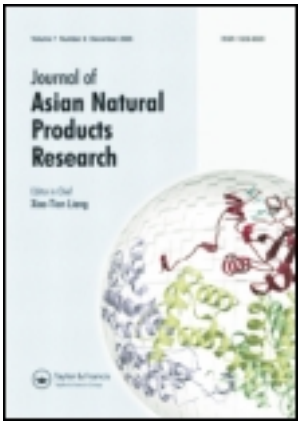

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Two new triterpenoid glycosides from the leaves of *Anthocephalus chinensis*

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Nine compounds were isolated from the leaves of *Anthocephalus chinensis* by column chromatography on silica gel and Sephadex LH-20, and their structures were elucidated by spectroscopic techniques as clethric acid-28-*O*-β-D-glucopyranosyl ester (**1**), mussaendoside T (**2**), β-stigmasterol (**3**), hederagenin (**4**), ursolic acid (**5**), clethric acid (**6**), 3β,6β,19α,24-tetrahydroxyurs-12-en-28-oic acid (**7**), mussaendoside I (**8**), and cadambine (**9**). Compounds **1** and **2**, and **7** and **8** were isolated from the plants of this genus for the first time, and compounds **1** and **2** were new triterpenoid glycosides.

Keywords: *Anthocephalus chinensis*; triterpenoid glycosides; clethric acid; mussaendoside; cadambine

1. Introduction

Anthocephalus chinensis (Lam.) Rich. ex Walp. (family Rubiaceae) is abundant and distributed widely in southern Asia and southern China. *A. chinensis* has many bioactivities, such as antioxidant, hypoglycemic, hypolipidemic, and so on [1]. In search for the active compounds from *A. chinensis*, more than 50 compounds have been isolated from its barks, leaves, roots, and seeds, such as triterpenoid glycosides, iridoids, and alkaloids [2–23]. Up to now, there are few reports about the study on the leaves of *A. chinensis*, except for two new alkaloids anthocephalusine A and 3β-isodihydrocadambine 4-oxide reported by Zhou *et al.* [10]. In this paper, we studied the leaves of *A. chinensis* further to search for natural products to reduce blood pressure and to use this medicinal plant resource in a sustainable way, which led to the isolation and characterization of two new compounds clethric acid-28-*O*-β-D-glucopyranosyl ester (**1**) and mussaendoside T (**2**), as well as seven known ones, i.e. β-stigmasterol (**3**), hederagenin (**4**), ursolic acid (**5**), clethric acid (**6**), 3β,6β,19α,24-tetrahydroxyurs-12-en-28-oic acid (**7**), mussaendoside I (**8**), and cadambine (**9**).

2. Results and discussion

Compound **1** was isolated as a yellow crystal, and its ESI-MS showed a pseudomolecular ion at *m/z* 689 $[M + Na]^+$. Its molecular formula was established as $C_{36}H_{58}O_{11}$ by HR-ESI-MS at *m/z* 689.3879 $[M + Na]^+$. The UV spectrum showed absorption maxima at 280 and 203 nm, whereas the IR spectrum showed strong absorptions at 3424 and 1729 cm^{-1}, indicating the presence of hydroxyl and

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carboxylic ester groups. Acid hydrolysis of **1** afforded aglycone and D-glucose, which were identified by GC analysis. The ^{1}H NMR spectrum of **1** (Table 1) indicated the presence of five methyl groups at δ_H 0.74 (s), 0.92 (s), 0.93 (d, $J = 2.9$ Hz), 1.19 (s), and 1.33 (s). The DEPT and ^{13}C NMR spectra of **1** exhibited the signals of a carboxyl group at δ_C 178.6 (s), an oxygenated quaternary carbon at δ_C 73.7 (s), an oxygenated methine at δ_C 70.7 (d), two oxymethyls at δ_C 64.9 (t) and 69.0 (t), and a C=C double bond at δ_C 129.6 (d) and 139.5 (s). The ^{1}H and ^{13}C NMR spectral data of **1** were similar to those of clethric acid [24], except for the sugar moiety. The NMR spectra indicated that the sugar is a glucose, and the anomeric proton at δ_H 5.32 (d, $J = 8.1$ Hz) demonstrated the β-configuration of the glucose. The β-D-glucose was linked to COOH (C-28) as C-28 shifted toward upfield from δ_C 180.8 to δ_C 178.6, compared with clethric acid, and the anomeric carbon located at δ_C 95.8, which was further demonstrated by a HMBC correlation of H-1′ at δ_H 5.32 with C-28 at δ_C 178.6. Therefore, the structure of **1** was determined as clethric acid-28-*O*-β-D-glucopyranosyl ester (Figure 1). The complete assignments of ^{1}H and ^{13}C NMR spectral data for compound **1** are shown in Table 1.

Compound **2** was obtained as a colorless crystal, and showed FAB-MS at *m/z* 819.6 $[M + Na]^+$. Its molecular formula was established as $C_{42}H_{68}O_{14}$ by HR-ESI-MS at *m/z* 819.4503 $[M + Na]^+$. The UV spectrum showed absorption maxima at 281 and 203 nm, whereas the IR spectrum showed strong absorptions at 3406 and 1696 cm^{-1}, indicating the presence of hydroxyl and carboxyl groups. Acid hydrolysis of **2** liberated aglycone and two D-glucoses, which were identified by GC analysis. The ^{1}H NMR spectrum of **2** (Table 1) indicated the presence of seven methyl groups at δ_H 0.83 (s), 1.06 (s), 1.10 (s), 1.15 (d, $J = 6.6$ Hz), 1.26 (s), 1.50 (s), and 1.73 (s). The DEPT and ^{13}C NMR spectra of **2** showed the signals of a carboxyl group at δ_C 181.2 (s), an oxygenated quaternary carbon at δ_C 73.0 (s), an oxygenated methine at δ_C 89.6 (d), and a C=C double bond at δ_C 128.3 (d) and 140.2 (s). The ^{1}H and ^{13}C NMR spectral data of **2** were similar to those of mussaendoside I [25], while the main difference was due to the sugar moiety. The NMR spectra indicated that there were two D-glucoses, and the anomeric protons at δ_H 4.89 (d, $J = 7.7$ Hz) and 5.38 (d, $J = 7.7$ Hz) demonstrated the β-configuration of two glucoses. The two β-D-glucoses were (1 → 2) linked as indicated by a HMBC correlation of H-1‴ at δ_H 5.38 with C-2″ at δ_C 83.0, and the sugar moiety was attached to C-3, as indicated by a HMBC correlation of H-1″ at δ_H 4.89 with the signal at δ_C 89.6 (C-3). Therefore, the structure of **2** was determined and named as mussaendoside T (Figure 1). The complete assignments of ^{1}H and ^{13}C NMR spectral data for compound **2** are shown in Table 1.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a SGW X-4 micromelting point apparatus. Optical rotations were taken on a JASCO P-1020 spectropolarimeter. UV spectra were recorded on a UV-2401PC spectrophotometer. IR spectra were recorded on a Bruker Tensor27 spectrometer. 1D and 2D NMR spectra were measured on Bruker AV-400 MHz and DRX-500 MHz spectrometers using TMS as the internal standard. ESI-MS and HR-ESI-MS data were obtained on an API QSTAR Pulsar spectrometer. FAB-MS was recorded on an Autospec Ultima-TOF spectrometer. Silica gel F254 plates (SiO_2; Qingdao Haiyang Chemical Co. Ltd., Qingdao, China) were used for analytical TLC. For column chromatography (CC), silica gel (Qingdao Haiyang Chemical Co. Ltd.), macroporous resin (Shandong Lukang Pharmaceutical

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** (CD_3OD, 400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR) and **2** (C_5D_5N, 500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR).

	1		**2**	
Position	δ_H	δ_C	δ_H	δ_C
1	α 1.27 (1H, m) β 1.36 (1H, m)	34.1	α 0.85 (1H, m) β 1.44 (1H, m)	39.1
2	α 1.62 (1H, m) β 2.59 (1H, m)	26.5	α 1.50 (1H, m) β 2.10 (1H, m)	27.2
3	4.07 (1H, dd, $J = 11.7, 4.1$ Hz)	70.7	3.27 (1H, dd, $J = 11.7, 4.1$ Hz)	89.6
4		46.2		39.8
5	1.65 (1H, m)	45.3	0.75 (1H, m)	56.2
6	1.42 (2H, m)	19.6	1.49 (1H, m) 1.29 (1H, m)	18.8
7	α 1.39 (1H, m) β 1.57 (1H, m)	34.1	α 1.34 (1H, m) β 1.56 (1H, m)	33.7
8		41.2		40.6
9	1.83 (1H, m)	48.5	1.74 (1H, m)	47.9
10		37.8		37.1
11	α 1.33 (1H, m) β 2.00 (1H, m)	24.9	α 1.25 (1H, m) β 1.98 (1H, m)	24.2
12	5.32 (1H, br t)	129.6	5.59 (1H, br t)	128.3
13		139.5		140.2
14		42.7		42.3
15	α 0.99 (1H, m) β 1.81 (1H, m)	29.6	α 1.26 (1H, m) β 2.31 (1H, m)	29.5
16	α 1.19 (1H, m) β 1.70 (1H, m)	27.2	α 2.05 (1H, m) β 2.25 (1H, m)	26.6
17		50.0		48.6
18	2.50 (1H, s)	54.9	3.04 (1H, s)	54.9
19		73.7		73.0
20	1.33 (1H, m)	42.9	1.51 (1H, m)	42.6
21	α 1.53 (1H, m) β 1.87 (1H, m)	26.3	α 1.35 (1H, m) β 1.88 (1H, m)	26.8
22	α 1.60 (1H, m) β 1.77 (1H, m)	38.3	α 2.04 (1H, m) β 2.13 (1H, m)	38.7
23	α 3.70 (1H, d, $J = 9.1$ Hz) β 3.90 (1H, d, $J = 9.1$ Hz)	69.0	1.26 (3H, s)	28.4
24	α 3.57 (1H, d, $J = 5.4$ Hz) β 3.70 (1H, d, $J = 5.4$ Hz)	64.9	0.83 (3H, s)	15.7
25	0.92 (3H, s)	16.1	1.06 (3H, s)	17.4
26	0.74 (3H, s)	17.5	1.10 (3H, s)	17.0
27	1.33 (3H, s)	24.8	1.73 (3H, s)	25.0
28		178.6		181.2
29	1.19 (3H, s)	27.2	1.50 (3H, s)	27.4
30	0.93 (3H, d, $J = 2.9$ Hz)	16.7	1.15 (3H, d, $J = 6.6$ Hz)	17.0
28-Glc				
1′	5.32 (1H, d, $J = 8.1$ Hz)	95.8		
2′	3.32 (1H, dd, $J = 9.5, 8.1$ Hz)	73.8		
3′	3.34 (1H, m)	78.5		
4′	3.35 (1H, m)	71.1		
5′	3.40 (1H, dd, $J = 5.0, 2.0$ Hz)	78.2		
6′	α 3.68 (1H, dd, $J = 12.0, 5.0$ Hz) β 3.80 (1H, dd, $J = 12.0, 2.0$ Hz)	62.4		
3-Glc				
1″			4.89 (1H, d, $J = 7.7$ Hz)	105.3
2″			4.30 (1H, dd, $J = 8.5, 7.7$ Hz)	83.0

Table 1 – *continued*

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
3″			4.25 (1H, m)	78.2
4″			4.24 (1H, m)	71.9
5″			4.35 (1H, dd, $J = 5.5, 2.0$ Hz)	78.6
6″			α 4.37 (1H, dd, $J = 12.0, 5.5$ Hz) β 4.55 (1H, dd, $J = 12.0, 2.0$ Hz)	63.0
3-Glc′				
1‴			5.38 (1H, d, $J = 7.7$ Hz)	105.8
2‴			4.12 (1H, dd, $J = 9.3, 7.7$ Hz)	77.1
3‴			3.95 (1H, m)	78.3
4‴			4.13 (1H, m)	71.8
5‴			4.35 (1H, dd, $J = 5.5, 2.0$ Hz)	78.6
6‴			α 4.37 (1H, dd, $J = 12.0, 5.5$ Hz) β 4.55 (1H, dd, $J = 12.0, 2.0$ Hz)	63.0

Co. Ltd., Shandong, China), and Sephadex LH-20 (GE Healthcare, New York, NY, USA) were used.

3.2 Plant material

The leaves of *A. chinensis* were collected in Xishuangbanna, Yunnan Province of China and air-dried. The plant was identified by Prof. Qi-Shi Song, Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Sciences. A voucher specimen (035783) of this plant is deposited in the Herbarium of XTBG, China.

3.3 Extraction and isolation

The air-dried leaves of *A. chinensis* (68 kg) were ground and refluxed three times with 90% MeOH. The extracts were combined and concentrated, and the residue (2.4 kg) was suspended in H_2O, and then successively partitioned with petroleum ether, chloroform, and *n*-butanol, respectively. The petroleum ether fraction (159.7 g) was subjected to CC (SiO_2; petroleum ether–Me_2CO 100:0–80:20) to afford two fractions (Fr. 1 and Fr. 2). Fr. 1 (58.6 g) was separated by repeated CC on silica gel, eluting with petroleum ether–Me_2CO

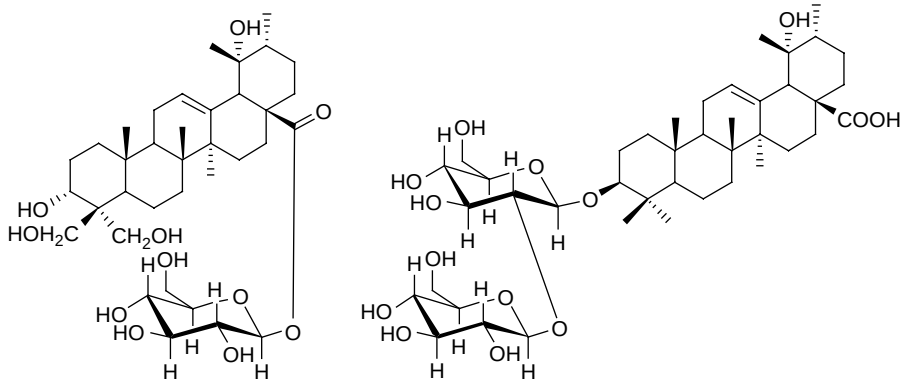

Figure 1. The structures of compounds **1** and **2**.

(95:5–90:10) to afford two subfractions, and subfraction 2 (29.8 g) was then purified by Sephadex LH-20 ($CHCl_3$–MeOH 1:1) to obtain **3** (26.3 g). The chloroform fraction (38.8 g) was subjected to CC (SiO_2; $CHCl_3$–MeOH 95:5–50:50) to afford five fractions (Fr. 1–Fr. 5). Fr. 1 (10.9 g) was separated by repeated CC on silica gel, eluting with $CHCl_3$–MeOH (98:2–95:5) to give two subfractions, and subfractions 1 (6.9 g) and 2 (1.8 g) were then purified by Sephadex LH-20 ($CHCl_3$–MeOH 1:1) to obtain **5** (6.5 g) and **6** (1.5 g), respectively; Fr. 2 (1.7 g) provided **7** (66.8 mg) after being chromatographed over silica gel, eluting with $CHCl_3$–MeOH (95:5–90:10) and purified by Sephadex LH-20 ($CHCl_3$–MeOH 1:1); Fr. 3 (2.4 g) was subjected to a silica gel column with gradient elution ($CHCl_3$–MeOH 90:10–80:20) to afford three subfractions, and subfractions 1 (123.5 mg) and 2 (521.0 mg) were then purified by Sephadex LH-20 ($CHCl_3$–MeOH 1:1) to obtain **2** (79.9 mg) and **9** (345.2 mg), respectively. The *n*-butanol fraction (649.7 g) was separated by macroporous resin (gradient MeOH/H_2O 10%, 30%, 50%, 80%, and 100%, Me_2CO) to afford six fractions (Fr. 1–Fr. 6). Fr. 3 (108.3 g) was subjected to CC (SiO_2; $CHCl_3$–MeOH 95:5–50:50) to afford five fractions (Fr. 3.1–Fr. 3.5). Fr. 3.1 (17.6 g) was separated by repeated CC on silica gel, eluting with $CHCl_3$–MeOH (98:2–95:5) to give three subfractions, and subfractions 1 (980.4 mg), 2 (11.8 g), and 3 (276.3 mg) were then purified by Sephadex LH-20 ($CHCl_3$–MeOH 1:1) to obtain **4** (749.2 mg), **5** (9.7 g), and **6** (202.4 mg), respectively; Fr. 3.3 (21.2 g) was subjected to a silica gel column with gradient elution ($CHCl_3$–MeOH 90:10–80:20) to afford three subfractions, and subfractions 1 (437.6 mg), 2 (329.1 mg), and 3 (12.3 g) were then purified by Sephadex LH-20 ($CHCl_3$–MeOH 1:1) to obtain **1** (257.0 mg), **8** (286.5 mg), and **9** (9.7 g), respectively.

*3.3.1 Clethric acid-28-O-β-D-glucopyranosyl ester (**1**)*

Yellow crystal; mp 152–154°C; $[\alpha]_D^{13.1}$ −8.6 ($c = 0.30$, MeOH); UV (MeOH) λ_{max} (log ϵ) nm: 280 (3.30), 203 (4.15); IR (KBr) ν_{max} (cm^{-1}): 3424, 2929, 1729, 1640, 1455, 1381, 1268, 1228, 1075, 1032, 930, 589; ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectral data, see Table 1; ESI-MS: *m/z* 689 $[M + Na]^+$; HR-ESI-MS: *m/z* 689.3879 $[M + Na]^+$ (calcd for $C_{36}H_{58}O_{11}Na$, 689.3877).

*3.3.2 Mussaendoside T (**2**)*

Colorless crystal; mp 214–216°C; $[\alpha]_D^{13.1}$ −0.46 ($c = 0.30$, MeOH); UV (MeOH) λ_{max} (log ϵ) nm: 281 (3.29), 203 (4.06); IR (KBr) ν_{max} (cm^{-1}): 3406, 2930, 2875, 1696, 1641, 1514, 1456, 1377, 1271, 1163, 1076, 896, 633, 585; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectral data, see Table 1; FAB-MS: *m/z* 819.6 $[M + Na]^+$; HR-ESI-MS: *m/z* 819.4503 $[M + Na]^+$ (calcd for $C_{42}H_{68}O_{14}Na$, 819.4507).

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