Indole Alkaloids from the Leaves of *Anthocephalus chinensis*

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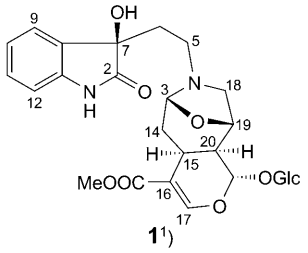
Anthocephalusine A (**1**) and 3β-isodihydrocadambine 4-oxide (**2**) were isolated from the leaves of *Anthocephalus chinensis* (Rubiaceae), together with five known compounds. The structures were established by spectroscopic methods including 2D-NMR analyses.

Introduction. – The genus *Anthocephalus* is a member of the tribe Naucleeae in the family Rubiaceae and distributed widely in south Asia and the south of China. Known as wild cinchona and popular in India as 'Kadamb', its bitter and pungent bark was used in ayurvedic medicine for blood diseases and dysentery [1].

To seek bioactive substances for blood diseases from *A. chinesis*, we isolated from its leaves two new glycosidic monoterpenoid indole alkaloids **1** and **2**, together with the five known compounds cadambine [2], 3α-dihydrocadambine [2], isodihydrocadambine [3], cadamine [4], and 3β-dihydrocadambine **3** [5].



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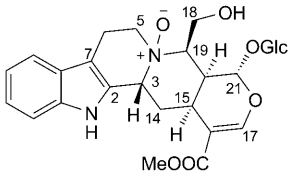

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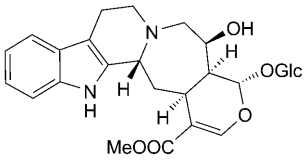

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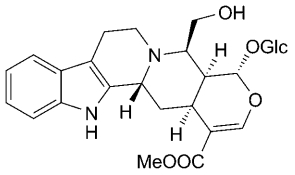

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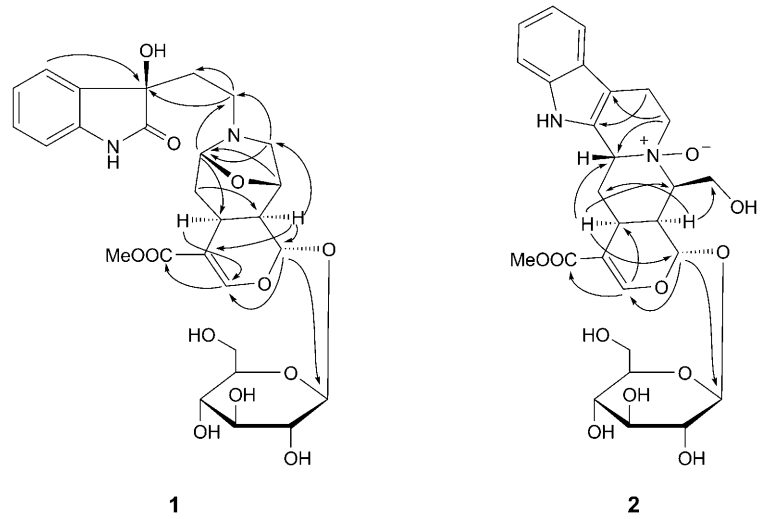
Results and Discussion. – Compound **1**, named anthocephalusine A, was obtained as an optically active colorless powder. Its UV spectrum was characteristic of an indole

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

chromophore with absorption maxima at 209, 236, and 286 nm, while the IR spectrum showed bands due to amide (1711 cm^{-1}) and carboxylic ester (1625 cm^{-1}) functions. Its molecular formula $C_{27}H_{34}N_2O_{12}$, indicating twelve degrees of unsaturation, was determined on the basis of HR-ESI-MS data (*m/z* 579.2200 ($[M+1]^+$)). The ^{1}H- and ^{13}C-NMR spectra of **1** (*Table*) were similar to those of 3β-dihydrocadambine (**3**) [5], which was obtained in a large amount and identified in our work. The HMBC data (*Fig.*) were in complete accord with the structure **1**. The relative configuration of **1** was determined from a 2D-ROESY NMR experiment which established a similar relative configuration as that of **3**, except for a reversed configuration of C(3), which possibly resulted from an S_N1 substitution reaction at C(3). The absolute configuration at C(7) was elucidated to be (*R*), since a positive *Cotton* effect in the region 300–260 nm and a negative curve in the region 260–230 nm were observed in the CD spectrum of **1** [6].



1 **2**

Figure. *HMBC of compounds* **1** *and* **2**

The ^{13}C-NMR spectrum of **1** gave a total of 27 C-atom resonances (one Me, five CH_2, fifteen CH, and six quaternary C-atoms). COSY and HMQC Experiments revealed the presence of the following partial structure: $NCH_2CHCHCHCH_2CH$ and NCH_2CH_2, which was further confirmed by a HMBC experiment. One major difference between the NMR data of **1** and **3** was the absence of signals of the C(2)=C(7) bond in **1** and their replacement by two quaternary C-atom signals, one at δ(C) 76.6 and another at δ(C) 181.8 (amide), and the downfield shift for C(3) of **1** (δ(C) 94.8), indicating the cleavage of the C(2)–C(3) bond and the bonding of C(3) to both an N- and O-atom. A signal at δ(C) 76.6 was assigned to C(7) on the basis of the HMBC cross-peaks C(7)/H–C(9), CH_2(6), and CH_2(5). The HMBC cross-peaks from a CH C-atom at δ(C) 74.3 (C(19)) to H–C(3) (δ(C) 94.8 C(3)) suggested the linkage between C(19) and C(3) by an O-atom.

Compound **2**, *i.e.*, 3β-isodihydrocadambine 4-oxide, was obtained as an optically active white powder. The UV spectrum showed absorption maxima at 221, 279, and 290 nm, while the IR spectrum suggested the presence of a carboxylic ester function

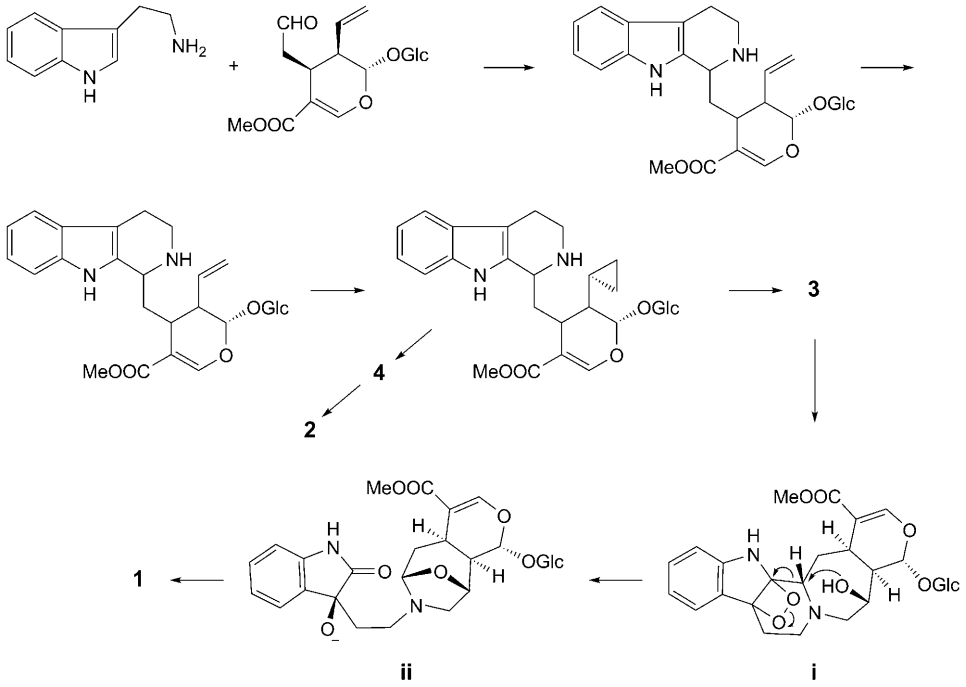
Table. *^1H-* (500 MHz) *and ^{13}C-NMR* (125 MHz) *Data of Alkaloids* **1** *and* **2** *in CD_3OD*[1]). δ in ppm, J in Hz.

	1		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)
C(2)	181.8 (*s*)		129.3 (*s*)	
H–C(3)	94.8 (*d*)	4.66 (br. *s*)	77.3 (*d*)	4.94(br. *s*)
CH_2(5)	54.2 (*t*)	2.68–2.70 (*m*), 2.50–2.55 (*m*)	73.1 (*t*)	3.70–3.75 (*m*), 3.90–3.93 (*m*)
CH_2(6)	37.9 (*t*)	2.12–2.15 (*m*)	19.9 (*t*)	3.11–3.14 (*m*)
C(7)	76.9 (*s*)		107.9 (*s*)	
C(8)	132.7 (*s*)		127.4 (*s*)	
H–C(9)	130.5 (*d*)	7.25 (*dd*, J = 7.8, 1.0)	119.1 (*d*)	7.48 (*dd*, J = 7.9, 1.0)
H–C(10)	123.6 (*d*)	7.06 (*dt*, J = 7.8, 1.0)	120.7 (*d*)	7.03 (*dt*, J = 7.9, 1.0)
H–C(11)	125.1 (*d*)	7.36 (*dt*, J = 7.8, 1.0)	123.5 (*d*)	7.09 (*dt*, J = 7.9, 1.0)
H–C(12)	111.3 (*d*)	6.90 (*dd*, J = 7.8, 1.0)	112.6 (*d*)	7.33 (*dd*, J = 7.9, 1.0)
C(13)	142.9 (*s*)		138.9 (*s*)	
CH_2(14)	37.3 (*t*)	2.00–2.05 (*m*), 1.34–1.38 (*m*)	26.9 (*t*)	2.50–2.55 (*m*), 3.23–3.25 (*m*)
H–C(15)	25.7 (*d*)	2.97–3.00 (*m*)	30.1 (*d*)	2.85–2.90 (*m*)
C(16)	111.1 (*s*)		109.3 (*s*)	
H–C(17)	154.0 (*d*)	7.48 (*s*)	156.1 (*d*)	7.70 (*s*)
CH_2(18)	58.0 (*t*)	2.97–3.00 (*m*), 2.67–2.70 (*m*)	59.0 (*t*)	3.73–3.75 (*m*)
H–C(19)	74.3 (*d*)	4.70–4.73 (*m*)	70.1 (*d*)	4.58 (*m*)
H–C(20)	41.6 (*d*)	1.59–1.61 (*m*)	45.5 (*d*)	1.91 (*m*)
H–C(21)	97.4 (*d*)	5.52 (*d*, J = 9.5)	96.5 (*d*)	5.54 (*d*, J = 9.4)
*C*OOMe	168.9 (*s*)		171.1 (*s*)	
COO*Me*	51.7 (*q*)	3.69 (*s*)	52.5 (*q*)	3.87 (*s*)
Glc:				
H–C(1′)	101.4 (*s*)	4.72 (*d*, J = 9.5)	100.4 (*d*)	4.78 (*d*, J = 9.4)
H–C(2′)	74.7 (*d*)	3.21–3.23 (*m*)	74.4 (*d*)	3.25–3.26 (*m*)
H–C(3′)	78.1 (*d*)	3.35–3.38 (*m*)	78.3 (*d*)	3.24–3.26 (*m*)
H–C(4′)	71.6 (*d*)	3.29–3.31 (*m*)	70.8 (*d*)	3.38–3.40 (*m*)
H–C(5′)	77.9 (*d*)	3.27–3.29 (*m*)	77.9 (*d*)	3.35–3.38 (*m*)
CH_2(6′)	62.8 (*t*)	3.83 (*d*, J = 12.0), 3.63 (*d*, J = 12.0)	62.1 (*t*)	3.83 (*d*, J = 11.9), 3.69 (*d*, J = 11.9)

(1679 cm^{-1}). The molecular formula was determined to be $C_{27}H_{35}N_2O_{11}$ by the HR-ESI-MS (*m*/*z* 563.2247 ($[M+1]^+$)), which was different from that of 3β-isodihydrocadambine (**4**) [7] by the presence of one additional O-atom in **2**. The ^{13}C-NMR spectrum (*Table*) gave a total of 27 C-atom resonances (one Me, five CH_2, fifteen CH, and six quaternary C-atoms). The ^{1}H- and ^{13}C-NMR spectra were in most respects rather similar to that of **4**, which was one of the major indole alkaloids present in the leaves of *A. chinensis*, except for the notable downfield shifts of C(3), C(5), and C(19) (δ(C) 77.3, 73.1, and 70.1, resp.), suggesting that they were adjacent to an N(4)-oxide moiety. Detailed analyses of the 1D and 2D NMR data (HSQC and HMBC (*Fig.*)) allowed us to assign all H- and C-atoms. The relative configuration of **2** was determined from a 2D-ROESY NMR experiment which showed that the relative configuration of **2** was consistent with that of **4**.

The mechanism of formation of these glycosidic indole alkaloids has been mentioned [8]. A plausible biogenetic pathway to **1** and **2** is proposed as shown in the *Scheme*. Oxidation of the C(2)=C(7) bond could yield a peroxy intermediate **i**.

Scheme. *Proposed Biogenetic Pathway to compounds* **1** *and* **2**



Subsequent attack by the O-atom at C(3) would lead to the opening of the C(2)–C(3) bond and the inversion of the relative configuration at C(3) yielding intermediate **ii** which would react with H^+ to give **1**.

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Experimental Part

General. Solvents were distilled before use. TLC and columm chromatography (CC): plates precoated with silica gel F_{254} and *H* (SiO_2; *Qingdao Haiyang Chemical CO. Ltd.*, Qingdao, China), resp. Optical rotations: *Horiba SEAP-300* spectropolarimer. UV Spectra: *UV-210A* spectrophotometer; λ_{max} in nm. IR Spectra: *BioRad FTS-135* spectrometer; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-AM-500 MHz* spectrometer; δ in ppm, *J* in Hz. EI- and HR-ESI-MS: *VG-AUTO-Spec-3000* spectrometer; in *m*/*z* (%).

Plant Material. The leaves of *A. chinensis* were collected in Xishuangbanna, Yunnan Province of China, in February 2005 and air-dried. The plant was identified by Prof. *De-Ding Tao*, Chinese Academy of Science. A specimen of this plant was deposited with the Kunming Institute of Botany, Kunming, China.

Extraction and Isolation. The dried leaves of *A. chinensis* (5.0 kg) were ground and refluxed three times with 95% EtOH. After solvent evaporation, the residue was extracted with 2% HCl soln. The acid-soluble fraction was washed with $CHCl_3$, then basified to pH 10 with 25% NH_3 soln., and extracted with $CHCl_3$ to give the crude alkaloid fraction (5.0 g). The crude alkaloid fraction was separated by CC (SiO_2,

increasing proportions of MeOH in $CHCl_3$): *Fractions 1–3*. *Fr. 1* contained no alkaloid and was not further separated. *Fr. 2* was subjected to repeated CC (SiO_2, $CHCl_3$/MeOH 5:1): 3α-dihydrocadambine (100 mg), 3β-dihydrocadambine (**3**; 250 mg), and isodihydrocadambine (200 mg). *Fr. 3* was separated by reversed-phase CC (*RP-18*, gradient 30% – 50% *v*/*v*) and then by CC (SiO_2, $CHCl_3$/MeOH 100:15): anthocephalusine A (**1**; 10 mg) and 3β-isodihydrocadambine 4-oxide (**2**; 20 mg).

Anthocephalusine A (=*(1*S*,4a*S*,6*R*,9*S*,9a*S*)-7-[2-[(3*R*)-2,3-Dihydro-3-hydroxy-2-oxo-1*H*-indol-3-yl]ethyl]-1-(β*-D*-glucopyranosyloxy)-1,4a,5,6,7,8,9,9a-octahydro-6,9-epoxypyrano[3,4-*d*]azepine-4-carboxylic Acid Methyl Ester*; **1**): Colorless, amorphous powder. $[\alpha]_D^{19.2} = +64.2$ ($c = 0.54$, MeOH). UV (MeOH): 209, 236, 286. IR (KBr): 3428, 2923, 2853, 1711, 1625, 1078. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 578 (100, $[M+1]^+$). HR-ESI-MS: 579.2200 ($[M+1]^+$, $C_{27}H_{35}N_2O_{12}^+$; calc. 579.2190).

3β-Isodihydrocadambine 4-Oxide (=rel-*(4*R*,4a*R*,5*S*,13*S*,14a*R*)-4-(β-D-Glucopyranosyloxy)-4a,5,7,8,13,13b,14,14a-octahydro-5-(hydroxymethyl)-4*H*-indolo[2,3-*a*]pyrano[3,4-*g*]quinolizine-1-carboxylic Acid Methyl Ester 6-Oxide*; **2**): Colorless, amorphous powder. $[\alpha]_D^{19.2} = -40.1$ ($c = 0.776$, MeOH). UV (MeOH): 221, 279, 290. IR (KBr): 3425, 2923, 2853, 1679, 1633, 1076. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 563 (100, $[M+1]^+$). HR-ESI-MS: 563.2247 ($[M+1]^+$, $C_{27}H_{35}N_2O_{11}^+$; calc. 563.2241).

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