Two New Indole Alkaloids from the Bark of *Anthocephalus chinensis*

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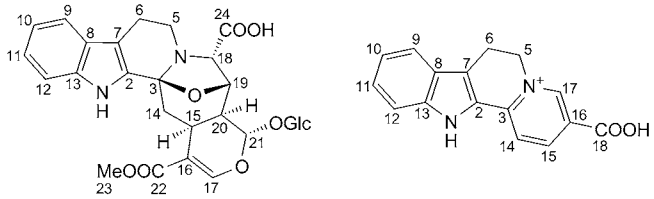
Two new indole alkaloids, cadambinic acid (**1**) and isonauclefidine (**2**), were isolated from the bark of *Anthocephalus chinensis*. The structures were identified by spectroscopic methods, and the compounds were tested for their biological activities. Both compounds showed weak cytotoxicities against A549, BGC-823, and HeLa cells in biological assays.

Introduction. – The genus *Anthocephalus* is a member of the tribe Naucleaceae in the family Rubiaceae, which is abundant and distributed widely in southern Asia and southern China. Their barks have been used to treat uterine complaints, blood disease, leprosy, and dysentery in 'Ayurvedo' of ancient Indian medicine [1]. In Indonesia, the plant is used to treat malaria [2]. These medicinal functions have attracted attention in medical circles of many countries. Many compounds have been isolated from these plants, such as alkaloids [3–9] and triterpenoid glycosides [10–14]. During our search for bioactive components from *A. chinensis*, we isolated two new indole alkaloids, **1** and **2**, from the bark. Here we report the isolation and structure elucidation of these new compounds (*Fig. 1*), including the results of their biological assays.



1 **2**

Fig. 1. *Structures of compounds* **1** *and* **2**[1])

Results and Discussion. – Cadambinic acid (**1**) was isolated as a yellow amorphous powder. The molecular formula was determined as $C_{28}H_{32}N_2O_{12}$ on the basis of the $[M+Na]^+$ peak in the HR-ESI-MS spectrum at m/z 611.1852.

[1]) Arbitrary numbering. For systematic names, see *Exper. Part*.

The UV spectrum was characteristic of an indole chromophore with absorption maxima at 225 and 364 nm, while the IR spectrum showed bands due to indole N–H (3431 cm^{-1}), COOH (3490 – 3225 cm^{-1}), aromatic C–H (3173 cm^{-1}), saturated C–H (2910 cm^{-1}), saturated carboxylic acid (1712 cm^{-1}), and carboxylic ester (1631 cm^{-1}) groups. The ^{1}H- and ^{13}C-NMR spectra of **1** were similar to those of cadambine [15], which was one of the major indole alkaloids present in the bark of *A. chinensis*. A major difference was that the signals of C(5), C(6), and C(18)[1]) (δ(C) 52.6, 23.4, and 63.4, resp.) were significantly shifted downfield, and the values for the shifts of H–C(5), H–C(6), and H–C(18) (δ(H) 3.44 (H_a–C(5)), 3.21 (H_b–C(5)), 2.86 (H_a–C(6)), 2.74 (H_b–C(6)), and 3.50 (H–C(18))) differed remarkably as well. This led to the assumption that C(18) was adjacent to a C=O moiety, which was elucidated by an HMBC of H–C(18) to δ(C) 174.3. The signal at δ(C) 174.3 was assigned to C(24) (*Fig. 2*). The relative configuration of **1** was determined from a ROESY experiment which established a similar relative configuration as that of cadambine [15], except for the configuration at C(18). H_b–C(5) was determined to be in α-position from the H_b–C(5) to H–C(19) ROESY correlation, the ROESY correlation from H_a–C(5) to H–C(18) indicated that H_a–C(5) and H–C(18) were in β-position (*Fig. 3*). Thus, the absolute configuration at C(18) was (*S*). Therefore, the structure of **1** was determined as shown in *Fig. 1*, and named cadambinic acid. The complete assignments of ^{1}H- and ^{13}C-NMR data for compound **1** are shown in *Table 1*.

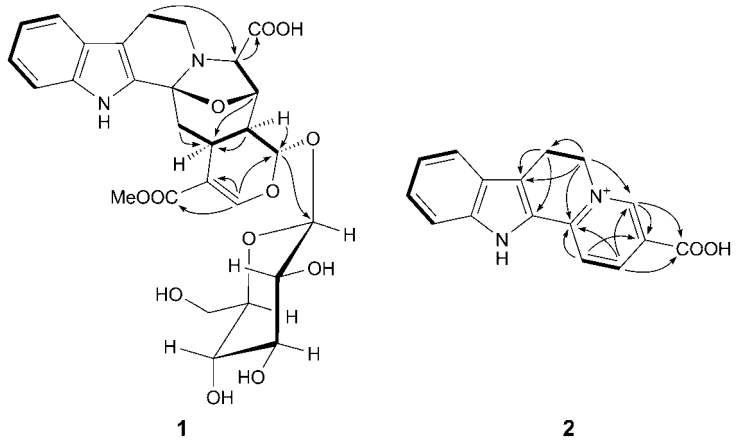


Fig. 2. *Key HMBC* (→) *and COSY* (—) *correlations of compounds* **1** *and* **2**

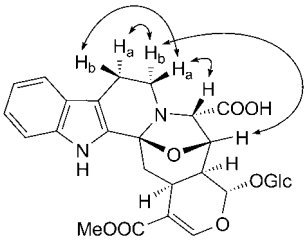


Fig. 3. *Key ROESY correlations in compound* **1**

Table 1. *NMR Data of Cadambinic Acid* **1** *in (D$_6$)DMSO* (500 MHz for ^{1}H, 125 MHz for ^{13}C)

	δ(H)	δ(C)	COSY (H → H)	HMBC (H → C)	ROESY (H → H)
H–N(1)	10.9 (*s*)			2, 7, 8, 13	
C(2)		133.7			
C(3)		89.5			
H$_a$–C(5)	3.44 (m_c[a]))	52.6	5b, 6a, 6b	3, 14	6b, 18
H$_b$–C(5)	3.21 (*d*, *J* = 9.0)		5a, 6a, 6b	14, 18, 19	6a, 19
H$_a$–C(6)	2.86 (*dd*, *J* = 7.5, 15.0)	23.4	5a, 5b, 6b	2, 7, 18	5b, 19
H$_b$–C(6)	2.74 (*dd*, *J* = 3.0, 15.0)		5a, 5b, 6a	2, 7, 18	5a
C(7)		110.5			
C(8)		126.1			
H–C(9)	7.41 (*d*, *J* = 7.8)	118.4	10	7, 11, 13	10
H–C(10)	6.94 (*dd*, *J* = 7.2, 7.8)	118.3	9, 11	8, 12	9, 11
H–C(11)	7.03 (*dd*, *J* = 7.2, 8.1)	121.0	10, 12	9, 13	10, 12
H–C(12)	7.28 (*d*, *J* = 8.1)	111.4	11	8, 10	11
C(13)		136.1			
H$_a$–C(14)	2.47 (m_c[a]))[b])	40.7		20	15
H$_b$–C(14)	1.91 (br. *d*, *J* = 11.0)		15	3, 15	21
H–C(15)	3.32 (m_c[a]))	25.4	14b, 20	14, 16, 17, 21	14a
C(16)		109.9			
H–C(17)	7.54 (*s*)	152.2		15, 16, 21, 22	
H–C(18)	3.50 (*d*, *J* = 4.6)	63.4	19	24	5a
H–C(19)	4.62 (*d*, *J* = 6.1)	72.2	18	3, 15	5b, 6a, 20
H–C(20)	1.68 (*t*, *J* = 7.0)	39.8	15, 21	14, 15, 21	19
H–C(21)	5.57 (*d*, *J* = 9.2)	96.0	20	1′	14b
C(22)		166.7			
Me(23)	3.59 (*s*)	51.0		22	
C(24)		174.3			
Glc					
H–C(1′)	4.63 (*d*, *J* = 7.7)	99.8	2′	21	3′, 5′
H–C(2′)	3.05 (*d*, *J* = 8.6)	73.2	1′, 3′	1′, 3′	4′
H–C(3′)	3.18 (*d*, *J* = 9.0)	76.6	2′, 4′	2′, 4′	1′, 5′
H–C(4′)	3.09 (*d*, *J* = 9.9)	70.0	3′, 5′	3′, 5′, 6′	2′
H–C(5′)	3.16 (*d*, *J* = 9.0)	77.0	4′, 6′b	4′	1′, 3′
H$_a$–C(6′)	3.62 (*d*, *J* = 11.5)	61.1	6′b		6′b
H$_b$–C(6′)	3.32 (m_c[a]))		5′, 6′a		6′a

[a]) Centered *multiplet* from HSQC spectrum. [b]) Overlap with (D$_6$)DMSO resonance.

Isonauclefidine (**2**) was obtained as yellow needles, and the molecular formula was established as $C_{16}H_{13}N_2O_2^+$ by means of the M^+ peak in the HR-ESI-MS spectrum at *m*/*z* 265.0977.

The UV spectrum showed absorption maxima at 206, 324, and 392 nm, while the IR spectrum suggested the presence of an indole N–H function (3424 cm^{-1}), COOH (3424 – 3075 cm^{-1}), aromatic C–H (3075 cm^{-1}), saturated C–H (2920 and 2851 cm^{-1}), and unsaturated carboxylic acid (1635 cm^{-1}) groups. The up-field region of the ^{1}H-NMR showed two CH$_2$ groups (δ(H) 4.96 (*t*, *J* = 7.4), 3.38 (*t*, *J* = 7.4)), the down-field region showed seven aromatic H-atoms (δ(H) 7.14, 7.36, 7.51, 7.70, 8.23, 8.81, 9.40). The signal at δ(H) 12.5 had no correlation signal in HSQC, and, therefore, was assigned

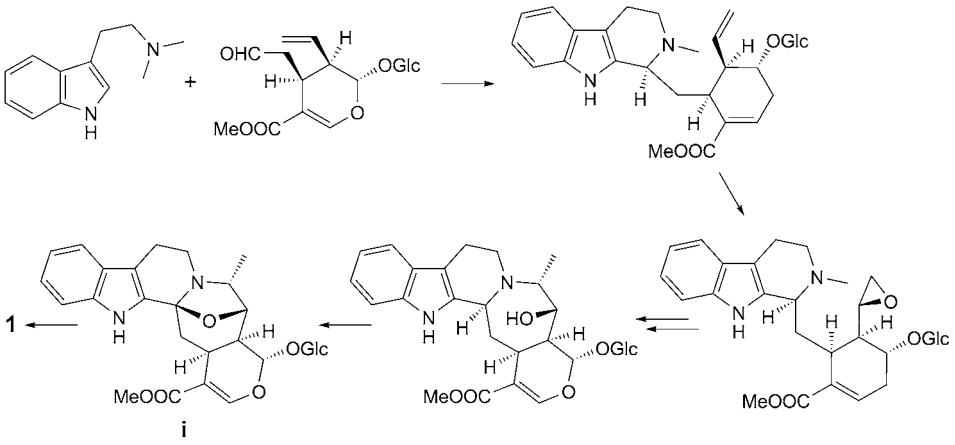
to NH. According to DEPT, in the down-field region of the ^{13}C-NMR were found signals for one CO group (δ(C) 163.9 (*s*)) and for 13 olefinic C-atoms (δ(C) 113.3 (*d*), 120.6 (*s*), 121.1 (*d*), 121.5 (*d*), 121.5 (*d*), 125.1 (*s*), 125.5 (*s*), 126.1 (*s*), 127.7 (*d*), 140.7 (*s*), 145.0 (*d*), 145.6 (*s*), 147.6 (*d*)); in the up-field region, signals for two CH_2 groups (δ(C) 56.5 (*t*), 19.3 (*t*)) were found. Thereof, the signal at δ(C) 56.5 could originate from a C-atom adjacent to a polar group. From HSQC, δ(C) 19.3, 56.5, 121.1, and 145.0 were corresponded to δ(H) 3.38, 4.96, 8.23, and 8.81, respectively. The COSY correlation of the signals δ(H) 3.38 and 4.96 and of δ(H) 8.23 and 8.81 indicated the presence of each a $-CH_2CH_2-$ and a –CH=CH– group. The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of nauclefidine [16][17]. This fact let us propose that **2** had the same skeleton as nauclefidine. The ^{1}H-NMR signals at δ(H) 8.23 (*d*, $J = 8.6$, 1 H), 8.81 (*d*, $J = 8.6$, 1 H), and 9.40 (*s*, 1 H), as well as the ^{13}C-NMR signals at δ(C) 121.1 (*d*), 126.1 (*s*), 145.0 (*d*), 147.6 (*d*), and 163.9 (*s*) were markedly shifted to the downfield region, which implied that a more strong polar group was adjacent to the N-atom in the six-membered heterocycle, compared to nauclefidine. HR-ESI-MS, DEPT, ^{1}H- and ^{13}C-NMR revealed that **2** had only one COO group (δ(C) 163.9 (*s*)), and that the N-containing six-membered heterocycle was mono-substituted. Therefore, we could suggest that –COOH was adjacent to the quaternary C-atom (δ(C) 126.1 (*s*)), which was further confirmed by the HMBC (*Fig. 2*). The HMBC between δ(H) 4.96 and δ(C) 147.6 (*d*) inferred that δ(C) 147.6 (*d*) was connected with N^+, and the location of the –COOH group at C(16)[1]) (δ(C) 126.1 (*s*)) was confirmed by the HMBCs between δ(H) 9.40 and δ(C) 163.9 (*s*), and 126.1 (*s*). Therefore, the structure of **2** was determined as shown in *Fig. 1*, and named isonauclefidine. Compound **2** has been reported as a synthetic compound [18], but not as a natural product. The complete assignments of the ^{1}H- and ^{13}C-NMR data for compound **2** are shown in *Table 2*. It's counter ion is Br^- as shown in the HR-ESI-MS spectrum.

Table 2. *NMR Data of Isonauclefidine* **2** *in (D$_6$)DMSO* (400 MHz for ^{1}H, 100 MHz for ^{13}C)

	δ(H)	δ(C)	COSY (H → H)	HMBC (H → C)
H–N(1)	12.5 (*s*)			2, 7, 8, 13
C(2)		125.5		
C(3)		145.6		
CH_2(5)	4.96 (*dd*, $J = 7.4$, 7.4)	56.5	6	3, 6, 7, 17
CH_2(6)	3.38 (*dd*, $J = 7.4$, 7.4)	19.3	5	2, 3, 5, 7
C(7)		120.6		
C(8)		125.1		
H–C(9)	7.70 (*d*, $J = 8.2$)	121.5	10	7, 11, 13
H–C(10)	7.14 (*dd*, $J = 7.5$, 8.2)	121.5	9, 11	8, 12
H–C(11)	7.36 (*dd*, $J = 7.5$, 8.4)	127.7	10, 12	9, 10, 12, 13
H–C(12)	7.51 (*d*, $J = 8.4$)	113.3	11	8, 10
C(13)		140.7		
H–C(14)	8.23 (*d*, $J = 8.6$)	121.1	15	3, 16
H–C(15)	8.81 (*d*, $J = 8.6$)	145.0	14	3, 17, 18
C(16)		126.1		
H–C(17)	9.40 (*s*)	147.6		5, 16, 18
C(18)		163.9		

The reported mechanism of formation of cadambine postulates that it was biosynthesized from secologanin *via* strictosidine [19]. We assume that compound **1** is biosynthesized from *N*,*N*-dimethyltryptamine and secologanin. Attack by the O-atom at C(3) could yield intermediate **i**, subsequent oxidation of the Me(24) would yield **1**. A biogenetic pathway to **1** is proposed as shown in *Scheme 1*.

Scheme 1. *Proposed Biogenetic Pathway to Compound* **1**



The structure of compound **2** is similar to nauclefidine, which was biomimetically formed from strictosamide or vincoside lactam [17]. Intermediate **ii** could be converted to **iii**. Reduction at C(17) could yield **iv**, further oxidation of the aldehyde group at C(18) would yield **2**. A plausible biogenetic pathway to **2** is proposed as shown in *Scheme 2*.

Compounds **1** and **2** were tested for cytotoxicity (A549, BGC-823, and HeLa cells) at 10 μg/ml by sulforhodanine B (SRB) assay [20]. Compound **1** exhibited 1.2, 13.8, and 12.9% growth inhibition against A549, BGC-823, and HeLa cells, respectively, while compound **2** showed 10.0, 19.6, and −15.2%. The data demonstrated that both compounds showed only little cytotoxicity against A549, BGC-823, and HeLa cells in biological assays, on the contrary, compound **2** promoted the growth of HeLa cells.

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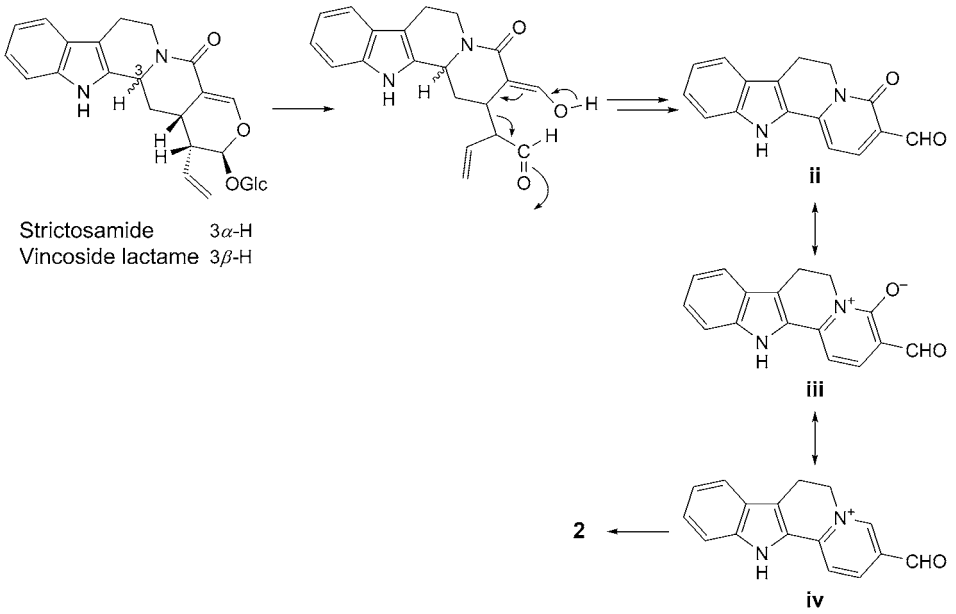
Experimental Part

General. TLC and column chromatography (CC): silica gel F_{254} plates (*Qingdao Haiyang Chemical CO. Ltd.*, Qingdao, P. R. China) and silica gel F_{254} (SiO_2; *Qingdao Haiyang Chemical CO. Ltd.*, Qingdao, P. R. China); macroporous resin (*Shandong Lukang Pharmaceutical Co., Ltd.*, Shandong, China); *MCI* gel (*Mitsubishi Chemical Corp.*, Tokyo, Japan). M.p.: *SGW X-4 Micro*-melting point apparatus. Optical rotations: *Jasco P-1020* spectropolarimer. UV Spectra: *UV-2401PC* spectrophotometer; λ in nm. IR

Scheme 2. *Proposed Biogenetic Pathway to Compound* **2**



Spectra: *Bruker Tensor27* spectrometer; ν in cm^{-1}. 1D-NMR and 2D-NMR spectra: *Bruker AV-400 MHz* and *DRX-500 MHz* spectrometers; δ in ppm, J in Hz. ESI-MS and HR-ESI-MS: *API QSTAR Pulsar* spectrometer; in m/z (%).

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

Extraction and Isolation. The dried bark of *A. chinensis* (100 kg) was ground and refluxed three times with 90% EtOH. After solvent evaporation, the residue (10 kg) was extracted with petroleum ether, $CHCl_3$, and BuOH. The BuOH fraction was separated by macroporous resin (gradient MeOH/H_2O 50% to 70% *v*/*v*) and *MCI* to give three fractions, *Frs. 1–3*. *Fr. 2* was further separated by CC (SiO_2; $CHCl_3$/MeOH 70:30) to give cadambinic acid (**1**; 870 mg). *Fr. 3* was further separated by CC (SiO_2; $CHCl_3$/MeOH 80:20) to give isonauclefidine (**2**; 80 mg).

Cadambinic Acid (*=(4S,4aS,5S,6S,14bS,15aS)-4-(β-*D*-Glucopyranosyloxy)-4,4a,5,6,9,14,15,15a-octahydro-1-(methoxycarbonyl)-8*H*-5,14b-epoxypyrano[4'',3'':4',5']azepino[1',2':1,2]pyrido[3,4-*b*]indole-6-carboxylic Acid*; **1**). Yellow amorphous powder (MeOH). M.p. 280–282°. $[\alpha]_D^{24.8} = -124.7$ ($c = 0.003$, DMSO). UV (MeOH): 225, 364. IR (KBr): 3490, 3431, 3225, 3173, 2910, 1712, 1631, 1568, 1402, 1164, 1109, 1092, 1040, 993, 739. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 611 ($[M+Na]^+$). HR-ESI-MS: 611.1852 ($C_{28}H_{32}N_2NaO_{12}^+$; calc. 611.1853).

Isonauclefidine (*=3-Carboxy-7,12-dihydro-6*H*-indolo[2,3-a]quinolizin-5-ium*; **2**). Yellow needle crystals (MeOH). M.p. 235–237°. $[\alpha]_D^{27.3} = +10.0$ ($c = 0.002$, MeOH/$CHCl_3$ 1:1). UV (MeOH): 206, 324, 392. IR (KBr): 3424, 3075, 2920, 2851, 1635, 1550, 1364, 1332, 1282, 747. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 265 (M^+). HR-ESI-MS: 265.0977 ($C_{16}H_{13}N_2O_2^+$; calc. 265.0972). It's counter ion is Br^-.

Biological Assay. The isolated compounds (**1** and **2**) were subjected to cytotoxic evaluation against A549 (human lung carcinoma), BGC-823 (human lung carcinoma), and HeLa (human cervical carcinoma) cell lines at 10 μg/ml by SRB method [20]. The cells were subjected to samples for 48 h. The positive control was taxol.

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