Cardenolides and Cardiac Aglycone from the Stem Bark of *Trewia nudiflora*

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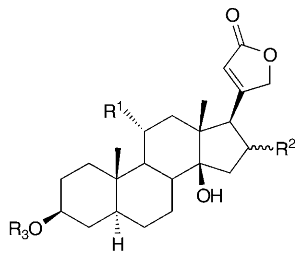
Five new cardenolides and one new cardiac aglycone, *i.e.*, (5α)-sarmentogenin 3-(α-L-rhamnopyranoside) (**1**), (5α)-sarmentogenin (**2**), 11-oxouzarigenin 3-(α-L-rhamnopyranoside) (**3**), (5α)-gitoxigenin 3-(α-L-rhamnopyranoside) (**4**), (5α)-oleandrigenin 3-(α-L-rhamnopyranoside) (**5**), and (5α)-oleandrigenin 3-[β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranoside] (**6**), together with two known cardenolides, *i.e.*, frugoside (=(3β,5α)-3-[(6-deoxy-β-D-allopyranosyl)oxy]-14,19-dihydroxycard-20(22)-enolide) and (17α)-ascleposide (=(3β,5α,17α)-3-[(6-deoxy-α-D-allopyranosyl)oxy]-14-hydroxycard-20(22)-enolide), were isolated from the stem bark of *Trewia nudiflora* L. (Euphorbiaceae) collected in Xishuangbanna, Yunnan Province, China. Their structures were established by spectroscopic studies. Cardenolides were first found in the genus *Trewia* (Euphorbiaceae).

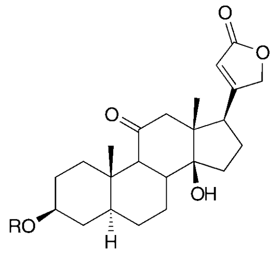
1. Introduction. – *Trewia nudiflora* L. (Euphorbiaceae) is a tropical plant mainly distributed in India, Malaysia, and the south of China [1], and has been used in folk herb medicines [2]. The seed of *T. nudiflora* is rich in trewiasine and a series of new maytansinoids [3–5]. The seed also contains highly unusual glyceride oil [6], several novel *ent*-atisane diterpenes [7], and pyridinone alkaloids [2]. The occurrence of cardenolides in *T. nudiflora* has not been reported previously. During our investigation on the chemical constituent of *T. nudiflora*, however, a cardiac aglycone and seven cardenolides were isolated from the stem bark. This paper describes the isolation and structural elucidation of these compounds.

2. Results and Discussion. – The AcOEt-soluble fraction of the 85% EtOH extraction from the stem bark of *T. nudiflora* was successively chromatographed over silica gel, *Sephadex LH-20* and *RP-18* to afford the new compounds **1**–**6** besides the known cardenolides frugoside (=(3β,5α)-3-[(6-deoxy-β-D-allopyranosyl)oxy]-14,19-dihydroxycard-20(22)-enolide) and (17α)-ascleposide (=(3β,5α,17α)-3-[(6-deoxy-α-D-allopyranosyl)oxy]-14-hydroxycard-20(22)-enolide). The new compounds were very similar to each other according to their ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*).

Compound **1** was isolated as an amorphous powder. Its molecular formula was determined to be $C_{29}H_{44}O_9$ by ^{13}C-DEPT-NMR and negative-ion FAB-MS (*m/z* 535 $[M-H]^-$). The ^{1}H-NMR spectra of **1** revealed the presence of characteristic signals for cardenolide: Further spectral data (^{13}C-NMR (*Table 2*), ROESY, HMBC, HMQC, ^{1}H,^{1}H-COSY) yielded sufficient data to define the structure of **1** as (5α)-sarmentogenin 3-(α-L-rhamnopyranoside) (**1**).



1 R^1 = OH R^2 = H R^3 = Rha
2 R^1 = OH R^2 = H R^3 = H
4 R^1 = H R^2 = OH R^3 = Rha
5 R^1 = H R^2 = MeCOO R^3 = Rha
6 R^1 = H R^2 = MeCOO R^3 = Rha4Glc



3 R = Rha

In the ^{1}H-NMR of **1** (*Table 1*), two angular Me groups at δ(H) 0.98 and 1.10 (2*s*), a CH_2(21) moiety at δ(H) 5.25 and 5.30, and an olefinic proton H−C(22) at δ(H) 6.32 were characteristisc of a cardenolide [8]. The ^{13}C-NMR DEPT spectrum (*Table 2*) showed 29 signals, including 3 Me, 9 CH_2, 12 CH, and 5 quaternary C-atoms (including one lactonic C=O). The ^{1}H- and ^{13}C-NMR spectra of **1** were very similar to those of affinoside S-IX [8], except for the signal of C(19) shifted upfield to δ(C) 12.5. Comparing the ^{13}C-NMR data of **1** with those of affinoside S-IX and uzarigenin 3-sulfate [9] suggested that H−C(5) of **1** was located on the α-side. In addition, the ROSEY plot showed the ^{1}H,^{1}H correlations δ(H) 4.07–4.10 (H−C(11))/1.10 (Me(18)) and 0.98 (Me(19)), suggesting that H−C(11) was on the β-side (*Fig.*). The ^{1}H,^{13}C-HMBC spectra exhibited along-range correlation of the anomeric H−C(1′) (δ(H) 5.51) with C(3) (δ(C) 75.8), indicating that the sugar unit was linked to C(3) (*Fig.*). The ^{13}C-NMR data of the sugar moiety revealed the presence of a substituted α-L-rhamnopyranose unit from the signals at δ(C) 99.5 (C(1′)), 72.9 (C(2′)), 73.0 (C(3′)), 74.2 (C(4′)), 69.8 (C(5′)), and 18.7 (C(6′)), and the α-L-configuration was deduced from the anomeric H−C(1′) signal at δ(H) 5.51 with a *J* of 1.0 Hz and the C(5′) signal at δ(C) 69.8 [10][11].

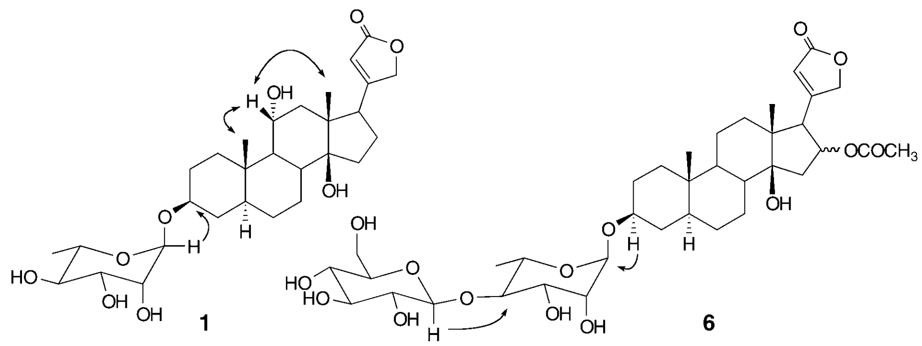


Figure. *Key HMBC* (→) *and NOESY* (↔) *correlations for* **1** *and* **6**

Compound **2** was obtained as white powder. Its molecular formula was deduced as $C_{23}H_{34}O_5$ on the basis of ^{13}C-DEPT-NMR and positive-ion EI-MS data, which showed a peak at *m*/*z* 372 ($[M-H_2O]^+$). The ^{1}H- and ^{13}C-NMR data of **2** (*Tables 1* and *2*) were similar to those of uzarigenin 3-sulfate [9] and the aglycone moiety of **1**. So the structure of **2** was determined to be (5α)-sarmentogenin (**2**).

Table 1. *^{1}H-NMR Data* for *Compounds* **1**–**8** δ in ppm, J in Hz.

	1[a][b]	**2**[a][b]	**3**[a][c]	**4**[c][d]	**5**[c][d]	**6**[c]
$CH_2(1)$	1.41–1.48 (*m*), 3.10–3.14 (*m*)	1.45–1.52 (*m*), 3.14–3.18 (*m*)	1.06–1.11 (*m*), 2.59–2.61 (*m*)	1.00–1.05 (*m*), 1.66–1.69 (*m*)	0.85–0.93 (*m*), 1.75–1.79 (*m*)	1.05–1.12 (*m*), 1.76–1.79 (*m*)
$CH_2(2)$	1.68–1.74 (*m*), 2.03–2.08 (*m*)	1.60–1.66 (*m*), 2.02–2.06 (*m*)	1.64–1.69 (*m*), 1.96–1.98 (*m*)	1.32–1.39 (*m*), 1.59–1.55 (*m*)	1.28–1.36 (*m*), 1.52–1.55 (*m*)	1.36–1.40 (*m*), 1.53–1.58 (*m*)
H–C(3)	3.80–3.88 (*m*)	3.92–3.96 (*m*)	3.74–3.80 (*m*)	3.54–3.57 (*m*)	3.31–3.35 (*m*)	3.57–3.62 (*m*)
$CH_2(4)$	1.26–1.33 (*m*), 1.65–1.73 (*m*)	1.23–1.29 (*m*), 1.64–1.70 (*m*)	1.18–1.24 (*m*), 1.64–1.69 (*m*)	1.26–1.30 (*m*), 1.50–1.55 (*m*)	1.23–1.29 (*m*), 1.52–1.55 (*m*)	1.21–1.25 (*m*), 1.53–1.58 (*m*)
H–C(5)	1.05–1.10 (*m*)	1.06–1.11 (*m*)	0.89–0.94 (*m*)	1.03–1.08 (*m*)	0.83–0.90 (*m*)	1.07–1.12 (*m*)
$CH_2(6)$	1.14–1.19 (*m*), 1.28–1.33 (*m*)	1.33–1.38 (*m*), 1.16–1.23 (*m*)	1.11–1.19 (*m*), 1.29–1.33 (*m*)	–	–	–
$CH_2(7)$	1.17–1.22 (*m*), 2.36–2.39 (*m*)	1.16–1.23 (*m*), 2.38–2.40 (*m*)	1.29–1.33 (*m*), 2.36–2.39 (*m*)	–	–	–
H–C(8)	1.78–1.84 (*m*)	1.81–1.87 (*m*)	2.19–2.23 (*m*)	1.49–1.55 (*m*)	1.52–1.55 (*m*)	1.53–1.58 (*m*)
H–C(9)	1.28–1.33 (*m*)	1.33–1.38 (*m*)	1.96–1.98 (*m*)	1.03–1.08 (*m*)	0.87–0.95 (*m*)	0.95–1.04 (*m*)
H–C(11) or $CH_2(11)$	4.07–4.10 (*m*)	4.13–4.16 (*m*)	–	0.86–0.94 (*m*), 1.32–1.38 (*m*)	0.85–0.90 (*m*), 1.28–1.36 (*m*)	0.86–0.91 (*m*), 1.32–1.40 (*m*)
$CH_2(12)$	1.84–1.92 (*m*), 1.92–1.97 (*m*)	1.84–1.89 (*m*), 1.92–1.97 (*m*)	2.19–2.23 (*m*), 2.46–2.48 (*m*)	1.32–1.38 (*m*), 1.49–1.55 (*m*)	1.28–1.36 (*m*), 1.52–1.55 (*m*)	1.32–1.40 (*m*), 1.53–1.58 (*m*)
$CH_2(15)$	1.89–1.96 (*m*), 2.17–2.22 (*m*)	1.89–1.96 (*m*), 2.13–2.21 (*m*)	2.05–2.11 (*m*), 2.36–2.39 (*m*)	1.76–1.82 (*m*), 2.53–2.57 (*m*)	1.75–1.79 (*m*), 2.66–2.72 (*m*)	1.76–1.79 (*m*), 2.69–2.75 (*m*)
H–C(16) or $CH_2(16)$	1.92–1.97 (*m*), 2.03–2.08 (*m*)	1.94–1.20 (*m*), 2.04–2.10 (*m*)	2.05–2.11 (*m*), 2.05–2.11 (*m*)	4.59–4.63 (*m*)	5.44–5.48 (*m*)	5.44–5.46 (*m*)
H–C(17)	2.90–2.93 (*m*)	2.91–2.94 (*m*)	2.73–2.74 (*m*)	3.30–3.34 (*m*)	3.12–3.18 (*m*)	3.20–3.25 (*m*)
Me(18)	1.10 (*s*)	1.06 (*s*)	1.03 (*s*)	0.82 (*s*)	0.83 (*s*)	0.94 (*s*)
Me(19)	0.98 (*s*)	1.11 (*s*)	1.03 (*s*)	0.92 (*s*)	0.89 (*s*)	0.83 (*s*)
$CH_2(20)$	5.25 (*dd*, J = 18.1, 1.5), 5.30 (*dd*)	5.26 (br. *s*), 5.30 (br. *s*)	5.00 (*dd*, J = 18.1, 1.4), 5.17 (*dd*)	5.11 (*dd*, J = 17.3, 1.6), 5.14 (*dd*)	4.82 (*dd*, J = 16.0, 1.6), 4.96 (*dd*)	4.81 (br. *s*), 4.98 (br. *s*)
H–C(22)	6.32 (*s*)	6.10 (*s*)	6.14 (s)	5.93 (*s*)	5.97 (*s*)	5.97 (*s*)
Ac					1.93 (*s*)	1.93 (*s*)
Sugar moieties						
H–C(1′)	5.51 (*d*, J = 1.0)		5.48 (br. *s*)	4.82 (*d*, J = 1.3)	4.83 (br. *s*)	4.82 (br. *s*)
H–C(2′)	4.51 (*d*, J = 3.4)		4.50–4.53 (*m*)	3.72–3.73 (*m*)	3.27–3.31 (*m*)	3.25–3.31 (*m*)
H–C(3′)	4.55 (*d*, J = 4.8)		4.50–4.53 (*m*)	3.55–3.57 (*m*)	3.73 (*d*, J = 1.6)	3.84–3.87 (*m*)
H–C(4′)	4.30–4.35 (*m*)		4.28 (br. *s*)	3.30 (br. *s*)	3.62–3.64 (*m*)	3.57–3.62 (*m*)
H–C(5′)	4.29–4.33 (*m*)		4.28 (br. *s*)	3.62–3.65 (*m*)	3.62–3.64 (*m*)	3.68–3.71 (*m*)
Me–C(6′)	1.66 (*d*, J = 5.6)		1.68 (*d*, J = 5.3)	1.22 (*d*, J = 6.3)	1.24 (*d*, J = 6.3)	1.32 (*d*, J = 6.1)
H–C(1″)						4.57 (*d*, J = 7.8)
H–C(2″)						3.20–3.28 (*m*)
H–C(3″)						3.20–3.28 (*m*)
H–C(4″)						3.69 (br. *s*)
H–C(5″)						3.34–3.37 (*m*)
$CH_2(6″)$						3.57–3.62 (*m*), 3.84–3.87 (*m*)

[a]) In (D_5)pyridine. [b]) At 400 MHz. [c]) At 500 MHz. [d]) In MeOD.

Table 2. *^{13}C-NMR Data for Compounds* **1**–**8**. δ in ppm.

	1[a][b]	**2**[a][b]	**3**[a][c]	**4**[c][d]	**5**[c][d]	**6**[b][d]
C(1)	39.6 (*t*)	39.8 (*t*)	36.6 (*t*)	38.3 (*t*)	38.2 (*t*)	38.2 (*t*)
C(2)	30.3 (*t*)	32.9 (*t*)	29.6 (*t*)	30.4 (*t*)	30.8 (*t*)	30.3 (*t*)
C(3)	75.8 (*d*)	70.5 (*d*)	75.5 (*d*)	77.3 (*d*)	77.2 (*d*)	77.3 (*d*)
C(4)	35.2 (*t*)	33.6 (*t*)	34.1 (*t*)	35.2 (*t*)	35.1 (*t*)	35.1 (*t*)
C(5)	45.1 (*d*)	45.6 (*d*)	44.5 (*d*)	45.6 (*d*)	45.5 (*d*)	45.4 (*d*)
C(6)	29.7 (*t*)	29.8 (*t*)	28.6 (*t*)	29.9 (*t*)	29.8 (*t*)	29.8 (*t*)
C(7)	28.4 (*t*)	28.5 (*t*)	28.6 (*t*)	28.6 (*t*)	28.3 (*t*)	28.3 (*t*)
C(8)	41.4 (*d*)	41.4 (*d*)	43.1 (*d*)	42.7 (*d*)	42.5 (*d*)	42.5 (*d*)
C(9)	55.7 (*d*)	55.8 (*d*)	60.3 (*d*)	51.0 (*d*)	50.8 (*d*)	50.8 (*d*)
C(10)	37.9 (*s*)	37.9 (*s*)	36.2 (*s*)	37.0 (*s*)	36.9 (*s*)	36.9 (*s*)
C(11)	67.8 (*d*)	67.9 (*d*)	209.6 (*d*)	22.0 (*d*)	22.0 (*t*)	21.9 (*t*)
C(12)	50.3 (*t*)	50.7 (*t*)	55.1 (*t*)	40.9 (*t*)	39.9 (*t*)	39.9 (*t*)
C(13)	51.2 (*s*)	50.3 (*s*)	53.3 (*s*)	51.3 (*s*)	51.4 (*s*)	51.4 (*s*)
C(14)	84.2 (*s*)	84.2 (*s*)	83.4 (*s*)	85.5 (*s*)	84.8 (*s*)	83.6 (*s*)
C(15)	33.6 (*t*)	32.9 (*t*)	33.5 (*t*)	43.7 (*t*)	41.3 (*t*)	41.3 (*t*)
C(16)	27.3 (*t*)	27.3 (*t*)	27.1 (*t*)	73.1 (*d*)	75.9 (*d*)	76.1 (*d*)
C(17)	50.6 (*d*)	51.3 (*d*)	50.2 (*d*)	59.6 (*d*)	57.4 (*d*)	57.3 (*d*)
C(18)	17.3 (*q*)	17.6 (*q*)	17.7 (*q*)	17.1 (*q*)	16.4 (*q*)	16.4 (*q*)
C(19)	12.5 (*q*)	12.7 (*q*)	12.5 (*q*)	12.5 (*q*)	12.5 (*q*)	12.5 (*q*)
C(20)	175.5 (*s*)	175.5 (*s*)	174.2 (*s*)	173.6 (*s*)	171.6 (*s*)	171.6 (*s*)
C(21)	73.8 (*t*)	73.8 (*t*)	73.6 (*t*)	77.8 (*t*)	77.5 (*t*)	77.6 (*t*)
C(22)	117.8 (*d*)	117.8 (*d*)	118.2 (*d*)	120.6 (*d*)	121.8 (*d*)	121.8 (*d*)
C(23)	174.5 (*s*)	174.5 (*s*)	173.8 (*s*)	177.3 (*s*)	172.1 (*s*)	172.1 (*s*)
Ac					176.5 (*s*), 20.9 (*q*)	176.8 (*s*), 20.9 (*q*)
Sugar moieties						
C(1′)	99.5 (*d*)		99.5 (*d*)	99.7 (*d*)	99.6 (*d*)	99.5 (*d*)
C(2′)	72.9 (*d*)		72.9 (*d*)	72.5 (*d*)	72.4 (*d*)	71.4 (*d*)
C(3′)	73.0 (*d*)		72.9 (*d*)	72.8 (*d*)	72.8 (*d*)	72.4 (*d*)
C(4′)	74.2 (*d*)		74.2 (*d*)	74.5 (*d*)	74.1 (*d*)	83.6 (*d*)
C(5′)	69.8 (*d*)		69.9 (*d*)	69.9 (*d*)	69.8 (*d*)	68.5 (*d*)
C(6′)	18.7 (*q*)		18.7 (*q*)	18.0 (*q*)	18.0 (*q*)	18.1 (*q*)
C(1″)						105.7 (*d*)
C(2″)						75.9 (*d*)
C(3″)						78.1 (*d*)
C(4″)						72.6 (*d*)
C(5″)						78.1 (*d*)
C(6″)						62.7 (*t*)

[a]) In (D_5)pyridine. [b]) At 100 MHz. [c]) At 125 MHz. [d]) In MeOD.

Compound **3**, colorless crystals (from MeOH), had the molecular formula $C_{29}H_{42}O_9$ as determined by HR-ESI-MS (*m/z* 557.2735 ([*M*+Na]$^+$). The ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*), HMQC, HMBC, and ^{1}H,^{1}H-COSY data and comparison with those of **1** determined compound **3** to be 11-oxouzarigenin 3-(α-L-rhamnopyranoside) (**3**).

The ^{13}C-NMR spectra of **3** showed 29 peaks. Its ^{1}H- and ^{13}C-NMR data were similar to those of **1**, except for the presence of a carbonyl signal at δ(C) 209.6 (C(11)). The HMBC correlation δ(H) 2.46–2.48 (H−C(12))/δ(C) 209.6 (C(11)) supported that OH−C(11) of **1** was oxidized to a C=O group in **3**. In addition, the HMBC correlations between the anomeric H−C(1′) of **3** at δ(H) 5.48 and C(3) at δ(C) 75.5 (*d*) confirmed that the sugar moiety was attached to C(3), and the sugar unit of **3** was the same as in **1**.

Compound **4**, isolated as a white powder, was established to have the molecular formula $C_{29}H_{44}O_9$ by FAB-MS (*m/z* 535 $[M-H]^-$) and ^{13}C-DEPT-NMR data. The ^{1}H- and ^{13}C-NMR spectra of **4** (*Tables 1* and *2*) were very similar to those of **1**, except for an OH group that was linked to C(16) in **4** by comparison with **1** and strospeside [12], a gitoxigenin glycoside. So the structure of **4** was determined to be (5*α*)-gitoxigenin 3-(*α*-L-rhamnopyranoside) (**4**).

Compound **5** was isolated as white powder. Its molecular formula was determined to be $C_{31}H_{46}O_{10}$ by the HR-ESI-MS, which showed a quasi-molecular peak at *m/z* 577.3011 ($[M-H]^-$). The structure of **5** was assigned to be that of (5*α*)-oleandrigenin 3-(*α*-L-rhamnopyranoside) (**5**) by comparison of its ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) with those of **4** and (5*α*)-oleandrigenin glycosides [13].

The ^{1}H-NMR spectra of **5** (*Table 1*) displayed 4 Me signals at δ(H) 0.83 (*s*), 0.89 (*s*), 1.24 (*d*, $J=6.3$), and 1.93 (*s*). The signal *s* at δ(H) 1.93 arose from a Me group linked to a C=O group because of its signal at lower field. Comparison of the ^{1}H- and ^{13}C-NMR data (*Table 2*) of **5** with those of **4** and (5*α*)-oleandrigenin glycoside [13] established that OH−C(16) of **4** was acetylated in **5**. The sugar unit was the same as that of **1** (see ^{13}C-NMR data).

Compound **6** was found to posses the molecular formula $C_{37}H_{56}O_{15}$ by negative-ion HR-ESI-MS (*m/z* 739.3548 ($[M-H]^-$), which was confirmed by the FAB-MS (*m/z* 739 ($[M-H]^-$) and ^{13}C-DEPT-NMR data. The aglycone moiety was the same as that of compound **5**. The ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*), HMBC and ROSEY (*Fig.*) and ^{1}H,^{1}H-COSY data and comparison with those of cryptostigmin II [10] determined the structure of **6** to be (5*α*)-oleandrigenin 3-[*O*-*β*-D-glucopyranosyl-(1 → 4)-*α*-L-rhamnopyranoside] (**6**).

The ^{13}C-NMR signals of the sugar moiety of **6** revealed the presence of a terminal *β*-D-glucopyranose unit in addition to an *α*-L-rhamnopyranose unit with the signals at δ(C) 99.5 (C(1′)), 71.4 (C(2′)), 72.4 (C(3′)), 83.6 (C(4′)), 68.5 (C(5′)), and 18.1 (C(6′)). The downfield shift of the C(4′) signal to δ(C) 83.6 as compared to the corresponding signal of **1** and **3**–**5** indicated the 1 → 4 linkage between the terminal glucose and the internal rhamnose unit. The *β*-D-form of the glucopyranose unit was determined by the *d* of the anomeric H−C(1″) at δ(H) 4.57 (*d*, $J=7.8$), while the *α*-L-configuration of the rhamnose unit was established from the upfield shift of its C(5′) at δ(C) 68.5. The HMBC plot displayed correlations between H−C(3) and C(1′) of the rhamnopyranose unit, and between the anomeric H−C(1″) of the glucopyranose residue and C(4′) of the rhamnopyranose unit (*Fig.*) [9][10].

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Experiment Part

1. *General.* TLC: precoated plates (*Si gel G*) from *Qingdao Marine Chemical Factory*, Qingdao, P. R. China. Column chromatography (CC): silica gel (200–300 mesh) from *Qingdao Marine Chemical Factory*, reversed-phase C_{18} silica gel from *Merk*, *Sephadex-LH-20* from *Amershan Bioscience*. Optical rotations: *Jasco DIP-370* digital polarmeter; MeOH soln. NMR Spectra: *Inova-400* and *Bruker AM-400* or *DRX-500* spectrometers; $SiMe_4$ as internal standards, δ in ppm, *J* in Hz. MS: *VG-Auto-Spec-3000* and *Thermo-Finnigan LCQ-Advantage* spectrometer; in *m/z* (rel.%).

2. *Plant Material.* The stem bark of *Trewia nudiflora* was collected in Xishuangbanna, Yunnan Province, P. R. China. A voucher specimen (No. 20159, K. M. Feng) is deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Science.

3. *Extraction and Isolation.* The air-dried stem bark of *Trewia nudiflora* (8.8 kg) was ground and extracted with 80% EtOH (4×) at r.t. After evaporation, the residues were suspended in H_2O and then extracted successively with petroleum ether and AcOEt. The AcOEt extract was subjected to CC (silica gel, $CHCl_3$, $CHCl_3$/MeOH, MeOH) to give *Fractions Et.1–Et.8*. *Fr. Et.1* was subjected to CC (silica gel, $CHCl_3/Me_2O$ 10:1): **2** (8 mg). *Fr. Et.3* was further separated into *Fr. Et.3.1–Et.3.8*. *Fr. Et.3.2* was subjected to CC (silica gel, AcOEt/MeOH 100:6): *frugoside* (10 mg). *Fr. Et.3.3* was subjected to CC (silica gel, AcOEt/MeOH 100:8) and then purified by CC (*Sephadex LH-20*, MeOH): **5** (5 mg) and **6** (9 mg). *Fr. Et.3.5* was separated by CC (*Sephadex LH-20*, MeOH; C_{18}, MeOH/H_2O 40:60 and 60:40): *Fr. Et.3.5.1* and *Et.3.5.2*. *Fr. Et.3.5.1* was recrystallized from MeOH: **3** (10 mg). *Fr. Et.3.5.2* was further purified by CC (silica gel AcOEt/MeOH 100:2): **4** (5 mg). *Fr. Et.3.6* was subjected to CC (silica gel $CHCl_3$/MeOH 9:1) and then purified by CC (C_{18}, MeOH/H_2O 45:55): **1** (15 mg). *Fr. Et.3.7* was subjected to CC (silica gel $CHCl_3/Me_2CO$ 3:2): *(17α)-ascleposide* (10 mg).

(5α)-Sarmentogenin 3-(α-L-Rhamnopyranoside) (=*(3β,5α,11α)-3-[(6-Deoxy-α-L-mannopyranosyl)oxy]-11,14-dihydroxycard-20(22)-enolide*; **1**): Amorphous powder. $[\alpha]_D^{20} = -5.2$ ($c = 0.42$, C_5H_5N). ^1H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 535 (100, $[M-H]^-$).

(5α)-Sarmentogenin (=*(3β,5α,11α)-3,11,14-Trihydroxycard-20(22)-enolide*; **2**): White powder. $[\alpha]_D^{20} = +8.0$ ($c = 0.30$, C_5H_5N). ^1H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 372 (14, $[M-H_2O]^+$).

11-Oxouzarigenin 3-(α-L-Rhamnopyranoside) (=*(3β,5α)-3-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14-hydroxy-11-oxocard-20(22)-enolide*; **3**): Colorless crystal (from MeOH). M.p. 262°. $[\alpha]_D^{20} = -55.2$ ($c = 0.29$, MeOH). ESI-MS: 579 (100, $[M+HCOOH]^-$), 535 (35, $[M+H]^+$). HR-ESI-MS: 557.2735 ($[M+Na]^+$; calc. 557.2726).

(5α)-Gitoxigenine 3-(α-L-Rhamnopyranoside) (=*(3β,5α,16β)-3-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14,16-dihydroxycard-20(22)-enolide*; **4**): White powder. $[\alpha]_D^{20} = -9.4$ ($c = 0.47$, C_5H_5N). ^1H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 535 (27, $[M-H^-$).

(5α)-Oleandrigenin 3-(α-L-Rhamnopyranoside) (=*(3β,5α,16β)-16-(Acetyloxy)-3-[(6-deoxy-α-L-mannopyranosyl)oxy]-14-hydroxycard-20(22)-enolide*; **5**). White powder. M.p. 262°. $[\alpha]_D^{20} = -66.7$ ($c = 0.24$, MeOH). FAB-MS: 577 (46, $[M-H]^-$). HR-ESI-MS: 577.3011 ($[M-H]^-$; calc. 557.3012).

(5α)-Oleandrigenin 3-[O-β-D-Glucopyranosyl-(1 → 4)-α-L-rhamnopyranoside] (=*(3β,5α,16β)-16-(Acetyloxy)-3-{[O-β-D-glucopyranosyl-(1 → 4)-6-deoxy-α-L-mannopyranosyl]oxy}-14-hydroxycard-20(22)-enolide*; **6**). Amorphous powder. $[\alpha]_D^{20} = -76.0$ ($c = 0.32$, MeOH). FAB-MS: 739 (100, $[M-H]^-$). HR-ESI-MS: 739.3548 ($[M-H]^-$; calc. 739.3540).

Frugoside [14]: White powder. ^{13}C-NMR (100 MHz, MeOD): 35.7 (C(1)); 30.8 (C(2)); 74.2 (C(3)); 32.8 (C(4)); 45.9 (C(5)); 29.5 (C(6)); 28.7 (C(7)); 43.1 (C(8)); 51.5 (C(9)); 40.6 (C(10)); 24.0 (C(11); 41.5 (C(12)); 51.2 (C(13)); 86.5 (C(14)); 33.4 (C(15)); 28.1 (C(16)); 52.2 (C(17)); 16.5 (C(18)); 60.0 (C(19)); 177.3 (C(20)); 75.4 (C(21)); 117.8 (C(22)); 178.5 (C(23)); 99.8 (C(1')); 72.5 (C(2')); 72.9 (C(3')); 77.4 (C(4')); 69.9 (C(5')); 18.0 (C(6')); data in accord with frugoside the published ones [14]. FAB-MS: 536 (100, $[M-H]^-$).

(17α)-Asclepioside [15]: Amorphous powder. ^{13}C-NMR (100 MHz; MeOD): 38.3 (C(1)); 30.4 (C(2)); 77.2 (C(3)); 34.1 (C(4)); 45.6 (C(5)); 30.0 (C(6)); 28.7 (C(7)); 42.5 (C(8)); 51.1 (C(9)); 37.5 (C(10)); 22.5 (C(11)); 40.8 (C(12)); 51.0 (C(13)); 86.3 (C(14)); 35.2 (C(15)); 28.0 (C(16)); 52.0 (C(17)); 16.4 (C(18)); 12.5 (C(19)); 178.4 (C(20)); 75.3 (C(21)); 117.8 (C(22)); 177.2 (C(23)); 99.6 (C(1')); 72.4 (C(2')); 72.8 (C(3')); 74.1 (C(4')); 69.8 (C(5')); 18.0 (C(6')); data in accord with published ones [15]. ESI-MS: 521 (100, $[M+H]^+$).

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