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PHYTOECDYSTEROIDS OF *Silene linicola*

N. Z. Mamadalieva,[1] L. N. Zibareva,[2] and Z. Saatov[1]

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Seven ecdysteroids isolated from the aerial part of Silene linicola *are identified using ^{1}H and ^{13}C NMR methods. Their yield from raw material was determined.*

Key words: *Silene linicola,* ecdysteroids, ^{1}H and ^{13}C NMR spectra.

The search for new physiologically active compounds, especially from natural sources, is of great interest because such compounds are readily eliminated from the organism and are ecologically purer. Biologically active compounds may act specifically and may intensify peroxide oxidation of lipids by altering the physicochemical systems that regulate cellular metabolism.

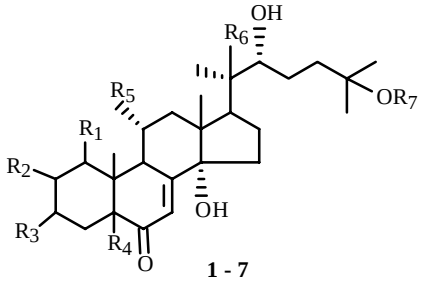
Plants of the *Silene* genus (Caryophyllaceae) are promising as ecdysone-containing plants. This family differs from others by having many species that contain ecdysone and high levels and a wide variety of ecdysteroids [1, 2]. Among representatives of the *Silene* genus, ecdysteroids have been found in over 100 species [2, 3] and isolated from 22 plants.

We studied a new ecdysteroid source, *S. linicola* C. C. Gmelin and determined its ecdysteroid profile.

Ecdysteroids were first observed in seeds of this species [4] by the literature method [5]. The plant was grown on an industrial plot at the Siberian Botanical Garden of Tomsk State University, where raw material for production of preparations (ecdystene) based on phytoecdysteroids was grown.

It was shown that ecdysterone occurs in the greatest amount in the aerial part of 1-year plants during flowering (0.93%). The ecdysteroid content is variable in various organs of this plant. It occurs in highest amounts in reproductive organs (1.05 and 1.55% in buds and flowers, respectively) [6].

TLC of the alcohol extract from *S. linicola* indicates that it contains at least 12 phytoecdysteroids of various structure. Using column chromatography, we isolated seven ecdysteroids from the aerial organs of *S. linicola* collected in 1997: viticosterone E (**1**) [7], 2-deoxyecdysterone (**2**) [8], α-ecdysone (**3**) [9], polypodine B (**4**), ecdysterone (**5**) [7], turkesterone (**6**) [10], and integristerone A (**7**) [7].



1 - 7

1: $R_1 = R_4 = R_5 = H$; $R_2 = R_3 = R_6 = OH$; $R_7 = Ac$
2: $R_1 = R_2 = R_4 = R_5 = R_7 = H$; $R_3 = R_6 = OH$
3: $R_1 = R_4 = R_5 = R_6 = R_7 = H$; $R_2 = R_3 = OH$
4: $R_1 = R_5 = R_7 = H$; $R_2 = R_3 = R_4 = R_6 = OH$
5: $R_1 = R_4 = R_5 = R_7 = H$; $R_2 = R_3 = R_6 = OH$
6: $R_1 = R_4 = R_7 = H$; $R_2 = R_3 = R_5 = R_6 = OH$
7: $R_1 = R_2 = R_3 = R_6 = OH$; $R_4 = R_5 = R_7 = H$

1) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75; 2) Tomsk State University, Siberian Botanical Garden. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 225-227, May-June, 2002. Original article submitted May 27, 2002.

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TABLE 1. ^{13}C and ^{1}H NMR Spectra of Turkesterone (**6**) (C_5D_5N, δ, ppm, TMS)

C Atom	XC ^{13}C	XC ^{1}H
1	39.94	3.44 1.99
2	68.89	4.62
3	68.21	4.22
4	32.90	2.03 1.80
5	52.51	3.04
6	203.91	-
7	122.32	6.30
8	164.23	-
9	42.82	3.87
10	39.57	-
11	68.44	4.58
12	44.21	3.03 2.68
13	48.23	-
14	84.29	-
15	31.91	2.23 1.93
16	21.64	2.48 2.13
17	50.06	3.10
18	18.93	1.27
19	24.90	1.33
20	76.87	-
21	21.63	1.59
22	77.56	3.87
23	27.53	2.12; 1.82
24	42.64	2.25; 1.81
25	69.58	-
26	30.11	1.37
27	30.06	1.37

Ecsyteroids **1**-**5** and **7** were identified using IR, PMR, and mass spectroscopies and by comparison with authentic samples (see Experimental).

Ecdysteroid **6** was identified using ^{1}H and ^{13}C NMR spectra (Table 1) [10].

The results indicated that **6** was turkesterone, which has been isolated previously from *Ajuga turkestanica* [11]. It is isolated from a plant of the *Silene* genus for the first time.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument at working frequencies 500.13 and 125.27 MHz, respectively, in C_5D_5N at 30°C with TMS standard. Two-dimensional spectra were recorded using standard Bruker methods. The accuracy of the ^{1}H and ^{13}C chemical shifts were 0.01 ppm; of $^{1}H/^{1}H$ spin—spin coupling constants, 0.2 Hz.

IR spectra were recorded on a Perkin—Elmer System 2000 FT IR Fourier spectrometer in KBr pellets; mass spectra, in a MS-25 RF (Kratos) instrument with direct sample introduction at accelerating potential 4 kV, ionizing potential 70 V, and ionization chamber temperature 250°C. The sample temperature was varied from 180 to 270°C.

Isolation of Ecdysteroids. The aerial part of *S. linicola* was collected during fruiting in June 1997. Air-dried ground material (3 kg) was extracted with ethanol (15 L). The extract was condensed, diluted with water, and filtered. After additional distillation of ethanol in vacuo, the aqueous remainder was treated with $CHCl_3$. Ecdysteroids from the aqueous remainder were exhaustively extracted by ethylacetate and then butanol. After evaporation of solvents in vacuo, we obtained ethylacetate

(12 g) and butanol (30 g) fractions.

The ethylacetate fraction was chromatographed over aluminum-oxide (0.5 kg) and then silica-gel columns. Elution in both instances used $CHCl_3$—CH_3OH (15:1) to produce viticosterone E (**1**, 24 mg) (the yield here and hereafter is calculated for air-dried material, 0.0008%), $C_{29}H_{46}O_8$. After crystallization from acetone, **1** had mp 195-196°C, $[\alpha]_D^{22}$ +59.2±2° (*c* 0.5, CH_3OH). IR spectrum (KBr, ν, cm^{-1}): 3430-3450 (OH), 1670 (7-en-6-keto), 1730, 1275 (ester).

Mass spectrum, *m/z* (%): 504 $[M - H_2O]^+$ (0.03), 486 (0.1), 462 (0.3), 444 (4), 426 (32), 411 (12), 408 (10), 393 (11), 363 (44), 345 (100), 327 (94), 309 (20), 301 (32), 300 (20), 173 (41), 143 (20), 125 (25), 107 (21), 99 (40), 81 (42), 69 (37).

PMR spectrum (C_5D_5N, δ, ppm): 1.07 (3H, s, H-19), 1.21 (3H, s, H-18), 1.42 and 1.49 (6H, s, H-26/27), 1.58 (3H, s, H-21), 4.18 (2H, m, H-2,3), 3.65 (1H, m, H-9), 3.81 (1H, m, H-22), 6.15 (1H, br.s, H-7) [7].

Further elution of the column with the same system isolated 2-deoxyecdysterone (**2**), 320 mg, 0.017%, $C_{27}H_{44}O_6$, mp 254-256°C (aqueous ethanol), $[\alpha]_D^{22}$ +82.0±2° (*c* 0.5, CH_3OH). IR spectrum (KBr, ν, cm^{-1}): 3370 (OH), 1645 (7-en-6-keto).

Mass spectrum, *m/z* (%): 464 $[M]^+$ (1), 446 (3), 428 (10), 413 (8), 410 (14), 395 (6), 392 (6), 377 (5), 347 (35), 329 (90), 312 (33), 311 (28), 303 (9), 302 (11), 297 (13), 295 (14), 285 (30), 284 (35), 234 (15), 233 (15), 99 (100), 81 (73).

PMR spectrum (C_5D_5N, δ, ppm): 1.05 (3H, s, H-19), 1.22 (3H, s, H-18), 1.58 (3H, s, H-21), 1.38 (6H, s, H-26/27), 3.52 (1H, m, H-9), 4.11 (1H, m, H-3), 3.85 (1H, m, H-22), 6.22 (1H, br.s, H-7) [8].

Elution of the column with $CHCl_3$—CH_3OH (9:1) gave α-ecdysone (**3**), 30 mg, 0.001%, $C_{27}H_{44}O_6$, mp 236-238°C (aqueous methanol), $[\alpha]_D^{22}$ +63.6° (*c* 0.83, methanol). IR spectrum (KBr, ν, cm^{-1}): 3400-3460 (OH), 1665 (7-en-6-keto).

Mass spectrum, *m/z* (%): 446 $[M - H_2O]^+$ (0.2), 431 (0.7), 428 (1.6), 418 (2), 413 (3), 348 (8), 315 (8), 301 (4), 300 (3), 279 (52), 250 (50), 99 (100), 81 (99).

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 0.74 (3H, s, H-18), 1.07 (3H, s, H-19), 1.24 (3H, d, J = 6, H-21), 1.37 (6H, s, H-26/27), 3.52 (1H, m, H-9), 4.10 (1H, m, H-22), 4.10 and 4.22 (2H, m, H-2 and H-3), 6.18 (1H, br.s, H-7) [9].

Further elution of the column with the same solvent isolated polypodine B (**4**), 2.22 g, 0.073%, $C_{27}H_{44}O_8$, mp 252-254°C (acetone), $[\alpha]_D^{20}$ +94.2±2° (*c* 0.50, CH_3OH). IR spectrum (KBr, ν, cm^{-1}): 3400 (OH), 1673 (7-en-6-keto).

Mass spectrum, *m/z* (%): 478 $[M - H_2O]^+$ (0.03), 463 (0.1), 460 (0.2), 445 (0.3), 427 (0.4), 424 (0.4), 409 (0.7), 379 (6), 361 (100), 360 (86), 344 (4), 343 (4), 325 (5), 316 (10), 99 (12), 81 (13), 69 (15).

PMR spectrum (C_5D_5N, δ, ppm): 1.14 (3H, s, H-19), 1.20 (3H, s, H-18), 1.57 (3H, s, H-21), 1.36 (6H, s, H-26/27), 3.60 (1H, m, H-9), 3.82 (1H, m, H-22), 4.17 and 4.27 (2H, m, H-2 and H-3), 6.19 (1H, br.s, H-7) [7]. Use of the same solvent gave ecdysterone (**5**), 2 g, 0.067%, $C_{27}H_{44}O_7$, mp 241-242°C (acetone), $[\alpha]_D^{20}$ +58.9±2° (*c* 0.3, CH_3OH). IR spectrum (KBr, ν, cm^{-1}): 3435 (OH), 1665 (7-en-6-keto).

Mass spectrum, *m/z* (%): 480 $[M]^+$ (0.03), 462 (1), 446 (14), 444 (2), 411 (4), 408 (10), 393 (5), 363 (10), 345 (33), 327 (20), 301 (16), 300 (12), 161 (5), 143 (10), 125 (8), 107 (6), 99 (100), 81 (34), 69 (21).

PMR spectrum (C_5D_5N, δ, ppm): 1.07 (3H, s, H-19), 1.20 (3H, s, H-18), 1.57 (3H, s, H-21), 1.38 (6H, s, H-26/27), 3.42 (1H, m, H-9), 4.23 (2H, m, H-2,3), 6.20 (1H, br.s., H-7) [7].

The butanol fraction of ecdysteroids (30 g) was chromatographed over a silica-gel column. Elution by $CHCl_3$—CH_3OH (4:1) gave ecdysterone (**5**), 9 g, 0.3% and turkesterone (**6**), 25 mg, 0.00083%, $C_{27}H_{44}O_8$, amorph., $[\alpha]_D^{20}$ + 52.0±2° (*c* 1.46, CH_3OH).

IR spectrum (KBr, ν, cm^{-1}): 3300-3500 (OH), 1660 (Δ^7-6-keto).

Mass spectrum, *m/z* (%): 460 $[M - H_2O]^+$ (3), 442 (20), 427 (9), 424 (30), 409 (20), 406 (15), 391 (7), 379 (9), 361 (40), 343 (50), 326 (50), 301 (40), 143 (50), 126 (75), 125 (40), 99 (100), 81 (80), 69 (50) [10].

^1H and ^{13}C NMR spectra are listed in Table 1.

Changing to $CHCl_3$—CH_3OH—H_2O (4:1:0.1) produced integristerone A (**7**), 450 mg, 0.0015%, $C_{27}H_{44}O_8$, mp 246-248°C (ethylacetate—methanol), $[\alpha]_D^{22}$ +36.2±2° (*c* 0.32, CH_3OH). IR spectrum (KBr, ν, cm^{-1}): 3400 (OH), 1660 (7-en-6-keto).

Mass spectrum, *m/z* (%): 478 $[M - H_2O]^+$ (3), 460 (4), 445 (10), 442 (18), 427 (11), 409 (7), 391 (4), 379 (73), 374 (5), 368 (17), 361 (74), 343 (100), 325 (43), 316 (17), 309 (7), 283 (41), 143 (60), 135 (61), 99 (35), 81 (34).

PMR spectrum (C_5D_5N, δ, ppm): 1.19 (3H, s, H-18), 1.40 (3H, s, H-19), 1.39 (6H, s, H-26/27), 1.58 (3H, s, H-21), 3.57 (1H, m, H-9), 3.77 (1H, m, H-22), 4.30 (3H, m, H-1,2,3), 6.17 (1H, br.s, H-7) [7].

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