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Phytoecdysteroids from *Silene praemixta*

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ABSTRACT

The waste products of the production of ecdysterone (3) from *Silene praemixta* M. Pop. (Caryophyllaceae) were investigated. The column chromatography of the technological waste has allowed to isolating 2-desoxyecdysone (1), 2-deoxyecdysterone (2), ecdysterone (3) and 22-O-*β*-D-glucopyranoside of 2-deoxyecdysone (4). Glucoside 4 was found for the first time in the plant *Silene praemixta.* Ecdysteroids 1, 2 prevailed in the sum. Compounds 3 and 4 were minor components of the waste products. The identification of compounds 1-4 was carried out by using the modern methods of 1D and 2D NMR spectroscopy: ^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, HSQC, NOESY, IR spectroscopy and ESI MS.

KEYWORDS

Phytoecdysteroid; *Silene praemixta*; Ecdysteron

1. INTRODUCTION

It has earlier been reported that ecdysterone accumulates in the leaves (2.5%), roots (0.34%) and inflorescences (1.7%) of *Silene praemixta* species [1].

The genus of *Silene* (Caryophyllaceae) is widely distributed in Uzbekistan and it is known as the rich source of phytoecdysteroids. The one main of them is the ecdysterone, which is possessed as an anabolic effect for human body [2]. The ecdysterone has the ability to adapt organism to experimental effects of environment and stimulate the operability [3].

The "Ecdisten" preparation is produced from the medicinal plants *Raponticum* and *Silene* at the Institute of the Chemistry of Plant Substances (Tashkent, Uzbekistan Academy of Sciences).

2. RESULTS

In the present paper the chemical composition of *Silene praemixta* plant was investigated. We described the isolation and structure elucidation of ecdysteroids from the waste product of this manufacture.

Four ecdysteroids were isolated and identified from *Silene praemixta* (**1-3**) and 22-O-*β*-D-glucopyranoside of 2-deoxyecdysone (**4**) (**Figure 1**).

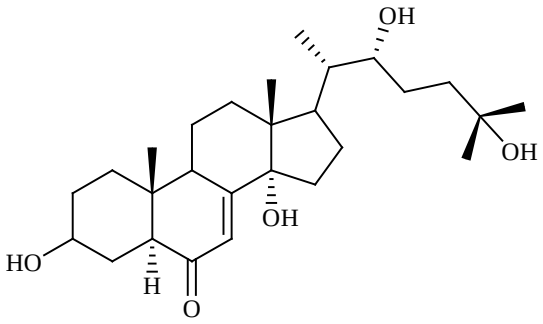
Part of the waste extract (6.0 g) was subjected to column chromatography on silica gel with gradient elution by CH_3Cl-MeOH (10:1), CH_3Cl-MeOH-H_2O (70:12:1) and (70:23:4) to afford **1-4.** Steroids **1-2** were the main compounds of the plant extract while **3-4** compounds were found in scarce amount.

The identification of isolated compounds was carried out by using 1D and 2D NMR spectroscopy techniques: ^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, HSQC, NOESY, IR spectroscopy and ESI MS. This spectral data identified steroid **1** as 2-deoxyecdysone, steroid **2** as 2-deoxyecdysterone, steroid **3**-ecdysterone [4-7] and compound **4** as 22-O-*β*-D-glucopyranoside of 2-deoxyecdysone [8].

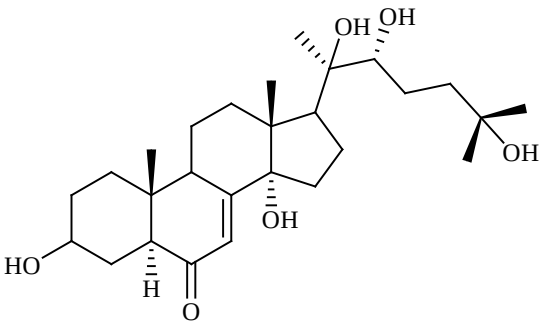
3. DICUSSION

Using infrared spectrophotometer data we obtained information regarding presence of hydroxyl groups (3387 - 3409 sm^{-1}) and 6-ketochromaphore (1639 - 1652 sm^{-1}) of tested compounds according to ecdysteroids skeleton. The mail role in the structure determination of isolated compounds plays NMR analysis which shows presence of signals of methyl groups at 0.64 and 1.57 ppm, corresponding to CH_3-18, CH_3-19 (CH_3-21 represented as doublets in area 0.88 - 1.6 ppm). Signals of CH_3-18 determined by presence of Δ^7-6-keto-14*α*-oxygroup, and signals at C-7 in current system shown in area 5.75 - 6.25 ppm. All mentioned data distribute identification of isolated compounds **1-4** as 2-deoxy-*α*-ecdysone, 2-deoxy-

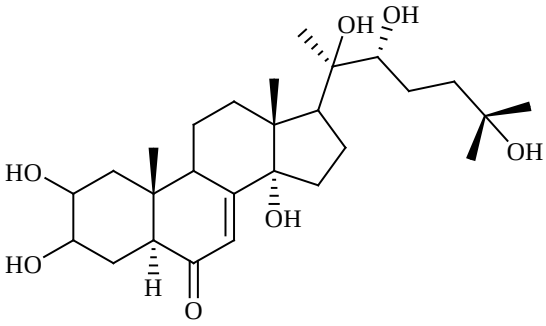
ecdysterone, ecdysteron (3), 22-O-*β*-D-glucopyranosid-2-deoxy-*α*-ecdysone. All chemical shifts of mentioned compounds correspond to literature data [4-8].



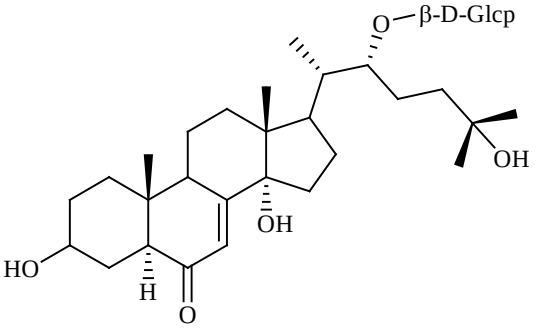
1. 2-Deoxy- *α*-ecdysone



2. 2-Deoxyecdysterone



3. Ecdysteron



4. 22-O-β-D-Glucopyranosid-2-deoxy-α-ecdysone

Figure 1. Isolated ecdysteroids from *Silene praemixta.*

4. EXPERIMENTAL

General Methods

^{1}H, ^{13}C NMR (**Table 1**) spectra were run in CD_3OD, C_5D_5N using TMS as internal reference on a Bruker Avance DRX 600 MHz spectrometer. ESI-MS spectra were recorded on a PE Q-STAR electrospray, ionization time of flight-tandem-mass spectrometry spectrometer. Silica gel 60 PF 254 was used for TLC. Infrared spectra were taken on Perkin Elmer Spectrum 100 FTIR sprectrophotometer, which are conformed with literature data.

2-Deoxy-*α*-ecdysone (1)-$C_{27}H_{44}O_5$

^{1}H NMR 2-deoxy-*α*-ecdysone (400 MHz, C_5D_5N, *δ*, ppm, J/Hz, 0-TMS): 0.73 (CH_3-18, s), 1.05 (CH_3-19, s), 1.30 (CH_3-21, d, ^{3}J = 6.6), 1.39 (CH_3-26 and CH_3-27, s), 2.97 (H-17, m), 3.50 (H-9, m), 4.07 (H-22, m), 4.15 (H-3, w.s), 5.95 (H-7, d, 4).

^{1}H NMR 2-deoxy-*α*-ecdysone (400 MHz, CD_3OD, *δ*, ppm, J/Hz, 0-HMDS): 0.66 (CH_3-18, s), 0.88 (CH_3-21, d, ^{3}J = 6.8), 0.90 (CH_3-19, s), 1.13 (CH_3-26, s), 1.14 (CH_3-27, s), 2.36 (H-5, dd, 12, 4), 3.14 (H-9, m), 3.54 (H-22, w.d), 3.92 (H-3, m), 5.75 (H-7, d, 2.3).

IR spectrum 2-deoxy-*α*-ecdysone (KBr, ν_{max}, sm^{-1}): 3387, 1640.

ES-MS Positive ion mode of 2-desoxy-*α*-ecdysone: 471.2 $[M + Na]^+$.

ES-MS negative ion mode of 2-desoxy-*α*-ecdysone 447.1 $[M - H]^-$.

2-Deoxyecdysterone (2)-$C_{27}H_{44}O_6$

^{1}H NMR 2-deoxyecdysterone (600 MHz, C_5D_5N, *δ*, ppm, J/Hz, 0-TMS): 1.06 (CH_3-19, s), 1.24 (CH_3-18, s), 1.37 (CH_3-26 and CH_3-27, s), 1.60 (CH_3-21, s), 3.04 (H-17, t, 9), 3.54 (H-9, m), 3.89 (H-22, w.d, 10.2), 4.12 (H-3, w.s), 6.25 (H-7, s).

^{1}H NMR 2-deoxyecdysterone (400 MHz, CD_3OD, *δ*, ppm, J/Hz, 0-HMDS): 0.82 (CH_3-18, s), 0.90 (CH_3-19, s), 1.13 (CH_3-21 and CH_3-26, s), 1.14 (CH_3-27, s), 2.05 (H-12, td, 12.9, 5), 2.35 (H-5 and H-17, m), 3.15 (H-9, m), 3.27 (H-22, m), 3.93 (H-3, m), 5.73 (H-7, d, 2.4).

IR-spectroscopy of 2-deoxyecdysterone (KBr, ν_{max}, sm^{-1}): 3388, 1639.

ES-MS Positive ion mode of **2**-deoxyecdysterone 487.3 $[M + Na]^+$.

ES-MS negative ion mode of **2**-deoxyecdysterone 463.0 $[M - H]^-$.

Ecdysteron (3)-$C_{27}H_{44}O_7$

^{1}H NMR ecdysteron (600 MHz, C_5D_5N, *δ*, ppm J/Hz, 0-TMS): 1.06 (CH_3-19, c), 1.22 (CH_3-18, s), 1.36 (CH_3-26 and CH_3-27, s, 1.59 (CH_3-21, s), 3.00 (H-5 and H-17, m), 3.59 (H-9, m), 3.87 (H-22,w.d, 10.2), 4.18 (H-2, m), 4.22 (H-3, w.s), 6.25 (H-7, d, 1.8).

^{1}H NMR ecdysteron (400 MHz, CD_3OD, *δ*, ppm, J/Гц, 0-HMDS): 0.83 (CH_3-18, s), 0.90 (CH_3-19, s), 1.126 (CH_3-21, s), 1.134 (CH_3-26, s), 1.38 (CH_3-27, s), 2.32

Table 1. ^{13}C NMR spectra compounds of **1-4** (C_5D_5N, CD_3OD, δ, ppm, 0-TMS).

C atom	DEPT	Compound						
		1		**2**		**3**		**4**
		δ_C C_5D_5N	δ_C CD_3OD	δ_C C_5D_5N	δ_C CD_3OD	δ_C C_5D_5N	δ_C CD_3OD	δ_C C_5D_5N
1	CH_2					37.98	37.38	
2	CH_2(CH)	29.02	28.97	28…	28.95	68.13	68.71	29.09
3	CH	64.02	65.46	64…	65.42	68.04	68.52	64.04
4	CH_2	33.14	33…	33.15	33.23	32.45	32.83	33.10
5	CH	52.75	52…	52…	52…	51.40	51.78	
6	C	203....		203…		203.49	206.47	206.53
7	CH	121.86	121.78	121.50	121.96	121.64	122.13	121.35
8	C	166.09	168.20	166.45	168.48	166.11	167.98	166.06
9	CH					34.39	35.11	
10	C	36.99	37.56	36.98	37.61	38.65	39.27	36.98
11	CH_2	21.05				21.47	21.50[a]	21.41
12	CH_2	31.80	32.30	32.20	32.73	31.98	32.51	31.86
13	C	48.33	48…	48…		48.08		48.33
14	C	83.99	85.33	84.34	85.45	84.15	85.24	84.11
15	CH_2	31.64	31.90	31.65	31.64	31.77	31.77	31.60
16	CH_2	25.57	25.37	21.55	21.55	21.09	21.50[a]	24.53
17	CH	48.33		50.12	50.55	50.08	50.54	48.33
18	CH_3	15.81	16.21	17.90	18.04	17.88	18.04	15.92
19	CH_3	24.36	24.41	24.40	24.38	24.46	24.39	24.34
20	CH(C)	43.05	43.45	76.83	77.93	76.82	77.93	41.07
21	CH_3	13.65	13.29	21.70	21.03	21.69	21.06	14.31
22	CH_2	73.94	75.27	77.54	78.43	77.53	78.43	85.39
23	CH_2	26.74	27.06	27.49	27.35	27.47	27.35	26.66
24	CH_2	42.53	42.24	42.66	42.38	42.65	42.38	41.34
25	C	69.63	71.41	69.51	71.30	69.51	71.30	69.72
26	CH_3	30.05	29.14	30.00	29.00	29.99	28.99	29.41
27	CH_3	30.21	29.56	30.14	29.67	30.12	29.68	30.61
				β-D-Glcp				
1	CH							106.92
2	CH							75.71
3	CH							78.79
4	CH							72.09
5	CH							78.06
6	CH_2							63.19

Chemical shifts with the whole numbers are not indicated in spectra. Chemical shifts for absent signals are not found. Signals with the same characters cover mutually inside of the column.

(H-5 and H-17, m), 3.09 (H-9, m), 3.30 (H-22, m), 3.76 (H-2, m), 3.88 (H-3, m), 5.75 (H-7, d, 2.5).

IR-spectrum ecdysteron (KBr, ν_{max}, sm^{-1}): 3409, 1652.

ES-MS Positive ion mode of ecdysteron: 503.3 $[M + Na]^+$.

ES-MS negative ion mode of ecdysteron: 479.1 $[M - H]^-$.

22-O-*β*-D-Glucopyranosid-2-deoxy-*α*-ecdysone (4)-$C_{33}H_{54}O_{10}$

Spectroscopy ^1H NMR 22-O-*β*-D-Glucopyranosid-2-deoxy-*α*-ecdysone (400 MHz, C_5D_5N, *δ*, ppm, J/Hz, 0-HMDS): 0.64 (CH_3-18, s), 0.91 (CH_3-19, s), 1.08 (CH_3-21, d, ^3J = 6.7), 1.19 (CH_3-26, s), 1.23 (CH_3-27, s), 3.36 (H-9, m), 3.86 (H-5 Glu, m), 3.98 (H-3, H-22 and H-2-Glu, m), 4.15 (H-3 Glu and H-4 Glu, m), 4.30 (H-6 Glu, dd, ^2J = 11.4, ^3J = 5.4), 4.47 (H-6' Glu, dd, ^2J = 11.4, ^3J = 2.7), 4.92 (H-1 Glu, d, ^3J = 7.7), 6.07 (H-7, d, ^3J = 2).

5. CONCLUSION

Compound 22-O-*β*-D-Glucopyranosid-2-deoxy-*α*-ecdysone (**4**) was isolated from *Silene praemixta* M. Pop. for the first time.

ACKNOWLEDGEMENTS

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