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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),