromatic protons
1	3.99	6.10	3.40	3.73	1.10	0.93	1.46	1.26	--
6	5.22	6.12	3.40	3.80	1.12	0.92	1.50	1.25	7. 37(3H), 8.12(2H)
7	3.70	5.98	--	3.70	1.07	0.79	1.45	1.25	--

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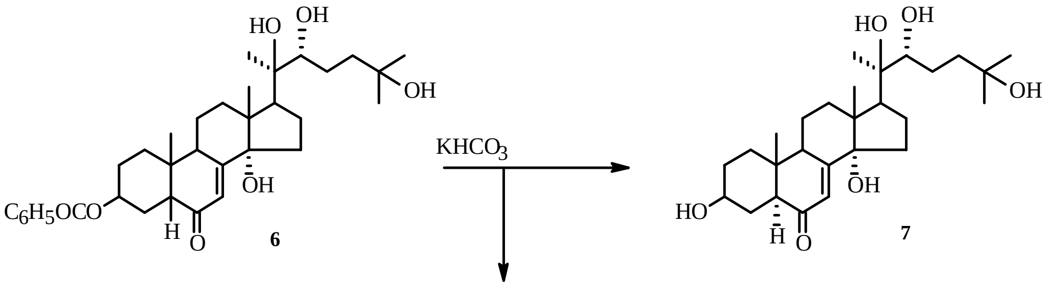
*Signals of methyl protons are singlets. In all instances H-7 appears as a broad singlet. Other signals are broad multiplets.



6

7

2

The signal for the 19-methyl group in the PMR spectrum of ecdysteroid **7** is noticeably shifted to strong field (Table 1) compared with that of ecdysteroid **2** (Δδ = 0.14 ppm). The chemical shifts of the remaining signals are practically unchanged. This diamagnetic shift suggests [5, 6] that ecdysteroid **7** is 5α-2-dehydroxyecdysterone. An authentic sample of this compound was obtained via alkaline hydrolysis of ecdysteroid **2**. Compound **7** was identical to it. Thus, ecdysteroid **6** is 2-dehydroxyecdysterone-3-O-benzoate.

EXPERIMENTAL

Mixtures of $CHCl_3$—CH_3OH [15:1 (1), 9:1 (2), 4:1 (3)] were used for column and thin-layer chromatography.

PMR spectra were obtained on a BS-567A instrument (100 MHz, Tesla, C_5D_5N, δ, 0 = HMDS); mass spectra, on an MS-25RF instrument (Kratos) with direct sample introduction at accelerating potential 4 kV, ionization potential 70 V, ionization chamber temperature 250°C, and sample ampul temperature from 180 to 270°C. IR spectra were recorded on a Perkin—Elmer System 2000 FT IR Fourier spectrometer in KBr pellets.

Isolation of Phytoecdysteroids. Roots of *Silene wallichiana* Klotsch were collected in June, 1984, in Uvabsai region (village Malguzar, Republic of Uzbekistan). Dried and ground material (2.5 kg) was exhaustively extracted with ethanol (10 l). The extract was concentrated and diluted with water. The resulting precipitate was removed. The ethanol was evaporated. The aqueous solution was treated first with $CHCl_3$ and then with ethylacetate. The solvents were evaporated under vacuum.

The ethylacetate extract (15 g) was chromatographed on an Al_2O_3 column (0.5 kg) with elution by system 2. Yield of **1** (4 g, 0.16%) (yields are calculated per dry weight of material), $C_{27}H_{44}O_5$, mp 234-235°C (aqueous ethanol), $[\alpha]_D^{23}$ +93.3 ± 2° (*c* 1.0, methanol) [7, 8]. Further elution of the column with the same system gave **2** (2 g, 0.08%), $C_{27}H_{44}O_6$, mp 254-256°C (aqueous ethanol), $[\alpha]_D^{23}$ +82.0 ± 2° (*c* 0.50, methanol) [7, 8].

Elution of the column with system 1 gave **3** (1 g, 0.04%), $C_{34}H_{48}O_8$, mp 203-205°C (methanol—water), $[\alpha]_D^{23}$ +45.0 ± 2° (*c* 1.0, methanol) [9].

Elution of the column with system 3 gave **4** (2 g, 0.08%), $C_{27}H_{44}O_7$, mp 241-242°C (methanol—acetone), $[\alpha]_D^{23}$ +58.9 ± 2° (*c* 0.3, methanol) [4, 10].

Subsequent elution of the column with the same system gave **5** (425 mg, 0.017%), $C_{27}H_{44}O_8$, mp 246-248°C (ethylacetate—methanol), $[\alpha]_D^{23}$ +36.2 ± 2° (*c* 1.0, methanol) [11].

2-Dehydroxyecdysterone-3-O-benzoate (6). Elution of the column with system 2 gave fractions containing **6**. Rechromatography of the eluates using system 1 gave **6** (120 mg, 0.0048%), $C_{34}H_{48}O_7$, mp 210-212°C (aqueous ethanol),

$[\alpha]_D^{23}$ +72.3 ± 2° (*c* 0.5, methanol). IR spectrum (KBr, ν, cm^{-1}): 3400-3440 (OH), 1665 (Δ^7-6-ketone), 1733, 1290 (ester), 1610, 720 (benzene ring).

Mass spectrum, *m/z*: 514 $[M - 3H_2O]^+$, 410, 386, 347, 340, 338, 337, 329, 311, 303, 279, 256, 234, 233, 122, 105, 99, 83, 81, 77, 69.

Alkaline Hydrolysis of 6. A solution of ecdysteroid **6** (50 mg) in methanol (5 ml) was treated with $KHCO_3$ (30 ml) in water (3 ml). The reaction mixture was held at 37-38°C in a thermostat for three days, diluted with water, neutralized, and extracted with ethylacetate. The aqueous solution was acidified with dilute (1:1) HCl and extracted with ethylacetate to give benzoic acid (7 mg), mp 122°C. The solvent was evaporated. The solid was chromatographed on a silica-gel column with elution by system 1 to give **7** (20 mg), $C_{27}H_{44}O_6$, mp 235-237°C (methanol—water). IR spectrum (KBr, ν, cm^{-1}): 3447 (OH), 1670 (Δ^7-6-ketone).

Mass spectrum, *m/z* 428 $[M - 2H_2O]^+$, 429, 410, 395, 330, 329, 312, 286, 285, 99, 81, 69.

Further elution of the column with the same system gave ecdysteroid **2** (27 mg), mp 254-255°C (aqueous ethanol), identical with an authentic sample [7, 8] according to TLC and mixed melting point.

5α-2-Dehydroxyecdysterone (7) from 2. A solution of ecdysteroid **2** (100 mg) in methanol (10 ml) was treated with $KHCO_3$ (80 mg) in water (2 ml). The reaction mixture was held at room temperature for three days, neutralized with acetic acid, evaporated (CH_3OH) to half the volume, diluted with water, and extracted with ethylacetate. The ethylacetate extract was evaporated to dryness. The solid was chromatographed on a silica-gel column with elution by system 1 to give 5α-2-dehydroxyecdysterone (35 mg), $C_{27}H_{44}O_6$, mp 235-237°C (aqueous methanol).

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