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2-DEHYDRO-3-*EPI*-20-HYDROXYECDYSONE FROM *FROELICHIA FLORIDA*

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Key Word Index—*Froelichia floridana*; Amaranthaceae; seeds; 2-dehydro-3-*epi*-20-hydroxyecdysone; ecdysteroid; phytoecdysteroid.

Abstract—A new phytoecdysteroid, 2-dehydro-3-*epi*-20-hydroxyecdysone, together with 20-hydroxyecdysone have been isolated by bioassay/RIA-directed HPLC analyses of a methanol extract of the seeds of *Froelichia floridana*. The structure of the novel ecdysteroid was determined unambiguously by UV, LSIMS, and a combination of 1D and 2D NMR techniques. The biological activity in the *Drosophila melanogaster* B_{II} cell bioassay ($ED_{50} = 4.0 \times 10^{-7}$ M) is considerably lower than that of 20-hydroxyecdysone ($ED_{50} = 7.5 \times 10^{-9}$ M).

INTRODUCTION

Froelichia floridana (Nutt.) Moq., widely known as "Florida snake-cotton" or "Cottontails" is a herbaceous annual, native to the U.S.A. and has become naturalised in Australia [1]. This species has not been investigated phytochemically before. As part of our continuing search for new phytoecdysteroids and also for new plant sources for phytoecdysteroids [2–13], we now report on the isolation and identification of a novel phytoecdysteroid, 2-dehydro-3-*epi*-20-hydroxyecdysone (**1**), as well as the known ecdysteroid 20-hydroxyecdysone (**2**) from the seeds of *F. floridana*.

RESULTS AND DISCUSSION

Ecdysteroid agonist/antagonist bioassay [14]- and ecdysteroid-specific RIA [15]-guided, and photodiode-array detector-assisted HPLC analyses of a methanol extract of the seeds of *F. floridana* yielded a novel phytoecdysteroid, 2-dehydro-3-*epi*-20-hydroxyecdysone (**1**) and a known one, 20-hydroxyecdysone (20E) [16]. 20E was readily identified by direct comparison of its HPLC and spectroscopic characteristics with those published in the literature and with a sample previously isolated in our laboratories.

The novel compound (**1**) was characterised by spectroscopic means. The positive response in the bioassay and RIA, and UV absorption spectrum readily identified compound **1** as an ecdysteroid. The LSIMS (−ve ion mode) of compound **1** revealed the molecular mass 478 compatible with the empirical formula $C_{27}H_{42}O_7$. The ^1H NMR spectrum (Table 1) of **1** showed all the signals arising from the protons on the side-chain, and the B, C, and D rings of a 20-hydroxyecdysone skeleton [16]. The striking differences, compared to 20E, were the absence of an oxymethine signal (either H-2 or H-3) and the splitting pattern (*dd*, J = 7.2, 11.0) of the remaining deshielded oxy-

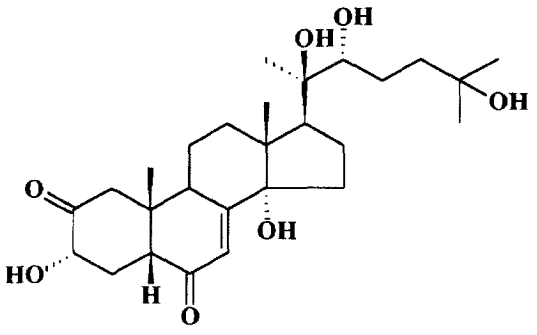

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Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data of **1** (coupling constant *J*, Hz in parentheses)

Carbon no.		δ_H	δ_C
1	α	2.35 *d* (13.7)	49.0
	β	2.70 *d* (13.7)	
2		–	210.0
3	β	4.54 *dd* (7.2, 11.0)	74.6
4	α	2.00 *m*	36.0
	β	2.40 *m*	
5	β	2.80 *dd* (3.9, 13.7)	55.6
6		–	ND
7		6.17 *d* (2.3)	120.9
8		–	ND
9	α	3.25 *m*	35.8
10		–	42.7
11		1.70	20.6
		1.88	
12	α	2.05	31.5
	β	2.45	
13		–	48.0
14		–	83.1
15	α	1.95	31.3
	β	2.10	
16		2.46	21.1
		2.15	
17	α	2.90 *t* (9.7)	49.7
18 (Me)	β	1.15 *s*	17.6
19 (Me)	β	1.06 *s*	22.7
20		–	76.5
21 (Me)	α	1.52 *s*	21.4
22	β	3.83 *t* (9.4)	77.3
23		1.72	27.3
		2.14	
24		2.25	42.4
		1.82	
25		–	69.3
26 (Me)		1.36 *s*	29.9
27 (Me)		1.36 *s*	29.8

Spectrum taken in pyridine-d_5.
ND: Could not be detected either from ^{13}C PENDANT or from HMBC experiments.

methine (δ 4.54) from the A-ring. The extent of deshielding of this oxymethine signal indicated the presence of a carbonyl group in its neighbourhood.

Table 3. ^{1}H–^{1}H NOE interactions in **1** obtained from a series of NOE difference spectra

Irradiated signals (δ_H)	Enhanced signals (δ_H)
1.06 (Me-19, β)	2.70 (H-1, β), 2.80 (H-5, β)
1.15 (Me-18, β)	2.10 (H-15, β or H-12, β)*
1.52 (Me-21, α)	1.15 (Me-18, β), 2.90 (H-17, α)
2.70 (H-1, β)	1.06 (Me-19, β), 2.35 (H-1, α)
2.80 (H-5, β)	1.06 (Me-19, β), 2.40 (H-4, β), 4.54 (H-3, β)
4.54 (H-3, β)	2.40 (H-4, β), 2.80 (H-5, β)

Spectra obtained in pyridine-d_5.
*Very weak enhancement.

At this stage, two probable positions for the hydroxyl and a carbonyl group are apparent: –OH at C-2 and C=O at C-3, or –OH at C-3 and C=O at C-2. Analysing the ^{1}H–^{1}H COSY 45 spectrum, the correlations amongst H-3 ↔ H_2-4 ↔ H-5 confirmed the latter of these two possibilities and this was also similar to the ^{1}H–^{1}H COSY correlations found for (5α)-2-dehydro-20-hydroxyecdysone [17]. A combination of ^{13}C PENDANT [18] (Table 1), ^{1}H–^{13}C HMQC and ^{1}H–^{13}C HMBC (Table 2) experiments established the assignments of all ^{1}H and ^{13}C (signals for C-6 and C-8 could not be detected owing to paucity of the sample) of the molecule. A series of NOE difference spectra (Table 3) determined the relative stereochemistry of the molecule, especially NOE amongst Me-19 (δ 1.06), H-5 (δ 2.80) and H-3 (δ 4.54) confirmed that the hydroxyl at C-3 has an α-orientation, and that Me-19, H-5 and H-3 are on the β-face of the molecule. Thus, the structure of this new ecdysteroid was unequivocally determined as **1**. Compound **1** possessed agonistic activity in the *Drosophila* B_{II} cell assay, but its potency ($ED_{50} = 4.0 \times 10^{-7}$ M) was 53-fold lower than that of 20E ($ED_{50} = 7.5 \times 10^{-9}$ M).

Table 2. ^{1}H–^{13}C–HMQC direct correlation (^1J) and ^{1}H–^{13}C–HMBC long-range correlation (^2J and ^3J) in **1**

Proton	δ^{13}C		
	^1J	^2J	^3J
H_2-1	49.0 (C-1)	42.7 (C-10), 210.0 (C-2)	55.6 (C-5), 74.6 (C-3)
H-3	74.6 (C-3)		
H_2-4	36.0 (C4)		
H-5	46.4 (C-5)		
H-7	120.9 (C-7)		35.8 (C-9), 84.0 (C-14)
H-9	35.8 (C-9)		
H_2-11	20.6 (C-11)		
H_2-12	31.5 (C-12)		
H_2-15	31.3 (C-15)		
H_2-16	21.1 (C-16)		
H-17	49.7 (C-17)		
H-22	77.3 (C-22)		
H_2-23	27.3 (C-23)		
H_2-24	42.4 (C-24)		
Me-18	17.6 (C-18)	48.0 (C-13)	31.5 (C-12), 49.7 (C-17), 84.0 (C-14)
Me-19	20.3 (C-19)	42.7 (C-10)	49.0 (C-1), 55.6 (C-5), 35.8 (C-9)
Me-21	21.6 (C-2 1)	76.5 (C-20)	49.7 (C-17), 77.3 (C-22)
Me-26	29.9 (C-26)	69.3 (C-25)	29.8 (C-27), 42.4 (C-24)
Me-27	29.8 (C-27)	69.3 (C-25)	29.9 (C-26), 42.4 (C-24)

Spectrum obtained in pyridine-d_5.

The presence of phytoecdysteroids has earlier been reported from several genera of the family Amaranthaceae [5, 13]. This is the first report on the occurrence of phytoecdysteroids in *F. floridana*. Interestingly, ecdysteroids are present in both the seeds (1.35 mg ecdysone equivalents/g with the DBL-1 antiserum) and in the cotton surrounding the seeds (0.25 mg ecdysone equivalents/g). 2-Dehydroajugalactone and 3-dehydroajugalactone have been previously isolated from *Ajuga reptans* [19] and 22-dehydroecdysteroids have been identified from *A. reptans* and *A. iva* [19, 20]. Phytoecdysteroids possessing a 3α-hydroxyl group have been found in *Blechnum vulcanicum* (3-*epi*-2-deoxyecdysone [21]), *Tinospora callipes* (3-*epi*-2-deoxy-20-hydroxyecdysone [22]) and *Serratula tinctoria* (3-*epi*-poststerone and 3-*epi*-rubrosterone [23]).

EXPERIMENTAL

UV MeOH; NMR: on a Bruker AVANCE DRX400 instrument using standard Bruker microprograms. The chemical shifts are expressed in ppm; LSIMS (–ve ion mode); glycerol matrix using a Cs^+ primary ion beam on a VG Quattro triple quadrupole mass spectrometer (VG Biotech, Altrincham); Sep-Pak Vac 35 cc (10 g) C_{18} cartridge (Waters) were used for initial fractionation of extract. HPLC: (a) preparative/semipreparative — Gilson model 806 HPLC coupled with Gilson UV-Visible detector, (b) analytical-Gilson model 811 HPLC coupled with Gilson 160 diode array detector and using Gilson Unipoint computer program; RP, NP, RP-prep. RP-anal. and NP-semiprep.-1 and NP-semiprep.-2 stand, respectively, for reversed-phase, normal-phase, preparative C_8 column (Technoprep 10C8), Spherisorb 5 ODS-2 analytical C_{18} column, Zorbax Silica semipreparative column and APEX II diol semipreparative column throughout this text. Chromatographic separations were monitored at 242 nm.

Radioimmunoassay

RIA was performed according to the procedure described previously [15] using ecdysteroid-specific antisera, DBL-1 and Black, which were donated by Professor Koolman (University of Marburg). The cross-reactivities of these antisera with a number of phytoecdysteroids are given elsewhere [24].

Bioassay

The biological activities (ecdysteroid agonist or antagonist) of the extract, HPLC fractions and of compound **1** were determined with a microplate-based bioassay using the *Drosophila melanogaster* B_{II} cell line [16].

Plant material

The seeds of *F. floridana* were purchased from B and T World Seeds, Paguignan, 34210 Olonzac. A voucher specimen has been retained at the Department of Biological Sciences, University of Exeter.

Extraction

Seeds (with attached cotton: 6.5 g) were ground and extracted four times (4 × 24 h) with 5 × 750 mL of MeOH at 55°C with constant stirring using a magnetic stirrer. Extracts were pooled together and made into 70% aq. methanolic solution. After being defatted with *n*-hexane, the extract was concentrated using a rotary evaporator at a maximum temperature of 45°C.

Isolation of compounds

Sep-Pak fractionation of the concentrated extract (redissolved in 10% aq. MeOH) using MeOH–H_2O step-gradient, followed by bioassay/RIA revealed the presence of ecdysteroids in the 60% MeOH–H_2O fraction which was then subjected to HPLC using RP-prep. column (isocratic elution with 55% MeOH–H_2O, 5 mL/min) to yield a mixture of 1 and 20E. NP-HPLC analyses (NP-semiprep.-1 column, isocratic elution with CH_2Cl_2–isoPrOH–H_2O 125:40:3, 2 mL/min) of this mixture resulted in the isolation of those two compounds (RTs 31 min for 20E, 15 min for **1**). Compound **1** was further purified on NP-Semi-prep.-2 column (4% MeOH in CH_2Cl_2, 2 mL/min).

2-*Dehydro*-3-*epi*-20-*hydroxyecdysone* (**1**) (0.15 mg)

Amorphous: UV λ_{max} nm (log ε): 242.4 (4.106). ^1H and ^{13}C NMR (Table 1). LSIMS: *m/z* 569 $[M–H + glycerol]^-$ and 477 $[M–H]^-$.

20-*Hydroxyecdysone* (22.0 mg)

Amorphous: HPLC, UV, ^1H NMR and ^{13}C NMR data as reported [16].

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REFERENCES

1. USDA-ARS-GRIN database (1997) http://www.ars-grin.gov/cgi-bin/npgs/html/tax_search.-pl?froelichia.
2. Volodin, V., Alexeeva, L., Kolegova, N. A., Sarker, S. D., Šik, V., Lafont, R. and Dinan, L., *Biochemical Systematics and Ecology*, 1998, **26**, 459.

3. Savchenko, T., Whiting, P., Sarker, S. D. and Dinan, L., *Biochemical Systematics and Ecology*, 1998, **26,** 337.
4. Sarker, S. D., Savchenko, T., Šik, V., Rees, H. H. and Dinan, L., *Biochemical Systematics and Ecology*, 1998, **26,** 135.
5. Savchenko, T., Whiting, P., Sarker, S. D. and Dinan, L., *Biochemical Systematics and Ecology*, 1998, **26,** 131.
6. Savchenko, T., Whiting, P., Sarker, S. D. and Dinan, L., *Biochemical Systematics and Ecology*, 1997, **25,** 623.
7. Allson, B., Whiting, P., Sarker, S. D., Underwood, E., Šik, V., Dinan, L. and Rees, H. H., *Biochemical Systematics and Ecology*, 1997, **25,** 255.
8. Sarker, S. D., Savchenko, T., Whiting, P., Šik, V., Lafont, R. and Dinan, L., *Biochemical Systematics and Ecology*, 1997, **25,** 367.
9. Sarker, S. D., Savchenko, T., Whiting, P., Šik, V., Rees, H. H. and Dinan, L., *Biochemical Systematics and Ecology*, 1997, **25,** 473.
10. Sarker, S. D., Girault, J.-P., Lafont, R. and Dinan, L., *Phytochemistry*, 1997, **44,** 513.
11. Sarker, S. D., Savchenko, T., Whiting, P., Lafont, R. and Dinan, L., *Biochemical Systematics and Ecology*, 1996, **24,** 803.
12. Sarker, S. D., Dinan, L., Girault, J.-P., Lafont, R. and Watennan, P. G., *Journal of Natural Products*, 1996, **59,** 789.
13. Sarker, S. D., Girault, J.-P., Lafont, R. and Dinan, L., *Biochemical Systematics and Ecology*, 1996, **24,** 177.
14. Clément, C. Y., Bradbrook, D. A., Lafont, R. and Dinan, L., *Insect Biochemistry and Molecular Biology*, 1993, **23,** 187.
15. Dinan, L., *Phytochemical Analysis*, 1992, **3,** 132.
16. Lafont, R. and Wilson, I., *The Ecdysone Handbook*. Chromatographic Society, Nottingham, 1996.
17. Girault, J.-P., Blais, C., Beydon, P., Rolando, C. and Lafont, R., *Archives of Insect Biochemistry and Physiology*, 1989, **10,** 199.
18. Homer, J. and Perry, M. C., *Journal of the Chemical Society, Chemical Communications*, 1994, 373.
19. Calcagano, M.-P., Camps, F., Coll, J., Melé, E. and Sanchez-Baeza, F., *Tetrahedron*, 1996, **52,** 10137.
20. Wessner, M., Champion, B., Girault, J.-P., Kaouadji, N., Saidi, B. and Lafont, R., *Phytochemistry*, 1992, **31,** 3785.
21. Russel, G. B., Greenwood, D. R., Lane, G. A., Blunt, J. W. and Munro, M. G. H., *Phytochemistry*, 1981, **20,** 2407.
22. Song, C. and Xu, R., *Chinese Chemical Letters*, 1991, **2,** 13.
23. Rudel, D., Bathori, M., Gharbi, J., Girault, J.-P., Racz, I., Melis, K., Szendrei, K. and Lafont, R., *Planta Medica*, 1992, **58,** 358.
24. Dinan, L., *European Journal of Entomology*, 1995, **92,** 271.