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Aminocadambines A and B, two novel indole alkaloids from *Neolamarckia cadamba*

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ABSTRACT

Two novel monoterpenoid indole alkaloids, aminocadambines A (**1**) and B (**2**), characterized by tetrahydrofuran and 1,2,3,4-tetrahydropyridine rings, were isolated from the leaves of *Neolamarckia cadamba*. Their structures were elucidated on the basis of spectroscopic and computational methods. The absolute configuration of **1** was established by CD analysis. A plausible biosynthetic pathway for **1** and **2** is proposed.

Neolamarckia cadamba, previously named *Anthocephalus chinensis*, is a member of the tribe Neolamarckia in the family Rubiaceae and is distributed widely in the South Asia.[1] Phytochemical studies of this genus have led to discoveries of indole alkaloid glycosides as the main constituents.[2] These diverse indole alkaloids and their biogenetic pathways have stimulated considerable interest in recent years since the indole alkaloids originate from the condensation of tryptophan with secologanin.[3] In our previous research, several new alkaloids were isolated from *N. cadamba*.[4] In a continuing effort to search for structurally and biologically significant metabolites from this genus, two novel indole alkaloids aminocadambines A (**1**) and B (**2**) were isolated from the leaves of *N. cadamba*. Both **1** and **2** possess an unprecedented polycyclic system featuring a tetrahydrofuran unit and a 1,2,3,4-tetrahydropyridine ring. In this Letter, the isolation and structure elucidation of **1** and **2** are described.

The air-dried and powdered leaves of *N. cadamba* (25 kg) were refluxed three times with 95% EtOH, and the extract was partitioned between EtOAc and 1% HCl solution. After having basified to pH 10 with saturated Na_2CO_3, the aqueous layer was further extracted with EtOAc and *n*-BuOH successively. The *n*-BuOH soluble material was then subjected to ion-exchange chromatography to enrich the alkaloid. The crude alkaloid fraction (30 g) was then chromatographed on RP-18 silica gel, eluting with H_2O–MeOH (from 10:1 to 0:10) to afford five fractions (I–V). Fraction II (10 g)

Table 1
^1H, ^{13}C and DEPT NMR data of **1** and **2**

No.	**1**[a]		**2**[b]	
	δ_H (mult, *J*, Hz)	δ_C	δ_H (mult, *J*, Hz)	δ_C
2	–	128.4 (s)	–	128.0 (s)
3	4.84 (1H, d, 8.5)	59.7 (d)	4.81 (1H, br.d, 12.1)	59.6 (d)
5α	3.88 (1H, m)	58.5 (t)	3.95 (2H, m)	59.1 (t)
5β	4.04 (1H, m)			
6	3.20 (2H, m)	19.5 (t)	3.13 (2H, m)	19.5 (t)
7	–	105.7 (s)	–	106.0 (s)
8	–	127.0 (s)	–	126.9 (s)
9	7.51 (1H, d, 7.7)	119.4 (d)	7.47 (1H, d, 7.5)	119.2 (d)
10	7.08 (1H, t, 7.7)	120.8 (d)	7.04 (1H, t, 7.5)	120.8 (d)
11	7.17 (1H, t, 7.7)	123.5 (d)	7.13 (1H, t, 7.5)	123.5 (d)
12	7.36 (1H, d, 7.7)	112.6 (d)	7.31 (1H, d, 7.5)	112.5 (d)
13	–	138.6 (s)	–	138.7 (s)
14α	2.03 (1H, m)	30.9 (t)	1.98 (1H, m)	30.8 (t)
14β	2.42 (1H, m)		2.32 (1H, m)	
15	3.28 (1H, m)	27.2 (d)	3.19 (1H, m)	26.8 (d)
16	–	105.3 (s)	–	105.7 (s)
17	7.81 (1H, s)	144.6 (d)	7.76 (1H, s)	146.3 (d)
18α	3.90 (1H, m)	59.6 (t)	3.83 (1H, m)	59.8 (t)
18β	3.38 (1H, m)		3.30 (1H, m)	
19	4.56 (1H, m)	68.8 (d)	4.54 (1H, d, 6.5)	68.8 (d)
20	2.38 (1H, m)	42.7 (d)	2.21 (1H, m)	43.2 (d)
21	5.53 (1H, d, 3.3)	89.1 (d)	5.63 (1H, d, 3.0)	87.1 (d)
22	–	168.7 (s)	–	168.1 (s)
23	3.78 (3H, s)	51.9 (q)	3.76 (3H, s)	51.9 (q)
1′	–	177.2 (s)	–	180.3 (s)
2′	4.15 (1H, q, 7.4)	66.3 (d)	2.23 (2H, m)	34.9 (t)
3′	1.68 (3H, d, 7.4)	17.7 (q)	1.93 (2H, m)	27.4 (t)
4′	–	–	3.56 (2H, m)	56.3 (t)

[a] Data were recorded in CD_3OD + DMSO-d_6 (4:1) at 400 MHz (^1H) and 100 MHz (^{13}C).
[b] Data were recorded in CD_3OD at 400 MHz (^1H) and 100 MHz (^{13}C).

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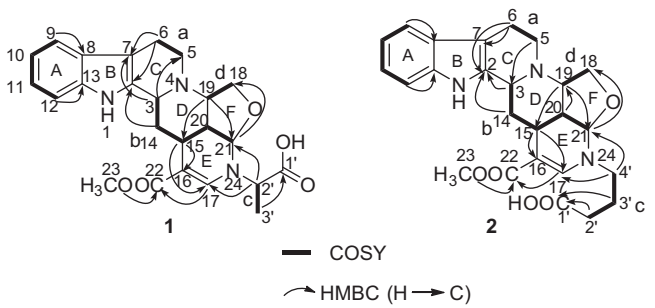


Figure 1. Selected 2D NMR correlations for **1** and **2**.

was purified by silica gel column chromatographies to afford compounds **1** (10 mg) and **2** (7 mg).

Aminocadambine A (**1**),[5] a white amorphous powder, had the molecular formula $C_{24}H_{27}N_3O_5$ according to its HRESIMS at *m/z* 438.2015 $[M+H]^+$ (calcd 438.2028), with 13° of unsaturation. The IR spectrum suggested the presence of NH and/or OH (3439 cm^{-1}) functionality and carbonyl (1627 cm^{-1}) group(s). The UV spectrum showed the existence conjugation based on the absorption at 219 and 273 nm. The ^{13}C NMR and DEPT spectra (Table 1) revealed 24 carbon signals due to 7 quaternary carbons, 11 methines, 4 methylenes, and 2 methyl groups. Among them, the eight sp^2 carbon signals (δ_C 128.4 (s, C-2), 105.7 (s, C-7), 127.0 (s, C-8), 119.4 (d, C-9), 120.8 (d, C-10), 123.5 (d, C-11), 112.6 (d, C-12), 138.6 (s, C-13)), and characteristic signals in the aromatic region of the ^1H NMR spectrum (Table 1) indicated the presence of an indole moiety.

^1H–^1H COSY data revealed that **1** possessed four fragments (**a**): (C-5–C-6), (**b**): (C-3–C-15, C-15–C-20, and C-20–C-21), (**c**): (C-2′–C-3′), and (**d**): (C-18–C-19), as shown in Figure 1. Analysis of the HMBC correlations of **1** established the connections among the four fragments, the indole moiety, a quaternary carbon, and the nitrogen atoms. HMBC correlations of H-6 to C-7 and C-2 suggested that unit (**a**) was connected to the indole moiety at C-7. The connectivity of units (**a**) and (**b**) via a nitrogen atom was indicated by HMBC correlation of H-3/C-5 and the chemical shifts of C-3 (δ_C 59.7) and C-5 (δ_C 58.5). Correlations of H-15 with C-16 and C-17 in the HMBC spectrum indicated the linkage of structure (**b**) and the olefinic unit [C-16 (δ_C 105.3) and C-17 (δ_C 144.6)]. Furthermore, HMBC correlations from H-2′ to C-17 and C-21 suggested that the three carbon atoms (C-2′, 17, and 21) were linked to another nitrogen atom to form the six-membered ring E. In addition, a carboxylic methyl ester group was appended to ring E at C-16 by the HMBC correlations of H_3-23/C-22 and H-17/C-22. Moreover, a carboxylic acid was located at C-2′ by the HMBC correlation between H_3-3′ and the carbonyl C-1′ (δ_C 177.2). According to the 13° of unsaturation of **1**, one more ring was still required for the structure. HMBC correlations of H-19/C-21 and H-21/C-18 enabled the linkage of fragments (**b**) and (**d**) via an oxygen atom to form tetrahydrofuran ring F. Thus, the planar structure of **1** was assigned as depicted, with an unprecedented 6/5/6/6/5/6 ring system containing tetrahydrofuran and 1,2,3,4-tetrahydropyridine rings.

The relative configuration of compound **1** was determined by the analysis of ROESY data and molecular modeling analysis (Fig. 2). The ROESY correlations of H-15/H-19, H-19/H-14α revealed the α-orientation of H-15, H-19, whereas the correlation

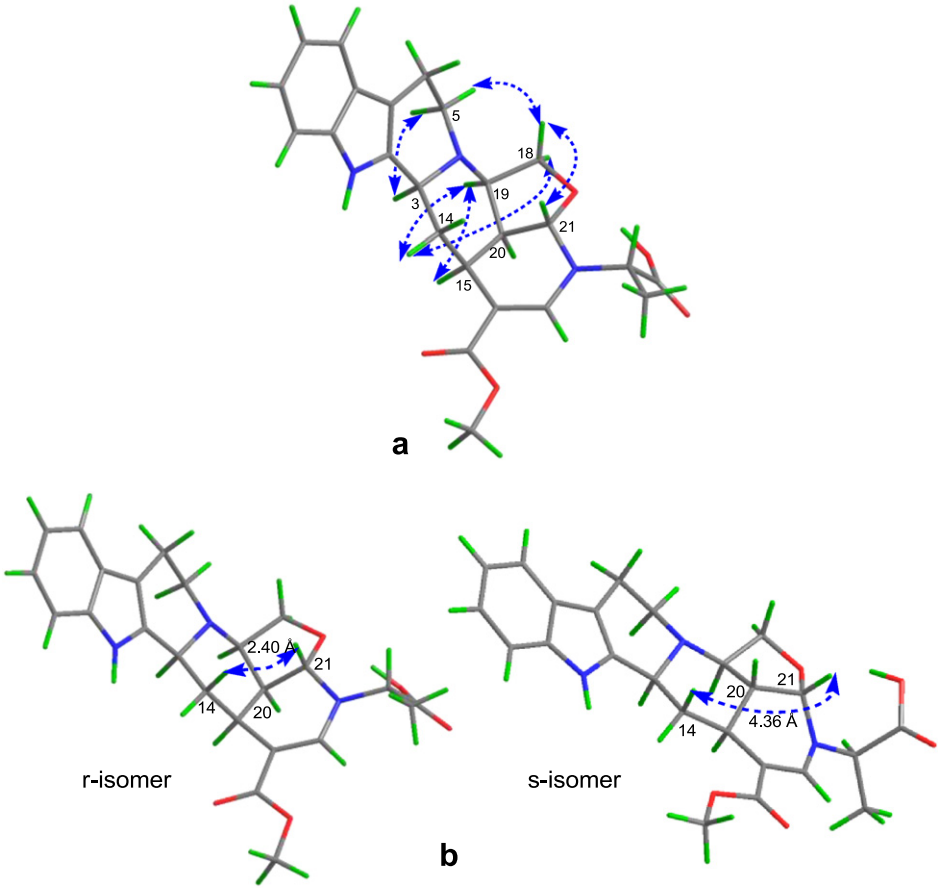


Figure 2. (a) Key ROESY correlations of **1**. (b) DFT-calculated of two isomers (*r*-isomer and *s*-isomer) of **1**, corresponding to the α- and β-orientations of H-20, respectively. Distances between H-14β and H-21 are given.

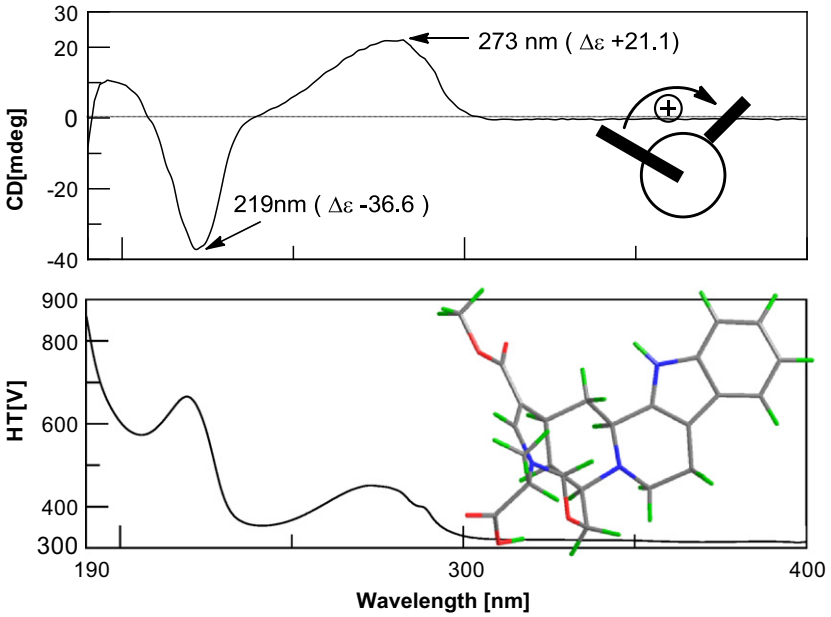
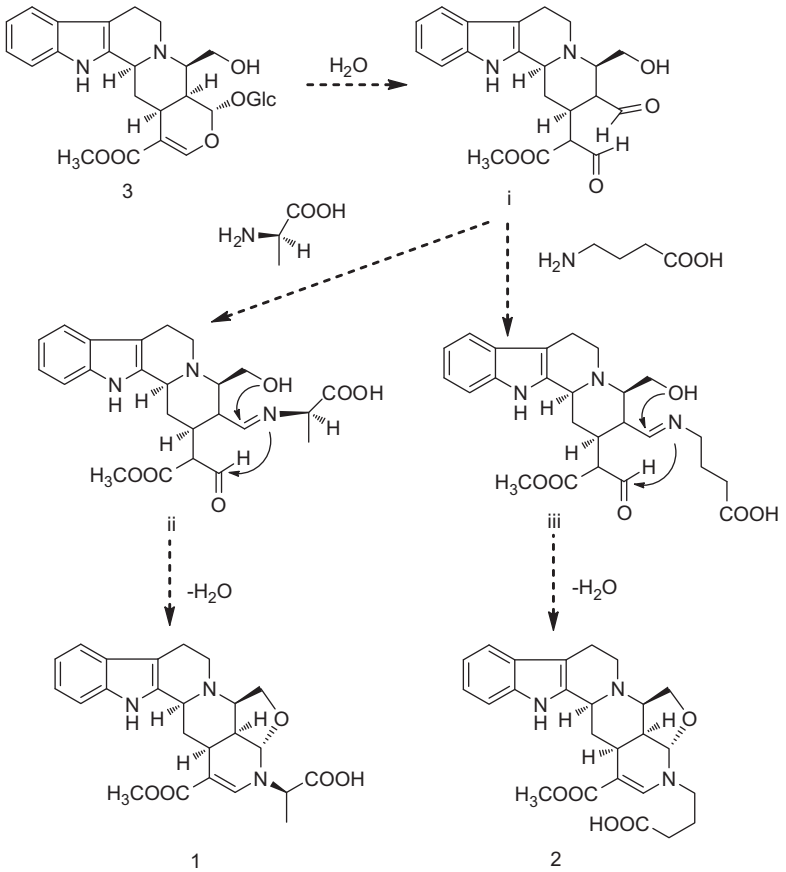


Figure 3. CD and UV spectra of **1** (in MeOH); arrows denote the electric transition dipole of the two chromophores.

of H-14β/H-21 suggested that H-21 was β-oriented. Furthermore, the observation of ROESY correlations of H-21/H-18β, H-18β/H-5β, and H-5α/H-3 indicated that H-3 was α-oriented. However, the orientation of H-20 could not be deduced directly from insufficient evidence in ROESY spectrum.

In order to determine the relative configuration at C-20, models of the *r*- and *s*-isomer of **1** were investigated by a quantum chemistry approach in GAUSSIAN 03 package at B3LYP/6-311G(d,p) level.[6] The resulted minimum energy conformations of both isomers (Fig. 2) gave a distance between H-21 and H-14β, of 2.40 Å for



Scheme 1. Biogenetic pathway proposed for **1** and **2**.

r-isomer and 4.36 Å for the other. Combined with the ROESY correlation of H-14β/H-21, the relative configuration of C-20 was assigned.[7] Thus, the relative configuration of **1** was elucidated as depicted.

The absolute configuration of **1** was assisted with CD exciton chirality method.[8] The CD spectrum of **1** showed a negative Cotton effect at λ_{max} 219 nm ($\Delta\varepsilon$ −36.6) and a positive Cotton effect at λ_{max} 273 nm ($\Delta\varepsilon$ +21.1) due to the exciton coupling between the two different chromophores of the α,β-unsaturated carboxylic methyl ester group and the indole ring, indicating that the transition dipole of the two chromophores was oriented in a clockwise manner (Fig. 3). The absolute configuration of **1** was therefore assigned as 3*S*, 15*S*, 19*R*, 20*S*, and 21*S*. Additionally, the configuration of C-2′ was supposed to be *R*, since the natural amino acids found in the plant are *R*-type.

The molecular formula of aminocadambine B (**2**)[9] was established as $C_{25}H_{29}N_3O_5$ based on its HRESIMS data. The similarity of the NMR data (Table 1) between compounds **2** and **1** indicated that both compounds possessed the same skeleton except for the substituent group at N-24. The substituent group in compound **2** was as shown based on ^1H–^1H COSY cross-peaks of H-2′/H-3′ and H-3′/H-4′ along with the HMBC correlation of H-2′/C-1′ (δ_C 180.3). The substituent group was located at N-24 due to the HMBC correlation of H-4′/C-17 (Fig. 1). The relative stereochemistry of the polycyclic ring system in **2** was measured to be the same as **1** since both compounds showed nearly identic chemical shifts for the central core and similar correlation signals in the ROESY spectrum.

A plausible biogenetic pathway for **1** and **2** was proposed as shown in Scheme 1. The biogenetic precursor of **1** and **2** would be 3α-isodihydrocadambine (**3**) which was also obtained from the title plant.[4] Hydrolysis of the glucoside of **3** could produce dialdehyde intermediate i, which could be followed by double Mannich-like condensation with an alanine or a γ-aminobutyric acid. The reaction afforded the novel alkaloid **1** and **2** directly. Normally, indole alkaloids mainly originate from the condensation of tryptophan with secologanin.[3a] However, compounds **1** and **2** are considered to be formed biogenetically from the corresponding glycosidic indole alkaloids by incorporating an L-alanine and a γ-aminobutyric acid into their E ring, respectively. The double Mannich-like condensation[10] is inferred to be the key step for these amino acid conjugations. Since any amino acid has not been used in the whole experimental process, these two alkaloids could be considered as the novel natural products, and they are the first to be reported that indole alkaloids could be further modified by amino acids.

The cytotoxic activities of **1** and **2** against the human tumor cell lines (HL-60, SMMC-7721, A-549, MCF-7, SW480) were evaluated. However, the results indicated that both **1** and **2** were inactive against the above cancer cells with IC_{50} >40 μM.[11]

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5. *Aminocadambine A* (**1**): white amorphous powder; $[\alpha]_D^{26}$ = +105.8 (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.53), 273 (4.27) nm; IR (KBr) ν_{max}: 3439, 2949, 1679, 1627, 1453, 1439, 1389, 1368, 1324, 1223, 1187, 1161, 1090, 1000, 890 and 747 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 438 $[M+H]^+$; HRESIMS *m*/*z* 438.2015 (calcd for $[M+H]^+$ 438.2028).
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9. *Aminocadambine B* (**2**): white amorphous powder; $[\alpha]_D^{26}$ = −3.8 (*c* 0.29, MeOH); UV (MeOH) λ_{max} (log ε) 205 (5.15), 272 (4.12) nm; IR (KBr) ν_{max}: 3424, 2950, 1685, 1629, 1570, 1453, 1440, 1390, 1321, 1224, 1186, 1157, 1095, 1066, 1011, 830 and 748 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 452 $[M+H]^+$; HRESIMS *m*/*z* 452.2163 (calcd for $[M+H]^+$ 452.2185).
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11. Cisplatin was used as a positive control compound, and the IC_{50} values against the cell lines (HL-60, SMMC-7721, A-549, MCF-7, SW480) were 0.75, 15.4, 13.6, 25.5, and 18.6 μM, respectively.