Monoterpenoid indole alkaloids from *Alstonia rostrata*

Mei-Fen Bao,a,c Chun-Xia Zeng,b Yan Qu,a Ling-Mei Kong,a Ya-Ping Liu,a Xiang-Hai Cai,a,* and Xiao-Dong Luo,a,*

aState Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

bSouthwest China Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

cGraduate University of Chinese Academy of Sciences, Beijing 100049, China

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Abstract: Four new monoterpenoid indole alkaloids, alstrostines C–F together with thirteen known alkaloids were isolated from the leaves and twigs of *Alstonia rostrata*. All structures of new compounds were elucidated based on NMR, FTIR, UV, and MS spectroscopic data. Alstrostines C–E might originate from keto-enol tautomerism of preakummicine during biogenetic pathway of akummicine.

Keywords: monoterpenoid indole alkaloid, alstrostines C–F, *Alstonia rostrata*, Apocynaceae

Introduction

Monoterpenoid indole alkaloids (MIAs), including more than 2000 compounds, are classified to five types, corynanthe, strychnos, iboga, aspidosperma, and yohimbinoid, which play a very important role in natural medicinal history. The genus *Alstonia* of Apocynaceae is rich of monoterpenoid indole alkaloids, and eight species of the genus are distributed in Yunnan province. The phytochemical constituents of *Alstonia* sp. have been investigated intensively with anticancer, antibacterial, antifertility, and antitussive activity being reported. We reported new alkaloids from *A. scholaris*, and *A. yunnanensis*, and *A. mairei* recently. As part of systematic phytochemical research on Yunnan endemic resources, another species, *A. rostrata*, named *Winchia calophylla* in *Flora of China*, was also investigated. Previous studies on the stem bark and root of *A. rostrata*, collected from Yunnan Province of China, have reported a number of indole alkaloids and some nonalkaloid compounds. In the current study, separation of total alkaloids led to seventeen MIAs besides alstrostines A and B. In this paper, we will describe the isolation and structural elucidation of other four new alkaloids alstrostines C–F (1–4) together with thirteen known isolates, 19,20-dihydroakuammicine (5), echitamidine (6), 12-methoxyechitamidine (7), 19-oxo-12-methoxyechitamidine, vallesiachotamine (9), isovallesiachotamine (10), deacetylakuammiline (11), 17-O-acetyl-N⁴-demethylechitamine (12), N⁴-demethylechitamine (13), akuammidine (14), 6,7-secoangustilobine (15), undulifoline (16), tabersonine (17). The biogenetic pathway of the new alkaloids was proposed. In addition, all compounds were tested for their cytotoxicity against five human cancer cell lines, but no activity was found (IC₅₀ > 40 μM).

Results and Discussion

The MeOH extract of *A. rostrata* leaves and twigs was partitioned between H₂O and EtOAc after acid-alkali treating, and column chromatography was used to separate the...
alkaloidal fraction into seventeen alkaloids.

Alkaloid 1 gave positive reactions with Dragendorff" reagent and had a molecular formula of C_{22}H_{26}N_{4}O_{4} by HRESIMS at m/z 399.1919 [M + H]^+]. The maximum UV absorptions at 214, 275, and 326 nm of 1 pointed to an indole alkaloid with a β-anilinacetyl system in agreement with the FTIR absorption bands at 3453, 1705, and 1646 cm⁻¹. In the 1H NMR spectrum of 1, three signals [δ_H 6.98 (d, J = 8.0 Hz, H-9), 7.13 (t, J = 8.0 Hz, H-10), 7.03 (d, J = 8.0 Hz, H-11)] revealed the presence of a mono-substituted A ring in MIA.⁶³ The 13C NMR and DEPT spectra of 1 indicated signals for a dihydroindole ring [δC 128.5 (s, C-13), 139.7 (s, C-8), 112.3 (s, C-11), 113.4 (d, C-9), 126.7 (d, C-10), 148.3 (s, C-12), 55.0 (s, C-7)]. Moreover, 1 also possessed three methylens [δC 53.8 (C-5), 41.6 (C-6), 48.5 (C-21)], three methines [δC 68.5 (C-19), 59.9 (C-9), 31.5 (C-15)], one methyl (δC 20.9, C-18), and one sp² quaternary carbon (δC 152.0) besides confirming presence of methyl β-anilinacetyl conjugation [δC 170.5 (s), 160.0 (d), 53.8 (q), 120.1 (s)]³. The quaternary carbon signal at δC 152.0 correlated with H-3 (δ_H 3.75) and H-6 (δ_H 1.65 and 2.82) in the HMBC spectrum was assigned to C-2. Detailed analysis of 13C NMR and DEPT data revealed 1 might be belong to akummicine-type alkaloids. Further NMR comparison with those of 12-methoxyechitamidine (7)³ indicated that 1 was similar to 7 with exception for an additional methine (δC 160.0 and δH 9.12) in 1. The methine proton showed HMBC correlation to C-2, suggesting that the methine was connected with C-2 by an oxygen bridge with consideration of its molecular formula.

The UV spectrum of 2 and 3 also indicated the absorption bands of indole rings with a β-anilinacetyl system together with FTIR spectrum. Compound 2 was found to possess a molecular formula of C_{22}H_{26}N_{4}O_{4} as evidenced by HRESIMS at m/z 397.1763 [M + H]^+], an additional degree of unsaturation to 1. The 1H NMR spectra of 2 also displayed the signals for mono-substituted indole ring [δ_H 7.39 (1H, d, J = 7.8 Hz, H-11), 7.16 (t, J = 7.8 Hz, H-10)]. Its UV and 13C NMR and DEPT data showed similar pattern to 1 (see Table 1) except that the methine of C-19 was disappeared, instead a new signal of carbonyl group (δC 207.2, s) was present in 2. Its HMBC correlations could support it, in which δH 2.24 (H-18) was correlated with δC 207.2 (C-19) and 50.6 (C-18). Compound 3 possessed a molecular formula of C_{22}H_{26}N_{4}O_{4} based on the HRESIMS. The 1H and 13C NMR spectra of 3 displayed similarity to 1 except for two methine signals at δC 68.5 (C-19) and 47.3 (C-20) in 1 were substituted by double bond signals (δC 136.1 and 119.8) in 3, suggesting that 3 was a dehydration product of 1.

Configuration of alkaloids 1–3 was determined by NMR and ROESY spectra together with their biogenetic pathway. The chemical shift of C-19 in 1 was deshielded (δC 6.26 ppm) similar to N⁴-demethyl-12-methylalstogustine and N⁴-demethylalstogustine, relative to 12-methoxyechitamidine and echitamidine due to intramolecular H-bonding between the nitrogen atom and the C-19-OH.⁶⁴ The ROEVS correlations of H-3 with H-15 and H-20 in 1–3 placed them on the same sides. The double bond C-19/20 of 3 was determined as E according the ROEVS correlation of H-3 with H-19. In the biogenetic pathway of akummicine,¹⁰ the keto-enol tautomerism among O=C-C=C=S of preakuammicine would give two reaction routes, which led to alstrostines C–E and akummicine, respectively.

### Table 1. 13C NMR spectroscopic data for compounds 1–4 (1–3 in acetonitrile, 4 in methanol, δ in ppm, J in Hz)

| Carbon | 1  | 2  | 3  | 4  |
|--------|----|----|----|----|
| 1      | 1   | 1   | 1   | 1   |
| 2      | 2   | 2   | 2   | 2   |
| 3      | 3   | 3   | 3   | 3   |
| 4      | 4   | 4   | 4   | 4   |

(Fig. 2). Hence, configurations of alstrostines C–E were same to akummicine, as shown in Fig. 1.

**Figure 2.** Proposed biogenetic pathway of alstrostines C–E skeleton

HRESIMS at m/z 603.2553 [M + H]^+ defined molecular formula of compound 4 as C_{13}H_{16}N_{2}O_{4}. The IR spectrum of 4 implied the presence of hydroxyls (3424 cm⁻¹), double bonds (1627 cm⁻¹), and carbonyl groups (1703 cm⁻¹). Its UV spectrum showed the characteristic absorption bands of indole alkaloids at 226 and 282 nm in agreement with 1H NMR signals at δH 7.39 (1H, d, J = 7.8 Hz), 7.28 (1H, d, J = 7.8 Hz), 7.03 (1H, d, J = 7.8 Hz), 7.13 (t, J = 7.8 Hz), 7.03 (d, J = 7.8 Hz), 6.98 (d, J = 7.8 Hz), 7.16 (t, J = 7.8 Hz), 7.39 (1H, d, J = 7.8 Hz).
Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were recorded on a Shimadzu double-beam 210A spectrophotometer. IR (KBr) spectra were obtained on Bio-Rac FTS-135 infrared spectrophotometer. $^1$H, $^13$C and 2D NMR spectra were recorded on an AM-400 and DRX-500 MHz NMR spectrometer with TMS as an internal standard. MS data were obtained on an API Qstar Pulsar I spectrometer. Silica gel (200–300 mesh) for column chromatography (CC) and GF$_254$ for TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, China and sprayed with Dragendorff’s reagent.

Table 2. $^1$H NMR spectroscopic data for compounds 1–4 (1–3 in acetone-$d_6$, 4 in methanol-$d_4$, $\delta$ in ppm, $J$ in Hz)

| C     | $\delta_H$ (1) | $\delta_H$ (2) | $\delta_H$ (3) | $\delta_H$ (4) |
|-------|----------------|----------------|----------------|----------------|
| N$_2$ | 3.75 (1H, br. s) | 3.78 (1H, t, 2.6) | 3.88 (1H, t, 2.6) | 9.65 (1H, br. s) |
| 5     | 2.82 (2H, m)    | 2.82 (1H, m); 2.98 (1H, m) | 2.79 (1H, m); 2.98 (1H, m) | 4.07 (1H, t, 6.0) |
| 6     | 1.65 (1H, m); 2.82 (1H, m) | 1.68 (1H, m); 2.85 (1H, m) | 3.10 (1H, m); 3.37 (1H, m) | 3.08 (1H, m); 3.12 (1H, m) |
| 9     | 6.98 (1H, d, 8.0) | 7.00 (1H, d, 7.9) | 7.02 (1H, d, 7.4) | 2.78–2.81 (1H, m); 2.46 (1H, d, 4.8, 4.5) |
| 10    | 7.13 (1H, t, 8.0) | 7.16 (1H, t, 7.9) | 7.15 (1H, t, 7.4) | 7.39 (1H, d, 7.8) |
| 11    | 7.03 (1H, d, 8.0) | 7.05 (1H, d, 7.9) | 7.07 (1H, d, 7.4) | 7.01 (1H, t, 7.8) |
| 12    |                |                |                | 6.90 (1H, t, 7.8) |
| 14    | 1.47 (2H, m)    | 1.52 (1H, m); 2.11 (1H, m) | 1.50 (1H, m); 2.06 (1H, m) | 1.86 (2H, m) |
| 15    | 3.01 (1H, m)    | 3.30 (1H, m) | 3.69 (1H, m) | 3.63 (1H, m) |
| 17    | 9.12 (1H, s)    | 9.06 (1H, s) | 9.12 (1H, s) | 7.45 (1H, s) |
| 18    | 1.11 (3H, d, 6.2) | 2.42 (3H, s) | 1.72 (3H, d, 7.2) | 5.75 (2H, m) |
| 19    | 3.44 (1H, m)    |                | 5.45 (1H, q, 7.2) | 5.36 (1H, m) |
| 20    | 1.65 (1H, m)    | 2.90 (1H, m) |                | 2.70 (1H, m) |
| 21    | 2.03 (1H, m); 2.80 (1H, m) | 2.53 (1H, m); 2.80 (1H, m) | 3.10 (1H, d, 13.4); 3.37 (1H, d, 13.4) | 5.49 (1H, d, 5.5) |
| 23    |                |                |                | 3.47 (1H, d, 16.5); 3.60 (1H, d, 16.5) |
| 12-O Me | 3.95 (3H, s) | 3.93 (3H, s) | 3.98 (3H, s) | 3.68 (3H, s) |
| 22-COO Me | 3.67 (3H, s) | 3.60 (3H, s) | 3.67 (3H, s) | 3.65 (3H, s) |
| 24-COO Me |                |                |                | 4.74 (1H, d, 7.8) |
| 1'    |                |                |                | 3.27 (1H, overlap) |
| 2'    |                |                |                | 3.43 (1H, overlap) |
| 3'    |                |                |                | 3.35 (1H, m) |
| 4'    |                |                |                | 3.35 (1H, overlap) |
| 5'    |                |                |                | 3.81 (1H, d, 12.8); 3.63 (1H, overlap) |

All alkaloids 1–17 were tested for their ability to prevent the cytopathic effects of cancer in breast cancer SK-BR-3, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, pancreatic cancer PANC-1, and lung cancer A-549 cells, and their cytotoxicity was measured in parallel with the determination of antitumor activity using cisplatin as the positive control. Unfortunately, none of them showed positive activity (IC$_{50}$ > 40 μM).

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were recorded on a Shimadzu double-beam 210A spectrophotometer. IR (KBr) spectra were obtained on Bio-Rac FTS-135 infrared spectrophotometer. $^1$H, $^13$C and 2D NMR spectra were recorded on a AM-400 and DRX-500 MHz NMR spectrometer with TMS as an internal standard. MS data were obtained on an API Qstar Pulsar I spectrometer. Silica gel (200–300 mesh) for column chromatography (CC) and GF$_254$ for TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, China and sprayed with Dragendorff’s reagent. C18 silica gel (20–45 μm) was bought from Fuji Chemical Ltd., Japan. MPLC was employed Buchi pumps system coupled with glass column (15×230 and 26×460 mm, respectively). HPLC was performed using Waters 600 pumps coupled with analytical and semipreparative sunfire C18 silica gel (20–45 mesh) for column chromatography (CC) and sprayed with Dragendorff’s reagent.

Plant Material. Alstonia rostrata C. E. C. Fischer was collected in Apr. 2010 in Simao of Yunnan Province, China, and identified by Dr. Chun-Xia Zeng. Voucher specimen (Cai100613) deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, the Chinese Academy of Sciences.

Extraction and Isolation. Air-dried leaves and twigs (8.0 kg) of A. rostrata was crushed and extracted with EtOH (20 L×3). After removal of the EtOH under reduced pressure, the residue was dissolved in 1% HCl, and partitioned with EtOAc for three times. The acidic solution was subsequently basified using ammonia water to pH 8–9, and partitioned with EtOAc for three times, affording a two-phase mixture including the aqueous phase, EtOAc/organic phase (total alkaloids). The
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Electronic Supplementary Material
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