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PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS *Silene*. XVII. TOMENTESTERONE FROM *Silene tomentella*

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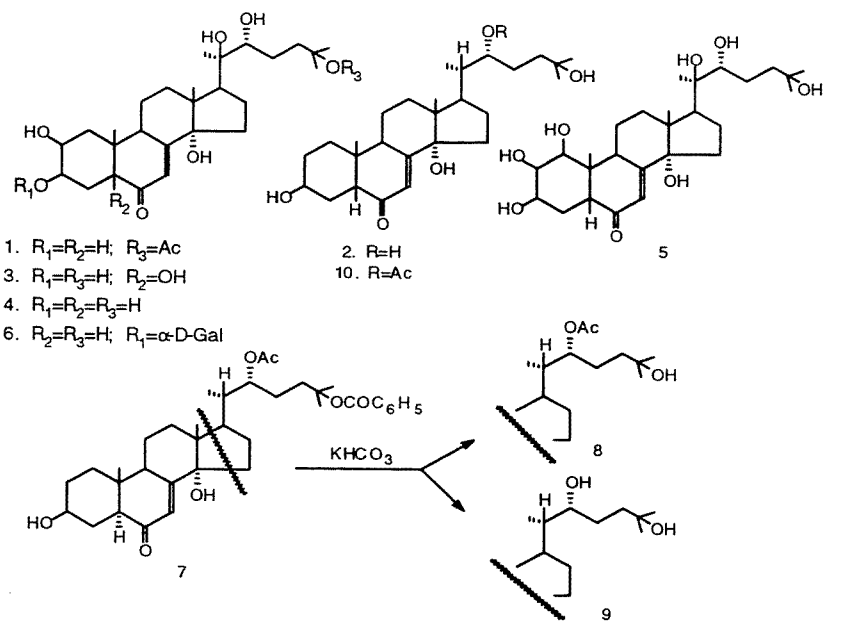
The new ecdysteroid tomentesterone A has been isolated from the epigeal part of Silene tomentella *Schischk. Its structure has been established by spectral studies and chemical transformations.*

Continuing the investigation of ecdysteroids from plants of the genus *Silene* (fam. Caryophyllaceae) [1], we have studied the phytoecdyteroids of *S. tomentella* Schischk. From the epigeal organs of this plant, in addition to the known compounds viticosterone E (**1**), 2-deoxy-α-ecdysone (**2**), polypodin B (**3**), ecdysterone (**4**), integristerone A (**5**), and sileneoside D (**6**), we have isolated a new ecdysteroid (**7**). The mass spectrum of (**7**) showed intense peaks of ions with *m/z* 332, 331, 284, 234, 231, 122 ($C_7H_6O_2$), and 105 (C_7H_5O), while the IR spectrum had absorption bands characteristic of a benzene ring (1610, 1590, 715 cm^{-1}). In the PMR spectrum there were the signals of five aromatic protons at (ppm) 7.44 (3H, m) and 8.19 ppm (2H, m) and a 3H singlet at 2.14 ppm. These spectral characteristics showed that compound (**7**) belonged to the 2-deoxyecdysteroid series [2] and that the molecule contained one benzoate group and one acetate group.



A comparative analysis of the characteristics of the PMR spectra of ecdysteroids (**7**) and (**2**) showed their closeness, with the exception of the chemical shifts of the CH_3-19, -21, -26/27, and H-22 lines. It can be seen from the chemical shifts of the protons and groups (Table 1) that in the case of the ecdysteroid (**7**), as compared with (**2**), the CH_3-19 signal had undergone a diamagnetic shift by 0.18 ppm. A similar situation is observed on passing from the 5β-2-deoxyecdysteroid (**2**) to

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TABLE 1. Chemical Shifts (δ, ppm) and SSCCs (J) of the Protons of 2-Deoxy-α-ecdysone (**2**), Tomentesterone A (**7**), 5α-2-Deoxy-α-ecdysone 22-Acetate (**8**), 5α-2-Deoxy-α-ecdysone (**9**), and 2-Deoxy-α-ecdysone 22-Acetate (**10**) (C_5D_5N; 0 — TMS)

Protons	Compound				
	2	**7**	**8**	**9**	**10**
H-3	4.13	4.10	4.12	4.05	4.10
H-22	4.13	5.34	5.33	4.05	5.27
H-7	6.16	6.19 J=3 Hz	6.14 J=3 Hz	6.18 J=3 Hz	6.15
CH_3-18	0.75	0.74	0.72	0.73	0.75
CH_3-19	1.07	0.89	0.90	0.93	1.03
CH_3-21	1.31 J=6.5 Hz	1.13 J=6.5 Hz	1.13 J=6 Hz	1.27 J=6 Hz	1.14 J=6 Hz
CH_3-26/27	1.43	1.60	1.35	1.41	1.33
H_{Ar}	–	7.48 (3H) 8.18 (2H)	–	–	–
OAc	–	2.14 (3H)	2.08 (3H)	–	2.08 (3H)

Note: The signal of the CH_3-21 group was a doublet, and those of the other methyl groups singlets. The H-7 protons in compounds (**7**), (**8**), and (**9**) appeared in the form of doublets and in the other case in the form of broadened singlets, while the other signals were broadened multiplets.

its 5α- isomer (**9**), which we have shown previously [1, 3]. From this, we drew the conclusion that rings *A* and *B* of the steroid part of ecdysteroid (**7**) are *trans*-linked with one another, and H-5 atom has the α-orientation. In actual fact, from the products of the alkaline hydrolysis of (**7**) we isolated two compounds, (**8**) and (**9**), one of which, (**9**), we identified as 5α-2-deoxy-α-ecdysone [1, 3]. The IR spectrum of the other hydrolysis product, (**8**), had characteristic absorption bands at 1735 and 1260 cm^{-1}, and in the PMR spectrum the resonance lines of one acetyl group and of H-22, H_3C-21, and H_3C-26/27 appeared at 2.08, 5.33, 1.13, and 1.35 ppm, respectively. When these characteristics are compared with those given in Table 1 for (**7**), we see the absence of the signals of aromatic protons from the spectrum of (**8**), retention of the chemical shifts of H-22 and H_3C-21, and a 0.25 ppm diamagnetic shift of the singlet from H_3C-26/27. In the light of the chemical shifts of the corresponding protons of the groups in 5α-deoxy-α-ecdysone and 2-deoxy-α-ecdysone 22-acetate (**10**) [4-6], these facts unambiguously showed that compound (**8**) is 5α-2-deoxy-α-ecdysone 22-acetate.

A comparative analysis of all the spectral characteristics given in Table 1 permitted the conclusion that tomentesterone A (**7**) is 5α-2-deoxy-α-ecdysone 22-O-acetate 25-O-benzoate.

EXPERIMENTAL

Thin-layer chromatography (TLC) was conducted in a fixed layer of silica gel containing 7% of gypsum that had been passed through a sieve with 0.08 mm apertures, and on Silufol plates. For column chromatography we used KSK silica gel and silica gel L (Czech Republic). The solvent systems used were chloroform–methanol, 1) (15:1), 2) (9:1), 3) (4:1), and 4) (50:1); and chloroform–methanol–water (4:1:0.1) (5). In TLC the ecdysteroids were revealed by spraying with vanillin–sulfuric acid followed by heating at 110-120°C for 2-5 min [7].

Mass spectra were taken on an MX-1310 instrument at an ionizing voltage of 50 V and a temperature of 100-140°C, while UR spectra were recorded on a UR-20 spectrometer and UV spectra on a Hitachi instrument. PMR spectra were obtained on a BS-567 A (100 MHz, Tesla) spectrometer in C_5D_5N relative to TMS.

Isolation of the Main Phytoecdysteroids. The epigeal part of the plant *Silene tomentella* (Schischk) was gathered in May, 1984, in the environs of Lake Surkul', Dzhambulskaya province, Republic of Uzbekistan. The dried and comminuted raw material (15 kg) was extracted with 80 liters of ethanol. The extract was concentrated and diluted with water, and the precipitate was removed. The ethanol was evaporated off in vacuum, and the aqueous residue was treated with hexane. The ecdysteroids were extracted from the purified aqueous fraction first with ethyl acetate and then with butanol.

The dry residue obtained after the ethyl acetate had been distilled off was chromatographed on a column of alumina, with elution by system 2. This led to the isolation of 262 mg of a mixture of four substances (for their subsequent treatment,

see below). Further elution with the same system gave known ecdysteroids — viticosterone E (500 mg) and 2-deoxy-α-ecdysone (3 g).

When the column was then eluted with system 3, 240 mg of polypodin B and 30 mg of ecdysterone were obtained.

The total material remaining after the evaporation of the butanol extract was chromatographed on a column of silica gel. Elution with system 3 enabled an additional amount of ecdysterone to be obtained.

The elution of the column with system 5 yielded 700 mg of integristerone A and 250 mg of sileneoside A.

The yields of the ecdysteroids (% on the weight of the air-dry raw material) were as follows: viticosterone E, 0.0033; 2-deoxy-α-ecdysone, 0.02; polypodin B, 0.0016; ecdysterone (total yield), 0.2; integristerone A, 0.0047; sileneoside D, 0.0017.

Viticosterone E (1), $C_{29}H_{46}O_8$, mp 195-196°C (from acetone), $[\alpha]_D^{20}$ + 59.2 ± 2° (*c* 0.50; methanol). IR spectrum (KBr, ν, cm^{-1}): 3430-3450 (OH), 1670 (7-ene-6-keto grouping), 1730, 1275 (ester group).

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 (95), 309 (20), 301 (32), 300 (20), 173 (41), 143 (20), 125 (25), 107 (21), 99 (40), 81 (42), 69 (37).

PMR spectrum (δ, ppm): 1.07 (s, CH_3-19), 1.21 (s, CH_3-18), 1.42 and 1.49 (s, CH_3-26/27), 1.58 (s, CH_3-21), 1.92 (OAc), 4.18 (m, H-2.3), 3.65 (m, H-9), 3.81 (m, H-22), 6.15 (br.s, H-7).

Compound (**1**) was identified from spectral indices and by a direct TLC comparison (system 2) with an authentic specimen of viticosterone E [8, 9].

2-Deoxy-α-ecdysone (2), $C_{27}H_{44}O_5$, mp 235-236°C (from aqueous ethanol), $[\alpha]_D^{23}$ + 93.2 ± 2° (*c* 0.50; methanol). UV spectrum (C_2H_5OH, λ_{max}, nm): 245 (lgε 4.10). IR spectrum (KBr, ν, cm^{-1}): 3440 (OH), 1645 (7-ene-6-keto grouping).

Mass spectrum, *m/z* (%): 448 (M 2), 430 (13), 415 (14), 412 (11), 402 (16), 397 (17), 379 (4), 361 (7), 343 (6), 332 (33), 314 (28), 303 (3), 294 (16), 285 (12), 284 (12), 235 (27), 234 (50), 233 (32), 99 (100), 81 (63).

The facts given permitted the assumption that ecdysteroid (**2**) was 2-deoxy-α-ecdysone [10, 11].

Polypodine B (3), $C_{27}H_{44}O_8$, after recrystallization from acetone had mp 252-254°C $[\alpha]_D^{20}$ + 94.2 ± 2° (*c* 0.50; methanol). IR spectrum (KBr, ν, cm^{-1}): 3400(OH), 1673(7-ene-6-keto grouping). UV spectrum (C_2H_5OH, λ_{max}, nm): 244 (lgε 3.9).

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 (δ, ppm): 1.16 (s, CH_3-19), 1.19 (s, CH_3-18), 1.56 (s, CH_3-21), 1.37 (s, CH_3-26/27), 3.61 (m, H-9), 3.83 (m, H-22), 4.23 (m, H-2.3), 6.20 (br.s, H-7).

From its spectral characteristics, and also according to a direct TLC comparison with an authentic specimen, compound (**3**) was identified as polypodine B [9, 12].

Ecdysterone (4), $C_{27}H_{44}O_7$, mp 241-242°C (from acetone), $[\alpha]_D^{20}$ + 63.2 ± 2° (*c* 6.30; methanol). UV spectrum (C_2H_5OH, λ_{max}, nm): 245 (lgε 4.01). IR spectrum (KBr, ν, cm^{-1}): 3435 (OH), 1665 (7-ene-keto grouping).

Mass spectrum, *m/z* (%): 480 (M; 0.03), 462 (1), 444 (2), 446 (14), 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 (δ, ppm): 1.07 (s, CH_3-19), 1.20 (s, CH_3-18), 1.57 (s, CH_3-21), 1.38 (s, CH_3-26/27), 4.23 (m, H-2.3), 6.20 (br.s, H-7).

A comparison of the above constants and spectral characteristics with information in the literature [9, 13] permitted ecdysteroid (**4**) to be identified as ecdysterone.

Integristerone A (5), $C_{27}H_{44}O_8$ with mp 246-248°C (from ethyl acetate methanol), $[\alpha]_D^{20}$ + 36.1 ± 2° (*c* 0.43; methanol). UV spectrum (C_2H_5OH, λ_{mass}, nm): 245 (lgε 4.00). IR spectrum (KBr, ν, cm^{-1}): 3400 (OH), 1660 (7-ene-6-keto grouping).

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 (δ, ppm): 1.19 (s, CH_3-18), 1.40 (s, CH_3-19), 1.39 (s, CH_3-26/27), 1.58 (s, CH_3-21), 3.57 (m, H-9), 3.77 (m, H-22), 4.30 (m, H-1,2,3), 6.17 (br.s, H-7).

The facts given, and also a direct comparison (TLC) with an authentic specimen, enabled compound (**5**) to be identified as integristerone A [9].

Sileneoside D (6), $C_{33}H_{54}O_{12}$, after recrystallization from methanol–acetone had mp 240-242°C; $[\alpha]_D^{20}$ + 91.4 ± 2° (*c* 0.76; methanol). UV spectrum (C_2H_5OH, λ_{mass}, nm): 247 (lgε 4.00). IR spectrum (KBr, ν, cm^{-1}): 3380-3430 (OH), 1650 (7-ene-6-keto grouping).

IR spectrum, *m/z* (%): 624 (M-H_2O; 0.5), 606 (0.8), 588 (5), 570 (1), 514 (0.8), 490 (1), 473 (0.6), 462 (1), 444 (1), 426 (11), 408 (3), 393 (1), 375 (1), 363 (5), 345 (55), 327 (13), 309 (10), 300 (11), 145 (10), 143 (12), 99 (100), 81 (56), 69 (54).

PMR spectrum (δ, ppm): 0.99 (s, CH_3-19), 1.22 (s, CH_3-18), 1.38 (s, CH_3-26/27), 1.61 (s, CH_3-21), 3.50 (br.m, H-9), 3.88 (br.m, H-22), 4.09 (br.m, H-2,3), 5.61 (d, H-1′, ^3J = 3.9 Hz), 6.23 (br.s, H-7).

The physicochemical constants and spectral characteristics of ecdysteroid (**6**) showed its identity with sileneoside D [14].

Isolation of Tomentesterone A (7). The mixture of four ecdysteroids obtained from the ethyl acetate fraction and subjected to preliminary purification (262 mg) was rechromatographed on a silica gel column. Elution with system 1 led to the successive isolation of 100 mg (0.0007%) of tomentesterone A (**7**) and 60 mg of ecdysterone B.

Tomentesterone A (7), $C_{36}H_{50}O_7$, mp 139-140°C (methanol–water). IR spectrum (KBr, ν, cm^{-1}): 3420 (OH), 1645 (Δ^7-6-keto grouping), 1720, 1730, 1290, 1250, 1225 (ester group), 1610, 1590, 715 (benzene ring).

Mass spectrum, *m/z* (%): 576 (M-H_2O; 0.8), 566 (0.5), 474 (46), 472 (10), 452 (46), 444 (46), 439 (9), 412 (46), 397 (46), 379 (11), 332 (30), 331 (35), 284 (100), 234 (13), 231 (12), 122 (50), 105 (45), 99 (46), 81 (47).

Alkaline Hydrolysis of Tomentesterone A (7). A solution of 50 mg of ecdysteroid (**7**) in 10 ml of methanol was treated with 60 mg of potassium bicarbonate in 4 ml of water. The reaction mixture was kept in a thermostat at 38°C for two days. Then it was 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 silica gel column. Elution with system 4 gave 16 mg of 5α-2-deoxy-α-ecdysone 22-acetate (**8**), $C_{29}H_{46}O_{46}$, mp 158-160°C (methanol–water), $[\alpha]_D^{25}$ +51.4±2° (*c* 0.20; methanol). IR spectrum (KBr, ν, cm^{-1}) 3440 (OH); 1735, 1260 (ester group); 1660 (7-ene-6-keto grouping); M^+ 490.

Further elution with the same mixture of solvents gave 12 mg of 5α-2-deoxy-α-ecdysone (**9**), $C_{27}H_{44}O_5$, mp 225-227°C (methanol–water) [1].

REFERENCES

1. Z. Saatov, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 852 (1987).
2. Ya. V. Rashkes and N. K. Abubakirov, Khim. Prir. Soedin., 518 (1980).
3. M. Kh. Dzhukharova, Z. Saatov, and N. D. Abdullaev, Khim. Prir. Soedin., 199 (1985).
4. Z. Saatov, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 439 (1986).
5. J. P. Girault, M. Bathori, E. Varga, K. Szendrei, and R. Lafont, J. Nat. Prod., **53**, 279 (1990).
6. Z. Saatov, N. D. Abdullaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 363 (1990).
7. M. H. Galbraith and D. H. S. Horn, Aust. J. Chem., **22**, 1045 (1969).
8. H. Rimpler, Tetrahedron Lett., 329 (1969).
9. Z. Saatov, M. B. Gorovits, N. D. Abdullaev, B. Z. Usmanov, and N. K. Abubakirov, Khim. Prir. Soedin., 738 (1981).
10. Y. K. Chong, M. H. Galbraith, and D. H. S. Horn, J. Chem. Soc., Chem. Commun., 1217 (1970).
11. Z. Saatov, B. Z. Usmanov, and N. K. Abubakirov, Khim. Prir. Soedin., 793 (1979).
12. J. Jizba, V. Herout, and F. Šorm, Tetrahedron Lett., 5139 (1967).
13. B. Z. Usmanov, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 535 (1971).
14. Z. Saatov, N. D. Abdullaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 741 (1984).