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Ecdysteroids and a sucrose phenylpropanoid ester from *Froelichia floridana*

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ABSTRACT

Phytoecdysteroid glycosides (**1**–**5**) and a phenylpropanoid ester of sucrose (**6**) were isolated from the whole plant of *Froelichia floridana*, along with eight known compounds including three ecdysteroids (**7**–**9**), four flavonoids (**10**–**13**), and one phenolic compound (**14**). Structures were determined using a combination of spectroscopic techniques. Compounds **1**, **2** and **6**–**14** were tested *in vitro* for their activity against human DNA topoisomerase I. Compound **13** (diosmetin) showed marginal inhibition against topoisomerase I with IC_{50} of 130 μM in conjunction with low intercalation ability.

1. Introduction

Froelichia Moench (cottonweed or snake-cotton) is a genus of the family Amaranthaceae with 16 species of annuals, perennials, and shrubs native to the Western Hemisphere (McCauley, 2004). Although the genus has a wide range from the southern extremes of Canada to Northern Argentina and Uruguay (McCauley, 2004), there is no report available on any medicinal uses of cottonweeds. The *Froelichia* genus is probably best known as a roadside weed, as thrives in environments with little competition (Flora of North America Editorial Committee, 1993). To date, there is only one report on chemical investigations of the genus, with two ecdysteroids (insect molting hormones) identified (Sarker et al., 1998).

Froelichia floridana (Nuttall) Moquin-Tandon is an annual herbaceous species, commonly known as "Florida snake-cotton" or "plains snake-cotton" (Flora of North America Editorial Committee, 1993). This species is native to North America and to the West Indies of the Caribbean and has also been naturalized in Australia (USDA ARS, 2008). It can be found on open sand prairies, edges of woodlands in sandy soils, roadsides, and railroad rights-of-way (Flora of North America Editorial Committee, 1993). Interestingly, the species has been listed as an endangered species in the Ohio Rive area of southeastern Ohio (McCauley and Ungar, 2002), but as invasive in both Nebraska and the Great Plains according to authoritative sources (USDA NRCS, 2008). From the seeds of *F. floridana*, Sarker et al. (1998) isolated 20-hydroxyecdysone and 2-dehydro-3-*epi*-20-hydroxyecdysone. As part of our continuing research on the attempted discovery of novel bioactive agents from native Texas plants, the isolation and structure elucidation of 14 compounds are reported, including five new ecdysteroid glycosides and one new phenylpropanoid ester of sucrose from *F. floridana*. All compounds except **3**–**5** were tested *in vitro* for the activity against human DNA topoisomerase I (Topo I).

2. Results and discussion

The EtOH extract of the whole plant of *F. floridana* was repeatedly subjected to silica gel, Sephadex LH-20, or ODS column chromatographic purification steps, followed by preparative HPLC and TLC to afford compounds **1**–**14** (Fig. 1). Compounds **1**–**6** were new, whereas compounds **7**–**14** were known previously. All were identified by analysis of their physical and spectroscopic data and comparison with literature values. The known compounds included three phytoecdysteroids: 20-hydroxyecdysone (**7**) (Zhu et al., 2001), blechnoside B (**8**) (Suksamrarn et al., 1986), achyranthesterone A (**9**) (Meng et al., 2005), four flavonoids: laricitin 3-*O*-β-D-glucopyranoside (**10**) (Braca et al., 2001; Zhao et al., 2007), kaempferol 3-*O*-β-D-(6-*O*-*p*-*E*-coumaroyl)-glucopyranoside (**11**) (Tsukamoto et al., 2004), daidzin (**12**) (Kinjo et al., 1987), and diosmetin (**13**) (Timmermann et al., 1979; Yu and Yang, 1999) and one phenylpropanoid: 3,4-dihydroxyphenyl caffeate (**14**) (Zhang et al., 2008).

Compound **1** was obtained as a colorless amorphous powder and had a $[M + Li]^+$ ion peak at *m*/*z* 617.3881 in the HRESIMS, corresponding to a molecular formula of $C_{33}H_{54}O_{10}$. The UV absorption spectrum gave an unusually broad peak with a maximum absorption at 246 nm, which was characteristic of an α, β-unsaturated ketone (Ikekawa et al., 1980; Sadikov et al., 2000; Jayasinghe

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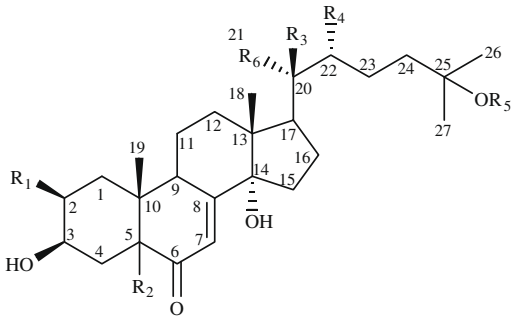
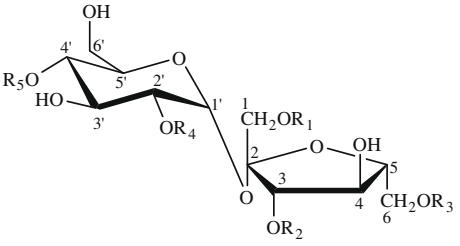
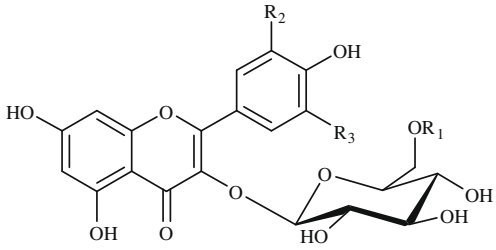


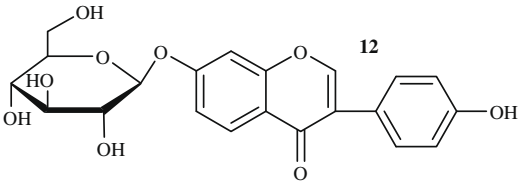
1 R_1=H; R_2=H-β; R_3=OH; R_4=H; R_5=Glc; R_6=CH_3
2 R_1=H; R_2=H-β; R_3=H; R_4=H; R_5=Glc^2-Glc^1; R_6=CH_3
3 R_1=H; R_2=H-β; R_3=H; R_4=H; R_5=Glc; R_6=CH_3
4 R_1=H; R_2=H-α; R_3=H; R_4=H; R_5=Glc^2-Glc^1; R_6=CH_3
5 R_1=H; R_2=OH-β; R_3=H; R_4=H; R_5=Glc^2-Glc^1; R_6=CH_3
7 R_1=OH; R_2=H-β; R_3=OH; R_4=OH; R_5=H; R_6=CH_3
8 R_1=H; R_2=H-β; R_3=H; R_4=OH; R_5=Glc; R_6=CH_3
9 R_1=OH; R_2=H-β; R_3=OH; R_4=OH; R_5=H; R_6=CH_2OH

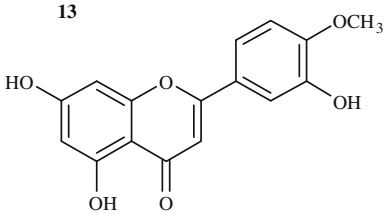


6 R_1=R_5=Ac; R_2=R_3=R_4=*p*-*E*-coumaroyl



10 R_1=H; R_2=OCH_3; R_3=OH;
11 R_1=*p*-*E*-coumaroyl; R_2=H; R_3=H





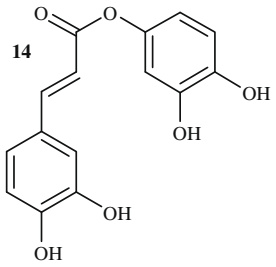


Fig. 1. Compound structures from *Froelichia floridana*.

et al., 2003; Hunyadi et al., 2004; Liktor-Busa et al., 2007). By analyzing the ^{1}H and ^{13}C NMR spectroscopic data, it was determined that **1** was an ecdysone analog with a monosaccharide moiety (Table 1). Five methyls observed at δ_H 0.73 (Me-18), 0.85 (Me-19), 1.13 (Me-21), 1.15 (Me-26 and Me-27) in the ^{1}H NMR spectrum of **1** were unambiguously assigned by HMBC correlations (Fig. 2). The ^{13}C NMR spectrum of **1** had one oxymethine at δ_C 62.8 (C-3) and three oxyquaternary carbons at δ_C 85.0 (C-14), 73.6 (C-20), and 76.5 (C-25), which were also assigned by HMBC correlations (Fig. 2). The Me-19/H_β-11, Me-19/H_β-1, and Me-19/H-5 correlations in the NOESY spectrum were indicative of a *cis*-A/B ring junction, whereas the H_β-11/Me-18, H_β-15/Me-18, and H_α-12/H-17 cross-peaks indicated a *trans*-C/D ring junction; at the same time it was verified that H-17 was in the α-position (Fig. 3). In addition, the coupling constants of the H-17 signal (*t*, *J* = 9.0 Hz) established the β-configuration of the side-chain at C-17 according to previously reported (Girault et al., 1996; Suksamrarn and Yingyongnarongkul, 1996). The NOESY correlation of H_α-2 with H-9 and the small NOE for H_α-2 to H-3 indicated that H-9 was α-oriented and OH-3 was β-oriented. The orientation of the 3β-OH group was also supported by the chemical shift of C-3 at δ_C 62.8 ppm, whereas a 3α-OH configuration usually gives a downfielded signal that is shifted 3–5 ppm (Lafont et al., 2008). Moreover, a NOE for Me-18 to Me-21 was observed, in agreement with a 20*R* configuration (Fig. 3) (Pongrácz et al., 2003). Thus, the aglycone of **1** was assigned as 2,22-dideoxy-20-hydroxyecdysone. Acid hydrolysis of **1** yielded a sugar, identified as D-glucose by co-TLC analysis and by measurement of optical rotation (Zhang et al., 2006). The monosaccharide moiety was deduced as glucopyranose with a β-anomeric configuration from its ^{13}C NMR spectroscopic data and the anomeric proton coupling constant (*J* = 7.7 Hz, see Table 1) (Maria et al., 2005). As observed in the HMBC spectrum of **1**, a key HMBC correlation of the anomeric proton of sugar at δ_H 4.26 (d, *J* = 7.7 Hz) with C-25 (δ_C 76.5) of the aglycone confirmed that the glucose was attached to the C-25 position (Fig. 2). Accordingly, compound **1** was established as 2,22-dideoxy-20-hydroxyecdysone 25-*O*-β-D-glucopyranoside.

Compound **2**, a colorless amorphous powder, was assigned a molecular formula of $C_{39}H_{64}O_{14}$ by HRESIMS, which exhibited a $[M + Li]^+$ ion peak at *m*/*z* 763.4463. The UV spectrum was consistent

Table 1
NMR spectroscopic data of **1**–**5** (**1** and **2** in DMSO-d_6, **3**–**5** in CD_3OD-d_4).

Position	**1**		**2**		**3**		**4**		**5**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1α	29.1	1.49 *m*	28.8	1.42 *m*	29.9	1.62 *m*	38.0	1.85 *m*	25.5	1.83 *m*
1β		1.22 *m*		1.26 *m*		1.43 *m*		1.39 *m*		1.45 *m*
2α	28.2	1.63 *m*	27.7	1.56 *m*	29.0	1.80 *m*	31.0	2.10 *m*	29.2	1.96 *m*
2β		1.32 *m*		1.43 *m*		1.65 *m*		1.39 *m*		1.77 *m*
3β	62.8	3.84 *br. s* ($W_{1/2}$ = 15.6)	62.0	3.78 *br. s* ($W_{1/2}$ = 15.7)	65.6	3.98 *br. s* ($W_{1/2}$ = 15.8)	71.8	3.61 *m*	67.1	4.09 *br.s* ($W_{1/2}$ = 16.2)
4α	32.1	1.37 *m*	32.2	1.32 *m*	33.4	1.31 *m*	31.4	1.78 *m*	36.7	1.61 *m*
4β						1.57 *m*		2.11 *m*		2.03 *m*
5	50.8	2.27 *dd* (12.1, 3.7)	50.7	2.19 *dd* (12.2, 3.3)	52.5	2.42 *dd* (12.1, 4.0)	54.7	2.36 *dd* (12.3, 3.5)	81.0	
6[a]	203.4		205		207		203.4		203	
7	120.3	5.63 *d* (3.0)	120.4	5.59 *s*	121.8	5.81 *s*	123.3	5.85 *d* (2.7)	120.4	5.86 *s*
8	165.8		167.0		168.6		166.7		167	
9	32.1	3.07 *m*	31.7	3.07 *m*	37.7	3.34 *m*	47.6	2.75 *m*	38.1	3.38 *m*
10	36.1		36.5		43.4		39.7		43.4	
11α	20.1	1.47 *m*	20.6	1.37 *m*	21.8	1.53 *m*	21.8	1.58 *m*	22.4	1.55 *m*
11β		1.59 *m*		1.51 *m*		1.74 *m*		1.77 *m*		1.70 *m*
12α	30.7	1.99 *dd* (11.8, 7.8)	30.9	1.86 *m*	32.4	2.08 *m*	32.2	2.07 *m*	32.3	2.09 *m*
12β		1.66 *m*		1.53 *m*		1.77 *m*		1.73 *m*		1.76 *m*
13	48.6		49.1		48.3		47.8		47.9	
14[a]	85.0		84.0		86.1		85.8		86.0	
15α	29.9	1.50 *m*	30.8	1.42 *m*	31.9	1.58 *m*	32.0	1.58 *m*	32.0	1.58 *m*
15β		1.79 *m*		1.73 *m*		1.95 *m*		1.92 *m*		1.93 *m*
16α	20.7	1.82 *m*	26.9	1.86 *m*	28.0	2.06 *m*	28.0	2.03 *m*	27.9	2.01 *m*
16β		1.67 *m*		1.19 *m*		1.42 *m*		1.40 *m*		1.40 *m*
17	51.2	2.17 *t* (9.0)	50.4	1.79 *t* (9.0)	52.0	1.97 *t* (9.5)	52.0	1.92 *m*	52.1	1.94 *m*
18	17.7	0.73 *s*	15.8	0.50 *s*	16.4	0.71 *s*	16.5	0.72 *s*	16.4	0.73 *s*
19	23.8	0.85 *s*	24.1	0.78 *s*	24.5	0.97 *s*	13.4	0.86 *s*	17.2	0.89 *s*
20	73.6		35.4	1.28 *m*	37.0	1.50 *m*	37.1	1.47 *m*	37.0	1.48 *m*
21	26.6	1.13 *s*	19.2	0.79 *d* (6.7)	19.6	0.96 *d* (6.4)	19.8	0.97 *d* (6.5)	19.6	0.97 *d* (6.5)
22a	44.5	1.33 *m*	36.7	1.25 *m*	38.0	1.45 *m*	38.0	1.46 *m*	38.0	1.42 *m*
22b		1.26 *m*		0.87 *m*		1.02 *m*		1.10 *m*		1.05 *m*
23a	18.2	1.38 *m*	20.6	1.99 *m*	21.8	1.62 *m*	21.8	1.56 *m*	21.8	1.45 *m*
23b		1.27 *m*		1.25 *m*		1.47 *m*		1.32 *m*		1.30 *m*
24a	42.1	1.38 *m*	42.3	1.37 *m*	43.7	1.56 *m*	43.9	1.53 *m*	43.8	1.55 *m*
24b				1.25 *m*		1.46 *m*		1.42 *m*		1.44 *m*
25	76.5		78.0		79.2		79.9		79.8	
26	26.3	1.15 *s*	25.7	1.08 *s*	26.9	1.25 *s*	26.4	1.27 *s*	26.4	1.27 *s*
27	26.2	1.15 *s*	26.9	1.08 *s*	27.1	1.25 *s*	27.3	1.27 *s*	27.2	1.29 *s*
Glc-1	97.1	4.26 *d* (7.7)	95.9	4.38 *d* (7.4)	98.8	4.45 *d* (7.8)	97.4	4.59 *d* (8.5)	97.3	4.59 *d* (8.1)
Glc-2	73.3	2.90 *m*	82.1	3.14 *d* (8.4)	75.4	3.13 *m*	82.7	3.41 *m*	82.6	3.42 *m*
Glc-3	77.0	3.13 *m*	76.7	3.34 d (8.3)	78.4	3.36 *m*	78.4	3.53 *m*	78.3	3.55 *m*
Glc-4	70.3	3.01 *m*	70.4	3.04 *m*	71.9	3.30 *m*	71.3	3.54 *m*	71.7	3.63 *m*
Glc-5	76.5	3.04 *m*	77.3	3.07 *m*	77.7	3.24 *m*	78.7	3.27 *m*	78.5	3.27 *m*
Glc-6	61.2	3.76 *d* (11.0)	61.3	3.60 *d* (11.6)	63.0	3.82 *dd* (11.7, 2.3)	63.1	3.84 *d* (11.5)	63.1	3.85 *m*
		3.63 *m*		3.43 *m*		3.65 *m*		3.69 *m*		3.68 *m*
Glc′-1			104.3	4.33 *d* (7.7)			105.5	4.58 *d* (8.1)	105.4	4.58 *d* (8.1)
Glc′-2			75.4	2.96 *t* (8.4)			76.5	3.22 *m*	76.4	3.24 *m*
Glc′-3			76.3	3.13 *m*			77.7	3.38 *m*	77.6	3.39 *m*
Glc′-4			70.2	3.07 *m*			71.6	3.31 *m*	71.7	3.28 *m*
Glc′-5			76.3	3.06 *m*			77.9	3.24 *m*	77.8	3.26 *m*
Glc′-6			61.4	3.55 *d* (11.6)			62.9	3.81 *d* (11.8)	62.0	3.83 *m*
				3.37 *m*				3.64 *m*		3.66 *m*

[a] Assignments were based on COSY, HMQC, and HMBC experiments.

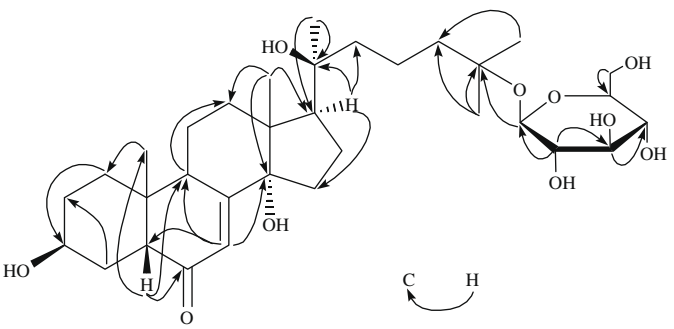


Fig. 2. Key HMBC correlations of **1**.

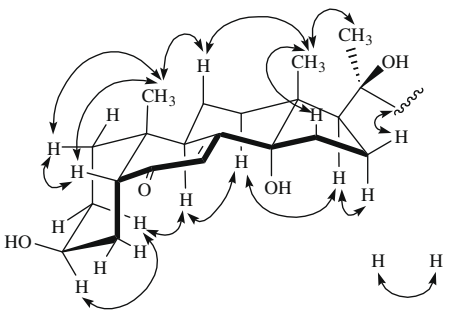


Fig. 3. Relative stereochemistry of **1**. Arrows show characteristic NOE steric proximities.

with the presence of a 7-en-6-one chromophore in ecdysteroids with a maximum value at 248 nm. The ^{13}C NMR spectrum of compound **2** showed 39 signals, of which 27 were assigned to the aglycone, the remaining 12 to two hexose units. Acid hydrolysis of **2** yielded D-glucose by co-TLC analysis with an authentic sample. Detailed NMR spectroscopic analysis showed that the aglycone of **2** was different from **1** only at the C-20 position. As shown in the ^1H and ^{13}C NMR spectra, the oxyquaternary carbon at δ_C 73.6 (C-20) in **1** was replaced by a methine at δ_C 35.4 in **2**, indicating that the aglycone of **2** was a 20-deoxy-derivative of 2,22-dideoxy-20-hydroxyecdysone. The methyl group at C-20 in **2** which appeared a doublet peak at δ_H 0.79 (J = 6.7 Hz) also supported this conclusion (Girault et al., 1990). The *cis* A/B and *trans* B/C and C/D ring junctions and C-20 (*R*) configuration were deduced from NOESY correlations, and comparison with NMR spectroscopic data of related compounds (Lafont et al., 2008). Therefore, the aglycone of **2** was defined as 2,22-dideoxyecdysone. The presence of two hexose units was indicated by two anomeric protons at δ_H 4.38 (d, J = 7.4 Hz) and 4.33 (d, J = 7.7 Hz) and two anomeric carbons (δ_C 95.9, 104.3) observed in the HMQC spectrum. Both sugars were assigned as β-glucopyranose based on their ^{13}C NMR spectroscopic data and two anomeric proton coupling constants (Table 1). The linkage and the sequence of the disaccharide moiety were deduced from HMBC correlations. The long-range correlation of δ_H 4.38 (Glc H-1) of the inner glucose (Glc) with δ_C 78.0 (C-25) of the ecdysteroid skeleton suggested that Glc was located at C-25 position, while the HMBC correlations δ_H 4.33 (Glc′ H-1) of the terminal glucosyl group (Glc′) with δ_C 82.1 (Glc C-2), and δ_H 3.14 (Glc H-2) with δ_C 104.3 (Glc′ C-1) demonstrated that the Glc′ was assigned the position. Thus, the structure of **2** was formulated as 2,22-dideoxyecdysone 25-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Compound **3**, a colorless amorphous powder, was established by HRMS (m/z 617.3250 $[M + Na]^+$) as $C_{33}H_{54}O_9$ with a maximal UV absorption at 247 nm. Detailed comparison of the NMR spectroscopic data of **3** with those of **2** indicated that both compounds had the same aglycone of a 2,22-dideoxyecdysone moiety, but differed in the sugar part (Table 1). Compared to 2, the NMR spectra of 3 displayed only one monosaccharide, which was assigned as β-glucopyranose by its NMR data (Table 1). Acid hydrolysis of **3** also afforded D-glucose, as determined by co-TLC analysis and by measurement of optical rotation. A key HMBC correlation between H-1 (δ_H 4.45, d, J = 7.8 Hz) of glucose and C-25 (δ 79.2) established the position of glycosilation at C-25. Full assignments of the ^{13}C and ^1H NMR signals were achieved by a combination of ^1H, ^{13}C, COSY, HMQC, and HMBC spectroscopic analysis. Consequently, **3** was elucidated as 2,22-dideoxyecdysone 25-*O*-β-D-glucopyranoside.

Compound **4** had the molecular formula of $C_{39}H_{64}O_{14}$ as **2**. Extensive NMR analysis indicated that compounds **4** and **2** were structurally different in terms of the orientation of H-5 (Table 1). The configuration of H-5 of **4** could be determined from the chemical shift of C-19 (Kim et al., 1988; Russell et al., 1981). The orientation of 5α-H for **4** was suggested by the chemical shift of C-19 (δ 13.4), while the C-19 with a 5β-H configuration was usually downfield shifted 10–11 ppm (Kim et al., 1988; Russell et al., 1981). The stereochemistry of 5α-H of **4** was further confirmed by H_α-9/H-5 and H_α-1/H-5 correlations in the NOESY spectrum. The ^{13}C NMR spectroscopic data also allowed for differentiation of **4** and **2** as the chemical shifts of ring A and B displayed significant differences (Table 1) (Lafont et al., 2008). Structure **4** was thus elucidated as (5α)-2,22-dideoxyecdysone 25-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Compound **5**, a colorless amorphous powder, exhibited a $[M + H]^+$ ion peak at m/z 773.3975 in the HRESIMS, 16 mass units higher than that of **2**, implying that **5** possessed an additional oxygen atom. Acid hydrolysis of **5** also afforded a D-glucose. Comparison of the ^{13}C NMR spectroscopic data between **5** and **2** (Table 1) indicated that the major difference was a methine at δ_C 50.7 (C-5) in **2** being replaced by an oxyquaternary at δ_C 81.0 in **5**. The HMBC correlations between H-7 and Me-19 with the signal at δ_C 80.1 established the presence of an OH substituent at C-5. Thus compound **5** was assigned as the 5-hydroxy derivative of **2**. The 5β-OH configuration was indicated by the upfield resonance for the Me-19 at δ_C 17.2 ppm (Jayasinghe et al., 2003; Tóth et al., 2008; Lafont et al., 2008). The similar NMR spectrum of rings C and D, the side chain, and the disaccharide chain in **5** and **2** suggested a similar structure and stereochemistry for these moieties (Tóth et al., 2008). Compound **5** was thus elucidated as 2,22-dideoxy-5β-hydroxyecdysone 25-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

The HRESIMS of compound **6**, a colorless amorphous powder, exhibited a $[M + Li]^+$ ion peak at m/z 871.2642 consisted with a molecular formula $C_{43}H_{44}O_{19}$. Alkaline hydrolysis of **6** yielded sucrose and *p*-coumaric acid, which were identified by co-HPLC and TLC with authentic samples. The presence of a sucrose moiety could be confirmed by its typical ^{13}C NMR spectroscopic data with eight oxymethines at δ_C 76.6 (C-3), 79.7 (C-4), 72.7 (C-5), 91.0 (C-1′), 72.3 (C-2′), 68.9 (C-3′), 68.8 (C-4′), 67.8 (C-5′), three oxymethylenes at δ_C 62.9 (C-1), 64.6 (C-6), 62.7 (C-6′), and one oxyquaternary carbon at δ_C 103.9 (C-2) (Takasaki et al., 2001). Three *p*-coumaroyl moieties in **6** were easily recognized by three pairs of *trans*-olefins at δ_H 6.32 (d, J = 15.9 Hz) and 7.50 (d, J = 15.9 Hz), 6.44 (d, J = 15.8 Hz) and 7.66 (d, J = 15.8 Hz), and 6.44 (d, J = 15.8 Hz) and 7.61 (d, J = 15.8 Hz), and three 1,4-disubstituted aromatic rings. In addition, the typical NMR signals of two acetyl groups at δ_H 1.84 and 2.05, and δ_C 20.3, 20.5, 169.4, and 170.2 were also observed. The attachment of the three *p*-coumaroyl moieties to C-3, C-6 and C-2′ was determined by HMBC correlations of H-3 (δ_H 5.53) of fructose with the ester carbonyl carbon of one *p*-coumaroyl group (δ_C 165.5), H-6 (δ_H 4.50) of fructose with δ_C 166.5 of the second *p*-coumaroyl group, and H-2′ (δ_H 5.27) of glucose with δ_C 166.0 of the third *p*-coumaroyl group. In the same way, the HMBC correlations of δ_H 4.13 (H-1) of fructose with one acetyl carbonyl carbon signal at δ_C 170.2 and δ_H 4.89 (H-4′) of glucose with δ_C 169.4 of the other acetyl group established that the two acetyl groups were linked to C-1 and C-4′ of sucrose, respectively. On the basis of above evidence, compound **6** was determined to be β-D-(1-*O*-acetyl-3,6-*O*-*p*-*E*-dicoumaroyl)-fructofuranosyl-α-D-(4′-*O*-acetyl-2′-*O*-*p*-*E*-coumaroyl)-glucopyranoside.

Compounds **1**, **2** and **6**–**14** isolated from *F. floridana* were screened for activity against human DNA Topo I. Compound **13** showed activity with IC_{50} value of 130 μM, while the other compounds proved inactive (IC_{50} > 500 μM). This compound was observed to completely inhibit Topo I relaxation activity at the concentration of 285 μM in conjunction with intercalation ability at 312 μM. Its activity was similar to luteolin (the positive control, IC_{50}: 108 μM), but much less than camptothecin, a well known Topo I poison (Webb and Ebeler, 2003). Our data showed that **13** inhibited Topo I catalytic activity by interacting directly with the free enzyme and preventing formation of the DNA-Topo I complex, but not stabilizing the intermediate DNA-Topo I covalent complex as camptothecin. Diosmetin (**13**) was previously found to poison human Topo II (Bandele and Osheroff, 2007), and here we report its inhibition on Topo I for the first time. Thus, **13** is a dual topoisomerase inhibitor as luteolin (Webb and Ebeler, 2004).

3. Conclusion

Eight ecdysteroids (compounds **1–5** and **7–9**) were isolated from *F. floridana* including five new phytoecdysteroid glycosides (**1–5**). HPLC analysis showed that **1**–**5** and **8** were found in stems and leaves, while **7** and **9** were detected in each part of the plant. All compounds except for **4** and **5** were tested *in vitro* for their activity

against DNA topoisomerase I. None of these ecdysteroids shows any activity in the Topo I assays. Of the four flavanoids isolated from the plant, only diosmetin (**13**) showed bioactivity against DNA Topo I.

Ecdysteroids are the steroid hormones found in animals and plants. There is a growing interest in ecdysteroids due to their important human pharmacological effects and insect-molting activity, particularly their potential as lead compounds for insecticide development (Dhadialla et al., 1998). Of 325 phytoecdysteroids isolated to date, 45 are ecdysteroid glycosides (Lafont et al., 2008). These ecdysteroid glycosides were isolated from Caryophyllaceae (20 compounds), ferns (12), Amaranthaceae (3), Malvaceae (3), Limnanthaceae (3), Menispermaceae (3), Liliaceae (2), Lamiaceae (1), and Melanthiaceae (1). The sugar moiety of the ecdysteroid glycosides may primarily be monosaccharide residue (glucose, galactose, xylose, or allose). Usually C-3 (31 compounds) and C-22 (10) are the positions of glycosylation in the plants with occasional C-25 (4), C-2 (2), and C-26 (1). Among these reported compounds, rhamnose is restricted to the fern *Polypodium* (Jizba et al., 1971; Kim et al., 1988; Kim and Kinghorn, 1989), xylose is only found in *Limnanthes* (Limnanthaceae) (Sarker et al., 1997; Meng et al., 2001), and allose is reported only in *Lilium* (Mimaki et al., 1989). Thus, it is possible that ecdysteroid glycosides may have taxonomic significance in some plant groups.

Froelichia is morphologically distinguished within the subfamily Gomphrenoideae of Amaranthaceae (McCauley and Ballard, 2007). It is the first report of ecdysteroid glycosides in the subfamily and the second in the family after *Pfaffia*. Its ecdysteroid glycosides (**1**–**5** and **8**) have sugar moieties attached at C-25. Ecdysteroid glycosides with sugar moieties at C-25 have only been reported in *Pfaffia* in the Amaranthaceae (Nishimoto et al., 1988), *Silene* in the Caryophyllaceae (Girault et al., 1990), and the fern *Blechnum* of Blechnaceae (Suksamrarn et al., 1986). Three compounds (**2**, **4**, and **5**) from *Froelichia* have unique disaccharide residues at C-25. The compounds of ecdysteroid glycosides with a disaccharide residue at C-3 were isolated from the fern *Polypodium* (Jizba et al., 1971; Kim et al., 1988), *Silene* (Baltayev, 1998), and *Limnathes* (Meng et al., 2001). To date, reported ecdysteroid glycosides show structural diversity in plants. With extensive ecdysteroid analysis in more plants, the taxonomic implications of phytoecdysteroids will be better understood.

4. Experimental

4.1. General experimental procedures

Optical rotation values were measured on a JASCO P-1010 polarimeter, whereas UV spectra were recorded in MeOH with a μQuant spectrophotometer (Bio-Tek Instruments, Inc.). NMR experiments were performed using a Bruker 600 MHz NMR instrument with data reported in δ ppm values and referenced to the solvent used, with HRESIMS being measured on a PE SCIEX QSTAR LC/MS/MS spectrometer. Octadecyl-functionalized silica gel, silica gel, Sephadex LH-20, and TLC plates were purchased from Aldrich Chemical Co. HPLC analysis was performed on a Hewlett Packard Series 1100 with a HP 1100 diode array detector using a Hypersil ODS column (150 × 4.6 mm, 5 μm, Supelco; flow rate, 1 ml/min; MeOH/H_2O (v/v) linear gradient, 2:98–98:2 in 35 min). Preparative HPLC was performed with an Acuflow Series III pump connected with an Acutect 500 UV/VIS detector using an Econosil ODS column (250 × 22 mm, 10 μm, Alltech). D-Glucose was purchased from Aldrich.

4.2. Plant material

Whole plants of *F. floridana* were collected in Nacogdoches, Texas, USA on September 14, 2006, and identified by Wanli Zhang. A voucher specimen (TX-Nac-20060914-#0001-wp) is deposited at the National Center for Pharmaceutical Crops at Stephen F. Austin State University, USA.

4.3. Extraction and isolation

Air-dried plant material (1 kg) was ground to a coarse powder and extracted with 9 L of EtOH/H_2O (95:5, v/v) at room temperature. The combined EtOH extracts were concentrated to give a residue (40 g) under reduced pressure. The residue was then suspended in MeOH/H_2O (500 ml, 1:1, v/v) and extracted successively with *n*-hexane (8.6 g) and EtOAc (17 g). The EtOAc extract exhibited Topo I inhibition properties with IC_{50} value of 15 μg/mL, and thus was subjected to further purification. Silica gel cc (500 g) of the EtOAc extract with gradient $CHCl_3$/MeOH (30:1, 15:1, 7:1, 5:1, 3:1 and 1:1, v/v, each 3000 ml) afforded seven fractions (1–7) on the basis of their TLC profiles. Fraction 2 was developed on preparative TLC (silical gel, $CHCl_3$/MeOH, 12:1) to give **13** (7 mg). Fraction 3 was applied to a Sephadex LH-20 column eluting with MeOH to afford four subfractions: Ia, Ib, Ic, and Id. Fraction Ia afforded **12** (4 mg) after preparative HPLC separation (MeOH/H_2O, 43:57, v/v). By preparative TLC (silica gel, $CHCl_3$/MeOH, 6:1), **11** (4 mg), **14** (3 mg) and **10** (3 mg) were purified from Ib, Ic, and Id, respectively. Compound **7** (400 mg) was obtained from fraction 4 by recrystallization in MeOH. After being combined, fractions 5 and 6 were further fractionated by ODS cc (100 g) eluting with MeOH/H_2O (15:85, 30:70, 50:50, and 75:25, v/v, each 700 ml), and 800 ml of 100% MeOH. According to the HPLC profiles, the eluents were combined to give four subfractions: IIa, IIb, IIc, and IId. Fraction IIa and IIb afforded compounds **8** (8.9 mg) and **1** (7 mg), respectively, by preparative HPLC (MeOH/H_2O, 50:50, v/v). HPLC isolation of IIc eluted with MeOH/H_2O (63:37, v/v) gave a mixture of **2**, **4**, and **5**. Further separation of this mixture was conducted on analytical HPLC (CH_3CN/H_2O, 31:69, v/v), which afforded **2** (70 mg), **4** (2.3 mg), and **5** (1.2 mg), respectively. Compound **3** (2.6 mg) was isolated from IId (preparative HPLC, CH_3CN/H_2O, 33:67, v/v). Compound **9** (22 mg) were purified by preparative HPLC from fraction 7 (MeOH/H_2O, 35:75, v/v). The rest parts of fraction 7 after removal of **9** gave **6** (3.6 mg) (preparative HPLC, MeOH/H_2O, 32:68, v/v). All fractions were detected at 254 nm, except for compound **6** (360 nm). The distribution of compounds **1**–**5** and **7**–**9** was also investigated by HPLC analysis in different plant parts (leaves, stems, flowers, and roots).

4.4. 2, 22-dideoxy-20-hydroxyecdysone 25-O-β-D-glucopyranoside (*1*)

Colorless amorphous powder. $[\alpha]_D^{21}$ + 26(*c* 0.07, MeOH); UV λ_{max}^{MeOH} nm: 246; For ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) spectroscopic data, see Table 1; HRESIMS *m*/*z* 617.3881 (calcd for $C_{33}H_{54}O_{10}Li$, 617.3877).

4.5. 2,22-dideoxyecdysone 25-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (*2*)

Colorless amorphous powder. $[\alpha]_D^{21}$ + 48 (*c* 0.92, MeOH); UV λ_{max}^{MeOH} nm: 248; For ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) spectroscopic data, see Table 1; HRESIMS *m*/*z* 763.4463 (calcd for $C_{39}H_{64}O_{14}Li$, 763.4456).

4.6. 2,22-deoxyecdysone 25-O-β-D-glucopyranoside (*3*)

Colorless amorphous powder. $[\alpha]_D^{21}$ + 14 (*c* 0.43, MeOH); UV λ_{max}^{MeOH} nm: 247; For ^{1}H NMR (600 MHz, CD_3OD-d_4) and ^{13}C NMR (150 MHz, CD_3OD-d_4) spectroscopic data, see Table 1; HRESIMS *m*/*z* 617.3250 (calcd for $C_{33}H_{54}O_9Na$, 617.3666).

*4.7. (5α)-2,22-dideoxyecdysone 25-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**4**)*

Colorless amorphous powder. $[\alpha]_D^{21}$ + 32 (*c* 0.38, MeOH); UV λ_{max}^{MeOH} nm: 248; For ^{1}H NMR (600 MHz, CD_3OD-d_4) and ^{13}C NMR (150 MHz, CD_3OD-d_4) spectroscopic data, see Table 1; HRESIMS *m*/*z* 757.4299 (calcd for $C_{39}H_{64}O_{14}H$, 757.4374).

*4.8. 2,22-dideoxy-5-hydroxyecdysone 25-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**5**)*

Colorless amorphous powder. $[\alpha]_D^{21}$ + 46 (*c* 0.11, MeOH); UV λ_{max}^{MeOH} nm: 248; For ^{1}H NMR (600 MHz, CD_3OD-d_4) and ^{13}C NMR (150 MHz, CD_3OD-d_4) spectroscopic data, see Table 1; HRESIMS *m*/*z* 773.3975 (calcd for $C_{39}H_{64}O_{15}H$, 773.4324).

*4.9. β-D-(1-O-acetyl-3,6-O-p-E-dicoumaroyl)-fructofuranosyl-α-D-(4′-O-acetyl-2′-O-p-E-coumaroyl)-glucopyranoside (**6**)*

Colorless amorphous powder. $[\alpha]_D^{21}$ + 33 (*c* 0.34, MeOH); UV λ_{max}^{MeOH} nm: 211, 231, 314; ^{1}H NMR (600 MHz, DMSO-d_6) sucrose moiety: δ_H 1.84 (3H, *s*, H-Ac-4′), 2.05 (3H, *s*, H-Ac-1), 3.45 (2H, *m*, H-1a and H-6′a), 3.71 (1H, *br. d*, *J* = 12.9 Hz, H-3′), 4.09 (1H, *dd*, *J* = 7.9, 8.1 Hz, H-4), 4.10 (1H, *m*, H-6′b), 4.13 (1H, *m*, H-1b), 4.29 (1H, *m*, H-5), 4.31 (1H, *m*, H-6a), 4.35 (1H, *d*, *J* = 9.8 Hz, H-5′), 4.50 (1H, *d*, *J* = 10.5 Hz, H-6b), 4.89 (1H, *t*, *J* = 9.7 Hz, H-4′), 5.27 (1H, *t*, *J* = 9.6 Hz, H-2′), 5.41 (1H, *s*, H-1′), 5.53 (1H, *d*, *J* = 7.9 Hz, H-3); *p*-coumaroyl moiety: δ_H 6.32 (1H, *d*, *J* = 15.9 Hz, H-8‴), 6.44 (2H, *d*, *J* = 15.8 Hz, H-8″ and H-8‴), 6.79–6.84 (6H, *m*, H-3″ and H-5″, and H- 3‴ and H-5‴, and H-3⁗ and H-5⁗), 7.50 (1H, *d*, *J* = 15.9 Hz, H-7⁗), 7.54 (2H, *d*, *J* = 8.5 Hz, H-2⁗ and H-6⁗), 7.58 (2H, *d*, *J* = 8.3 Hz, H-2‴ and H-6″), 7.62 (2H, *d*, *J* = 8.5 Hz, H-2″ and H-6″), 7.61 (1H, *d*, *J* = 15.8 Hz, H-7‴), 7.66 (1H, *d*, *J* = 15.8 Hz, H-7″); ^{13}C NMR (150 MHz, DMSO-d_6) sucrose moiety: δ_C 20.3 (*q*, Ac-4′ C-2), 20.5 (*q*, Ac-1C-2), 62.7 (*t*, C-6′), 62.9 (*t*, C-1), 64.6 (*t*, C-6), 67.8 (*d*, C-5′), 68.8 (*d*, C-4′), 68.9 (*d*, C-3′), 72.3 (*d*, C-2′), 72.7 (*d*, C-5), 76.6 (*d*, C-3), 79.7 (*d*, C-4), 91.0 (*d*, C-1′), 103.9 (*s*, C-2), 169.4 (*s*, Ac-4′ C-1), 170.2 (*s*, Ac-1 C-1); *p*-coumaroyl moiety: δ_C 113.7 (*d*, C-8″ and C-8‴), 113.8 (*d*, C-8⁗), 115.8 (*d*, C-3″ and C-5″, C-3‴ and C-5‴, and C-3⁗ and C-5⁗), 124.9 (*s*, C-1″), 125.4 (*s*, C-1‴ and C-1⁗), 130.4 (*d*, C-2‴ and C-6‴, C-2⁗ and C-6⁗), 132.7 (*d*, C-2″ and C-6″), 145.1 (*d*, C-7⁗), 145.3 (*d*, C-7‴), 145.5 (*d*, C-7″), 160.1 (*s*, C-4‴ and C-4⁗), 161.7 (*s*, C-4″), 165.5 (*s*, C-9″), 166.0 (*s*, C-9⁗), 166.5 (*s*, C-9‴); HRESIMS *m*/*z* 871.2642 (calcd for $C_{43}H_{44}O_{19}Li$, 871.2637).

*4.10. Acid hydrolysis of **1**-**5***

Solutions of compounds **1**–**5** (1 mg each) in 1 M HCl-dioxane (1:1, 1 ml) were individually heated to 80 °C, this being held for 2 h. After the dioxane was removed, each solution was extracted three times with $CHCl_3$–MeOH (7:3, 1 ml × 3). The aqueous layer of **1**–**5** afforded the monosaccharide portion, respectively, which was identified as glucose by comparison with authentic samples by direct co-TLC [$CHCl_3$–MeOH–H_2O (10:5:2)]. After purified by preparative TLC, the determination of the absolute configuration of glucose followed the procedure described in a previous paper (Zhang et al., 2006).

*4.11. Alkaline hydrolysis of **6***

Compound **6** (2 mg) was dissolved in 3% KOH–MeOH (2 ml) with the solution stirred at room temperature for 6 h. The reaction mixture was neutralized with 1 N HCl and then extracted with $CHCl_3$. The organic solutions were analyzed using an Agilent 1100 HPLC system with an Agilent Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm) (mobile phase: 10% MeOH in 0.1% acetic acid solution, flow rate: 1 ml/min, UV detector: 280 nm). By comparison of its retention time at 7.8 min and DAD UV absorption *o*-coumaric acid was identified (Yuan et al., 2007). Sucrose was identified by TLC in $CHCl_3$–MeOH–H_2O (6:4:1) using an authentic sample (Takasaki et al., 2001; Li et al., 2002).

4.12. Topoisomerase I assay

Compounds **1**–**2** and **6**–**14** were individually evaluated for their ability to inhibit Topo I activity following the protocol described by Webb and Ebeler (2003). Compounds were tested at concentrations of 30, 63, 125, and 312 μM, respectively. Camptothecin (100 μM), luteolin (312 μM), and ethidium bromide (0.4 μg/ml) were used as positive controls for poisoning, inhibition and intercalation, respectively. All compounds were analyzed for DNA Topo I inhibition, poisoning and intercalation activities using a gel electrophoresis assay. IC_{50} values were estimated by analyses of enzyme activity versus inhibitor concentration. Three replicates were used in the Topo I assay.

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