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Six new C_{21} steroidal glycosides from *Asclepias curassavica* L.

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ABSTRACT

Six new C_{21} steroidal glycosides, named curassavosides A–F (**3–8**), were obtained from the aerial parts of *Asclepias curassavica* (Asclepiadaceae), along with two known oxypregnanes, 12-O-benzoyldeacylmetaplexigenin (**1**) and 12-O-benzoylsarcostin (**2**). By spectroscopic methods, the structures of the six new compounds were determined as 12-O-benzoyldeacylmetaplexigenin 3-O-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**3**), 12-O-benzoylsarcostin 3-O-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**4**), sarcostin 3-O-β-D-oleandropyranosyl-(1 → 4)-β-D-canaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**5**), sarcostin 3-O-β-D-oleandropyranosyl-(1 → 4)-β-D-canaropyranosyl-(1 → 4)-β-D-canaropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**6**), 12-O-benzoyldeacylmetaplexigenin 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-canaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**7**), and 12-O-benzoylsarcostin 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-canaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**8**), respectively. All compounds (**1–8**) were tested for *in vitro* cytotoxicity; only compound **3** showed weak inhibitory activity against Raji and AGZY cell lines.

1. Introduction

Asclepias curassavica is said to be a toxic plant dating from ancient times in Latin America, and is a good source of the doubly linked cardenolide glycosides [1]. Previous studies mainly focused on the host–guest–predator relationship between *A. curassavica* and butterflies and other sucking insects. The monarch butterfly (*Danaus plexippus* L.) stores these cardioactive compounds in the adult body by feeding on the *Asclepias* genus, including *A. curassavica*, as a defense substance [2–4]. This plant is used as a cancer treatment in traditional medical practice, but only limited research has been carried out concerning the cytotoxic constituents. Calotropin isolated from this plant family has been reported as a potent cytotoxic agent against KB cells (IC_{50} 15 ng/mL) [5]. The phytochemical investigations on this plant have revealed the presence of the cardenolide glycosides, doubly linked cardenolide glycosides, pregnane steroids and triterpenoids [1,6–7], but no C_{21} steroidal glycosides have been reported. In our chemical investigation of the *A. curassavica*, six new C_{21} steroidal glycosides, curassavosides A–F (**3–8**), were obtained along with two known oxypregnanes, 12-O-benzoyldeacylmetaplexigenin (**1**) and 12-O-benzoylsarcostin (**2**), and this paper deals with the isolation,

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the structure elucidation and the cytotoxicity of these new compounds.

2. Experimental

2.1. General methods

FAB mass spectra were obtained on a VG Auto spec-3000 spectrometer and high-resolution ESI mass spectra were recorded on an API Qstar Pulsar LC/TOF instrument. NMR spectra were measured in C_5D_5N and recorded on a Bruker AM-400 (for ^1H NMR and ^{13}C NMR) and DRX-500 (for 2D NMR) instrument with TMS as internal standard. IR spectra were taken in KBr on a Bio-Rad FTS-135 infrared spectrophotometer. Optical rotations were measured in a JASCO DIP-370 digital polarimeter. UV spectra were measured using a Shimadzu UV-2401PC spectrophotometer. Separation and purification were performed by column chromatography on silica gel (200–300 mesh, Qingdao), RP-18 (Merck), MPLC (Büchi Pump Module C-605, Büchi Pump manager C-615, Büchi Fraction Collector C-660) and on semi-prep HPLC using an Agilent 1100 instrument (Zorbax column 9.4 mm × 250 mm, DAD).

2.2. Plant material

The aerial parts of *Asclepias curassavica* L. were collected in September 2005 from Xishaungbannan, Yunnan Province, China and identified by Prof. Guo-Da Tao at Xishuangbannan Tropical Botanical Garden, the Chinese Academy of Sciences (CAS). A voucher specimen (No. 200508) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Chinese Academy of Sciences.

2.3. Extraction and isolation

The dried powder of the aerial parts of *A. curassavica* (5 kg) was extracted with 75% ethanol under reflux (25 × 3 L, each 3 h). After removal of the solvent under vacuum, the resulting residue was partitioned between H_2O and EtOAc, and then between H_2O and *n*-BuOH. The EtOAc extract (110 g) was separated into 15 fractions (fraction 1 to fraction 15) through MPLC by elution with a gradient mixture of petroleum ether (PE)/EtOAc (1:0 → 0:1, v/v) and EtOAc/CH_3OH (6:1 → 4:1 → 0:1, v/v). Fraction 9 (23 g, PE/EtOAc 1:1) was subjected to repeated MPLC ((1) SiO_2, $CHCl_3/CH_3OH$ (40:1 → 9:1), and PE/EtOAc (9:1); (2) RP-18, CH_3OH/H_2O (30:70 → 40:60), and then to semi-prep HPLC (CH_3OH/H_2O 40:60 → 50:50) to afford **1** (34 mg), **2** (20 mg), **3** (24 mg), and **4** (10 mg). Fraction 11 (3.9 g, PE/EtOAc 0:1) was subjected to repeated MPLC ((1) SiO_2, $CHCl_3/CH_3OH$ 15:1; (2) RP-18, CH_3OH/H_2O 70:30), then to semi-prep HPLC (CH_3OH/H_2O 60:40) to yield **5** (25 mg), **6** (18 mg), **7** (23 mg), and **8** (10 mg).

2.3.1. 12-O-Benzoyldeacylmetaplexigenin (1)

$C_{28}H_{36}O_7$; white amorphous powder; EI-MS *m/z* (%): 441 $[M-COCH_3]^+$ (18), 319 $[M-\text{benzoic acid}-COCH_3]^+$ (30), 301 (35), 283 (40), 105 $[COC_6H_5]^+$ (100). ^1H NMR (C_5D_5N, 400 MHz): δ 1.39 (3H, s, H-19), 2.06 (3H, s, H-18), 2.36 (3H, s, H-21), 3.89 (1H, m, H-3), 5.35 (1H, overlap, H-6), 5.37 (1H, d, J = 4.2 Hz, H-12), 7.48 (2H, t, J = 7.3 Hz, 4′-H, 6′-H), 7.53 (1H, t, J = 7.3 Hz, 5′-H) and 8.28 (2H, d, J = 7.3 Hz, 3′-H, 7′-H). For ^{13}C NMR data, see Table 1.

2.3.2. 12-O-Benzoylsarcostin (2)

$C_{28}H_{38}O_7$; white amorphous powder; EI-MS *m/z* (%): 450 $[M-2\times H_2O]^+$ (25), 346 $[M-2\times H_2O-\text{benzoic acid}]^+$ (30); ^1H NMR (C_5D_5N, 400 MHz): δ 1.41 (3H, s, H-19), 1.24 (3H, d, J = 7.5 Hz, H-21), 2.23 (3H, s, H-18), 3.88 (1H, m, H-3), 4.11 (1H, q, J = 6.0 Hz, H-20), 5.38 (1H, brs, H-6), 5.41 (1H, overlap, H-12), 7.39 (2H, t, J = 7.3 Hz, 4′-H, 6′-H), 7.47 (1H, t, J = 7.3 Hz, 5′-H) and 8.57 (2H, d, J = 7.3 Hz, 3′-H, 7′-H). For ^{13}C NMR data, see Table 1.

2.3.3. Curassavoside A (3)

$C_{41}H_{58}O_{13}$; white amorphous powder; $[\alpha]_D^{20}$ −42.8 (*c* 0.23, C_5H_5N); UV (C_5H_5N) λ_{max} (log ε): 201.6 (2.95), 229.6 (2.84) nm; IR (KBr) υ_{max}: 3441, 2933, 1715, 1630, 1451, 1383, 1276, 1109, 1067 cm^{-1}; negative FAB-MS *m/z*: 757 $[M-H]^-$ (13), 636 $[M-H-C_6H_5COO]^-$ (5), 121 (100); HRESI-MS (negative) *m/z*: 757.3794 $[M-1]^-$ (calcd. for $C_{41}H_{57}O_{13}$ 757.3799). For ^1H NMR and ^{13}C NMR data, see Tables 1–3.

2.3.4. Curassavoside B (4)

$C_{41}H_{60}O_{13}$; white amorphous powder; $[\alpha]_D^{20}$ −30.9 (*c* 0.08, C_5H_5N); UV (C_5H_5N) λ_{max} (log ε): 202.2 (3.06), 229.8 (3.02) nm; IR (KBr) υ_{max}: 3441, 2933, 1710, 1632, 1451, 1383, 1279, 1164, 1067 cm^{-1}; negative FAB-MS *m/z*: 759 $[M-H]^-$ (13), 638 $[M-H-C_6H_5COO]^-$ (5), 121 (100); HRESI-MS (negative) *m/z*: 759.3970 $[M-1]^-$ (calcd. for $C_{41}H_{59}O_{13}$ 759.3955). For ^1H NMR and ^{13}C NMR data, see Tables 1–3.

2.3.5. Curassavoside C (5)

$C_{47}H_{78}O_{18}$; white amorphous powder; $[\alpha]_D^{20}$ −58.6 (*c* 0.05, C_5H_5N); IR (KBr) υ_{max}: 3432, 2933, 1649, 1451, 1379, 1279, 1164, 1104, 1060 cm^{-1}; negative FAB-MS *m/z*: 930 $[M]^-$ (100), 785$[M-H-ole]^-$ (5), 655 $[M-H-ole-can]^-$ (3); HRESI-MS (negative) *m/z*: 929.5089 $[M-1]^-$ (calcd. for $C_{47}H_{77}O_{18}$ 929.5109). For ^1H NMR and ^{13}C NMR data, see Tables 1–3.

2.3.6. Curassavoside D (6)

$C_{46}H_{76}O_{18}$; white amorphous powder; $[\alpha]_D^{20}$ −27.3 (*c* 0.17, C_5H_5N); IR (KBr) υ_{max}: 3427, 2933, 1654, 1449, 1383, 1279, 1164, 1096, 1061 cm^{-1}; negative FAB-MS *m/z*: 915 $[M-H]^-$ (100), 771 $[M-H-ole]^-$ (8), 511 $[M-ole-can-can]^-$ (6); HRESI-MS *m/z*: 915.4960 $[M-1]^-$ (calcd. for $C_{46}H_{75}O_{18}$ 915.4953). For ^1H NMR and ^{13}C NMR data, see Tables 1–3.

2.3.7. Curassavoside E (7)

$C_{60}H_{90}O_{24}$; white amorphous powder; $[\alpha]_D^{20}$ −51.2 (*c* 0.21, C_5H_5N); UV (C_5H_5N) λ_{max} (log ε): 201.8 (3.25), 229.6 (3.13) nm; IR (KBr) υ_{max}: 3441, 2933, 1715, 1636, 1451, 1383, 1277, 1164, 1100, 1067 cm^{-1}; negative FAB-MS *m/z*: 1194 $[M]^-$ (15), 1072 $[M-C_6H_5COOH]^-$ (5), 121 (100); HRESI-MS (negative) *m/z*: 1193.5745 $[M-1]^-$ (calcd. for $C_{60}H_{89}O_{24}$ 1193.5743). For ^1H NMR and ^{13}C NMR data, see Tables 1–3.

2.3.8. Curassavoside F (8)

$C_{60}H_{92}O_{24}$; white amorphous powder; $[\alpha]_D^{20}$ −15.4 (*c* 0.27, C_5H_5N); UV (C_5H_5N) λ_{max} (log ε): 201.4 (3.19), 229.6 (3.08) nm; IR (KBr) υ_{max}: 3432, 2933, 1711, 1654, 1451, 1383, 1279, 1164, 1101, 1068 cm^{-1}; negative FAB-MS *m/z*: 1196 $[M]^-$ (8),

Table 1 – ^{13}C NMR spectral data of the aglycone of compounds 1–8 (δ in ppm, C_5D_5N)

Carbon	1[a]	2[a]	3[b]	4[b]	5[a]	6[a]	7[a]	8[a]
1	39.2	39.1	39.2	39.2	39.1	39.1	39.2	39.2
2	32.0	32.1	29.8	29.8	30.0	30.0	29.8	29.8
3	71.6	71.6	77.6	77.7	77.8	77.8	77.6	77.7
4	43.3	43.4	38.9	38.9	39.3	39.3	38.9	38.9
5	140.3	140.0	139.4	139.3	139.2	139.2	139.3	139.3
6	118.5	118.9	119.1	119.2	119.8	119.8	119.2	119.2
7	33.9	35.0	34.7	35.0	35.4	35.3	34.7	35.0
8	74.5	74.3	74.3	74.3	74.1	74.1	74.3	74.3
9	44.5	44.2	44.5	44.4	44.5	44.5	44.4	44.4
10	37.4	37.3	37.4	37.3	37.4	37.4	37.4	37.3
11	25.1	25.7	25.0	25.7	29.1	29.1	25.0	25.7
12	74.1	75.4	74.0	75.4	70.8	70.8	74.0	75.4
13	58.4	57.2	58.4	57.2	58.7	58.6	58.4	58.4
14	89.6	88.9	89.6	88.6	89.0	89.0	89.6	89.6
15	34.8	34.3	33.8	34.2	34.6	34.6	33.8	34.3
16	33.3	32.9	33.3	32.9	34.3	34.3	33.3	32.9
17	92.5	88.7	92.5	88.8	88.9	88.9	92.5	88.8
18	10.9	11.8	10.8	11.8	11.4	11.4	10.8	11.8
19	18.4	19.5	18.1	19.5	18.7	18.7	18.1	19.5
20	210.4	70.9	210.3	70.9	73.2	73.2	210.3	71.0
21	27.9	18.3	27.8	18.2	17.8	17.8	27.8	18.2
12-O-Bez								
1′	165.4	165.3	165.3	165.3			165.3	165.3
2′	131.2	131.3	131.3	131.7			131.2	131.2
3′	130.0	129.9	129.9	130.5			128.8	128.8
4′	128.9	128.8	128.8	128.8			129.9	129.9
5′	133.4	133.3	133.3	133.3			133.3	133.3
6′	128.9	128.8	128.8	128.8			129.9	129.9
7′	130.0	129.9	129.9	130.5			128.8	128.8

[a] Measured at 100.40 MHz.
[b] Measured at 125.65 MHz.

1074 $[M-C_6H_5COOH]^-$ (5), 121 (100); HRESI-MS (negative) *m/z*: 1195.5927 $[M-1]^-$ (calcd. for $C_{60}H_{91}O_{24}$ 1195.5900). For ^{1}H NMR and ^{13}C NMR data, see Tables 1–3.

2.4. *Cytotoxicity testing*

In vitro cytotoxicity was determined against a panel of cell lines (Raji and AGZY) according to the previously described procedures [8].

3. Results and discussion

The EtOH extract of aerial parts of *A. curassavica* was partitioned between H_2O and EtOAc. The EtOAc portion was subsequently separated on normal- and reverse-phase silica gel column chromatography and on semi-prep HPLC to provide eight compounds (**1–8**). Each of the isolates was subjected to detailed spectroscopic analysis to establish their chemical structures. Compounds **1** and **2** were elucidated to be 12-*O*-benzoyldeacylmetaplexigenin and 12-*O*-benzoylsarcostin by comparison of the ^{1}H NMR and ^{13}C NMR spectral data with the reported data [9,10] (Fig. 1).

Curassavoside A (**3**) was obtained as a white amorphous powder. The negative HRESI-MS gave an $[M-H]^-$ peak at *m/z* 757.3794, corresponding to the molecular formula $C_{41}H_{58}O_{13}$ (calcd. 757.3799). IR spectrum showed the absorption bands for hydroxyl (3441 cm^{-1}), carbonyl (1715 cm^{-1}), and olefinic (1630 cm^{-1}) groups. The ^{13}C NMR spectrum of **3** showed 41 carbon signals, consisting of two olefinic carbons, six aromatic carbons, and one ester group, as well as five methyls, one methoxyl, nine methylenes, eleven methines, and six quaternary carbons. By step-by-step comparison the NMR data of the aglycone of **3** with those of 12-*O*-benzoyldeacylmetaplexigenin (**1**), the only significant differences included the downfield shift of C-3 (δ + 6.0 ppm), and the upfield shifts of C-2 and C-4 (δ −2.2 and −4.4 ppm, respectively) owing to the glycosidation effect. Therefore, the aglycose of **3** was considered to be 12-*O*-benzoyldeacylmetaplexigenin (**1**) and the sugar moiety was linked at C-3 hydroxyl group. The NMR (^{1}H, ^{13}C NMR, DEPT, HMQC, and HMBC) spectral data of **3** showed that it contained two anomeric carbon signals at δ_C 96.4, and 101.6, correlating with anomeric protons at δ_H 5.51 (brd, J = 9.5 Hz), 4.82 (dd, J = 9.7, 1.5 Hz), respectively, which suggested that there were two sugar units in this compound. The coupling constants of anomeric proton signals indicated that **3** have two sugar units with β-linkages. On the basis of the HMQC, HMBC, and ^{1}H-^{1}H COSY experiments as well as comparison with the literature [11], the ^{1}H and ^{13}C NMR spectrum of **3** suggested that the two sugar components were assigned to one β-D-digitoxopyranose [δ_C: 96.3 (C-1^I), 39.2 (C-2^I), 67.6 (C-3^I), 83.7 (C-4^I), 68.5 (C-5^I) and 18.6 (C-6^I)] and one β-D-oleandropyranose [δ_C: 101.6 (C-1^{II}), 37.0(C-2^{II}), 81.4 (C-3^{II}), 76.2

Table 2 – ^{13}C NMR spectral data of the sugar moiety of compounds 3–8 (δ in ppm, C_5D_5N)[a]

Carbon	3[b]	4[b]	5[c]	6[c]	7[c]	8[c]
	β-D-Digit	β-D-Digit	β-D-Digit	β-D-Digit	β-D-Digit	β-D-Digit
1^{I}	96.4	96.4	96.4	96.4	96.3	96.3
2^{I}	39.2	39.2	39.3	39.2[d]	39.2	39.2
3^{I}	67.6	67.6	67.6	67.6	67.6	67.6
4^{I}	83.7	83.7	83.7	83.7	83.7	83.7
5^{I}	68.5	68.5	68.5	68.5	68.5	68.5
6^{I}	18.6	18.6	18.6[d]	18.4	18.6[d]	18.6[d]
	β-D-Ole	β-D-Ole	β-D-Ole	β-D-Ole	β-D-Ole	β-D-Ole
1^{II}	101.6	101.6	101.5	101.6[e]	101.4	101.5
2^{II}	37.0	37.0	37.4	39.3[d]	37.1[e]	37.1[e]
3^{II}	81.4	81.4	79.0	69.8[f]	79.1[f]	79.1[f]
4^{II}	76.2	76.2	83.1	87.7	83.1[g]	83.1[g]
5^{II}	72.9	72.9	71.0	71.2[g]	71.6[h]	71.6[h]
6^{II}	18.7	18.7	18.7[d]	18.2[h]	18.7[d]	18.7[d]
OMe	57.0	57.0	57.5		57.4	57.5
			β-D-Can	β-D-Can	β-D-Can	β-D-Can
1^{III}			100.4	100.3[e]	100.3	100.3
2^{III}			39.9	39.6[d]	39.9	39.9
3^{III}			70.0	69.7[f]	70.0	70.0
4^{III}			88.3	87.7	88.1	88.1
5^{III}			71.6	70.9[g]	71.0	71.0
6^{III}			18.4	18.1[h]	18.4	18.4
			β-D-Ole	β-D-Ole	β-D-Ole	β-D-Ole
1^{IV}			101.3	101.3	101.0	101.0
2^{IV}			36.9	36.8	37.4[e]	37.4[e]
3^{IV}			81.2	81.1	78.9[f]	78.9[f]
4^{IV}			75.8	75.8	82.8[g]	82.8[g]
5^{IV}			73.2	73.2	71.8[h]	71.8[h]
6^{IV}			18.2	17.8	18.2	18.2
OMe			57.2	57.2	57.5	57.5
					β-D-Glc	β-D-Glc
1^{V}					104.5	104.5
2^{V}					75.7	75.7
3^{V}					78.6	78.6
4^{V}					72.3	72.3
5^{V}					78.3	78.4
6^{V}					63.0	63.0

Digit: digitoxopyranosyl; ole: oleandropyranosyl; can: canaropyranosyl; glc: glucopyranosyl.

[a] Assignments were determined using 2D NMR spectra.

[b] Measured at 125.65 MHz.

[c] Measured at 100.40 MHz.

[d–h] Interchangeable in the same column.

(C-4^{II}), 72.9 (C-5^{II}), 18.7 (C-6^{II}) and 57.0 (OMe)]. The sugar sequence of **3** was determined by the HMBC spectrum (Fig. 2), which the correlations of δ_H 5.51 (H-1^{I} of β-digitoxopyranose) with δ_C 77.6 (C-3), and δ_H 4.82 (H-1^{I} of β-oleandropyranose) with δ_C 83.7 (C-$4^{I}$of β-digitoxopyranose) were observed. Therefore, **3** was deduced to be 12-*O*-benzoyldeacylmetaplexigenin 3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranoside.

Curassavoside B (**4**) was suggested to have the molecular formula $C_{41}H_{60}O_{13}$, based on the HRESI-MS in the negative-ion mode. The aglycone of **4** was identified as 12-*O*-benzoylsarcostin (**2**) by careful comparison with NMR spectral data. The signals of major difference in the ^{13}C NMR data between **4** and **2** occurred for C-2, C-3, and C-4, by −2.3,+6.1, and −4.5 ppm, respectively. Therefore, the sugar moiety was linked to the C-3 hydroxyl group of the aglycone. Furthermore, the NMR assignments of **4** were made unambiguously on the basis of HMQC, HMBC, and ^{1}H-^{1}H COSY experiments. According to the consistence of the ^{1}H and ^{13}C NMR data of the sugar moieties in **3** and **4**, two compounds were considered to have the same sugar sequence. Thus, the structure of **4** was determined to be 12-*O*-benzoylsarcostin 3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranoside.

Curassavoside C (**5**), with the molecular formula $C_{47}H_{78}O_{18}$, determined by HRESI-MS, showed the signals of sarcostin [12] and the glycosidation shift indicated that **5** was glycosylated at the C-3 position. In the ^{1}H and ^{13}C NMR, four anomeric carbon and proton signals at δ_C 101.5, 101.3, 100.4, 96.4 and δ_H 4.76 (brd, J = 9.2 Hz), 4.80 (brd, J = 9.5 Hz), 4.90 (brd, J = 9.8 Hz), 5.50 (brd, J = 9.5 Hz) were observed, respectively, so all the glycosidic linkages are β-configuration as judged from the coupling constants. By the HMQC, HMBC, and ^{1}H-^{1}H COSY correlations, we assignabled all the carbon and proton

Table 3 – ^{1}H NMR spectral data of compounds 3–8 (δ in ppm, J in Hz, C_5D_5N)

Proton	3[a]	4[a]	5[b]	6[a]	7[b]	8[b]
3	3.86 (m)	3.91 (m)	3.89 (m)	3.93 (m)	3.93 (m)	3.92 (m)
6	5.27 (brs)	5.37 (brs)	5.33 (brs)	5.34 (brs)	5.27 (brs)	5.27 (brs)
9	1.78 (m)	1.82 (m)	1.58 (d, 9.6)	1.62 (d, 9.5)	1.76 (m)	1.82 (m)
12	5.29 (dd, 4.0,7.6)	5.37 (brs)	3.96 (brs)	3.93 (m)	5.28 (dd, 4.0, 7.6)	5.35 (brs)
18	2.07 (s)	2.21 (s)	1.92 (s)	1.95 (s)	2.06 (s)	2.21 (s)
19	1.32 (s)	1.33 (s)	1.48 (s)	1.39 (s)	1.29 (s)	1.29 (s)
20		4.08 (q, 6.5)	4.34 (q, 5.2)	4.34 (q, 7.5)		4.08 (brs)
21	2.35 (s)	1.26 (d, 6.0)	1.48 (d, 5.2)	1.50 (d, 7.5)	2.34 (s)	1.25 (d, 6.0)
12-Bez						
3′	8.28 (d, 7.4)	8.56 (d, 7.5)			8.28 (d, 7.3)	8.56 (d, 7.4)
4′	7.47 (t, 7.4)	7.39 (t, 7.5)			7.45 (t, 7.3)	7.38 (t, 7.4)
5′	7.53 (d, 7.4)	7.47 (t, 7.5)			7.51 (t, 7.3)	7.45 (t, 7.4)
6′	7.47 (t, 7.4)	7.39 (t, 7.5)			7.45 (t, 7.3)	7.38 (t, 7.4)
7′	8.28 (d, 7.4)	8.56 (d, 7.5)			8.28 (d, 7.3)	8.56 (d, 7.4)
	β-D-Digit	β-D-Digit	β-D-Digit	β-D-Digit	β-D-Digit	β-D-Digit
1^I	5.51(brd, 9.5)	5.52 (brd, 9.0)	5.50 (brd, 9.5)	5.50 (brd, 9.2)	5.51 (brd, 9.5)	5.51 (brd, 9.5)
2^I	2.12 (m), 2.46 (m)	2.12 (m), 2.46 (m)	2.06 (m) 2.43 (m)[c]	2.05 (m), 2.46 (m)[c]	2.06 (m), 2.42 (m)[c]	2.09 (m), 2.45 (m)[c]
3^I	4.70 (brs)	4.68 (brs)	4.64 (dd, 2, 9.5)	4.66 (brs)	4.63 (brs)	4.65 (brs)
4^I	3.59 (m)[c]	3.54 (m)[c]	3.56 (m)[d]	3.53 (m)[d]	3.52 (m)[d]	3.55 (m)[d]
5^I	4.38 (m)	4.38 (m)	4.33 (m)	4.34 (m)	4.33 (m)	4.33 (m)
6^I	1.49 (d, 7.0)	1.50 (d, 7.0)	1.47 (d, 7.0)	1.44 (d, 7.0)	1.46 (d, 7.0)	1.47 (d, 7.0)
	β-D-Ole	β-D-Ole	β-D-Ole	β-D-Ole	β-D-Ole	β-D-Ole
1^{II}	4.82 (dd, 9.7, 1.5)	4.81 (brd, 9.7)	4.76 (brd, 9.2)	4.82 (brd, 9.6)	4.72 (brd, 9.5)	4.76 (brd, 9.5)
2^{II}	1.68 (m), 2.55 (m)	1.67 (m), 2.54 (m)	1.70 (m), 2.57 (m)[e]	1.95 (m)[e], 2.56 (m)[f]	1.73 (m), 2.54 (m)[e]	1.69 (m), 2.48 (m)
3^{II}	3.66 (m)	3.69 (m)	3.64 (m)[f]	3.94 (m)[g]	3.62 (m)	3.66 (m)
4^{II}	3.44 (m)	3.45 (m)	3.43 (m)	3.23 (t, 9.2)	3.42 (t, 11.0)	3.44 (dd, 8.9,11.0)
5^{II}	3.57 (m)[c]	3.53 (m)[c]	3.51 (m)	3.64 (m)	3.70 (m)[f]	3.71 (m)[e]
6^{II}	1.51 (d, 6.5)	1.57 (d, 6.5)	1.33 (d, 6.5)	1.38 (d, 6.5)	1.31 (d, 6.5)	1.35 (d, 6.5)
OMe	3.45 (s)	3.45 (s)	3.49 (s)		3.53 (s)	3.55 (s)
			β-D-Can	β-D-Can	β-D-Can	β-D-Can
1^{III}			4.90 (brd, 9.8)	4.84 (brd, 9.6)	4.91 (brd, 9.8)	4.94 (brd, 9.7)
2^{III}			1.91 (m), 2.57 (m)[e]	1.91 (m)[e], 2.54 (m)[f]	1.89 (m), 2.55 (m)[e]	1.93 (m), 2.55 (m)
3^{III}			3.94 (m)	3.94 (m)[g]	3.95 (m)[g]	3.94 (m)
4^{III}			3.30 (t, 8.6)	3.26 (t, 8.8)	3.23 (m)	3.25 (t, 8.7)
5^{III}			3.56 (m)[d]	3.54 (m)[d]	3.49 (m)	3.51 (m)
6^{III}			1.35 (d, 6.5)	1.34 (d, 6.5)	1.37 (d, 6.5)	1.37 (d, 6.5)
			β-D-Ole	β-D-Ole	β-D-Ole	β-D-Ole
1^{IV}			4.80 (brd, 9.5)	4.79 (brd, 9.5)	4.72 (brd, 9.5)	4.76 (brd, 9.5)
2^{IV}			1.66 (m), 2.45 (m)[c]	1.72 (m), 2.43 (m)[c]	1.89 (m), 2.44 (m)[c]	1.80 (m), 2.45 (m)[c]
3^{IV}			3.54 (m)[d]	3.54 (m)[d]	3.52 (m)[d]	3.55 (m)[d]
4^{IV}			3.47 (m)	3.46 (m)	3.70 (m)[f]	3.71 (m)[e]
5^{IV}			3.66 (m)[f]	3.70 (m)	3.74 (m)	3.73 (m)
6^{IV}			1.48 (d, 6.5)	1.50 (d, 6.5)	1.64 (brs)	1.65 (brs)
OMe			3.46 (s)	3.46 (s)	3.49 (s)	3.51 (s)
					β-D-Glc	β-D-Glc
1^V					5.12 (d, 7.5)	5.07 (d, 7.7)
2^V					3.97 (m)[g]	3.98 (t, 8.3)
3^V					4.23 (overlap)	4.22 (t, 8.8)
4^V					4.21 (overlap)	4.16 (t, 9.0)
5^V					3.95 (m)[g]	3.96 (m)
6^V					4.51 (d, 11.0), 4.30 (m)	4.54 (d, 11.2) 4.35 (m)

Digit: digitoxopyranosyl; ole: oleandropyranosyl; can: canaropyranosyl; glc: glucopyranosyl.

[a] Measured at 500 MHz.

[b] Measured at 400 MHz.

[c–g] Interchangeable in the same column.

signals of the sugar moieties and the four sugar units were identified as β-D-oleandropyranose, β-D-canaropyranose, β-D-oleandropyranose, β-D-digitoxopyranose, respectively, by comparison of their ^{1}H and ^{13}C NMR data with those in the literature [11]. The sugar sequence of compound **5** was confirmed by the HMBC spectrum, which showed long-range correlations between (i) H-1^I of the β-D-digitoxopyranosyl (δ_H 5.50) and C-3 of the aglycone (δ_C

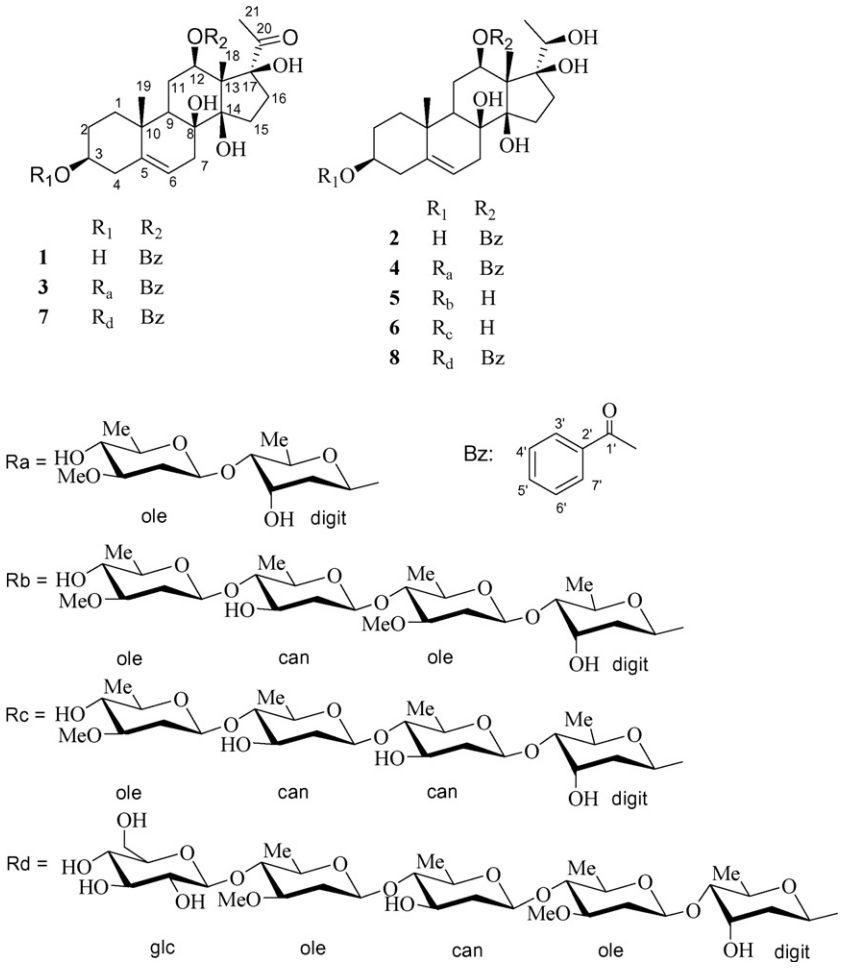

Fig. 1 – The chemical structures of compounds 1–8.

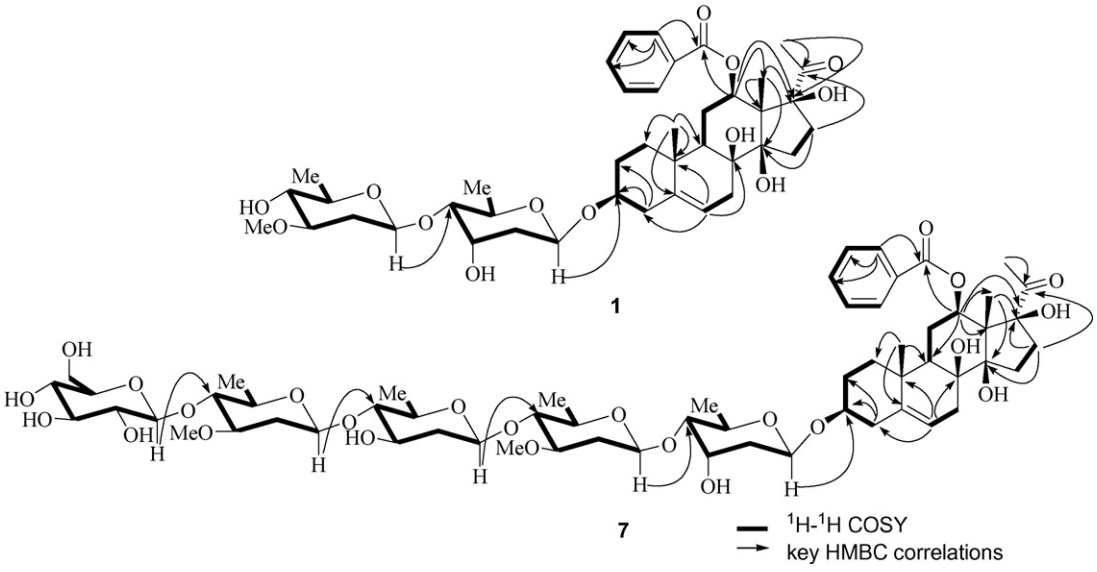

Fig. 2 – Structure units from ^{1}H-^{1}H COSY NMR spectra and key HMBC correlations of 1 and 7.

77.8); (ii) H-1^{II} of the β-D-oleandropyranosyl (δ_H 4.76) and C-4^{I} of the β-D-digitoxopyranosyl (δ_C 83.7); (iii) H-1^{III} of the β-D-canaropyranosyl (δ_H 4.90) and C-4^{II} of the β-D-oleandropyranosyl (δ_C 83.1); and (iv) H-1^{IV} of the β-D-oleandropyranosyl (δ_H 4.80) and C-4^{III} of the β-D-canaropyranosyl (δ_C 88.3). Thus, the sequence and linkage sites of the sugar units were established as ole-(1 → 4)-can-(1 → 4)-ole-(1 → 4)-dig-(1 → 3)-aglycone. Based on the above evidence, **5** was proved to be sarcostin 3-O-β-D-oleandropyranosyl-(1 → 4)-β-D-canaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranoside.

Curassavoside D (**6**) was established to have a molecular formula of $C_{46}H_{76}O_{18}$ by HRESI-MS (found 915.4960 $[M-H]^-$, calcd. 915.4953). The mass spectrum indicated that compound **6** was 14 mass units less than compound **5**. The ^1H and ^{13}C NMR spectra showed the signals of sarcostin and four monosaccharides the same as **5**, but the signal for one methoxyl of one sugar moiety disappeared. Based on the HMQC, HMBC, and ^1H-^1H COSY experiments, the four sugars, which four anomeric carbon signals at δ_C 101.6, 101.3, 100.3, 96.4 ppm were observed, were determined as one β-D-oleandropyranose, two β-D-canaropyranose, one β-D-digitoxopyranose and the inner β-D-oleandropyranose in **5** was replaced with β-D-canaropyranose in **6** by the result of correlation between H-1^{II} of the β-D-canaropyranose (δ_H 4.82) and C-4^{I} of the β-D-digitoxopyranosyl (δ_C 83.7) in HMBC experiment. Therefore, **6** was tentatively elucidated as sarcostin 3-O-β-D-oleandropyranosyl-(1 → 4)-β-D-canaropyranosyl-(1 → 4)-β-D-canaropyranosyl-(1 → 4)-β-D-digitoxopyranoside.

The molecular formula of curassavoside E (**7**) was determined as $C_{60}H_{90}O_{24}$ by HRESI-MS. The aglycone moiety was identified as 12-O-benzoyldeacylmetaplexigenin by comparing the spectroscopic data with compound **1** and the glycosidation shift of the aglycone carbon signals was observed at the C-3 (+6 ppm), C-2 (−2.2 ppm) and C-4 (−4.4 ppm) positions, indicating that the sugar chain was attached at the C-3 position. Five anomeric carbons were observed (δ_C 104.5, 101.4, 101.0, 100.3, and 96.3) revealing the presence of five sugar residues. By comparing the spectroscopic data of the sugar moiety in **7** with **5**, the structure of the sugar moiety in **7** corresponded to **5** except that one more β-D-glucopyranose was linked with the β-D-oleandropyranose. In the HMBC (Fig. 2), the long-range correlations between H-1^{V} of the β-D-glucopyranosyl (δ_H 5.12, d, J = 7.5 Hz) and C-4^{IV} of the β-D-oleandropyranosyl (δ_C 82.8) was observed. Thus, **7** was tentatively established as 12-O-benzoyldeacylmetaplexigenin 3-O-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1 → 4)-β-D-canaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranoside.

The negative HRESI-MS revealed the molecular formula of curassavoside F (**8**) was $C_{60}H_{92}O_{24}$. According to the consistence of the ^1H and ^{13}C NMR data of the sugar moieties in **7** and **8**, two compounds were considered to have the same sugar sequence. The aglycone moiety was identified as 12-O-benzoylsarcostin by comparing the spectroscopic data with compound **2** and the sugar chain was also attached at the C-3 position. Accordingly, **8** was tentatively elucidated as 12-O-benzoylsarcostin 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-canaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranoside.

The cytotoxic activity of all compounds (**1–8**) were evaluated against Raji and AGZY cell lines. However, only curassavoside A (**3**) exhibited weak activity against the above cell lines with IC_{50} value of 15.47 and 26.83 μg/ml, respectively. Other compounds were completely inactive in the selected cell lines with $IC_{50} > 100$ μg/ml.

4. Conclusion

Present phytochemical investigation has resulted in isolation and characterization of six new oxypregnane glycosides. To the best of our knowledge, this is the first report of oxypregnane glycosides having been isolated from *A. curassavica*, which is used as a cancer treatment in traditional medical practice. However, the cytotoxic experiment showed that only curassavoside A (**3**) exhibited weak cytotoxicity against Raji and AGZY cell lines. The above results and the literatures [5,6] suggest that the doubly linked cardenolide glycosides, such as calotropin, calactin, and asclepin, maybe are the major cytotoxic constituents of *A. curassavica*.

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