Phytochemistry Letters 16 (2016) 185–191

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Short communication

Sullivantosides A-F: Pregnane glycosides from *Asclepias sullivantii* L.

Juan J. Araya[a], Kelly Kindscher[b], Barbara N. Timmermann[c,*]

[a] Centro de Investigaciones en Productos Naturales, Escuela de Química; Instituto de Investigaciones Farmacéuticas, Facultad de Farmacia, Universidad de Costa Rica, San Pedro 11501-2060, Costa Rica
[b] Kansas Biological Survey, University of Kansas, Lawrence, KS 66047, USA
[c] Department of Medicinal Chemistry, University of Kansas, Lawrence, KS 66045, USA

ARTICLE INFO

Article history:
Received 3 February 2016
Received in revised form 5 April 2016
Accepted 14 April 2016
Available online xxx

Keywords:
Asclepias sullivantii
Pregnane glycosides
Asclepiadeaceae

ABSTRACT

Phytochemical investigation of the above-ground biomass of *Asclepias sullivantii* L. (Asclepiadaceae) afforded six new pregnane glycosides, named sullivantosides A-F **(1–6)**. The structures of **1–6** were elucidated through a variety of spectroscopic and spectrometric techniques (1D and 2D NMR; HRESIMS). To the best of our knowledge, this work represents the first phytochemical study of this species.

1. Introduction

Asclepias sullivantii—commonly known as "prairie milkweed"- is native to the United States and Canada, where in the northern states (Minnesota, Wisconsin, and Michigan) it is listed as a threatened species (USDA, 2015). This species is predominantly found in humid and relatively undisturbed lowland habitats such as the native prairies of Kansas, Missouri, and Illinois. Currently, there are 75 recognized species of *Asclepias* in the United States (USDA, 2015). Our ethnobotanical database lists over 400 specific uses of *Asclepias* species by over 60 Native American tribes, yet *A. sullivantii* is not one of these species. This maybe due to the fact that the species is rather uncommon and confined to such a small range, but there is evidence to suggest that it may have been used medicinally for a variety of treatments like similar and more common *Asclepias* species (Kindscher, 1987, 1992).

The *Asclepias* genus is known to contain cardiac and pregnane glycosides (Araya et al., 2012a; Warashina and Noro, 2003, 2009). As part of our ongoing effort to study the chemical diversity and medicinal potential of the flora from Kansas, the phytochemistry of *A. sullivantii* was investigated. To the best of our knowledge, this report represents the first known phytochemical investigation of the title plant.

2. Material and methods

2.1. General experimental procedures

Melting points were recorded with an OptiMelt automatic apparatus. IR spectra were obtained with a Thermo Nicolet Avatar 380 FT-IR. UV–vis spectra were recorded on a Varian Cary 50 Scan; ^{1}H NMR, ^{13}C NMR and 2D spectra were recorded with a Bruker Avance AV-III 500 with a dual carbon/proton cryoprobe. HRESIMS were conducted with an LCT Premier Waters Corp. (Milford, MA). Preparative HPLC separations were done using an Agilent 1100HPLC system with a Phenomenex Luna C18 column (5 μm, 250 21 mm), flow rate of 35 mL/min (aprox. 35 bar), injection volume of 800 μL (ca. 100 mg sample), and UV detection using multi-wavelength detector. Semi-preparative HPLC was conducted using an Agilent 1200HPLC system with a Phenomenex Luna C18 column (5 μm, 250 10 mm), flow rate of 4.5 mL/min (approx. 160 bar), injection volume of 50 μL (ca. 10 mg sample), and UV detection using diode array.

2.2. Plant material

Above-ground biomass (stems, leaves, and flowers) of *A. sullivantii* was collected on April 24, 2010, by Kelly Kindscher and Juan Araya. The plant material was collected 2 km northeast of Welda, Kansas at the University of Kansas, Anderson County Prairie Preserve. The GPS location of the collection site was LAT 38.18052 , LONG 95.27485 . Botanical identification was performed by Kelly Kindscher, and a botanical specimen was deposited in the McGregor Herbarium of the University of Kansas (Kindscher 4039).

* Corresponding author.
E-mail address: btimmer@ku.edu (B.N. Timmermann).

http://dx.doi.org/10.1016/j.phytol.2016.04.011
1874-3900/

2.3. Extraction and isolation

The collected fresh biomass (5.5 kg) was left to dry at room temperature (time period needed). The resulting dry material (1.1 kg) was then ground to a fine powder and extracted four times with MeOH-CH_2Cl_2 (1:1, v/v) at room temperature. The organic solvents (c.a. 10 L) were removed under reduced pressure to afford the crude extract (102.0 g, 9.1% w/w of dry weight). The organic extract was suspended in MeOH-H_2O (9:1, 1 L) and partitioned with hexanes (500 mL, three times) to give a hexanes fraction. The resulting aqueous layer was subjected to rotary evaporation to remove the dissolved MeOH, the volume adjusted to 500 mL with distilled H_2O, and successively partitioned with CH_2Cl_2 (500 mL, three times) and *n*-BuOH (500 mL, three times) to give CH_2Cl_2 and *n*-BuOH fractions, respectively. The CH_2Cl_2 fraction (20 g) was subjected to a large silica gel flash column chromatography (200 g) to afford 14 fractions (CH_2Cl_2-1 to CH_2Cl_2-14). Fraction CH_2Cl_2-13 (6.60 g) was separated using automatic flash chromatography (RP-C_{18} prepacked column, 200 g) using a linear gradient (from 20% MeOH to 100% MeOH in 45 min) to yield 7 subfractions (SF1-7). To remove pigmentation, the SF5 subfraction (2.20 g) was subjected to Sephadex LH-20 (500 g) column chromatography (MeOH as mobile phase). The resulting clean sample (1.10 g) was subjected to preparative HPLC (solvent A: distilled water, solvent B: acetonitrile, linear gradient 40%B to 55%B in 25 min, 100%B during 5 min, then 40%B for 5 min) that lead to in the isolation of the six new compounds, which were subsequently named sullivantosides A (**1**; 9.0 mg; Rt = 10.2 min), B (**2**; 8.1 mg; Rt = 10.8 min), C (**3**; 10.0 mg; Rt = 11.3 min), D (**4**; 6.1 mg; Rt = 12.4 min), E (**5**; 4.0 mg; Rt = 13.1 min), and F (**6**; 4.1 mg; Rt = 14.2 min). The *n*-BuOH extract (22.9 g) was suspended in H_2O (500 mL) and adsorbed in a MCI gel (500 g) column, washed with H_2O (2 L), and then eluted with H_2O-MeOH mixtures [(10% to 100% MeOH (v/v in H_2O) in 10% step increments (2L each fraction)] to afford a total of 10 fractions (1–10). Fractions *n*-BuOH 6–10 were subjected to Sephadex LH-20 (500 g) column chromatography (100% MeOH elution), followed by silica gel column chromatography [$CHCl_3$:MeOH 95:5 (v/v) or $CHCl_3$:MeOH 90:10 (v/v) elution], and finally HPLC chromatography (semi-preparative or preparative with CH_3CN-H_2O) elution. Using these separation steps, the common flavonoid rutin was isolated from fraction *n*-BuOH-6; whereas the known lignan 9′-*O*-butyl-3-*O*-demethyl-9-*O*-β-D-glucopyranosyl dehydrodiconiferylalcohol (6 mg) was obtained in conjunction with the known cardiac glycosides 3-*O*-β-D-glucopyranosyl-(1→4)-6-desoxy-β-D-allopyranosyl uzarigenin (80 mg) and 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl uzarigenin (5 mg), were obtained from the fraction *n*-BuOH-8.

2.4. Sullivantoside A (**1**)

Amorphous white powder; mp 141.7-143.0 C; $[\alpha]_D^{25}$ = 2.8 (*c* 0.38, MeOH); IR ν_{max} (film) cm ^{1}: 3389.3 (OH), 1708.7 (C=O), 1644.8 (C=O), 1156.6 (C O); HRESIMS *m/z*: 1195.6150 $[M+Na]^+$ (1195.6240 calc. for $C_{59}H_{96}NaO_{23}$) ^{1}H and ^{13}C NMR: see Tables 1–4.

2.5. Sullivantoside B (**2**)

Amorphous white powder; mp 153.7-154.9 C; $[\alpha]_D^{25}$ = +18 (*c* 0.5, MeOH); IR ν_{max} (film) cm ^{1}: 3389.7 (OH), 1708.5 (C=O), 1644.6 (C=O), 1156.9 (C O); HRESIMS *m/z*: 1181.6076 $[M+Na]^+$ (1181.6084 calc. for $C_{58}H_{94}NaO_{23}$) ^{1}H and ^{13}C NMR: see Tables 1–4.

2.6. Sullivantoside C (**3**)

Amorphous white powder; mp 158.9-160.0 C; $[\alpha]_D^{25}$ = +15.3 (*c* 0.45, MeOH); IR ν_{max} (film) cm ^{1}: 3389.3 (OH), 1708.7 (C=O), 1644.8 (C=O), 1156.6 (C O); HRESIMS *m/z*: 1330.7278 $[M+Na]^+$ (1330.7285 calc. for $C_{67}H_{110}NaO_{26}$) ^{1}H and ^{13}C NMR see Tables 1–4.

Table 1
^{13}C NMR (125 MHz, C_5D_5N) data for the aglycone part of sullivantosides A-F (**1–6**).

Atom	1	2	3	4	5	6
1	38.5, CH_2	38.5, CH_2	38.4, CH_2	38.6, CH_2	38.5, CH_2	38.6, CH_2
2	29.9, CH_2	29.9, CH_2	29.9, CH_2	30.0, CH_2	30.0, CH_2	29.9, CH
3	76.9, CH	76.9, CH	77.3, CH	77.0, CH	76.9, CH	77.2, CH_2
4	34.8, CH_2	34.8, CH_2	34.8, CH_2	34.9, CH_2	34.9, CH_2	34.9, CH_2
5	45.6, CH	45.6, CH	45.7, CH	45.8, CH	45.7, CH	45.8, CH
6	25.6, CH_2	25.6, CH_2	25.7, CH_2	25.8, CH_2	25.8, CH_2	25.9, CH_2
7	35.8, CH_2	35.8, CH_2	35.9, CH_2	36.1, CH_2	36.0, CH_2	36.8, CH_2
8	76.4, C	76.4, C	76.4, C	76.5, C	76.9, C	76.6, C
9	48.5, CH	48.1, CH	48.2, CH	48.8, CH	48.5, CH	48.8, CH
10	37.0, C	37.0, C	37.0, C	37.0, C	37.0, C	37.0, C
11	24.1, CH_2	24.1, CH_2	24.1, CH_2	27.9, CH_2	27.9, CH_2	28.0, CH_2
12	78.6, CH	78.6, CH	78.6, CH	75.2, CH	75.2, CH	75.2, CH
13	55.5, C	55.6, C	55.6, C	57.3, C	57.3, C	57.3, C
14	86.4, C	86.7, C	86.7, C	86.8, C	86.8, C	86.8, C
15	36.2, CH_2	36.2, CH_2	36.3, CH_2	36.8, CH_2	36.8, CH_2	36.8, CH_2
16	25.4, CH_2	25.3, CH_2	25.4, CH_2	25.5, CH_2	25.5, CH_2	25.5, CH_2
17	59.3, CH	59.5, CH	59.5, CH	59.1, CH	59.2, CH	59.0, CH
18	13.4, CH_3	13.4, CH_3	13.4, CH_3	12.4, CH_3	12.4, CH_3	12.4, CH_3
19	13.5, CH_3	13.5, CH_3	13.6, CH_3	13.7, CH_3	13.7, CH_3	13.7, CH_3
20	214.9, C	214.9, C	215.0, C	217.4, C	217.3, C	217.4, C
21	32.3, CH_3	32.3, CH_3	32.3, CH_3	32.8, CH_3	32.8, CH_3	32.8, CH_3
	12-Tg	**12-Tg**	**12-Tg**			
1	168.1, C	168.1, C	168.2, C			
2	129.7, C	129.7, C	129.7, C			
3	138.1, CH	138.1, CH	138.1, CH			
4	14.7, CH_3	14.7, CH_3	14.7, CH_3			
5	12.7, CH_3	12.7, CH_3	12.7, CH_3			

2.7. Sullivantoside D (**4**)

Amorphous white powder; mp 155.1-157.0 C; $[\alpha]_D^{25}$ = 1.6 (*c* 0.1, MeOH); IR ν_{max} (film) cm ^{1}: 3389.3 (OH), 1708.5 (C=O), 1644.5 (C=O), 1156.3 (C O); HRESIMS *m/z*: 1113.5798 $[M+Na]^+$ (1113.5821 calc. for $C_{55}H_{92}NaO_{23}$) ^{1}H and ^{13}C NMR: see Tables 1–4.

2.8. Sullivantoside E (**5**)

Amorphous white powder; mp 164.7-165.4 C; $[\alpha]_D^{25}$ = +15.5 (*c* 0.2, MeOH); IR ν_{max} (film) cm ^{1}: 3389.4 (OH), 1708.3 (C=O), 1644.7 (C=O), 1156.8 (C O); HRESIMS *m/z*: 1099.5668 $[M+Na]^+$ (1099.5665 calc. for $C_{53}H_{88}NaO_{22}$) ^{1}H and ^{13}C NMR: see Tables 1–4.

2.9. Sullivantoside F (**6**)

Amorphous white powder; mp 138.9-140.1 C; $[\alpha]_D^{25}$ = +20.3 (c. 0.3, MeOH); IR ν_{max} (film) cm ^{1}: 3389.2 (OH), 1708.4 (C=O), 1644.9 (C=O), 1156.5 (C-O); HRESIMS *m/z*: 1271.6756 $[M+Na]^+$ (1271.6764 calc. for $C_{62}H_{104}NaO_{25}$) ^{1}H and ^{13}C NMR: see Tables 1–4.

2.10. Acid hydrolysis of compounds

An aliquot of the fraction CH_2Cl_2-13 (approx. 100 mg) was hydrolyzed using 3 mL of 1 M HCl (dioxane:H_2O 1:1, v/v) for 4 h at 70 C. The resulting mixture was neutralized with 3 M NaOH and extracted three times with EtOAc. The aqueous layer was concentrated and the residue was fractionated in a small silica gel column using mixtures of $CHCl_3$, MeOH, and H_2O. The purified sugars were compared by TLC with authentic samples and the optical rotation values recorded in H_2O after equilibration during

Table 2
^{13}C NMR (125 MHz, C_5D_5N) data for the sugar moiety of sullivantosides A-F (**1**–**6**).

Atom	**1** D-Cym	**2** D-Cym	**3** D-Ole	**4** D-Cym	**5** D-Cym	**6** D-Ole
1′	96.3, CH	96.3, CH	98.0, CH	96.2, CH	96.2, CH	97.9, CH
2′	37.7,CH_2	37.7,CH_2	38.3,CH_2	37.7,CH_2	37.7,CH_2	38.3,CH_2
3′	78.4, CH	78.4, CH	79.6, CH	78.4, CH	78.4, CH	79.7, CH
4′	83.8, CH	83.8, CH	83.6, CH	83.8, CH	83.8, CH	83.6, CH
5′	69.3, CH	69.3, CH	72.2, CH	69.4, CH	69.3, CH	72.2, CH
6′	19.0CH_3	19.0CH_3	19.1CH_3	19.0CH_3	19.0CH_3	19.1CH_3
3′-OMe	59.3CH_3	59.2CH_3	57.6CH_3	59.3CH_3	59.2CH_3	57.6CH_3

Atom	**1** D-Cym	**2** D-Dig	**3** D-Ole	**4** D-Cym	**5** D-Dig	**6** D-Ole
1″	100.9, CH	100.9, CH	100.6, CH	100.9, CH	100.9, CH	100.6, CH
2″	37.4, CH_2	39.1, CH_2	38.2, CH_2	37.4, CH_2	39.1, CH_2	38.2, CH_2
3″	78.3, CH	67.8, CH	79.5, CH	78.3, CH	67.8, CH	79.5, CH
4″	83.5, CH	83.5, CH	83.6, CH	83.5, CH	83.5, CH	83.6, CH
5″	69.2, CH	68.9, CH	71.9, CH	69.4, CH	68.9, CH	72.0, CH
6″	18.9, CH_3	18.8, CH_3	19.1, CH_3	18.9, CH_3	18.8, CH_3	19.1, CH_3
3″-OMe	59.3, CH_3		57.8, CH_3	59.3, CH_3		57.8, CH_3

Atom	**1** D-Dig	**2** D-Dig	**3** D-Ole	**4** D-Dig	**5** D-Dig	**6** D-Ole
1‴	100.9, CH	100.3, CH	100.6, CH	100.8, CH	100.3, CH	100.6, CH
2‴	39.4, CH_2	39.3, CH_2	38.2, CH_2	39.4, CH_2	39.3, CH_2	38.2, CH_2
3‴	67.9, CH	67.9, CH	79.7, CH	67.9, CH	67.9, CH	79.7, CH
4‴	83.6, CH	83.6, CH	83.6, CH	83.6, CH	83.6, CH	83.6, CH
5‴	68.8, CH	68.8, CH	72.3, CH	68.8, CH	68.9, CH	72.3, CH
6‴	18.7, CH_3	18.7, CH_3	19.2, CH_3	18.8, CH_3	18.9, CH_3	19.2, CH_3
3‴-OMe			57.8, CH_3			57.8, CH_3

Atom	**1** D-Ole	**2** D-Ole	**3** D-Cym	**4** D-Ole	**5** D-Ole	**6** D-Cym
1⁗	101.8, CH	101.8, CH	98.9, CH	101.8, CH	101.8, CH	98.9, CH
2⁗	37.4, CH_2	37.6, CH_2	37.4, CH_2	37.6, CH_2	37.6, CH_2	37.6, CH_2
3⁗	79.6, CH	79.6, CH	78.5, CH	79.6, CH	79.6, CH	78.3, CH
4⁗	83.5, CH	83.4, CH	83.4, CH	83.5, CH	83.4, CH	83.4, CH
5⁗	72.3, CH	72.6, CH	69.5, CH	72.3, CH	72.3, CH	69.5, CH
6⁗	19.2, CH_3	19.2, CH_3	19.9, CH_3	19.2, CH_3	19.2, CH_3	19.9, CH_3
3⁗-OMe	57.6, CH_3	57.2, CH_3	59.2, CH_3	57.6, CH_3	57.3, CH_3	59.2, CH_3

Atom	**1** D-Glc	**2** D-Glc	**3** D-Ole	**4** D-Glc	**5** D-Glc	**6** D-Ole
1⁗′	104.8, CH	104.8, CH	102.2, CH	104.8, CH	104.9, CH	102.3, CH
2⁗′	76.1, CH	76.1, CH	37.8, CH_2	76.1, CH	76.1, CH	37.8, CH_2
3⁗′	79.1, CH	79.1, CH	79.7, CH	79.1, CH	79.1, CH	79.3, CH
4⁗′	72.4, CH	72.4, CH	83.3, CH	72.4, CH	72.4, CH	83.3, CH
5⁗′	78.6, CH	78.6, CH	72.0, CH	78.6, CH	78.6, CH	72.2, CH
6⁗′	63.4, CH_2	63.4, CH_2	19.2, CH_3	63.4, CH_2	63.4, CH_2	19.2, CH_3
3⁗′-OMe			57.6, CH_3			57.6, CH_3

Atom	**1**	**2**	**3** D-Glc	**4**	**5**	**6** D-Glc
1⁗″			104.4, CH			104.9, CH
2⁗″			76.1, CH			76.1, CH
3⁗″			79.1, CH			79.1, CH
4⁗″			72.4, CH			72.4, CH
5⁗″			78.5, CH			78.5, CH
6⁗″			63.4, CH_2			63.4, CH_2

24 h. The mixture of compounds afforded D-glucose $[\alpha]_D^{25}$ = +50.2 (*c* 0.1, H_2O), lit. +56 (Cornelius and Mahmmoud, 2009); D-cymarose $[\alpha]_D^{25}$ = +50.1 (c. 0.1, H_2O); lit. +51.6 (Tsukamoto et al., 1986); D-oleandrose $[\alpha]_D^{25}$ = 9.8 (c. 0.1 H_2O)); lit. 10.3 (Nakagawa et al., 1983); and D-digitixose $[\alpha]_D^{25}$ = +43.3 (c. 0.1 H_2O); lit. +45 (Abe et al., 1994).

3. Results and discussion

Six new pregnane glycosides were isolated, identified and named sullivantosides A-F. The new compounds belong to a structural class that are common among the plant family Asclepiadaceae representing an important contribution to the chemotaxonomic knowledge of the *Asclepias* genus. The ecological importance of this compound collection remain to be investigated. In addition, two known cardiac glycosides 3-*O*-β-D-glucopyranosyl-(1→4)-6-desoxy-β-D-allopyranosyl uzarigenin (**7**, 80 mg), 3-*O*-β-D-glucopyranosyl-(1→4)- β-D-glucopyranosyl uzarigenin (**8**, 5 mg) as well as the lignan 9′-*O*-butyl-3-*O*-demethyl-9-*O*-β-D-glucopyranosyl dehydrodiconiferylalcohol (**9**, 6 mg) were identified. Interestingly, the occurrence of compounds **7-9** in the "common milkweed" *Asclepias syriaca* were reported by our research group (Araya et al., 2012b) (Fig. 1).

Sullivantoside A (**1**) was obtained as a white, amorphous powder. The HRESIMS displayed a $[M+Na]^+$ ion at *m/z* 1195.6150 consistent with a molecular formula of $C_{59}H_{96}NaO_{23}$ (calc. 1195.6240). The ^{1}H NMR spectrum showed three methyl-singlet signals at δ 2.24 (s, Me-21), 1.54 (s, Me-18), and 1.18 (s, Me-19). The HMBC correlations observed of these three methyl groups indicated the presence of a pregn-20-one skeleton, including correlations between proton CH_3-21 and carbon resonances at δ 214.9 (C-20) and 59.3 (C-17); CH_3-18 and carbon resonances at δ 55.5 (C-12), 78.6 (C-13), 59.3 (C-17), and 86.4 (C-14); as well as CH_3-19 and carbon resonances at δ 38.5 (C-1), 37.0 (C-10), 45.6 (C-5), and 48.5 (C-9) (Fig. 2). This steroidal core was further confirmed by the ^{1}H,^{1}H-COSY correlations (Fig. 2). Moreover, in combination with the ^{1}H,^{1}H-COSY, HSQC, and HMBC spectra, the partial structure of the aglycone portion was revealed (Tables 1–4, Experimental data). In addition, an isolated spin system based on ^{1}H, ^{1}H-COSY corresponding to a tiglate group was determined to be a substitution at C-12 by the observed HMBC correlation between H-12 (δ 5.02, m) and carbonyl resonance of the tigloyl group at δ 168.1. Hence, the planar structure of the aglycone was deduced to be 12-tigloyl-8,14-dihydroy-pregn-20-one, and the NMR data were in good agreement with literature data observed for similar compounds (Kunert et al., 2006; Kunert et al., 2008). Analysis of the ROESY spectrum of **1** revealed the correlations of H-3/H-5, H-5/H9, H9/H-12 and H-12/H-17 (Fig. 3). Therefore, the relative configuration of this aglycone could be deduced as 12-β-tigloyl-8β, 14β-dihydroxy-pregn-20-one. In addition to the signals for the aglycone, five anomeric protons were also observed in the ^{1}H NMR spectrum at δ 5.35 (dd, *J* = 8.2, 1.5, H-1‴), 5.32 (dd, *J* = 9.5, 1.7H-1′), 5.14 (d, *J* = 8.0, H-1⁗′), 5.14 (br d, *J* = 8.0, H-1″) and 4.76 (dd, *J* = 9.8, 1.5, H-1⁗) suggesting the presence of equal number of sugars. The attachment of the sugar side chain to C-3 could be deducted from the HMBC correlations from H-1′ (δ 5.32, dd, *J* = 9.5, 1.7 Hz) to C-3 (δ 76.9) and H-3 (δ 3.90, m) to C-1′ (δ 96.3). Furthermore, the identification of four methyl doublet signals in the in the ^{1}H NMR spectrum [at δ 1.68 (d, *J* = 5.3 Hz, H-6⁗), 1.44 (d, *J* = 6.1 Hz, H-6″), 1.44 (d, *J* = 6.1 Hz, H-6′), and 1.34 (d, *J* = 6.3 Hz, H-6‴]) as well as three methoxy groups [at δ 3.65 (s, C-3′-OCH_3), 3.65 (s, C-3‴-OCH_3) and 3.54(s, C-3⁗-OCH_3)], suggested the presence of three 6-deoxy-3-methoxy and one 6-deoxy sugars which commonly occur in *Asclepias* as previously described (Araya et al., 2012a). Using ^{1}H,^{1}H DQFCOSY, ^{1}H,^{1}H-TOCSY, and HSQC-TOCSY spectra, the proton spin systems and the carbon resonances allowed for the full assignment of the sugar units (Tables 2 and 4). Comparison of the NMR data with those reported in the literature revealed the identity of the five sugars as two cymaroses, oleandrose, digitoxose, and glucose (Araya et al., 2012a,b; Warashina and Noro, 2003, 2009). The chemical shifts of C-4 of the four 6-deoxysugars were extremely close at δ 83.8 (C-4′), 83.5 (C-4″), 83.6 (C-4‴), and 83.5 (C-4⁗) resulting in the overlap of HMBC correlations. Therefore, the HMBC signal between H-4 of a sugar unit and anomeric carbon from adjacent sugar were used to elucidate the connectivity as follows: cymarosyl anomeric proton H-1′ (δ 5.32, dd, *J* = 9.5, 1.7 Hz) and aglycone C-3 (δ 76.9);

Table 3
^{1}H NMR (500 MHz, C_5D_5N) data for the aglycone part of sullivantosides A-F (**1**–**6**).

Atom	**1** (*J* in Hz)	**2** (*J* in Hz)	**3** (*J* in Hz)	**4** (*J* in Hz)	**5** (*J* in Hz)	**6** (*J* in Hz)
1	1.62, m	1.62, m	1.65, m	1.76, m	1.75, m	1.75, m
	0.91, m	0.91, m	0.92, m	0.98, m	0.96, m	0.96, m
2	2.02, m	2.02, m	2.04, m	2.04, m	2.03, m	2.03, m
	1.72, m	1.72, m	1.75, m	1.77, m	1.75, m	1.75, m
3	3.90, m	3.90, m	3.92 m	3.96, m	3.92, m	3.93, m
4	1.76, m	1.75, m	1.82, m	1.83, m	1.77, m	1.77, m
	1.44, m	1.42, m	1.49, m	1.50, m	1.44, m	1.45, m
5	1.00, m	1.00, m	1.05, m	1.05 m	1.01, m	1.01, m
6	1.81, m	1.80, m	1.88, m	1.88, m	1.84, m	1.82, m
	1.10, m	1.10, m	1.18, m	1.20, m	1.12, m	1.11, m
7	2.18, m	2.17, m	2.22, m	2.22, m	2.20, m	2.20, m
	1.41, br dd (10.5, 4.3)	1.40, br dd (10.5, 4.3)	1.42, br dd	1.43, m	1.42, br dd (10.5, 4.3)	1.41, br dd (10.5, 4.3)
9	1.34, m	1.35, br d (12.8)	1.37, m	1.31, br d (13.0)	1.29, m	1.29, br d (12.7)
11	2.18, m	2.17, m	2.20, m	2.33, m	2.31, m	2.32, m
	1.92, m	1.92, m	1.94, m	1.94, m	1.93, m	1.92, m
12	5.02, m	5.01, m	5.03, m	3.74, m	3.72, m	3.74, m
15	2.10, m	2.11, m	2.12, m	2.12, m	2.11, m	2.10, m
	1.98, m	1.98, m	1.99, m	2.00, m	1.98, m	1.98, m
16	2.22, m	2.23, m	2.24, m	2.19, m	2.19, m	2.18, m
	1.99, m	1.92, m	1.94, m	1.97, m	1.96, m	1.96, m
17	3.21, dd (9.2, 5.9)	3.19, dd (9.3, 5.8)	3.21, dd (9.2, 5.8)	3.87, dd (9.3, 5.4)	3.85, dd (9.5, 5.4)	3.85, dd (9.5, 5.4)
18	1.54, s	1.54, s	1.56, s	1.57, s	1.56, s	1.56, s
19	1.18, s	1.18, s	1.21, s	1.24, s	1.21, s	1.21, s
21	2.24, s	2.24, s	2.25, s	2.30, s	2.30, s	2.29, s
	12-An	**12-An**	**12-An**			
2						
3	7.14, m	7.14, m	7.14, m			
4	1.72, br d (7.2)	1.72, br d (7.2)	1.73, br d (7.2)			
5	1.98, br s	1.98, br s	1.99, br s			

oleandrosyl proton H-4‴′ (δ 3.69, m) and glucosyl anomeric carbon C-1⁗ (δ 104.8); and digitoxyl proton H-4‴ (δ 3.45, dd, *J* = 9.5, 2.5 Hz) and oleandrosyl anomeric carbon C-1‴′ (δ 101.8). As the NMR data suggested, the glucose unit was the fifth and terminal sugar, oleandrose was attached to glucose via 1→4 glycosidic linkage, digitoxose was 1→4 linked to oleandrose and one cymarose unit was directly attached to the aglycone, leaving the connectivity of one cymarose unit to be assigned. It should be noted that the anomeric carbons of the second cymarose C-1″ (δ 100.9) unit and digitoxose C-1‴ (δ 100.9) were undistinguishable (Table 2), however, knowing the identity of the sugar units led to the following sequence: Glc-Ole-Dig-Cym-Cym-aglycone. In addition, the β-linkages of the five sugars were established by the large coupling constants (*J* = 8.0–9.8 Hz) observed for the anomeric protons. Finally, the optical rotation of the purified monomeric sugars after acid hydrolysis established the absolute configuration D for all the sugars present in this compound (see Experimental Section). Therefore, the structure of **1** was determined to be 12-*O*-tygloyl-3β, 8β, 12β,14β-tetrahydroxypregn-20-one-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-digitoxypyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranose, and named sullivantoside A.

Sullivantoside B (**2**) was obtained as a white, amorphous powder. The HRESIMS displayed a $[M+Na]^+$ ion at *m*/*z* 1181.6076 consistent with the molecular formula $C_{58}H_{94}NaO_{23}$ (calc. 1181.6084). Analysis of the ^{1}H and ^{13}C NMR data permitted the identification of the same aglycone as in **1** (*vide supra*). In addition, five sugars were identified to be present in the structure on the basis of the five anomeric protons observed at δ 5.41 (dd, *J* = 9.6, 1.6, H-1‴), 5.35 (dd, *J* = 9.5, 1.6, H-1″), 5.33 (br d, *J* = 9.5, 1.6, H-1′), 5.14 (d, *J* = 7.8, H-1⁗) and 4.74 (dd, *J* = 9.7,1.6, H-1‴′). Sugar signals in the ^{1}H and ^{13}C NMR spectra were assigned using 2D NMR spectra and the identity of these five sugars were determined to be two digitoxoses, and single units of cymarose, oleandrose, and glucose. As previously described for **1**, the connectivity of the sugars was achieved through the HMBC correlations between cymarosyl anomeric proton H-1′ (δ 5.33, dd, *J* = 9.5, 1.6) and C-3 (δ 76.9), cymarosyl proton H-4″ (δ 3.56, dd, *J* = 9.6, 2.7) and digitoxyl-1 anomeric carbon C-1″ (δ 100.9), digitoxyl-1 proton H-4″ (3.50, dd, *J* = 9.6, 2.5) and digitoxyl-2 anomeric carbon C-1‴ (δ100.3), digitoxyl-2 proton H-4‴ (δ 3.43, dd, *J* = 9.6, 2.5) and oleandrosyl anomeric carbon C-1‴′ (101.8), as well as oleandrosyl proton H-4‴′ (δ 3.67, m) and glucosyl anomeric carbon C-1⁗ (δ 104.8). These data showed that glucose unit was the terminal sugar, oleandrose was attached to glucose via 1→4 glycosidic linkage, digitoxose was 1→4 linked to oleandrose, and cymarose was directly attached to the aglycone. As described previously, although the HMBC correlations were severely overlapped, knowing the identity of the sugar units, the position of the second digitoxose unit must be placed between the cymarose and previously assigned digitoxose units. Consequently, the structure of **2** was proposed to be 12-*O*-tygloyl-3β,8β,12β,14β-tetrahydroxypregn-20-one-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-digitoxypyranosyl-(1→4)-β-D-digitoxypyranosyl-(1→4)-β-D-cymaropyranoside.

Sullivantoside C (**3**) was obtained as a white, amorphous powder. The HRESIMS displayed a $[M+Na]^+$ ion at *m*/*z* 1330.7278 consistent with the molecular formula $C_{60}H_{98}NaO_{23}$ (calc. 1330.7285). The ^{1}H and ^{13}C NMR signals of **3** were almost superimposable to those of **1**, thus it was concluded that both structures shared the same 12-*O*-tygloyl-3β,8β,12β,14β-tetrahydroxypregn-20-one steroidal skeleton. The ^{1}H and ^{13}C NMR data of the aglycone was assigned using 2D NMR spectra (Tables 1 and 2). Different to the five sugars unit of compounds **1** and **2,** six anomeric protons were observed in the ^{1}H NMR spectrum of **3** at δ 5.30 (dd, *J* = 9.7, 1.6, H-1‴), 5.16 (d, *J* = 7.8, H-1⁗′), 4.93 (dd, *J* = 9.8, 1.6, H-1″), 4.91 (dd, *J* = 9.8, 1.6, H-1‴), 4.88 (dd, *J* = 9.7, 1.5, H-1′) and 4.71 (dd, *J* = 9.7, 1.6, H-1⁗). This side chain of six sugars was determined as one cymarose, four oleandroses, and one glucose by data analysis of ^{1}H and ^{13}C NMR, ^{1}H,^{1}H-DQFCOSY, ^{1}H,^{1}H-TOCSY, and

Table 4
^{1}H NMR (500 MHz, C_5D_5N) data for the sugar moiety of sullivantosides A-F (**1**–**6**).

Atom	**1** (*J* in Hz) D-Cym	**2** (*J* in Hz) D-Cym	**3** (*J* in Hz) D-Ole	**4** (*J* in Hz) D-Cym	**5** (J in Hz) D-Cym	**6** (*J* in Hz) D-Ole
1′	5.32, dd (9.5, 1.7)	5.33, dd (9.5, 1.6)	4.88, dd (9.7, 1.5)	5.34, dd (9.5, 1.7)	5.34, dd (9.5, 1.6)	4.90, dd (9.7, 1.7)
2′	2.34, m	2.34, m	2.50, m	2.36, m	2.35, m	2.51, m
	1.92, m	1.92, m	1.84, m	1.92, m	1.94, m	1.80, m
3′	4.11, m	4.13, m	3.63, m	4.10, m	4.13, m	3.63, m
4′	3.54, dd (9.5, 2.8)	3.56, dd (9.6, 2.7)	3.54, m	3.47, dd (9.5, 2.8)	3.56, dd (9.6, 2.7)	3.54, m
5′	4.28, m	4.28, dq (9.6, 6.3)	3.63, m	4.28, dq (9.5, 6.1)	4.30, dq (9.6, 6.3)	3.62, m
6′	1.44, d (6.1)	1.42, d (6.3)	1.53, d (5.4)	1.44, d (6.1)	1.42, d (6.3)	1.48, d (5.4)
3′-OMe	3.65, s	3.65, s	3.54, s	3.65, s	3.66, s	3.54, s

Atom	**1** (*J* in Hz) D-Cym	**2** (*J* in Hz) D-Dig	**3** (*J* in Hz) D-Ole	**4** (*J* in Hz) D-Cym	**5** (J in Hz) D-Dig	**6** (*J* in Hz) D-Ole
1″	5.14, br d (8.0)	5.35, dd (9.5, 1.6)	4.93, dd (9.8, 1.6)	5.15, br d (9.5)	5.36, dd (9.5, 1.6)	4.93, dd (9.8, 1.7)
2″	2.35, m	2.43, m	2.52, m	2.36, m	2.43, m	2.52, m
	1.87, m	1.98, m	1.80, m	1.87, m	2.01, m	1.81, m
3″	4.10, m	4.65, m	3.64, m	4.10, m	4.67, m	3.60, m
4″	3.50, m	3.50, dd (9.6, 2.5)	3.54, m	3.52, dd (9.8, 2.8)	3.52, dd (9.6, 2.5)	3.55, m
5″	4.19, dq (9.2, 6.1)	4.28, dq (9.6, 6.3)	3.64, m	4.19, dq (9.8, 6.3)	4.30, dq (9.6, 6.3)	3.62, m
6″	1.44, d (6.1)	1.38, d (6.3)	1.46, d (5.4)	1.44, d (6.1)	1.39, d (6.3)	1.46, d (5.4)
3″-OMe	3.65, s		3.58, s	3.68, s		3.58, s

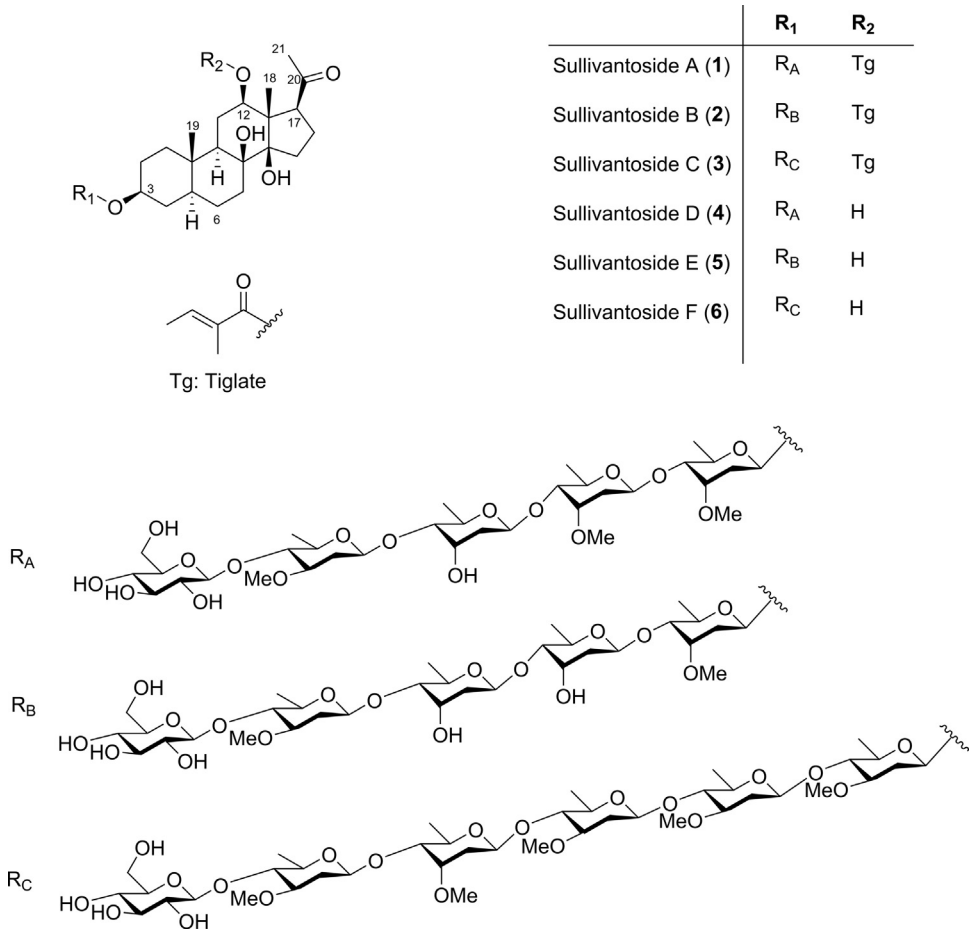
Atom	**1**(*J* in Hz) D-Dig	**2**(*J* in Hz) D-Dig	**3**(*J* in Hz) D-Ole	**4** (*J* in Hz) D-Dig	**5** (*J* in Hz) D-Dig	**6** (*J* in Hz) D-Ole
1‴	5.35, dd (8.2, 1.5)	5.41, dd (9.6, 1.6)	4.91, dd (9.8, 1.6)	5.35, dd (9.5, 1.5)	5.40, dd (9.6, 1.6)	4.93, dd (9.8, 1.7)
2‴	2.42, m	2.46, m	2.52, m	2.43, m	2.43, m	2.52, m
	1.99, m	1.98, m	1.80, m	1.97, m	2.01, m	1.81, m
3‴	4.62, m	4.66, m	3.63, m	4.63, m	4.62, m	3.60, m
4‴	3.45, dd (9.5, 2.5)	3.43, dd (9.6, 2.5)	3.54, m	3.50, dd (9.8, 2.5)	3.44, dd (9.6, 2.5)	3.55, m
5‴	4.30, dq (9.5, 6.3)	4.32, dq (9.6, 6.3)	3.64, m	4.30, dq (9.8, 6.3)	4.30, dq (9.6, 6.3)	3.62, m
6‴	1.34, d (6.3)	1.39, d (6.3)	1.47, d (5.4)	1.34, d (6.3)	1.40, d (6.3)	1.46, d (5.4)
3‴-OMe			3.58, s			3.58, s

Atom	**1** (*J* in Hz) D-Ole	**2** (*J* in Hz) D-Ole	**3** (*J* in Hz) D-Cym	**4** (*J* in Hz) D-Ole	**5** (*J* in Hz) D-Ole	**6** (*J* in Hz) D-Cym
1⁗	4.76, dd (9.8, 1.5)	4.74, dd (9.7, 1.6)	5.30, dd (9.7, 1.6)	4.76, dd (9.8, 1.5)	4.75, dd (9.7, 1.6)	5.31, dd (9.5, 1.6)
2⁗	2.48, m	2.45, m	2.32, m	2.48, ddd (12.5, 5.1, 1.6)	2.47, m	2.34, m
	1.66, m	1.63, m	1.82, m	1.66, m	1.65, m	1.83, m
3⁗	3.64, m	3.54, m	4.05, m	3.64, m	3.64, m	4.06, m
4⁗	3.69, m	3.67, m	3.45, m	3.70, m	3.68, m	3.50, m
5⁗	3.67, m	3.65, m	4.22, m	3.68, m	3.67, m	4.22, m
6⁗	1.68, d (5.3)	1.66, d (5.8)	1.41, d (6.3)	1.69, d (5.4)	1.67, d (5.8)	1.41, d (6.3)
3⁗-OMe	–	3.54, s	3.58, s		3.55, s	3.59, s

Atom	**1** (*J* in Hz) D-Glc	**2** (*J* in Hz) D-Glc	**3** (*J* in Hz) D-Ole	**4** (*J* in Hz) D-Glc	**5** (*J* in Hz) D-Glc	**6** (*J* in Hz) D-Ole
1⁗′	5.14, d (8.0)	5.14, d (7.8)	4.71, dd (9.7, 1.6)	5.16, d (8.0)	5.14, d (7.8)	4.71, dd (9.7, 1.5)
2⁗′	4.04, m	4.03, m	2.50, m	4.06, dd (8.3, 7.9)	4.01, m	2.51, m
			1.75, m			1.75, m
3⁗′	4.25, m	4.25, m	3.66, m	4.24, m	4.25, m	3.67, m
4⁗′	4.24, m	4.24, m	3.75, m	4.25, m	4.24, m	3.75, m
5⁗′	3.99, ddd (9.7, 5.3, 2.1)	3.98, ddd (9.8, 5.2, 2.7)	3.68, m	3.99, ddd (9.8, 5.4, 2.1)	3.98, ddd (9.8, 5.2, 2.7)	3.63, m
6⁗′	4.56, dd (11.4, 2.1)	4.57, dd (11.2, 2.7)	1.75, d (5.3)	4.57, dd (11.4, 2.1)	4.57, dd (11.2, 2.7)	1.53, d (5.3)
	4.38, dd (11.4, 5.3)	4.38, dd (11.2, 5.2)		4.40, dd (11.4, 5.4)	4.38, dd (11.2, 5.2)	
3⁗′-OMe			3.54, s			3.54, s

Atom	**1** (*J* in Hz)	**2** (*J* in Hz)	**3** (*J* in Hz) D-Glc	**4** (*J* in Hz)	**5** (*J* in Hz)	**6** (*J* in Hz) D-Glc
1⁗″			5.16, d (7.8)			5.16, d (7.8)
2⁗″			4.04, m			4.04, m
3⁗″			4.25, m			4.25, m
4⁗″			4.24, m			4.24, m
5⁗″			3.99, ddd (9.8, 5.4, 2.1)			3.99, ddd (9.8, 5.4, 2.1)
6⁗″			4.56, dd (11.4, 2.1)			4.57, dd (11.4, 2.1)
			4.39, dd (11.4, 5.4)			4.39, dd (11.4, 5.4)

HSQC-TOCSY spectra (Tables 2 and 4). The carbon-4 resonances of the five sugar units in the ^{13}C NMR spectrum collapsed very close to each other at δ 83.6 (C-4′), 83.6 (C-4″), 83.6 (C-4‴), 83.4 (C-4⁗), and 83.3 (C-4⁗′) making any HMBC correlation for these carbons inconclusive. Nonetheless, it was still possible to determine the sequence of the sugars by other key HMBC correlations: between olandrosyl anomeric proton H-1′ (δ 4.88) and carbon C-3 (δ 77.3) of the aglycone, the olandrosyl proton H-4⁗′ (δ 3.66, m) and the glucosyl anomeric carbon C-1⁗″ (δ 104.4), cymarosyl proton H-4⁗ (δ 4.05, m) and the oleandrosyl anomeric carbon C-1⁗′ (δ 102.2), leaving two oleandrose units to be assigned in positions 2 and 3 of the sugar moiety. In fact, the NMR data of oleandrose-



	R_1	R_2
Sullivantoside A (**1**)	R_A	Tg
Sullivantoside B (**2**)	R_B	Tg
Sullivantoside C (**3**)	R_C	Tg
Sullivantoside D (**4**)	R_A	H
Sullivantoside E (**5**)	R_B	H
Sullivantoside F (**6**)	R_C	H

Fig. 1. Structures of compounds **1–6**.

2 and oleandrose-3 units are virtually undistinguishable between one another (Tables 2 and 4). Hence, the sugar sequence was then established as Glc-Ole-Cym-Ole-Ole-Ole-aglycone. Therefore, the structure of **3** was determined to be 12-*O*-tygloyl-3β,8β,12β,14β-tetrahydroxypregn-20-one-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-d-oleandropyranose.

Sullivantoside D (**4**), an amorphous white powder, displayed an HRMS $[M+Na]^+$ ion at *m/z* 1113.5798 consistent with the molecular formula $C_{54}H_{90}NaO_{22}$ (calc. 1113.5821). Although the ^1H and ^{13}C NMR signals of **4** were similar to those of **1**, the most noticeable difference was the absence of signals for the tiglate group at C-12. Instead of the signals of δ 78.6 (C-12), δ 55.5 (C-13), and δ 86.4 (C-11) as well as the proton signal at δ 5.02 (m, H-12) in **1**, the carbon signals at δ 75.2 (C-12), 57.3 (C-13), and 27.9 (C-11) as well as the proton signal at 3.71 (m, H-12) were observed with significant shift in **4**. Aided by 2D NMR spectra, the structure of the aglycone was elucidated as 3β,8β,12β,14β-tetrahydroxypregn-20-one. In addition, five anomeric protons were identified in the ^1H NMR spectrum at δ 5.35 (dd, *J* = 9.5, 1.5, H-1‴), 5.35 (dd, *J* = 9.5, 1.5H-1′), 5.16 (d, *J* = 8.0, H-1⁗), 5.15 (br d, *J* = 9.5, H-1″) and 4.76 (dd, *J* = 9.8, 1.5, H-1⁗′). After assignment of the ^1H and ^{13}C NMR of each sugar unit using 2D NMR spectra, the sugar moiety in **4** was determined to be the same as in **1** since the chemical shifts were superimposable. Therefore, the structure of **4** was determined to be 3β,8β,12β,14β-tetrahydroxypregn-20-one-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-digitoxypyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranose

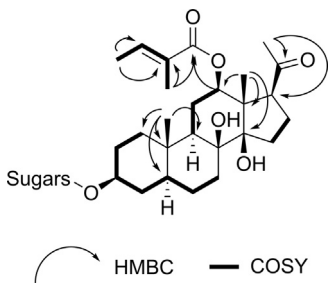


Fig. 2. Selected HMBC and $^1H,^1H$-COSY correlations observed for sullivantoside A (**1**).

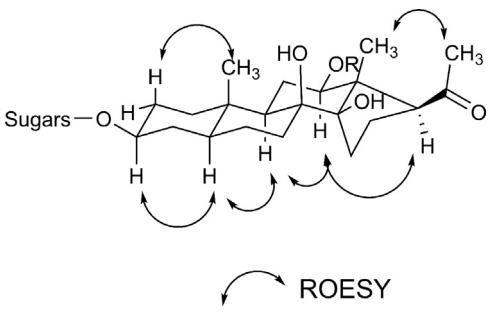


Fig. 3. Selected ROESY dipolar interactions observed for sullivantoside A (**1**).

Sullivantoside E (**5**), an amorphous white powder with a HRESIMS $[M+Na]^+$ ion at *m/z* 1199.5668 consistent with the molecular formula $C_{53}H_{88}NaO_{22}$ (calc. 1099.5665) was also found to have the same aglycone as that of compound **4** and the same sugar moiety as that of compound **2** by NMR data comparison. Consequently, compound **5** was determined to be 3β,8β,12β,14β-tetrahydroxypregn-20-one-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-digitoxypyranosyl-(1→4)-β-D-digitoxypyranosyl-(1→4)-β-D-cymaropyranoside.

Finally, sullivantoside F (**6**) was obtained as a white, amorphous powder. The HRESIMS displayed a $[M+Na]^+$ ion at *m/z* 1271.6756 consistent with the molecular formula $C_{55}H_{92}NaO_{22}$ (calc. 1271.6764). The aglycone was identified as the same as in **4** by comparison of the assigned ^1H and ^{13}C NMR data. Furthermore, the six sugars present in this compound were identical to those described for compound **3**. Hence, the identity of **6** was determined as 3β,8β,12β,14β-tetrahydroxypregn-20-one-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl (1→4)-β-D-oleandropyranose.

Supporting information

^1H NMR and ^{13}C NMR spectra of compounds **1**–**6**.

Acknowledgments

This study was supported by grant IND0061464 (awarded to B. N.T and K.K.) from the Kansas Bioscience Authority (KBA) and Center for Heartland Plant Innovations (HPI). The authors thank Q. Long, H. Loring, and M. Ferreira, botanists who worked at the University of Kansas for their assistance in plant collections and identifications. J.J.A. thanks the LASPAU-Fulbright program and the University of Costa Rica for financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2016.04.011.

References

Abe, F., Mohri, Y., Okabe, H., Yamauchi, T., 1994. Steroidal constituents from the roots and stems of *Asclepius fruiticosa*. Chem. Pharm. Bull. 42, 1777–1783.
Araya, J.J., Binns, F., Kindscher, K., Timmermann, B.N., 2012a. Verticillosides A-M: polyoxygenated pregnane glycosides from *Ascelpias verticillata* L. Phytochemistry 78, 179–189.
Araya, J.J., Kindscher, K., Timmermann, B.N., 2012b. Cardiac glycosides and other compounds from *Asclepias syriaca*. J. Nat. Prod. 75, 400–407.
Cornelius, F., Mahmmoud, Y.A., 2009. Interaction between cardiotonic steroids and Na: K-ATPase. Effects of pH and ouabain-induced changes in enzyme conformation. Biochemistry 48, 10056–10065.
Kindscher, K., 1987. Edible Wild Plants of the Prairie: An Ethnobotanical Guide. University Press of Kansas, Lawrence, Kansas.
Kindscher, K., 1992. Medicinal Wild Plants of the Prairie: an Ethnobotanical Guide. University Press of Kansas, Lawrence.
Kunert, O., Rao, B.V.A., Babu, G.S., Padmavathi, M., Kumar, B.R., Alex, R.M., Schuhly, W., Simic, N., Kuhnelt, D., Rao, A., 2006. Novel steroidal glycosides from two Indian *Caralluma* species: *C. stalagmifera* and *C. indica*. Helv. Chim. Acta 89, 201–209.
Kunert, O., Rao, V.G., Babu, G.S., Sujatha, P., Sivagamy, M., Anuradha, S., Rao, B.V.A., Kumar, B.R., Alex, R.M., Schuhly, W., Kuhnelt, D., Rao, G.V., Rao, A., 2008. Pregnane glycosides from *Caralluma adscendens* var. *fimbriata*. Chem. Biodivers. 5, 239–250.
Nakagawa, T., Hayashi, K., Wada, K., Mitsuhashi, H., 1983. Studies on the constituents of Asclepiadaceae plants. 52. The structures of 5 glycosides glaucoside-a glaucoside-b, glaucoside-c, glaucoside-d, and glaucoside e from the Chinese drug Pai-Chien *Cynanchum glaucescens* Hand-Mazz. Tetrahedron 39, 607–612.
Tsukamoto, S., Hayashi, K., Kaneko, K., Mitsuhashi, H., 1986. Studies on the constituents of Asclepiadaceae plants. 65. The optical resolution of D-cymaroses and L-cymaroses. Chem. Pharm. Bull. (Tokyo) 34, 3130–3134.
United States Department of Agriculture (USDA), 2015. Plants Database. . (accesed 11.01.15) http://plants.usda.gov/.
Warashina, T., Noro, T., 2003. Cardenolides from *Asclepias syriaca* L. Nat. Med. 57, 185–188.
Warashina, T., Noro, T., 2009. Acylated-oxypregnane glycosides from the roots of *Asclepias syriaca*. Chem. Pharm. Bull. (Tokyo) 57, 177–184.