

Mass spectrum, m/z (%): 566 (M-H₂O; 0.1), 548(0.2), 530(0.3), 515(0.2), 512(0.2), 462 (0.7), 444 (1), 426(6), 411(2), 408(6), 393(2), 363(2), 345(6), 329(2), 327(2), 301(8), 300 (9), 250(7), 122(44), 105(100), 99(22), 81(23), 77(50), 69(24), 55(24), 51(20).

Alkaline Hydrolysis of 5 α -Ecdysterone 22-O-Benzoate (VI). To 5 mg of the ecdysteroid (VI) in 5 ml of methanol was added 3 ml of a 1% aqueous solution of potassium bicarbonate. The reaction mixture was left in an atmosphere of nitrogen at 40°C for 5 days and was then diluted with water, neutralized, and extracted with ethyl acetate. The solvent was distilled off to dryness, and the residue was recrystallized from methanol to give 1.5 mg of the ecdysteroid (V) with mp 276-278°C, identified from its IR spectrum and R_f value in TLC (ethyl acetate-ethanol (9:1) system) with an authentic sample of 5 α -ecdysterone (for preparation, see below).

Benzoic acid was isolated from the aqueous solution after acidification with dilute hydrochloric acid (1:1) and extraction with ethyl acetate.

Isomerization of Ecdysterone (II) into 5 α -Ecdysterone (V). A solution of 225 mg of ecdysterone (II) in 25 ml of 90% methanol containing 0.6% of potassium bicarbonate was boiled in an atmosphere of nitrogen for 30 min. After cooling, the reaction products were neutralized with acetic acid, the methanol was evaporated off to half-volume, water was added, and the mixture was extracted with butanol. The butanolic extract was distilled off to dryness and the residue was chromatographed on a column of alumina. Elution with the chloroform-methanol (9:1) system yielded 20 mg of 5 α -ecdysterone (V), C₂₂H₄₄O₇, mp 276-278°C (methanol), $[\alpha]_D^{20} +54.6 \pm 2^\circ$ (c 0.34; methanol). Literature figures for substance (V) [3]: mp 278°C $[\alpha]_D^{20} +55.5^\circ$. When the washing of the column was continued with the same solvent system, the initial ecdysterone was isolated.

CONCLUSIONS

The new ecdysteroid 5 α -ecdysterone 22-O-benzoate has been isolated from the epigeal organs of the plant *Silene scabrifolia* Kom.

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PHYTOECDYSTEROIDS OF *Rhaponticum carthamoides*

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From the roots with rhizomes of the plant *Rhaponticum carthamoides* Willd) Iljin Compositae), in addition to integristerone A, ecdysterone, polypodin B, 2-deoxyecdysterone, and 24(28)-dehydromakisterone A, we have isolated the new compounds ecdysterone 3-2,3-monoacetone (I), ecdysterone 20,22-monoacetone (II) and rhapisterone (III): I - C₃₀H₄₈O₇, mp 232-233° (ethyl acetate-methanol) $[\alpha]_D^{20} +56.4 \pm 2^\circ$ (c 0.9; methanol); II - C₃₀H₄₈O₇, mp 227-229° (ethyl acetate-methanol), $[\alpha]_D^{20} +60.1 \pm 2^\circ$ (c 1.3; methanol); III - C₂₉H₄₆O₇, mp, 241-242° (ethyl acetate-methanol), $[\alpha]_D^{20} +30 \pm 2^\circ$ (c 0.1; dioxane). The structure of (III) was established on the basis of spectral characteristics as 2 β ,3 β ,14 α ,20R,22R,23 ξ -5 β -stigmast-7-en-6-one. Details of the PMR, mass, and IR spectra of all the compounds and of the CD of rhapisterone are given.

Even in our first publications on plant ecdysone-like compounds we showed that *Rhaponticum carthamoides* (Willd) Iljin is a rich source of a universal arthropod molting hormone—

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ecdysterone [1-3]. In addition to ecdysterone [4], several new compounds have been detected in *R. integrifolium* C. Winkl. [5-7]. In the present paper we present the results of further investigations on the phytoecdysteroids of *R. carthamoides*.

The air-dry roots (with rhizomes) of the plant were extracted with methanol. On TLC, the extract showed the presence of nine compounds of ecdysteroid nature. We have succeeded in isolating and identifying integristerone A [5], ecdysterone [8], polypodin B [9], 2-deoxyecdysterone [10], and 24(28)-dehydromakisterone A [7]. In addition to the substances listed, ecdysterone 2,3-monoacetone (I) and ecdysterone 20,22-monoacetone (II), and also a previously unknown compound, which we have called raphisterone (III), were detected.

The IR spectrum of compound (I) contained absorption bands of hydroxy groups ($3460-3500\text{ cm}^{-1}$) and of a keto group conjugated with a double bond (1655 cm^{-1}). Its UV spectrum ($\lambda_{\text{max}}^{\text{C}_2\text{H}_5\text{OH}}$ 244 nm) also confirmed the presence of a 7-en-6-keto group in the steroid nucleus.

The mass spectrum of compound (I) showed the peaks of the molecular ion with m/z 520. This is higher by 40 m.u., i.e., by the size of an isopropylidene group, than the molecular weight of ecdysterone. The peak of an ion with m/z corresponding in ecdysterone to the cleavage of the C-20-C-22 bond was absent. In place of it, a fairly strong peak appeared with m/z 403. The peaks of ions formed from the side chain with m/z 99 and 81 remained unchanged. It follows from this that the ecdysterone (I) was an ecdysterone acetone. The isopropylidene group was located in the steroid part of the molecule and occupied the single diol group at C-2 and C-3.

From its IR and UV spectra, compound (II) also belonged to the typical ecdysteroids. It had the same molecular weight as ecdysterone 2,3-monoacetone (I) and was a structural isomer of it. According to the results of mass-spectrometric fragmentation, in compound (II), the acetone group replaced the hydroxyls at C-20 and C-22.

The third new substance — raphisterone (III) — belonged to the group of phytoecdysteroids of the C-29 series. The IR spectrum of this compound had the bands of hydroxy groups ($3300-3500\text{ cm}^{-1}$), while at 1655 cm^{-1} absorption corresponding to a keto group conjugated with a double bond was observed. The UV spectrum contained an intense maximum at 244 nm ($\log \epsilon$ 4.07), which is characteristic for Δ^7 -6-ketosteroids.

The circular dichroism (CD) curve of raphisterone revealed a positive Cotton effect with $\Delta\epsilon = +1.52$ ($n \rightarrow \pi^*$ transition, 340 nm), and a negative Cotton effect with $\Delta\epsilon = -3.82$ ($\pi \rightarrow \pi^*$ transition, 248 nm), which showed the presence in the ecdysteroid (III) of a $5\beta(\text{H})$ -7-ene-6-keto-14-hydroxy grouping. The molecular peak was absent from the mass spectrum of compound (III). The high-mass region was characterized by the peaks of ions with m/z 490 ($M \pm \text{H}_2\text{O}$), 475, 472, 457, 454, 439, and 436. The fragments formed on the cleavage of the C-20-C-22 bond contained 363, 345, 327 m.u. An ion with m/z 300 corresponded to cleavage at the C-17-C-20 bond. Like the three fragments mentioned above, it was similar to the analogous ion in the mass spectrum of ecdysterone.

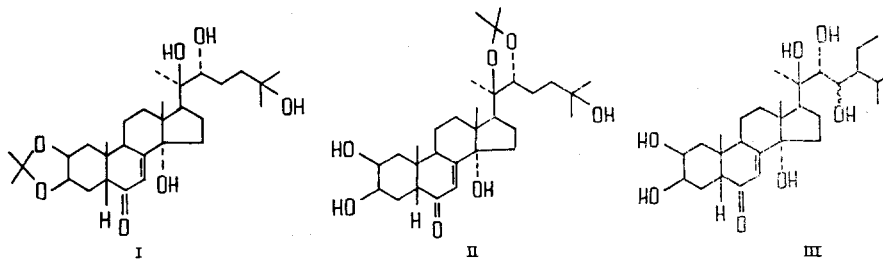
It is obvious that the difference between the structures of ecdysterone and raphisterone must be sought in the side chain.

Usually, in the mass spectra of ecdysteroids with side chains similar to that of ecdysterone cleavage of the C-20-C-22 bond leads, after dehydration, to a cyclic fragment with m/z 99. In the mass spectrum of episterone, this fragment was displaced by 28 m.u. (m/z 127), which presupposes the presence of an additional C_2H_4 group. After dehydration, this fragment formed an ion with m/z 109.

The PMR spectrum of compound (III) showed a two-proton signal at 4.12 ppm the nature of which resembled that of the signal of the two protons of ecdysterone located geminally to the secondary hydroxy groups at C-2 and C-3. We were therefore justified in assuming that in the new ecdysteroid the hydroxy groups were present in positions C-2 and C-3 of the steroid nucleus and had the β -orientation.

In the PMR spectrum of raphisterone, signals of the C-26 and C-27 methyl groups appeared in the form of a doublet at 1.13 ppm. The PMR spectrum bears this nature if there is a proton (H), and not a hydroxy group, at C-25. The absence of an OH group at C-25 was also shown by the peak of an ion with m/z 84 in the mass spectrum of compound (III). Such ions in the ecdysteroids are formed through cleavage of the C-23-C-24 bond, which indicates the presence in the C-24 position of a methyl or ethyl radical. Thus, the appearance of an ion with m/z 84 in combination with the PMR spectrum is evidence in favor of the assumption that an ethyl group is located at C-24.

The appearance on mass-spectrometric breakdown of a fragment with m/z 363 showed that in rhapisterone there is no proton at the C-20 carbon atom, and this conclusion is in harmony with the PMR spectrum, where the methyl group has a singlet nature. The proton at C-22 in rhapisterone gave a signal at 3.80 ppm with a doublet nature having a spin-spin coupling constant $^3J = 10$ Hz. The other hydrogen atom with which this proton interacts can only be an H atom at C-23. Consequently, there is another OH group at C-23.



The combination of the facts given above shows that rhapisterone has the most probable structure of 2 β ,3 β ,14 α ,20R,22R,23 ξ -hexahydroxy-5 β -stigmast-7-en-6-one.

EXPERIMENTAL

IR spectra were obtained on a UR-20 spectrophotometer (KBr). Mass spectra were taken on a MKh-1310 instrument fitted with a system for the direct introduction of the sample into the ion source, at an ionizing voltage of 50 V, a collector current of 60 μ A, and a temperature of the evaporator bulb and the ionization chamber of 170-190°C; and PMR spectra were taken on a JNM-4H-100 instrument, δ -scale, 0 - HMDS, temperature of the sample $22 \pm 2^\circ\text{C}$.

Isolation of the Phytoecdysteroids. The air-dry roots with rhizomes of *R. parthamoides* (22 kg) were extracted with methanol. According to preliminary TLC results, the extract showed the presence of nine spots of ecdysteroid nature. The methanol was evaporated to a volume of 1 liter and this residue was diluted with an equal amount of water. After the extraction of the hydrophobic compounds with hexane from the aqueous ethanolic fraction, the phytoecdysteroids were extracted with butanol. The butanolic fraction was evaporated in vacuum to dryness, giving 372.5 g of total extractive substances. Part (200 g) of this material was transferred to a column of Al_2O_3 (1400 g). Elution of the column with the chloroform-methanol (4:1) solvent system followed by concentration gave 10.2 g of purified total phytoecdysteroids. These combined phytoecdysteroids were separated on a column of SiO_2 in chloroform-ethanol (9:1) and (4:1) systems. The weakly-polar fractions of the ecdysterone-like substances were combined and rechromatographed on a column of silica gel in the chloroform-methanol (9:1) system. On elution of the column, the following phytoecdysteroids emerged successively: integristerone A (983 mg), ecdysterone (785 mg), polypodin B (87 mg), 2-deoxyecdysterone (65 mg), 24(28)-dehydromakisterone A (70 mg), ecdysterone 2,3-monoacetone (25 mg), ecdysterone 20,22-monoacetone (28 mg), and rhapisterone (32 mg).

The yield of phytoecdysteroids (as percentages of the weight of the air-dry raw material) were: integristerone A, 0.0083; ecdysterone, 0.066; polypodin B, 0.00043; 2-deoxyecdysterone, 0.00032; 24(28)-dehydromakisterone A, 0.00035; ecdysterone 2,3-monoacetone (I), 0.00012; ecdysterone 20,22-monoacetone (II), 0.00014; and rhapisterone (III), 0.00016.

Krasnov et al. [11] reported the isolation from the roots of *R. carthamoides* of ecdysterone and inokosterone. Inokosterone was not detected in our investigations.

Integristerone A - $\text{C}_{27}\text{H}_{44}\text{O}_8$, mp $243.5\text{--}245^\circ\text{C}$ (ethyl acetate-methanol), $[\alpha]_D^{20} +35.5 \pm 2^\circ$ (c 0.94; methanol) [5].

Ecdysterone - $\text{C}_{27}\text{H}_{44}\text{O}_7$, mp $236\text{--}237.5^\circ\text{C}$ (ethyl acetate-methanol), $[\alpha]_D^{20} +62.5 \pm 2^\circ$ (c 1.4; methanol) [4, 8].

Polypodin B - $\text{C}_{27}\text{H}_{44}\text{O}_8$, mp $258\text{--}259^\circ\text{C}$ (ethyl acetate-methanol), $[\alpha]_D^{20} +83.4 \pm 2^\circ$ (c 0.7; methanol) [9].

2-Deoxyecdysterone - $\text{C}_{27}\text{H}_{44}\text{O}_6$, mp $248\text{--}249.5^\circ\text{C}$ (ethyl acetate-methanol) $[\alpha]_D^{20} +75.4 \pm 2^\circ$ (c 1.02; methanol) [10].

24(28)-Dehydromakisterone A - $\text{C}_{27}\text{H}_{44}\text{O}_7$, mp $244\text{--}246^\circ\text{C}$ (ethyl acetate-methanol) [7].

The known phytoecdysterones that had been isolated were also identified by their IR, PMR, and mass spectra.

Ecdysterone 2,3-monoacetone (I) — $C_{30}H_{48}O_7$, mp 232–233°C (ethyl acetate–methanol), $[\alpha]_D^{20} +56.4 \pm 2^\circ$ (c 0.9; methanol); $\lambda_{C_2H_5OH}^{max}$ 244 nm (log ϵ 4.08); ν_{max}^{KBr} , cm^{-1} : 2460–3500 (OH); 1643 (Δ^7 -6-keto group). Mass spectrum, m/z (%): 520 (M^+) (1); 505(7), 502(2), 487(3), 484(2), 469(5), 466(4), 451(4), 403(72), 385(100), 371(7), 368(6), 360(7), 345(10), 327(36), 309(9), 269(13), 143(12), 99(36), 81(24). PMR spectrum (C_5D_5N): 0.88 (3 H, s, H-19), 1.04 (3 H, s, H-18), 1.23 (6 H, s, H-26 and H-27), 1.42 (3 H, s, H-21), 1.23 and 1.42 (6 H, s, isopropylidene group overlapping with methyl groups at C-26, C-27, and C-21), and 5.99 (H-7).

Ecdysterone 20,22-monoacetone (II) — $C_{30}H_{48}O_7$, mp 227–229°C (ethyl acetate–methanol) $[\alpha]_D^{20} +60.1 \pm 2^\circ$ (c 1.3; methanol), $\lambda_{C_2H_5OH}^{max}$ 243 nm (log ϵ 4.01); ν_{max}^{KBr} , cm^{-1} : 3400–3465 (OH); 1660 (Δ^7 -6-keto group). Mass spectrum (170°C, 50 V, m/z (%): 520 (M^+), 505(2), 502(1), 487(4), 484(4), 469(5), 445(4), 427(29), 409(13), 363(100), 353(15), 345(26), 329(10), 327(9), 320(4), 300(32), 201(13), 143(11), 99(19), and 81(26). PMR spectrum (C_5D_5N): 0.87 (3 H, s, H-19); 0.80 (3 H, s, H-18), 1.24 (6 H, s, H-26 and H-27); 1.24 and 1.33 (6 H, s, isopropylidene group overlapping with methyl groups at C-26 and C-27); 1.40 (3 H, s, H-21); and 6.07 (H-7).

Rhapisterone — $C_{28}H_{46}O_7$, mp 241–242°C (ethyl acetate–methanol), $[\alpha]_D^{20} +30 \pm 2^\circ$ (c 0.1; dioxane); $\lambda_{C_2H_5OH}^{max}$ 244 nm (log ϵ 4.07), ν_{max}^{KBr} , cm^{-1} : 3300–3500 (OH); 1655 (Δ^7 -6-keto group). CD (c 0.1; dioxane): $\Delta\epsilon +1.52$ (340 nm); $\Delta\epsilon -3.81$ (248 nm). Mass spectrum (180°C, 60 V, m/z (%): 490 ($M^+ - H_2O$) (1), 475(1), 472(3), 457(6), 454(13), 443(2), 439(2), 436(1), 363(75), 345(88), 327(30), 300(12), 250(12), 171(21), 127(100), 109(30), and 84(40). PMR spectrum (XL-200, Varian, C_5D_5N , 200 MHz, δ , 0 — HMDS, ppm): 0.92 (3 H, d, H-29) 0.89 (3 H s, H-19), 1.28 (3 H, s, H-18), 1.13 (6 H, at H-26 and H-27), 1.48 (3 H, s, H-21), 2.90 (H-5), 3.50 (2 H, m, H-9 and H-23), 3.80 (1 H, d, $^3J = 10$ Hz, H-22), 4.12 (2 H, m, H-2 and H-3), 6.16 (1 H, broadened singlet, H-7).

CONCLUSIONS

Eight phytoecdysteroids have been isolated from the roots with rhizomes of *Rhaponticum carthamoides* (Willd) Iljin (family Compositae) of which integristerone A, ecdysterone, polypodin B, 2-deoxyecdysterone, and 24(28)-dehydromakisterone A were known, while new ones proved to be ecdysterone 2,3-monoacetone, ecdysterone 20,22-monoacetone, and rhapisterol.

Rhapisterol has the structure of 2 β ,3 β ,14 α ,20R,22R,23 ξ -hexahydroxy-5 β -sigmast-7-en-6-one.

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