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

Ling-Li Liu a,b, Ying-Tong Di a, Qiang Zhang a, Xin Fang a, Feng Zhu a, Dong-Lin Chen c, Xiao-Jiang Hao a,* and Hong-Ping He a,*

a State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P.R. China
b School of Chinese Academy of Sciences, Beijing 100039, P.R. China

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N. 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 new 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 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 Na2CO3, the aqueous layer was further extracted with EtOAc and 1% HCl solution. After having basified to pH 10 with saturated Na2CO3, the aqueous layer was further extracted with EtOAc and 1% HCl solution. 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 H2O–MeOH (from 10:1 to 0:10) to afford five fractions (I–V). Fraction II (10 g)

<table>
<thead>
<tr>
<th>No.</th>
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<th>δC</th>
<th>No.</th>
<th>δH (mult, J, Hz)</th>
<th>δC</th>
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<td>5β</td>
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<td>4′</td>
<td>—</td>
<td>56.3(t)</td>
</tr>
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</table>

* Corresponding authors. Tel.: +86 871 522 3263; fax: +86 871 526 9684 (X.-J. Hao). E-mail addresses: haoxj@mail.kib.ac.cn (X.-J. Hao), hehongping@mail.kib.ac.cn (H.-P. He).

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Table 1
1H, 13C and DEPT NMR data of 1 and 2

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

Aminocadambine A (1), a white amorphous powder, had the molecular formula C_{24}H_{27}N_{3}O_{5} according to its HRESIMS at m/z 438.2015 [M+H]+ (calcd 438.2028), with 13/C_{176} 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 methyl- enes, and 2 methyl groups. Among them, the eight sp\(^{2}\) carbon signals (\(dC\) 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 \(^{1}H\) NMR spectrum (Table 1) indicated the presence of an indole moiety.

\(^{1}\)H–\(^{1}\)H 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 (\(dC\) 59.7) and C-5 (\(dC\) 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 (\(dC\) 105.3) and C-17 (\(dC\) 144.6)]. Furthermore, HMBC correlations of 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 H3-23/C-22 and H-17/C-22. Moreover, a carboxylic acid was located at C-20 by the HMBC correlation between H3-30 and the carbonyl C-1 (\(dC\) 177.2). According to the 13/C_{176} 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-14 showed the \(\alpha\)-orientation of H-15, H-19, whereas the correlation

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

Figure 2. (a) Key ROESY correlations of 1. (b) DFT-calculated of two isomers (\(r\)-isomer and \(s\)-isomer) of 1, corresponding to the \(\alpha\)- and \(\beta\)-orientations of H-20, respectively. Distances between H-14\(\beta\) and H-21 are given.
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/6-31G(d,p) level. The resulted minimum energy conformations of both isomers (Fig. 2) gave a distance between H-21 and H-14β, of 2.40 Å for

![CD and UV spectra of 1](image)
r-isomer and 4.36 Å for the other. Combined with the ROESY correlation of H-14/H/21, the relative configuration of C-20 was assigned. Thus, the relative configuration of 1 was elucidated as depicted.

The absolute configuration of 1 was assisted with CD exciton chirality method. The CD spectrum of 1 showed a negative Cotton effect at $\lambda_{\text{max}}$ 219 nm ($\Delta\varepsilon$ = -36.6) and a positive Cotton effect at $\lambda_{\text{max}}$ 273 nm ($\Delta\varepsilon$ = +21.1) due to the exciton coupling between the two different chromophores of the $\alpha\beta$-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 $S$, $Z$, $Z$, 18R, 20S, and 21S. 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) was established as C$_{25}$H$_{20}$N$_2$O$_5$ 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 $^1$H-1H COSY cross-peaks of H-2'/H-3' and H-3'/H-4' along with the HMBC correlation of H-2'/C-1' ($\delta_{C}$ 180.3). The substituent group was located at N-24 due to the HMBC correlation of H-2'/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 identical 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 3$\alpha$-isodihydrocadambine (3) which was also obtained from the title plant. Hydrolysis of the glucoside of 3 could produce dialdehyde intermediate i, which could be followed by double Mannich-like condensation with an alamine or a $\gamma$-aminobutyric acid. The reaction afforded the novel alkaloid 1 and 2 directly. Normally, indole alkaloids mainly originate from the condensation of tryptophan with secoligand. However, compounds 1 and 2 are considered to be formed biogenetically from the corresponding glycosidic indole alkaloids by incorporating an $\alpha$-amine and a $\gamma$-aminobutyric acid into their E ring, respectively. The double Mannich-like condensation 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, SMCC-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 $\mu$M.

Acknowledgments

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References and notes

5. Aminocadambine A (1): white amorphous powder; $\Delta \varepsilon_{290} = +105.8$ (c 0.22, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $e$) 219 (4.53), 273 (4.27) nm; IR (KBr) $\nu_{\text{max}}$, 3439, 2949, 1679, 1627, 1453, 1349, 1389, 1368, 1324, 1223, 1187, 1161, 1090, 1000, 890 and 747 cm$^{-1}$; $^{1}$H and $^{1}$C NMR data (Table 1); ESIMS m/z 438 [M+H]+; HRESIMS m/z 438.2015 (calcd for [M+H]+ 438.2028).
9. Aminocadambine B (2): white amorphous powder; $\Delta \varepsilon_{372} = -3.8$ (c 0.29, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $e$) 205 (5.15), 272 (4.12) nm; IR (KBr) $\nu_{\text{max}}$, 3424, 2950, 1685, 1629, 1570, 1453, 1440, 1390, 1321, 1224, 1188, 1157, 1095, 1066, 1011, 830 and 748 cm$^{-1}$; $^{1}$H and $^{13}$C NMR data (Table 1); ESIMS m/z 452 [M+H]+; HRESIMS m/z 452.2163 (calcd for [M+H]+ 452.2185).
11. Coquilin was used as a positive control compound, and the IC$_{50}$ values against the cell lines (HL-60, SMCC-7721, A-549, MCF-7, SW480) were 0.75, 15.4, 13.6, 25.5, and 18.6 $\mu$M, respectively.