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Picrinine-type Alkaloids from the Leaves of Alstonia scholaris

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【ABSTRACT】 **AIM:** To investigate the chemical constituents of Yunnan local medicinal plants *Alstonia scholaris*. **METHODS:** Silica gel column chromatography was used to isolate the constituents, and spectroscopic techniques (NMR, IR, UV and MS) were used for structural elucidation. **RESULTS:** Four picrinine-type monoterpenoid indole alkaloids, 5β-methoxyaspidophylline (**1**), picrinine (**2**), picralinal (**3**) and 5-methoxystrictamine (**4**) were obtained from the leaves of *Alstonia scholaris*. **CONCLUSION:** Compound **1** is a new monoterpenoid indole alkaloid.

【KEY WORDS】 *Alstonia scholaris*; Monoterpenoid indole alkaloid; Picrinine-type; 5β-Methoxyaspidophylline
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1 Introduction

The genus *Alstonia* (Apocynaceae) comprises about 60 species, 8 of which are distributed in China. 4 species of this genus have been found in Yunnan province[1]. The phytochemical constituents of *Alstonia* sp. have been investigated intensively. Untill now, more than 300 compounds have been isolated from this genus, most of them are monoterpenoid indole alkaloids[2]. This type of *Alstonia* alkaloids possess 19 (or 18) carbon atoms on the skeleton and reportedly have anticancer, antibacterial, antifertility, and antitussive activities[3-5]. The leaves of *A. scholaris* are used to treat chronic respiratory disease in "*Dai*" ethnopharmacy historically in Yunnan Province, China. Now the extract of the leaves has been developed to be a traditional Chinese medicine in China based on their traditional usage. As part of a continuing effort to search novel secondary metabolites from Yunnan local medicinal plants, we undertook phytochemical research on this plant. In this paper, we report a new alkaloid (**1**), together with 3 known picrinine-type alkaloids, picrinine (**2**)[6], picralinal (**3**)[6], and 5-methoxystrictamine (**4**)[7] (Fig 1).

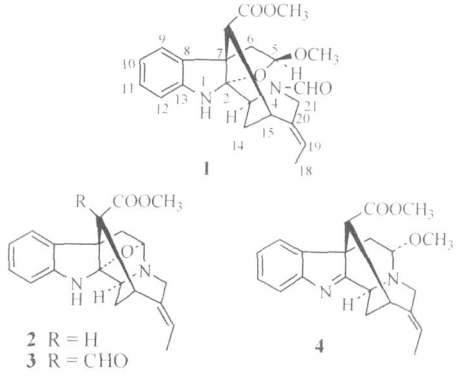

Fig 1 Compounds 1-4 from Alstonia scholaris

2 Results and Disscussion

Compound **1** was isolated as a white powder. Its molecular formula of $C_{22}H_{26}N_2O_5$ was established on the basis of HRESIMS analysis and its NMR data. Its UV spectrum showed absorption maxima at 204, 237, 295 nm, characteristic of a dihydroindole skeleton[8]. The IR spectra exhibited absorption bands for -NH (3 315 cm^{-1}), C=O (1 742 cm^{-1}), and benzene rings (1 651 cm^{-1}). The ^1H and ^{13}C NMR spectrum of **1** displayed signals for a substituted dihydroindole ring [δ_C 145.5 (s, C-13), 136.3 (s, C-3), 128.5 (d, C-8), 123.3 (d, C-9), 120.4 (d, C-10), 110.7 (d, C-11), 102.9

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(d, C-2), 52.4 (s, C-7), δ_H 7.14 (2H, overlap, H-9, 11), 6.83 (1H, t, J = 7.5 Hz, H-10), 6.72 (1H, d, J = 7.5 Hz, H-12)][6], an aldehyde group ([δ_C 164.5 (d) and δ_H 8.20 (s)], a methyl ester group [δ_C 171.7 (s), 51.6 (q)], five methines (δ_C 124.3, 107.1, 53.8, 53.2 30.5), three methenes (δ_C 44.5, 39.9, 30.6), a methoxy (δ_C 56.8) and a methyl group (δ_C 12.9). These NMR data of **1** were similar to those of aspidophylline A[8]. The main differences are: i) the NMR spectra of **1** displayed resonances due to an *O*-methyl group; ii) the C-5, C-6 resonances were shifted downfield from δ_C 69.1, 34.3 in aspidophylline A to δ_C 107.1, 39.9 in **1** respectively. The HMBC correlations from OCH_3 (δ_H 3.30) to C-5 (δ_C 107.1) confirmed the location of OCH_3 at C-5. The methoxy at C-5 was assignable to be β, other than α, because the coupling constants of H-6 (δ_H 4.89, t, J = 6.0 Hz) and chemical shift of 5-OMe (δ_H 3.30, s) were similar to those of isoalschomine (δ_H 4.83, dd, J = 6.0, 5.0 Hz, H-5; δ_H 3.38, s, 5-OMe) instead of alschomine (δ_H 5.01, d, J = 5.0 Hz, H-5; δ_H 3.00, s, 5-OMe) (Fig 2)[6]. Thus, **1** was determined to be 5β-methoxyaspidophylline.

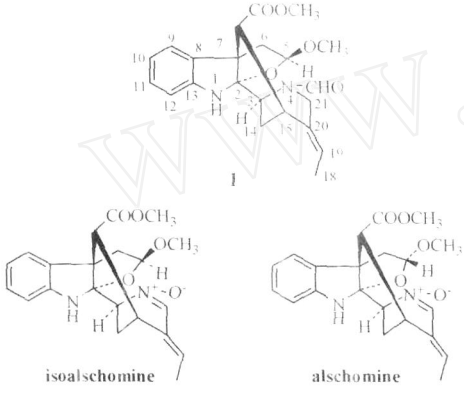

Fig 2 Structure of 1, isoalschomine and alschomine

3 Experimental

3.1 *General*

Optical rotations were measured using a Horiba SEAP-300 spectropolarimeter. The IR (KBr) spectra were obtained on a Brucker Tensor 27 infrared spectrophotometer. 1D and 2-D NMR spectra were recorded on a Bruker DRX-500 MHz NMR spectrometer with TMS as the internal standard. ESI-MS measurement was carried on an API QSTAR Pulsar 1 spectrometer. Silica gel (200-300 mesh) for column chromatography (C.C.) and GF254 for TLC were obtained from Qingdao Marine Chemical Factory (Qingdao, China). Spots on chromatograms were detected by spraying with Dragendorff's reagent.

3.2 *Plant Material*

The leaves of *Alstonia scholaris* were collected in Simao, Yunnan Province, China, 2004 and identified by Dr. Zeng Chun-Xia, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen has been deposited at Herbarium of Department of Taxonomy, Kunming Institute of Botany, the Chinese Academy of Sciences.

3.3 *Extraction and Isolation*

The dried and powdered leaves of *A. scholaris* (50 kg) were extracted with EtOH (150 L × 3) under reflux, and the solvent was evaporated in vacuo. The residue was dissolved in 1% HCl, and the acidic solution was adjusted to pH 9-10 with ammonia. The basic solution was partitioned with EtOAc to afford total alkaloids (EtOAc layer). Total alkaloids (450 g) were subjected to C.C. on silica gel eluted with $CHCl_3$-Me_2CO [from $CHCl_3$ to $CHCl_3$-Me_2CO (1∶1)] to afford 6 fractions (I-VI). Fraction III (94 g) was chromatographed on Si gel C.C. (1.5 kg) using $CHCl_3$-Me_2CO (97∶3) to give **4** (150 mg), **1** (15 mg). Fraction IV (129 g) was subjected to Si gel C.C. (2.0 kg) using $CHCl_3$-MeOH (95∶5) as eluent to obtain two parts (A and B). Part A (24.5 g) was separated on Si gel C.C. once again eluted with $CHCl_3$-Me_2CO (3∶1) to give **3** (2.8 g). Compound **2** (4.5 g) was isolated from Part B (16.4 g) by silica gel (500 g) eluted with $CHCl_3$-MeOH (97∶3).

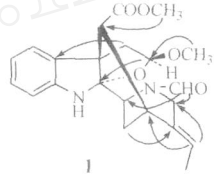

Fig 3 Key HMBC correlations of 1

4 Identification

5β-Methoxyaspidophylline (**1**): white power; $[\alpha]_D^{21}$ 21.8 °(*c* 0.55, MeOH); UV (CHCl$_3$) λ: 204 (log ε 4.52), 237 (log ε 4.04), 295 (log ε 3.58) nm; IR (KBr) ν: 3315, 1742, 1651 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.20 (1H, s, -CHO), 7.14 (2H, overlap, H-9, 11), 6.83 (1H, t, J = 7.5 Hz, H-10), 6.72 (1H, d, J = 7.5 Hz, H-12), 5.61 (1H, q, J = 8.0 Hz, H-19), 4.89 (1H, t, J = 6.0 Hz, H-5), 4.32 (2H, br s, H-21), 3.99 (1H, br s, H-3), 3.70 (3H, s, -$COOCH_3$), 3.43 (1H, br s, H-15), 3.30 (3H, s, 5-OCH_3), 3.09 and 2.54 (each 1H, dd, J = 15.0, 6.0 Hz, H-6), 2.67 (1H, d, J = 4.0 Hz, H-16), 2.28 and 2.08 (each 1H, m, H-14), 1.58 (3H, d, J = 8.0 Hz, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.7 (s, $COOCH_3$), 164.5 (d, -CHO), 145.6 (s, C-13), 136.3 (s, C-8), 128.6 (s, C-20), 128.5 (d, C-11), 124.3 (d, C-19), 123.3 (d, C-9), 120.4 (d, C-10), 110.7 (d, C-12), 107.1 (d, C-5), 102.9 (s, C-2), 56.8 (q, 5-OCH_3), 53.8 (d, C-16), 53.2 (d, C-3), 52.4

(s, C-7), 51.6 (q, $COOCH_3$), 44.5 (s, C-21), 39.9 (t, C-6), 30.6 (t, C-14), 30.5 (d, C-15), 12.9 (q, C-18); Positive ESI-MS *m/z* 421 ($[M+Na]^+$), HRESI-MS *m/z* 421.1742 (cacld. for $C_{22}H_{26}N_2O_5Na$, 421.1739).

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灯台树叶中鸭脚树叶碱型生物碱

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【摘　要】 目的:研究云南当地药材灯台树的化学成分。**方法**:运用硅胶柱色谱分离、光谱技术(核磁共振,红外,紫外和质谱)鉴定结构。**结果**:从灯台树叶中分离到4个鸭脚树叶碱类型的单萜吲哚生物碱:5β-methoxyaspidophylline(**1**),鸭脚树叶碱(**2**),鸭脚树叶醛(**3**),5-methoxystrictamine(4)。**结论**:**1**是一个新化合物。

【关键词】 灯台树;单萜吲哚生物碱;鸭脚树叶碱型;5β-methoxyaspidophylline

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