Ervahainanmine, a new indole alkaloid from the stems of *Ervatamia hainanensis*

Zha-Jun Zhan, Li-Wen Zhang and Wei-Guang Shan*

College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou, 310014, P.R. China

A new sarpagine-type alkaloid with N-oxide group, called ervahainanmine, and three known ones including coronaridine, 19(S)-heyneanineguan, and coronaridine hydroxyindolenineguan were isolated from the stems of *Evratamia hainanensis*. The structure was established by spectroscopic methods, especially 2D NMR techniques.

Keywords: *Evratamia hainanensis*, indole alkaloid, ervahainanmine

The terpenoid indole alkaloids, which arise by the condensation of tryptophan and secologanin, are outstanding among secondary plant metabolites in their structural intricacy and diversity. Because of the range of their structures and interesting bioactivities,[1-3] these alkaloids have attracted the attention of synthetic groups.[4-7] There are about 120 plant species in the genus *Ervatamia* (Apocynaceae) distributed in the tropical and subtropical areas of Asia and Australia. Fifteen plant species and five varieties of this genus grow in the south of China, and many of them have been used in traditional Chinese medicine or folklore medicine.[8] The roots of *Ervatamia hainanensis*, have long been used for the treatment of stomach ache, dysentery, rheumatic arthritis, hypertension, and viral hepatitis in traditional Chinese medicine.[8] Previous studies on this plant revealed the presence of indole alkaloids,[9-13] triterpenes[14] and other natural products. In our search for novel alkaloids, a new sarpagine-type alkaloid and three known alkaloids were isolated from the stems of *E. hainanensis*. We report here the isolation and structural determination of these compounds.

Ervahainanmine (**1**) was obtained as an amorphous powder. It had a molecular formula of $C_{19}H_{22}N_2O_2$ established on the basis of NMR and HRMS data, implying the existence of 10 degrees of unsaturation. The IR absorption band at 3356 cm^{-1} was attributed to an amino group. Examination of the NMR indicated the presence of an indole group in **1**, which was confirmed by the UV spectroscopic data. Nineteen carbon signals comprising five quaternary carbons, nine methines, four methylenes, and one methyl were observed in the ^{13}C NMR and DEPT spectra. The ^1H NMR spectrum (Table 1) of **1** revealed the presence of a tri-substituted double bond proton at δ 5.60 (1H, q, J = 7.0 Hz), a methyl signal at δ 1.75 (3H, d, J = 6.8 Hz), and four low-field signals assigned to a phenyl group. The NMR data mentioned above and UV data indicated **1** was a sarpagine-type indole alkaloid.[15]

Analysis of the ^1H NMR, ^{13}C NMR and HMQC spectra of **1** enabled us to assign all the protons to their bonding carbons. The two main fragments (C-3, C-5 to C-6, and C-14 to C-17; C-9 to C-12) were established by the ^1H–^1H COSY spectrum. The linkage of two structural fragments by other carbon or hetero-atom was finally made by an HMBC experiment (Fig. 2). The linkage of C-21, C-5, and C-3 to each other via the N^4 was established by the correlations of H-21/C-3, H-3/C-5, and H-5/C-21; the HMBC correlations of H-19/C-15 and H-19/C-21 indicated the connectivity of C-15 and C-21 via the quaternary carbon C-20; the linkage of C-2 and C-3 could be tentatively established by the HMBC correlations of H-14/C-2; the linkage of C-6 and C-7 was solved by the same method. The planar structure of **1** was thus established.

The ^1H and ^{13}C NMR data of **1** exhibited similarity with those of 16-epinormacusine B[16]. Typical difference in 1D NMR spectra of **1** was that the carbon signals attached N^4 were shifted downfield compared with those of 16-epinormacusine B. This was considered to be caused by the deshielding effect of N^4-oxide[17], which was also supported by its molecular composition. These data indicated that compound **1** had the same relative stereochemistry as 16-epinormacusine B, which was confirmed by the NOESY spectrum. The *E* geometry

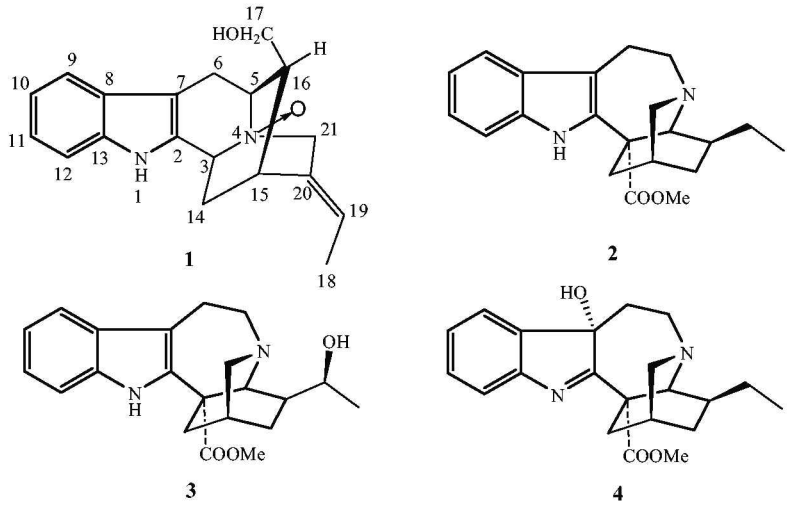

Fig. 1 Structures of alkaloids from *E. hainanensis*.

* Correspondent. E-mail:tianranyaowu@zjut.edu.cn

Table 1 NMR Data of **1** measured in CD_3OD at 400 MHz

No.	1	
	δ_H, *J* (Hz)	δ_C
2	–	129.8
3	4.12 (1H, brs)	64.4
5	4.90 (1H, m)	63.3
6	3.08 (1H, m)	18.6
	3.16 (1H, m)	
7	–	105.7
8	–	127.2
9	7.50 (1H, d, 7.9)	119.6
10	7.15 (1H, t, 8.0)	121.2
11	7.06 (1H, t, 8.0)	124.0
12	7.38 (1H, d, 8.1)	112.8
13	–	139.1
14	2.68 (1H, m)	32.7
	2.08 (1H, m)	
15	3.64 (1H, m)	30.4
16	2.83 (1H, m)	44.7
17	3.75 (1H, m)	61.6
17	3.90 (1H, m)	
18	1.75 (3H, d, 6.8)	13.2
19	5.60 (1H, q, 7.0)	123.3
20	–	129.3
21	4.06 (1H, d, 14.6)	66.3
	4.38 (1H, d, 14.6)	

of the ethylidene side chain of **1** was deduced from the observation of an NOE between Me-18 and H-15. The characteristic shielding of C-17 and C-6 of **1** compared to affinisine-N^4-oxide[16,18] indicated the 16S configuration in **1**. Hence, the structure of **1** was elucidated, and named as ervahainanmine.

The known alkaloids were identified as coronaridine (**2**), 19(S)-heyneaninegan (**3**),[19] and coronaridine hydroxyindolenineguan (**4**)[13] by comparison of their NMR data with those reported in the literature.

Experimental

UV spectra were measured on a Shimadizu UV-2450 spectrometer. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Thermo Nicolet 6700 spectrometer with KBr disks. NMR spectra were measured on a Bruker Avance-400 spectrometer with TMS as internal standard. ESIMS was recorded on a Agilent 6210 Lc/Tof Mass spectrometer. All solvents used were of analytical grade (Hangzhou Gaojing Fine Chemical Plant, Hangzhou, P. R. China). Silica gel (200–300 mesh) was used for column chromatography, and a precoated silica gel GF_{254} plate (Qingdao Haiyang Chemical Plant, Qingdao, P. R. China) was used for TLC.

Plant material: The stems of *E. hainanensis* were collected from the Hainan Province of P. R. China and identified by Prof. Shi-Man Huang in Hainan University. A voucher specimen (ZJUT 060712) has been deposited at Zhejiang University of Technology, People's Republic of China.

Extraction and isolation: The dry stems (4.6 kg) of *E. hainanensis* were extracted three times with 95% EtOH at room temperature. The extract was evaporated to dryness under reduced pressure to afford the residue (273 g), which was dissolved in water (3 L) to form a suspension, and then adjusted with 0.5 N H_2SO_4 to pH≈5. The acidic mixture was extracted with EtOAc (6 × 500 mL) to remove the non-alkaloid components. The aqueous phase was brought to pH≈10 by addition of 1 N Na_2CO_3 and partitioned with chloroform (6 × 500 mL) to give the crude alkaloids (4.7 g). The crude alkaloids were then subjected to a silica gel column chromatography and eluted with $CHCl_3$–MeOH (20:1–1:1) to collect four major fractions 1–3. Fraction 1 (0.8 g) was separated by column chromatography over silica gel and eluted with $CHCl_3$–MeOH (20:1) to yield alkaloids **2** (80 mg). Fraction 2 (0.4 mg) was purified by silica gel column chromatography eluted with $CHCl_3$–MeOH (10:1) to afford **3** (6.8 mg) and **4** (5.6 mg). Compound **1** (5.2 mg) was purified from fraction 3 (150 mg) by silica gel column chromatography eluted with $CHCl_3$–MeOH (6:1).

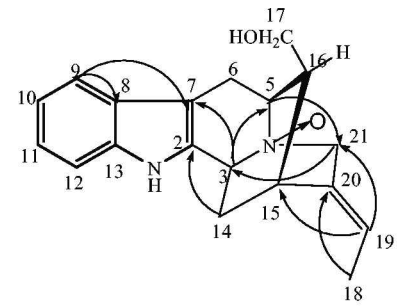

Fig. 2 Key HMBC correlations of **1**.

Eervahainanmine (**1**), amorphous powder, $[\alpha]^{20}_D$ +6.1° (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ε) 297 (4.01), 280 (3.56), 226 (4.41); IR (KBr): 3356, 2929, 1602, 1473, 1380, 1192, 1042, 740 cm^{-1}; ESIMS *m/z*: 333 $[M + Na]^+$; HR-ESIMS *m/z*: 333.1591 $[M + Na]^+$ (Calcd for $C_{19}H_{22}NaN_2O_2$ 333.1579). ^1H NMR and ^{13}C NMR data: see Table 1.

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