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RHAPISTERONE D 20-ACETATE FROM THE SEEDS OF *Leuzea carthamoides*

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In addition to makisterone A, the new ecdysteroid rhapisterone D 20-acetate has been isolated from the seeds of Leuzea carthamoides.

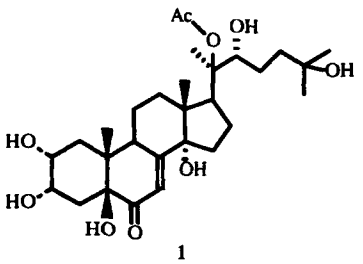
Continuing a study of plants of the genus *Rhaponticum* (synonym *Leuzea)* for the presence of ecdysteroids we have investigated the seeds of *Leuzea carthamoides* (Willd.) Iljin [1—4]. In the present paper we consider the determination of the structure of a weakly polar ecdysteroid (**1**) and the identification of a known ecdysteroid — makisterone A (**2**) — isolated from a plant of this genus for the first time.

In the IR spectrum of ecdysteroid (**1**), in addition to the absorption bands of hydroxy groups (3300—3400 cm^{-1}) and of a keto group conjugated with a double bond (1662 cm^{-1}), we observed the bands of an ester group (1715 and 1285 cm^{-1}).

The molecular ion was absent from the mass spectrum of ecdysteroid (**1**), while in the high-mass region there were the peaks of ions with *m/z* 520, 505, 502, 487, 478, 469, 460, and 451, corresponding to the splitting out from the molecular ion of water and methyl groups $[M - nH_2O - nCH_3]^+$. Cleavage of the C-20—C-22 bond of ecdysteroid (**1**) was characterized by an ion with *m/z* 421, the similar cleavage of a bond in rhapisterone D being shown by the peak of an ion with *m/z* 379 [3].

In the PMR spectrum of the new compound, in the region where olefinic protons usually resonate, there was a one-proton broadened singlet at 6.24 ppm that is characteristic for a proton at C-7 of an ecdysteroid. In addition, the spectrum contained the signals of protons geminal to hydroxy groups at C-2 (4.14 ppm), C-3 (4.25 ppm), and C-22 (3.84 ppm) and also an additional signal at 1.92 ppm, where signals corresponding to an —OAc group usually appear. These facts, together with the mass spectrum, where an ion with *m/z* 421 corresponds to cleavage of the C-20—C-22 bond, indicated the presence of an acetate group in the compound under investigation.

Cleavage of the steroid part of ecdysteroid (**1**) at the C-17—C-20 bond was characterized by an ion with *m/z* 316, which, together with the PMR spectrum, where protons geminal to hydroxy groups at C-2, C-3, and C-22 appeared in their characteristic positions, showed that the addition acetate group was located at C-20. Thus, compound (**1**) has the structure of rhapisterone D 20-acetate.



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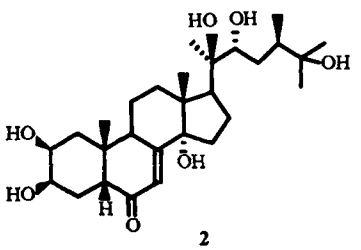

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Compound (**2**) that we had isolated was identified as makisterone A [5] on the basis of its mass spectrum and its PMR and ^{13}C NMR spectrum using the J-modulation method (Table 1).

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TABLE 1. ^{13}C NMR Spectrum of Makisterone A (δ, ppm)

C atom	δ_c	C atom	δ_c	C atom	δ_c
C-1	37.96	C-11	21.10	C-21	21.50
C-2	68.05	C-12	31.70	C-22	74.70
C-3	68.05	C-13	48.10	C-23	34.50
C-4	32.40	C-14	84.20	C-24	41.70
C-5	51.40	C-15	32.00	C-25	72.10
C-6	203.50	C-16	21.30	C-26	28.20
C-7	121.70	C-17	49.90	C-27	26.40
C-8	166.05	C-18	17.90	C-28	15.40
C-9	34.50	C-19	24.40		
C-10	38.60	C-20	76.90		

The action of makisterone A on the hibernation of the honey bee *Apis mellifera* when the queens are removed artificially and its action on the parasitic mite *Varroa jacobsoni* have been investigated.

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 injection of the specimen into the ion source, at an ionizing voltage of 40 V, a collector current of 50 μA, and a temperature of the evaporator bulb and the ionization chamber of 160°C.

Isolation of Rhapisterone D 20-Acetate (1). For the methods of extracting and purifying the ecdysteroids, see [4]. The mixture of ecdysteroids containing (1) (125 mg) was chromatographed on silica gel (SiO_2) and eluted in the chloroform—methanol (20:1) system, leading to the isolation of 12 mg (0.001%) of pure rhapisterone D 20-acetate. Rhapisterone D 20-acetate (1), $C_{29}H_{46}O_8$, mp 225—227°C (ethyl acetate—methanol), $[\alpha]_D^{20}$ +36.5±2° (*c* 0.7, methanol). IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3300-3400 (OH), 1662(Δ^7-6-keto group, 1715 and 1285 (ester). Mass spectrum, *m/z* (%): 520(2), 505(2), 502(3), 487(4), 478(3), 469(3), 460(3), 451(5), 421(78), 406(65), 403(100), 316(15), 301(6), 285(6), 99(45), 81(30). PMR spectrum (C_5D_5N, 400 Mhz, δ, ppm): 1.12 (CH_3, H-18, s); 1.18 (CH_3, H-19, s); 1.35 (CH_3-26, CH_3-27, s); 1.56 (CH_3, H-21, s); 1.92 (Ac, s), 2.95 (H-17, t); 3.62 (H-9, m); 3.84 (H-22, dd); 4.14 (H-2, q, J=3.5 Hz);4.25 (H-3, dt, J=12 and 4 Hz); 6.23 (H-7, br.s).

Isolation of Makisterone A (2). Continuing elution of the column in the chloroform—methanol (9:1) system led to the isolation of 15 mg (0.00125%) of makisterone A. Makisterone A (2) — $C_{28}H_{46}O_7$, mp 261—263°C (methanol—ethyl acetate). Mass spectrum *m/z*: 4.95, 476, 458, 440, 425, 422, 363, 345, 327, 300, 285, 267, 157, 157, 113, 95. PMR spectrum (C_5D_5N, 400 MHz, δ, ppm): 1.04 (CH_3, H-19, s); 1.05 (CH_3-28, d); 1.21 (CH_3-18, s); 1.28 and 1.30 (CH_3-26, CH_3-27, s); 1.56 (CH_3-21, s); 2.98 (H-17, t), 3.57 (H-9, m), 3.95 (H-22, dd), 4.19 (H-3, dt), 4.21 (H-2, q), 6.24 (H-7, br.s).

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