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Additional minor ecdysteroid components of *Leuzea carthamoides*

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ABSTRACT

Seventeen additional minor ecdysteroid compounds were isolated and identified from the roots of *Leuzea carthamoides* (Wild.) DC. Eight of them are new phytoecdysteroids: carthamoleusterone (**13**) is a new side-chain cyclo-ether with five-membered ring; 14-epi-ponasterone A 22-glucoside (**12**) is a rare and unusual natural 14β-OH epimer; 15-hydroxyponasterone A (**11**) is also new and rare with its C-15 substituted position, as well as 22-deoxy-28-hydroxymakisterone C (**18**) possessing secondary hydroxyl in position C-28 and 26-hydroxymakisterone C (**20**) with hydroxy groups in positions 25 and 26. New are also 1β-hydroxymakisterone C (**21**) and 20,22-acetonides of inokosterone (**8**) and integristerone A (**10**). Series of already known ecdysteroids: ecdysone (**1**), 20-hydroxyecdysone 2- and 3-acetates (**3** and **4**), turkesterone (**6**), inokosterone (**7**), 24-epi-makisterone A (**14**), and amarasterone A (**22**) are reported here as new constituents of *L. carthamoides*. Seven earlier reported *Leuzea* ecdysteroids: 20-hydroxyecdysone (**2**), ajugasterone C (**5**), integristerone A (**9**), 24(28)-dehydromakisterone A (**15**), 24(28)-dehydroamarasterone B (**16**), (24Z)-29-hydroxy-24(28)-dehydromakisterone C (**17**) and makisterone C (**19**) are also included because they are now better characterized.

1. Introduction

Leuzea carthamoides DC [syn. *Rhaponticum carthamoides* (Willd.) Iljin] served as a primary source for preparation various individual ecdysteroids [1,2] needed for our chemical [3–7] and biological [8–14] study. This plant served the purpose well, because of its high content of ecdysteroids in roots or seeds [15] and because it provides a large structure variability of ecdysone analogues isolated so far [1,2,16–19]. Moreover, *L. carthamoides* in the last two decades is cultivated as a medicinal plant on a large scale in the east and central Europe. This is why it was chosen to serve as a rich source of ecdysteroids, not only for chemical and biological studies, but also for production of various nutraceuticals (adjunctive functional food) [20,21] or cosmetic preparations [22]. Proposed use of phytoecdysteroids in cosmetics and dermatology [23] demanded to carry out further experiments requiring large quantity of active compounds. This urgently involved scaling up the production of 20-hydroxyecdysone and other ecdysteroids or ecdysteroid mixtures with fixed qualitative and quantitative compositions to several hundred grams amount. Such large-scale preparation displayed many ecdysteroid-containing separation fractions, turned into a disposable source of several already reported major and minor *Leuzea* ecdysteroids [1,2,17] in previously unattainable quantities, as well as a rich source of several new minor ecdysteroid constituents, undetectable in the previous low-scale separations.

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Table 1 – HPLC retention times of ecdysteroids 1–22 from *L. carthamoides* under various analytical conditions

Compound	Retention time [min]			
	System 1[a]	System 2[b]	System 3[c]	System 4[d]
Ecdysone (1)	39.9	41.7	52.3	53.0
20-Hydroxyecdysone (2)[e]	34.2	50.7	75.4	90.9
20-Hydroxyecdysone 2-acetate (3)	42.0	36.1	28.4	–
20-Hydroxyecdysone 3-acetate (4)	38.6	38.7	34.2	25.4
Ajugasterone C (5)[e]	39.2	32.3	33.2	45.9
Turkesterone (6)	26.9	76.8	–	–
Inokosterone (7)	35.1	57.5	64.9	48.1
Inokosterone 20,22-acetonide (8)	55.0	23.1	24.6	28.0
Integristeone A (9)[e]	31.0	78.5	–	–
Integristeone A 20,22-acetonide (10)	53.4	27.5	66.1	38.3
15-Hydroxyponasterone A (11)	41.3	28.9	24.8	38.3
14-epi-Ponasterone A 22-glucoside (12)	47.3	58.5	142.3	88.8
Carthamoleusterone (13)	40.2	30.2	32.1	–
24-epi-Makisterone A (14)	37.7	35.9	38.2	–
24(28)-Dehydromakisterone A (15)	38.1	35.9	30.1	23.7
24(28)-Dehydroamarasterone B (16)	41.4	42.6	44.2	48.1
(24Z)-29-Hydroxy-24(28)-dehydromakisterone C (17)[e]	33.6	74.6	129.4	90.8
22-Deoxy-28-hydroxymakisterone C (18)	41.2	62.3	86.6	96.7
Makisterone C (19)[e]	34.2	32.2	23.8	28.4
26-Hydroxymakisterone C (20)	37.9	64.5	80.6	78.8
1β-Hydroxymakisterone C (21)	39.8	58.0	61.3	39.7
Amarasterone A (22)	41.9	39.9	33.0	34.5

[a] *System 1*: Separon SGX C-18 column (5 μm, 250 mm × 4 mm i.d.) eluted with linear gradient of 10–70% methanol in water over 50 min at flow rate 0.6 ml min^{-1}.
[b] *System 2*: Silasorb 600 column (5 μm, 250 mm × 4 mm i.d.) eluted with *n*-hexane–ethanol–water (812:180:8) at flow rate 0.8 ml min^{-1}.
[c] *System 3*: Silasorb 600 column (5 μm, 250 mm × 4 mm i.d.) eluted with diethyl ether–acetonitrile–water (880:102:18) at 0.8 ml min^{-1}.
[d] *System 4*: Silasorb 600 column (5 μm, 250 mm × 4 mm i.d.) eluted with dichlormethane–isopropanol–water (84:15:1) at 0.8 ml min^{-1}.
[e] Previously reported ecdysteroids from *L. carthamoides* [1,2] used in this study as authentic standards for HPLC and reference compounds for structural analysis of new related ecdysteroids.

The identities of major compounds, 20-hydroxyecdysone (**2**), ajugasterone C (**5**), integristerone A (**9**), 29-hydroxy-24(28)-dehydromakisterone C (**17**) and makisterone C (**19**) were confirmed comparing their RP- and NP-HPLC retention times with authentic samples using Systems 1–4 (Table 1), and comparing their NMR data with the data reported earlier [1,2]. NMR data of compounds **2** and **5** published earlier [1] were now completed utilizing presently available advanced NMR methods. Majority of the previously obtained major and minor ecdysteroids [1,2], including some of those presented in this paper, were scheduled for ecdysteroid receptor mapping based on their interaction with the ligand-binding domain in the B_{II} bioassay [9–12,24]. Our results, published earlier [9], were taken over also to other models, using a homology modeling and docking approach [25].

The structures of minor constituents **1**, **3**, **4**, **6–8**, **10**, **11** (Fig. 1) and **12–18**, **20–22** (Fig. 2) were elucidated by analysis of their IR, mass, and NMR spectra (for ^{1}H and ^{13}C NMR data, see Tables 2–5). The ^{1}H and ^{13}C 1D spectra together with ^{1}H, ^{1}H-COSY and ^{1}H, ^{13}C-HMQC spectra were used for complete (or nearly complete) structure assignment of carbon and proton signals. Characteristic NMR data of compounds **1–7** and **9** (Tables 2 and 3) and **14–17**, **19** and **22** (Tables 4 and 5) correspond with the already published data of ecdysone (**1**) [26], 20-hydroxyecdysone 2-acetate (**3**) [27,28], 20-hydroxyecdysone 3-acetate (**4**) [27,28], turkesterone (**6**) [29,30], inokosterone (**7**) [31,32], integristerone A (**9**) [2,33], 24-epi-makisterone A (**14**) [34], 24(28)-dehydromakisterone A (**15**) [17,35], 24(28)-dehydroamarasterone B (**16**) [18], (24Z)-29-hydroxy-24(28)-dehydromakisterone C (**17**) [2], makisterone C (**19**) [2,17] and amarasterone A (**22**) [36], all summarized in the Ecdysone Handbook [20]. Compounds **1**, **3**, **4**, **6**, **7**, **14**, **20** and **22** are new in *L. carthamoides*, although their occurrence was already reported in other unrelated plants [20]. 24(28)-Dehydromakisterone A (**15**) was found also in the genetically related *Rhaponticum integrifolium* [35]. Some compounds were reported in earlier papers with rather incomplete NMR data, therefore we publish here their currently completed data (Tables 2–5). There were included herein also our reference NMR data of 20-hydroxyecdysone (**2**) and ajugasterone C (**5**), previously isolated as major constituents of *L. carthamoides* [1], but reported only with data obtained in unrelated solvent (hexadeuteroacetone).

Eight new natural ecdysteroid analogues **8**, **10–13**, **18**, **20** and **21** (Figs. 1 and 2) were found among the isolated minor constituents.

Compound **8** (inokosterone 20,22-acetonide), with the composition $C_{30}H_{48}O_7$ (HR-MS) and characteristic IR bands, exhibited ^{1}H and ^{13}C NMR spectra very similar to those of inokosterone **7** with two additional methyl signals in ^{1}H NMR spectrum (singlets at δ 1.39 and 1.32) and three extra signals in ^{13}C NMR spectrum (δ 107.98 (⟩C⟨), 29.36 and 27.19 (2× CH_3) indicating a presence of acetonide group. The only significant chemical shift differences between **8** and **7** observed for carbon atoms C-20 and C-22 ($\Delta\delta$ = 7.93 and 4.91) allow to place acetonide (isopropylidene) group unequivocally into position