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24(24¹)[*Z*]-DEHYDROAMARASTERONE B, A PHYTOECDYSTEROID FROM SEEDS OF *LEUZEA CARTHAMOIDES*

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Key Word Index—*Leuzea carthamoides*; *Rhaponticum carthamoides*; Compositae; ecdysteroid; phytoecdysteroid; 24(24¹)[*Z*]-dehydroamarasterone.

Abstract—A new phytoecdysteroid, 24(24¹)[*Z*]-dehydroamarasterone B, has been isolated from seeds of *Leuzea* (*Rhaponticum*) *carthamoides*. It has been unambiguously identified by CIMS, ^{13}C NMR and ^{1}H NMR spectroscopy. The biological activity of the ecdysteroid has been determined in the *Drosophila melanogaster* B_{II} bioassay. The ED_{50} (5.2×10^{-7} M) is 70-fold higher than that for 20-hydroxyecdysone (7.5×10^{-9} M). © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Certain species within the Compositae, including *Leuzea carthamoides* D.C. (syn. *Rhaponticum carthamoides* [Willd.] Iljin) [1–6], contain very high concentrations of insect steroid hormone analogues (phytoecdysteroids) [7], even though phytoecdysteroids are not of widespread occurrence in the Compositae in general (L. Dinan, unpublished data). While proceeding with a systematic study of the phytoecdysteroids of *Leuzea carthamoides*, we investigated the seeds for their phytoecdysteroid content [8–10]. We now report the isolation and identification of a new phytoecdysteroid, 24(24¹)[*Z*]-dehydroamarasterone B (**1**) from this source.

RESULTS AND DISCUSSION

The CI mass spectrum clearly established the molecular mass of the purified compound **1** as 506, solving for $C_{29}H_{46}O_7$. The UV spectrum was characteristic for an ecdysteroid, as was the fragmentation pattern in the mass spectrum. The ^{1}H NMR spectrum of **1** (Table 1) was very similar to that of 20-hydroxyecdysone [11] in all regions except for those signals originating from the side-chain (C-22–C-29). However, extra signals with respect to the ^{1}H NMR spectrum of 20-hydroxyecdysone [11] are observed; a triplet 1H at δ 5.45 in the ethylenic zone coupled to the multiplet 2H at δ 4.23 in the zone for hydroxymethene and a septuplet 1H (δ 2.87). Moreover, the upfield shift (0.2 ppm) of the 26-Me and 27-Me signals and their splitting to give doublets are indicative of a 25-deoxy compound. 2D COSY and TOCSY [12] experiments lead to correlation of the 26-Me and 27-Me signals with the septuplet 1H at δ 2.87, consequently assigned as H-25. The chemical shift of the H-25 signal and the absence of other correlations than with 26-Me and 27-Me signals, together with the correlation of H-22 with two downfield shifted H-23 at δ 2.41 and 1.95 are in accordance to the presence of an ethylenic double bond between C-24 and C-24¹. The signal at δ 5.45 is similar to one of those observed previously for C-24¹ in 24(24¹)-dehydromakisterone A [13]. A phase-sensitive NOESY experiment [14] carried out with a mixing time of 500 ms leads to the observation of NOEs between {H-25}H-24² {H-24²}26-Me/27-Me and between {H-24¹}H-22 {H-24¹}H-29 and {H-24¹}H-23 revealing a *Z*-stereochemistry of the C-24/C-24¹ double bond. The structures of the side-chain and of the steroid nucleus, are confirmed by ^{13}C NMR data (Table 2) obtained by PFG-HMQC and PFG-HMBC [15] ^{1}H-^{13}C 2D correlations. HMBC leads to ^{2}J and $^{3}J^{1}H$-^{13}C correlations from 26-Me/27-Me; H-25; H-23a; H-23b to the quaternary sp^2 C at δ 147.4 (assigned as C-24) and from H-25; H-23a; H-23b to the sp^2 CH at δ 124.3.

Purified **1** possesses agonistic activity in the B_{II} bioassay. The potency ($ED_{50} = 5.2 \times 10^{-7}$ M) was less

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Table 1. ^{1}H NMR data of 20-hydroxyecdysone and compound **1**

	1	20-Hydroxyecdysone
1-Hax	1.4 (*t*, 13)	1.4 (*t*, 13)
1-He	1.85	1.85
2-Hax	3.99 (*m*, $W_{1/2}$ = 22, 1H)	3.99 (*m*, $W_{1/2}$ = 22)
3-He	4.07 (*m*, $W_{1/2}$ = 8, 1H)	4.07 (*m*, $W_{1/2}$ = 8)
4-Hax	1.75	1.75
4-He	1.75	1.75
5-H	2.34 (1H)	2.36 (*t**)
7-H	5.99 (*d*, 2.5, 1H)	5.97 (*d*, 2.5)
9-Hα	3.11 (*m*, $W_{1/2}$ = 22)	3.11 (*m*, $W_{1/2}$ = 22)
11-Hax	1.75	1.75
11-He	1.85	1.85
12-Hax	1.73	1.73
12-He	1.95	1.95
15-Hα	1.65	1.7
15-Hβ	2.05 (*m*)	2.05 (*m*)
16-Hα	1.95	1.9
16-Hβ	1.85	1.75
17-H	2.39	2.34 (*t*)
22-H	3.72 (*d*, 10, 1H)	3.43 (*d*, 10)
23-Ha	2.41	1.3
23-Hb	1.95	1.65
24-Ha	—	1.75
24-Hb	—	1.50 (*t*, *d* 13,3,5)
25-H	2.87 (*sp*, 6.6, 1H)	—
28-H	5.45 (*t*, 7, X, 1H)	—
29-Ha/b	4.23 (*m*, AB, 2H)	—
18-Me	0.88 (*s*, 3H)	0.87 (*s*)
19-Me	1.00 (*s*, 3H)	1.00 (*s*)
21-Me	1.27 (*s*, 3H)	1.22 (*s*)
26-Me	1.04 (*d*, 6.6, 3H)	1.24 (*s*)
27-Me	1.04 (*d*, 6.6, 3H)	1.24 (*s*)

Spectra were obtained in D_2O; δ in ppm, reference $TSPD_4$, T = 300 K. ax—axial, e—equatorial.

than that of 20-hydroxyecdysone ($ED_{50} = 7.5 \times 10^{-9}$ M; [16]), makisterone A (24β-methyl-20-hydroxyecdysone; $ED_{50} = 1.3 \times 10^{-8}$ M), 24-epimakisterone A (24α-methyl-20-hydroxyecdysone; $ED_{50} = 2.2 \times 10^{-7}$ M) [17], 24(24^1)-dehydromakisterone A ($ED_{50} = 7.2 \times 10^{-8}$ M) or makisterone C (24β-ethyl-20-hydroxyecdysone; $ED_{50} = 2.0 \times 10^{-7}$ M). The absence of a C-25 hydroxyl group is associated with a significantly higher biological activity (e.g. 20-hydroxyecdysone vs ponasterone A [25-deoxy-20-hydroxyecdysone; $ED_{50} = 3.1 \times 10^{-10}$ M]) [16–18]. By deduction from the bioassay data presented above, it seems probable that the presence of an ethyl group at C-24, the introduction of a 24(24^1)[*Z*]-double bond and hydroxylation at C-24^2 each contributes significantly to the 1700-fold lower activity of **1** relative to ponasterone A.

EXPERIMENTAL

General. The UV spectrum was obtained in MeOH. CIMS was obtained on a Riber 10-10B apparatus (Nermag S.A.) in chemical desorption mode with NH_3 as the reagent gas. HPLC sepn was performed on Gilson equipment consisting of two model 303 pumps, a holochrome detector (set at 242 nm) and controlled by the Gradient Manager program.

NMR spectroscopy. The experiments were run at 500 MHz for ^{1}H, at 300 K, on a Bruker AMX 500 Spectrometer equipped with a Silicon Graphics workstation. Presaturation of the solvent was used for all the 1D and homonuclear 2D ^{1}H experiments. The sample was lyophilised twice and dissolved in D_2O. The errors on the chemical shifts are 0.01 ppm for ^{1}H and 0.1 ppm for ^{13}C. TSPD4, 3-(trimethylsilyl)[2,2,3,3-d_4] propionic acid (sodium salt), was used as internal reference for the proton and carbon shifts. A degassed sample was used for the ^{1}H NOESY experiments using a 2D phase-sensitive method (States-TPPI). ^{1}H NOESY experiments in D_2O were performed using a mixing time of 500 ms. Zero quantum coherence was suppressed by incorporation of a randomly positioned 180° pulse during the mixing time [19]. FIDs were acquired (32 scans) over 5102 Hz into a 2K data block for 256 incremental values of the evolution time and a relaxation delay of 2 s. The raw data were zero filled to a 2K × 2K matrix. Water suppression was

Table 2. ^{13}C NMR data of 20-hydroxyecdysone and compound **1**

		20-Hydroxyecdysone	**1**
C-1	CH_2	36.4	36.4
C-2	CH	68.3	68.3
C-3	CH	68.2	68.2
C-4	CH_2	32.3	32.3
C-5	CH	51.4	51.4
C-6	C	209.3	209.3
C-7	CH	122.1	122.1
C-8	C		
C-9	CH	34.9	34.9
C-10	C	39.1	39.1
C-11	CH_2	21.0	21.0
C-12	CH_2	32.0	32.0
C-13	C	48.3	48.5
C-14	C	86.1	86.3
C-15	CH_2	31.2	31.2
C-16	CH_2	21.0	21.0
C-17	CH	50.2	50.2
C-18	CH_3	18.0	18.1
C-19	CH_3	24.2	24.2
C-20	CH	79.4	79.0
C-21	CH_3	20.6	20.6
C-22	CH	78.4	75.7
C-23	CH_2	27.0	34.7
C-24	CH_2	41.6	147.4 (C)
C-25	C	72.6	30.4 (CH)
C-26	CH_3	28.3	21.7
C-27	CH_3	29.3	22.2
C-28	CH	—	124.3
C-29	CH_2	—	58.7

Spectra were obtained in D_2O. δ in ppm, reference $TPSD_4$, T = 300 K.

performed by a low power transmitter pulse of presaturation (70 dB) during relaxation delay and mixing time. One half-sinusoid (5% truncated) shape homospoil gradients of 10 G cm^{-1} were used during mixing time.

Extraction. Air-dried seeds of *Leuzea carthamoides* (1.2 kg) were milled and then extracted with MeOH. The combined extracts were evapd under vacuum at 40–45° to a vol. of 250 ml and this was then diluted with 375 ml H_2O. After extraction of the hydrophobic compounds by partitioning against hexane, the phytoecdysteroids were extracted into BuOH. The BuOH was removed under vacuum to give 30.4 g of crude material. After the isolation of known ecdysteroids, the frs containing 24(24[1])-dehydroamarasterone B (43.6 mg) were separated by RP-HPLC (Spherisorb ODS-2, 250 × 4.6 mm i.d., eluted with MeOH–H_2O [1:1] at 1 ml min^{-1}). Further purification was achieved on a silicic acid column (30 cm × 10 mm i.d.), eluted with $CHCl_3$–MeOH (9:1), yielding 5.3 mg of **1** (overall yield = 0.00004%).

Bioassay. The biological potency of the purified ecdysteroid was determined with a microplate-based bioassay using the *Drosophila melanogaster* B_{II} cell line [16].

24(24[1])[Z]-*Dehydroamarasterone B* (**1**). Amorphous UV λ_{max} nm: 243 nm. ^1H NMR (D_2O); see Table 1. ^{13}C NMR (D_2O); see Table 2. CIMS *m/z*: 524 $[M+H+NH_3]^+$, 507 $[M+H]^+$, 489 $[M+H-H_2O]^+$, 471 $[M+H-2H_2O]^+$, 453 $[M+H-3H_2O]^+$, 380 [524-{C-22–C-24}]$^+$, 363 $[M+H-\{C\text{-}22\text{–}C\text{-}24\}]^+$, 345.

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