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INDOLE ALKALOIDS FROM LEAVES AND STEMS OF *Hunteria zeylanica*

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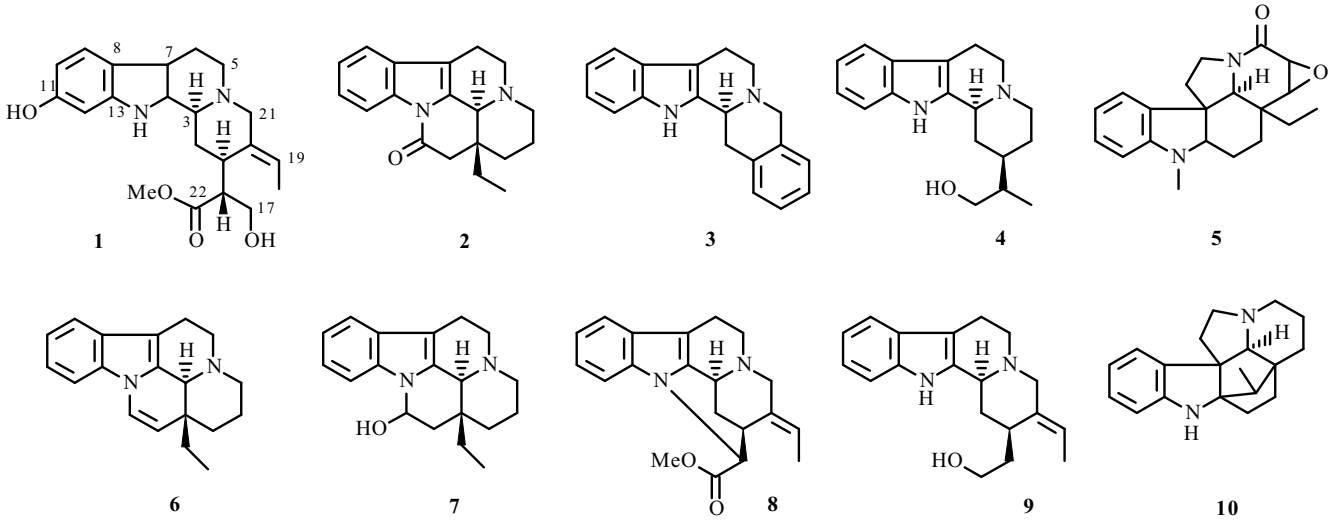
Investigation of the leaves and stems of Hunteria zeylanica *resulted in the isolation of one new indole alkaloid, 11-hydroxyrhazimanine (**1**), and nine known analogs **2–10**. Their structures were determined by 1D and 2D NMR analyses. Decarbomethoxydihydrogambirtannin (**2**), dihydroantirhine (**3**), and 3-oxomehranine (**4**) were isolated from* Hunteria *genus for the first time.*

Key words: *Hunteria zeylanica*, Apocynaceae, indole alkaloids, 11-hydroxyrhazimanine.

The genus *Hunteria* (Apocynaceae), which includes eight species, is distributed mainly in the tropical areas of Asia and Africa. *Hunteria zeylanica* (Retz.) is the only species endemic in Hainan Province, China. This plant has been used by the natives as medicine to cure yaws and reduce boils and skin irritations [1].

Previous chemical investigations on the *Hunteria* genus have led to the isolation of more than 45 indole alkaloids [2–8]. Nearly 30 of them were reported from *H. zeylanica*. In this paper, we describe the isolation and chemical characterization of 10 indole alkaloids from *H. zeylanica*, including one new compound, named 11-hydroxyrhazimanine (**1**), and nine known ones, i.e., decarbomethoxydihydrogambirtannin (**2**) [9], dihydroantirhine (**3**) [10], 3-oxomehranine (**4**) [11], eburnamonine (**5**) [12], eburnamenine (**6**) [12], eburnamine (**7**) [12], pleiocarpamine (**8**) [13], geissoschizol (**9**) [14], and tuboxenine (**10**) [15].



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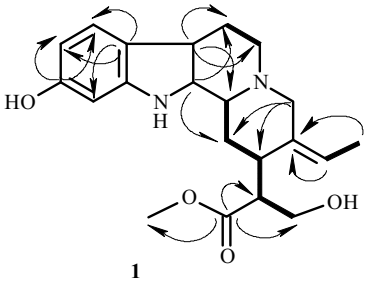
Compound **1** was obtained as a yellow amorphous powder. The molecular formula was deduced to be $C_{21}H_{26}N_2O_4$ on the basis of the pseudo-molecular ion peak at *m/z* 371.2066 ([M+H]$^+$ calcd for 371.1971) in HRESIMS. The IR spectrum exhibited the absorption bands for the hydroxyl group (3423 cm^{-1}) and the benzene moiety (1720, 1664, 1623, and 1454 cm^{-1}). The ^{1}H NMR spectrum showed characteristic signals of three aromatic protons at δ_H 6.72 (dd, J = 8.6, 2.4 Hz), δ_H 6.85 (d, J = 2.4 Hz) and δ_H 7.21 (dd, J = 8.6, 0.5 Hz), one methoxyl at δ_H 3.76, and one methyl at δ_H 1.66 (dd, J = 7.0, 1.5 Hz). The ^{13}C NMR and DEPT spectra displayed 21 signals ascribed to one carbonyl, ten carbons in the aromatic or olefinic region, three methines, five methylenes, one methoxyl, and one methyl. Seven of the total ten unsaturation degrees were occupied by one carbonyl carbon, four olefinic carbons, and a benzene ring. Thus, the remaining three degrees of unsaturation were attributed to the presence of three cyclic rings. All these data suggested a basic corynanthe indole skeleton in this molecule [16]. Furthermore, a partial structure (–N-CH-CH$_2$-CH-CH-CH$_2$-O-) and a propenyl group were constructed on the basis of the correlations in the ^{1}H–^{1}H COSY spectrum (bold lines in Fig. 1). The carbonyl group was located at C-16 based on the HMBC correlation between C-22/H-16 and C-22/H-17. The propenyl group was linked to C-20, which was also elucidated from the HMBC correlation between C-20/H-18 and C-20/H-19. These structural features of **1** were very close to those of rhazimanine [16]. Compound **1** was deduced to be a hydroxyl derivative by a detailed comparison with that of rhazimanine. Such deduction was based on its HRESIMS (an additional oxygen was displayed) and ^{1}H NMR data in the aromatic region, where an AMX system [with J_{AM} = 8.6 Hz (*ortho*), J_{MX} = 2.4 Hz (*meta*), J_{AX} = 0~0.5 Hz (*para*)] was clearly observed. The HMBC correlations of C-11/H-9, C-11/H-10, C-8/H-9, C-8/H-10, and C-8/H-12 confirmed further that the hydroxyl was fixed at C-11. All segments were finally connected by the key correlations in the HMBC spectrum (arrows in Fig. 1). The conformation and relative configuration of **1** was deduced to be the same as that of rhazimanine based on the comparison of their NMR data [17], as well as its biogenetic relationship. Therefore, the whole structure of **1** was established, named 11-hydroxyrhazimanine.



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— ^{1}H–^{1}H COSY

C→H HMBC correlations

Fig. 1. Important ^{1}H–^{1}H COSY and HMBC correlations in **1**.

In addition, nine known indole alkaloids **2–10** were also isolated from this plant. Their structures were determined by comparison of their NMR and MS spectroscopic data with those reported in the literature.

This is the first phytochemical study of *H. zeylanica* growing in Hainan. Ten indole alkaloids were isolated and identified. Among these compounds, **1** is a new alkaloid; **2**, **3**, and **4** have never been reported previously from this genus.

As the characteristic constituents, indole alkaloids exist generally in *Hunteria* plants. Some common chemical characters were shared by species *H. zeylanica* grown in different localities. For example, compounds **6**, **7**, **8**, and **10** can also be found in Ceylon *H. zeylanica* [2]. Compounds **7**, **8** and **9** were reported from this species growing in Kenya of Africa [3]. In addition, compound **6** exists also in Tailand *H. zeylanica* [4]. These data, to some extent, demonstrate the chemotaxonomic similarity among the same species from different habitats.

EXPERIMENTAL

General Experimental Procedures. Column chromatography (CC): commercial silica gel (Qing Dao Marine Chemical Industrials; 200–300 and 300–400 mesh). TLC: precoated silica gel GF$_{254}$ plates (Yan Tai Chemical Industrials). IR spectra: Nicolet Magna FT-IR 750 spectrophotometer. NMR spectra: Bruker AM-400 and Bruker AC-300 NMR spectrometers, chemical shifts, δ in ppm, with TMS as internal standard, and coupling constants, J in Hz, assignments supported by ^{1}H–^{1}H COSY, HSQC, ROESY, and HMBC experiments. EI-MS and HR-EI-MS spectra: Finnigan MAT-95 mass spectrometer in *m/z*. ESI-MS and HR-ESI-MS spectra: Micromass LC-MS-MS mass spectrometer in *m/z*.

Plant Material. The leaves and stems of *H. zeylanica* were collected in Hainan Province in March, 2006 and identified by Professor Jin-gui Shen of Shanghai Institute of Materia Medica. A voucher specimen (No. 2006010) has been deposited at the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. 5.0 kg air-dried leaves and stems of *H. zeylanica* were ground into powder and extracted with 95% aq. EtOH (20 L × 3) at room temperature. After evaporation of the collected percolate, the crude extract was acidified with dilute HCl (4%) and partitioned between EtOAc and H_2O. The aqueous part was then basified with aqueous NH_3 and extracted with EtOAc to afford 30 g of crude alkaloid. The crude alkaloid (30 g) was chromatographed over an MCI column and eluted with MeOH–H_2O gradiently (10–100%) to give six subfractions A–F. Subfraction D was subjected to chromatography column (CC) over silica gel and then Sephadex LH-20 gel, affording compounds **1** (23 mg), **6** (15 mg), **7** (15 mg), **8** (15 mg), **9** (15 mg), and **10** (15 mg). Similarly, subfraction E was also separated over silica gel and Sephadex LH-20 gel to give **2** (31 mg), **3** (12 mg), **4** (26 mg), and **5** (15 mg).

11-Hydroxyrhazimanine (1), pale yellow amorphous powder; IR (KBr, ν_{max}, cm^{-1}): 3423 (OH), 2929, 1720, 1664, 1623, 1454, 1386, 1207; ESIMS *m/z*: 371 $[M+H]^+$, 369 $[M–H]^+$; HREIMS *m/z*: 371.2066 ($[M+H]^+$ $C_{21}H_{27}N_2O_4$ calcd 371.1971); ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.66 (3H, dd, J = 7.0, 1.5, H-18), 2.08 (1H, m, H-14), 2.24 (1H, m, H-14), 2.46 (1H, m, H-16), 2.52 (1H, m, H-6), 2.94 (1H, m, H-6), 2.96 (1H, m, H-21), 2.98 (1H, m, H-5), 3.13 (1H, m, H-5), 3.15 (1H, m, H-15), 3.44 (2H, m, H-17), 3.55 (1H, m, H-21), 3.76 (3H, s, OCH_3), 4.22 (1H, dd, J = 9.1, 3.8, H-3), 5.65 (1H, d, J = 7.0, H-19), 6.72 (1H, dd, J = 8.6, 2.4, H-10), 6.85 (1H, d, J = 2.4, H-12), 7.21 (1H, dd, J = 8.6, 0.5, H-9); ^{13}C NMR (100 MHz, CD_3OD, δ): 177.1 (C-22), 151.8 (C-11), 136.0 (C-20), 135.8 (C-2), 133.2 (C-13), 129.7 (C-8), 125.2 (C-19), 112.9 (C-10), 112.3 (C-9), 107.2 (C-7), 103.7 (C-12), 63.7 (C-17), 54.5 (C-21), 54.3 (C-3), 52.7 (OCH_3), 52.3 (C-5), 51.5 (C-16), 34.6 (C-15), 31.9 (C-14), 19.4 (C-6), 13.9 (C-18).

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