

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

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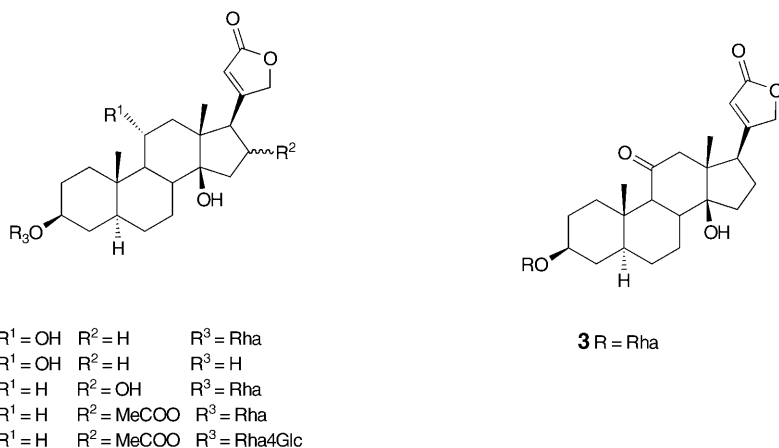
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Five new cardenolides and one new cardiac aglycone, *i.e.*, (5 α)-sarmentogenin 3-(α -L-rhamnopyranoside) (**1**), (5 α)-sarmentogenin (**2**), 11-oxozarigenin 3-(α -L-rhamnopyranoside) (**3**), (5 α)-gitoxigenin 3-(α -L-rhamnopyranoside) (**4**), (5 α)-oleandrigenin 3-(α -L-rhamnopyranoside) (**5**), and (5 α)-oleandrigenin 3-[β -D-glucopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranoside] (**6**), together with two known cardenolides, *i.e.*, frugoside (= (3 β ,5 α)-3-[(6-deoxy- β -D-allopyranosyl)oxy]-14,19-dihydroxycard-20(22)-enolide) and (17 α)-ascleposide (= (3 β ,5 α ,17 α)-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 (= (3 β ,5 α)-3-[(6-deoxy- β -D-allopyranosyl)oxy]-14,19-dihydroxycard-20(22)-enolide) and (17 α)-ascleposide (= (3 β ,5 α ,17 α)-3-[(6-deoxy- α -D-allopyranosyl)oxy]-14-hydroxycard-20(22)-enolide). The new compounds were very similar to each other according to their ¹H- and ¹³C-NMR spectra (*Tables 1* and *2*).

Compound **1** was isolated as an amorphous powder. Its molecular formula was determined to be C₂₉H₄₄O₉ by ¹³C-DEPT-NMR and negative-ion FAB-MS (*m/z* 535 [*M* – H][–]). The ¹H-NMR spectra of **1** revealed the presence of characteristic signals for cardenolide: Further spectral data (¹³C-NMR (*Table 2*), ROESY, HMBC, HMQC, ¹H,¹H-COSY) yielded sufficient data to define the structure of **1** as (5 α)-sarmentogenin 3-(α -L-rhamnopyranoside) (**1**).



In the $^1\text{H-NMR}$ of **1** (Table 1), two angular Me groups at $\delta(\text{H})$ 0.98 and 1.10 (2s), a $\text{CH}_2(21)$ moiety at $\delta(\text{H})$ 5.25 and 5.30, and an olefinic proton $\text{H-C}(22)$ at $\delta(\text{H})$ 6.32 were characteristic of a cardenolide [8]. The $^{13}\text{C-NMR}$ 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 $^1\text{H-}$ and $^{13}\text{C-NMR}$ spectra of **1** were very similar to those of affinoside S-IX [8], except for the signal of C(19) shifted upfield to $\delta(\text{C})$ 12.5. Comparing the $^{13}\text{C-NMR}$ data of **1** with those of affinoside S-IX and uzarigenin 3-sulfate [9] suggested that $\text{H-C}(5)$ of **1** was located on the α -side. In addition, the ROSEY plot showed the $^1\text{H,}^1\text{H}$ correlations $\delta(\text{H})$ 4.07–4.10 ($\text{H-C}(11)$)/1.10 (Me(18)) and 0.98 (Me(19)), suggesting that $\text{H-C}(11)$ was on the β -side (Fig.). The $^1\text{H,}^{13}\text{C-HMBC}$ spectra exhibited along-range correlation of the anomeric $\text{H-C}(1')$ ($\delta(\text{H})$ 5.51) with C(3) ($\delta(\text{C})$ 75.8), indicating that the sugar unit was linked to C(3) (Fig.). The $^{13}\text{C-NMR}$ data of the sugar moiety revealed the presence of a substituted α -L-rhamnopyranose unit from the signals at $\delta(\text{C})$ 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 $\text{H-C}(1')$ signal at $\delta(\text{H})$ 5.51 with a J of 1.0 Hz and the C(5') signal at $\delta(\text{C})$ 69.8 [10][11].

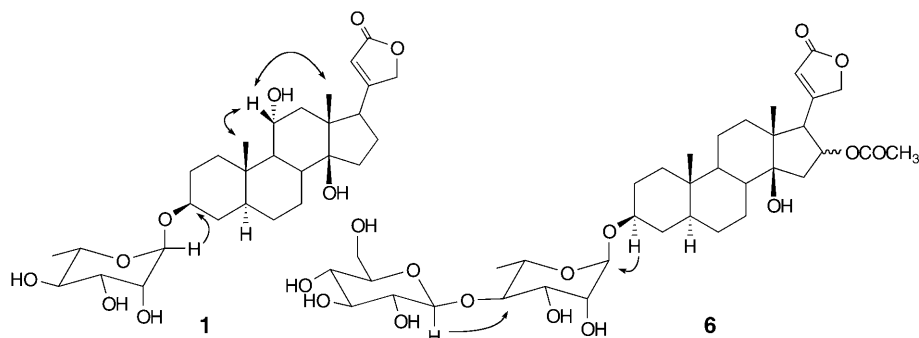


Figure. Key HMBC (\rightarrow) and NOESY (\leftrightarrow) correlations for **1** and **6**

Compound **2** was obtained as white powder. Its molecular formula was deduced as $\text{C}_{23}\text{H}_{34}\text{O}_5$ on the basis of $^{13}\text{C-DEPT-NMR}$ and positive-ion EI-MS data, which showed a peak at m/z 372 ($[\text{M}-\text{H}_2\text{O}]^+$). The $^1\text{H-}$ and $^{13}\text{C-NMR}$ 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 (5 α)-sarmentogenin (**2**).

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

^{a)} In (D₅)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 (<i>t</i>)	39.8 (<i>t</i>)	36.6 (<i>t</i>)	38.3 (<i>t</i>)	38.2 (<i>t</i>)	38.2 (<i>t</i>)
C(2)	30.3 (<i>t</i>)	32.9 (<i>t</i>)	29.6 (<i>t</i>)	30.4 (<i>t</i>)	30.8 (<i>t</i>)	30.3 (<i>t</i>)
C(3)	75.8 (<i>d</i>)	70.5 (<i>d</i>)	75.5 (<i>d</i>)	77.3 (<i>d</i>)	77.2 (<i>d</i>)	77.3 (<i>d</i>)
C(4)	35.2 (<i>t</i>)	33.6 (<i>t</i>)	34.1 (<i>t</i>)	35.2 (<i>t</i>)	35.1 (<i>t</i>)	35.1 (<i>t</i>)
C(5)	45.1 (<i>d</i>)	45.6 (<i>d</i>)	44.5 (<i>d</i>)	45.6 (<i>d</i>)	45.5 (<i>d</i>)	45.4 (<i>d</i>)
C(6)	29.7 (<i>t</i>)	29.8 (<i>t</i>)	28.6 (<i>t</i>)	29.9 (<i>t</i>)	29.8 (<i>t</i>)	29.8 (<i>t</i>)
C(7)	28.4 (<i>t</i>)	28.5 (<i>t</i>)	28.6 (<i>t</i>)	28.6 (<i>t</i>)	28.3 (<i>t</i>)	28.3 (<i>t</i>)
C(8)	41.4 (<i>d</i>)	41.4 (<i>d</i>)	43.1 (<i>d</i>)	42.7 (<i>d</i>)	42.5 (<i>d</i>)	42.5 (<i>d</i>)
C(9)	55.7 (<i>d</i>)	55.8 (<i>d</i>)	60.3 (<i>d</i>)	51.0 (<i>d</i>)	50.8 (<i>d</i>)	50.8 (<i>d</i>)
C(10)	37.9 (<i>s</i>)	37.9 (<i>s</i>)	36.2 (<i>s</i>)	37.0 (<i>s</i>)	36.9 (<i>s</i>)	36.9 (<i>s</i>)
C(11)	67.8 (<i>d</i>)	67.9 (<i>d</i>)	209.6 (<i>d</i>)	22.0 (<i>d</i>)	22.0 (<i>t</i>)	21.9 (<i>t</i>)
C(12)	50.3 (<i>t</i>)	50.7 (<i>t</i>)	55.1 (<i>t</i>)	40.9 (<i>t</i>)	39.9 (<i>t</i>)	39.9 (<i>t</i>)
C(13)	51.2 (<i>s</i>)	50.3 (<i>s</i>)	53.3 (<i>s</i>)	51.3 (<i>s</i>)	51.4 (<i>s</i>)	51.4 (<i>s</i>)
C(14)	84.2 (<i>s</i>)	84.2 (<i>s</i>)	83.4 (<i>s</i>)	85.5 (<i>s</i>)	84.8 (<i>s</i>)	83.6 (<i>s</i>)
C(15)	33.6 (<i>t</i>)	32.9 (<i>t</i>)	33.5 (<i>t</i>)	43.7 (<i>t</i>)	41.3 (<i>t</i>)	41.3 (<i>t</i>)
C(16)	27.3 (<i>t</i>)	27.3 (<i>t</i>)	27.1 (<i>t</i>)	73.1 (<i>d</i>)	75.9 (<i>d</i>)	76.1 (<i>d</i>)
C(17)	50.6 (<i>d</i>)	51.3 (<i>d</i>)	50.2 (<i>d</i>)	59.6 (<i>d</i>)	57.4 (<i>d</i>)	57.3 (<i>d</i>)
C(18)	17.3 (<i>q</i>)	17.6 (<i>q</i>)	17.7 (<i>q</i>)	17.1 (<i>q</i>)	16.4 (<i>q</i>)	16.4 (<i>q</i>)
C(19)	12.5 (<i>q</i>)	12.7 (<i>q</i>)	12.5 (<i>q</i>)	12.5 (<i>q</i>)	12.5 (<i>q</i>)	12.5 (<i>q</i>)
C(20)	175.5 (<i>s</i>)	175.5 (<i>s</i>)	174.2 (<i>s</i>)	173.6 (<i>s</i>)	171.6 (<i>s</i>)	171.6 (<i>s</i>)
C(21)	73.8 (<i>t</i>)	73.8 (<i>t</i>)	73.6 (<i>t</i>)	77.8 (<i>t</i>)	77.5 (<i>t</i>)	77.6 (<i>t</i>)
C(22)	117.8 (<i>d</i>)	117.8 (<i>d</i>)	118.2 (<i>d</i>)	120.6 (<i>d</i>)	121.8 (<i>d</i>)	121.8 (<i>d</i>)
C(23)	174.5 (<i>s</i>)	174.5 (<i>s</i>)	173.8 (<i>s</i>)	177.3 (<i>s</i>)	172.1 (<i>s</i>)	172.1 (<i>s</i>)
Ac					176.5 (<i>s</i>), 20.9 (<i>q</i>)	176.8 (<i>s</i>), 20.9 (<i>q</i>)
Sugar moieties						
C(1')	99.5 (<i>d</i>)		99.5 (<i>d</i>)	99.7 (<i>d</i>)	99.6 (<i>d</i>)	99.5 (<i>d</i>)
C(2')	72.9 (<i>d</i>)		72.9 (<i>d</i>)	72.5 (<i>d</i>)	72.4 (<i>d</i>)	71.4 (<i>d</i>)
C(3')	73.0 (<i>d</i>)		72.9 (<i>d</i>)	72.8 (<i>d</i>)	72.8 (<i>d</i>)	72.4 (<i>d</i>)
C(4')	74.2 (<i>d</i>)		74.2 (<i>d</i>)	74.5 (<i>d</i>)	74.1 (<i>d</i>)	83.6 (<i>d</i>)
C(5')	69.8 (<i>d</i>)		69.9 (<i>d</i>)	69.9 (<i>d</i>)	69.8 (<i>d</i>)	68.5 (<i>d</i>)
C(6')	18.7 (<i>q</i>)		18.7 (<i>q</i>)	18.0 (<i>q</i>)	18.0 (<i>q</i>)	18.1 (<i>q</i>)
C(1'')						105.7 (<i>d</i>)
C(2'')						75.9 (<i>d</i>)
C(3'')						78.1 (<i>d</i>)
C(4'')						72.6 (<i>d</i>)
C(5'')						78.1 (<i>d</i>)
C(6'')						62.7 (<i>t</i>)

^{a)} In (D₅)pyridine. ^{b)} At 100 MHz. ^{c)} At 125 MHz. ^{d)} In MeOD.

Compound **3**, colorless crystals (from MeOH), had the molecular formula C₂₉H₄₂O₉ as determined by HR-ESI-MS (*m/z* 557.2735 ([*M*+Na]⁺). The ¹H- and ¹³C-NMR (Tables 1 and 2), HMQC, HMBC, and ¹H,¹H-COSY data and comparison with those of **1** determined compound **3** to be 11-oxouzarigenin 3-(α -L-rhamnopyranoside) (**3**).

The ¹³C-NMR spectra of **3** showed 29 peaks. Its ¹H- and ¹³C-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 1H - 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 strosipeside [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 1H - and ^{13}C -NMR data (Tables 1 and 2) with those of **4** and (5 α)-oleandrigenin glycosides [13].

The 1H -NMR spectra of **5** (Table 1) displayed 4 Me signals at $\delta(H)$ 0.83 (s), 0.89 (s), 1.24 (d, $J = 6.3$), and 1.93 (s). The signal s at $\delta(H)$ 1.93 arose from a Me group linked to a C=O group because of its signal at lower field. Comparison of the 1H - 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 possess 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 1H - and ^{13}C -NMR (Tables 1 and 2), HMBC and ROSEY (Fig.) and $^1H, ^1H$ -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 \rightarrow 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 $\delta(C)$ 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 $\delta(C)$ 83.6 as compared to the corresponding signal of **1** and **3**–**5** indicated the 1 \rightarrow 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 $\delta(H)$ 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 $\delta(C)$ 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 *Amersham Bioscience*. Optical rotations: *Jasco DIP-370* digital polarimeter; 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 H₂O and then extracted successively with petroleum ether and AcOEt. The AcOEt extract was subjected to CC (silica gel, CHCl₃, CHCl₃/MeOH, MeOH) to give *Fractions Et.1–Et.8*. *Fr. Et.1* was subjected to CC (silica gel, CHCl₃/Me₂O 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; *C₁₈*, MeOH/H₂O 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 CHCl₃/MeOH 9:1) and then purified by CC (*C₁₈*, MeOH/H₂O 45:55): **1** (15 mg). *Fr. Et.3.7* was subjected to CC (silica gel CHCl₃/Me₂CO 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. $[\alpha]_{\text{D}}^{20} = -5.2$ ($c = 0.42$, C₅H₅N). ¹H- and ¹³C-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. $[\alpha]_{\text{D}}^{20} = +8.0$ ($c = 0.30$, C₅H₅N). ¹H- and ¹³C-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°. $[\alpha]_{\text{D}}^{20} = -55.2$ ($c = 0.29$, 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. $[\alpha]_{\text{D}}^{20} = -9.4$ ($c = 0.47$, C₅H₅N). ¹H- and ¹³C-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°. $[\alpha]_{\text{D}}^{20} = -66.7$ ($c = 0.24$, MeOH). FAB-MS: 577 (46, $[M - H]^{-}$). HR-ESI-MS: 577.3011 ($[M - H]^{-}$; calc. 577.3012).

(*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. $[\alpha]_{\text{D}}^{20} = -76.0$ ($c = 0.32$, MeOH). FAB-MS: 739 (100, $[M - H]^{-}$). HR-ESI-MS: 739.3548 ($[M - H]^{-}$; calc. 739.3540).

Frugoside [14]: White powder. ¹³C-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*)-*Ascleposide* [15]: Amorphous powder. ¹³C-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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