ORGANIC LETTERS

2013
Vol. 15, No. 3
590–593

Myriberine A, a New Alkaloid with an Unprecedented Heteropentacyclic Skeleton from *Myrioneuron faberi*

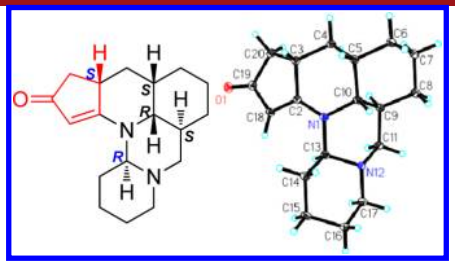
Sheng-Dian Huang,[†,‡,∥] Yu Zhang,[†,∥] Ming-Ming Cao,[†] Ying-Tong Di,[†] Gui-Hua Tang,[†] Zong-Gen Peng,[§] Jian-Dong Jiang,[§] Hong-Ping He,[*,†] and Xiao-Jiang Hao[*,†]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, P. R. China, University of Chinese Academy of Sciences, Beijing 100039, P. R. China, and Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 10050, P. R. China

haoxj@mail.kib.ac.cn; hehongping@mail.kib.ac.cn

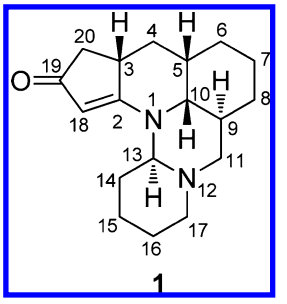
Received December 13, 2012

ABSTRACT



Myriberine A (1) possessing an unprecedented carbon skeleton was isolated from *Myrioneuron faberi*. The structure and absolute configuration of 1 were elucidated by a combination of spectroscopic data, X-ray crystallographic, and computational methods. Myriberine A (1) demonstrated inhibition against the hepatitis C virus (HCV) life cycle in vitro.

Myrioneuron alkaloids elaborated by plants of the genus *Myrioneuron* R. Br. (Rubiaceae) are a new family of structurally diverse natural products containing the decahydroquinoline (*cis*-DHQ) motif which are generally restricted to animal sources, such as amphibians or tunicates,[1] but rarely from plant sources.[2] Their unique structural features with striking similarities to *Nitraria* alkaloids have attracted great attention for total synthesis and biosynthetic research.[3,4] Previous chemical investigations on *M. nutans* Drake of Vietnam origin carried out by Bodo and co-workers resulted in the isolation of 10 *Myrioneuron* alkaloids that could be classified into six different carbon skeletons.[4] Some of them showed inhibition on KB cell proliferation and remarkable antimalarial activities.[4b,d,e]



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Myrioneuron faberi Hemsl., a suffruticose herb, is distributed mainly in the southern area of China.[5] Chemical constituents of *M. faberi* have not been previously

[†] Kunming Institute of Botany.
[‡] University of Chinese Academy of Sciences.
[§] Institute of Medicinal Biotechnology.
[∥] These authors contributed equally to this work.

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10.1021/ol3034065
Published on Web 01/15/2013

Table 1. ^{1}H and ^{13}C NMR Data for **1** in $CDCl_3$[a]

no.	δ_H (mult; *J*, Hz)	δ_C	no.	δ_H (mult; *J*, Hz)	δ_C
2		175.3 (s)	13	3.77 (dd, 9.0, 4.4)	77.0 (d)
3	2.86 (m)	39.1 (d)	14a	1.69 (m)[b]	26.2 (t)
4a	1.66 (m)	27.8 (t)	14b	1.72 (m)	
4b	1.66 (m)		15a	1.87 (m)	23.6 (t)
5	2.25 (m)	33.9 (d)	15b	1.43 (m)	
6a	1.70 (m)[b]	30.8 (t)	16a	1.61 (ddd, 12.4, 7.2, 4.0)	22.0 (t)
6b	1.74 (m)		16b	1.45 (ddd, 12.4, 5.2, 4.0)	
7a	1.54 (ddd, 13.2, 8.0, 4.0)	20.7 (t)	17a	2.91 (m)	54.0 (t)
7b	1.35 (dt, 13.2, 4.0)		17b	2.42 (td, 12.8, 2.8)	
8a	1.63 (m)	31.4 (t)	18	5.07 (s)	99.8 (d)
8b	1.20 (ddd, 16.0, 12.8, 4.0)		19		202.2 (s)
9	1.77 (ddd, 11.2, 7.2, 5.4)	36.9 (d)	20a	2.52 (dd, 16.8, 7.2)	40.5 (t)
10	3.62 (dd, 11.2, 5.6)	55.8 (d)	20b	2.14 (dd, 16.8, 5.2)	
11a	2.62 (dd, 11.6, 5.4)	54.9 (t)			
11b	2.50 (dd, 11.6, 7.2)				

[a] The ^{1}H NMR spectrum was recorded at 400 MHz and the ^{13}C NMR spectrum at 100 MHz. [b] Overlapped.

investigated. In the course of a research program aiming at the finding of bioactive alkaloids,[6] a new *Myrioneuron* alkaloid, myriberine A (**1**), has been isolated from the twigs and leaves of this plant.[7] Myriberine A possesses an unprecedented pentacyclic system with the parent compound conjugated to a C_3 unit and was characterized by five contiguous stereogenic centers including a 13*R* configuration that is uncommon within the *Myrioneuron* alkaloids family. Inspired by its structural similarity to matrine alkaloids,[3] myriberine A (**1**) was evaluated to inhibit the hepatitis C virus (HCV) life cycle with a good therapeutic index (CC_{50}/EC_{50}) of greater than 12.0 in vitro. In this report, the details of the structure elucidation of **1** as well as the inhibitory effect on the HCV life cycle of **1** are presented.

Myriberine A (**1**)[8] was obtained as colorless crystals. Its molecular formula was established as $C_{18}H_{26}N_2O$ by HREIMS data (*m*/*z* 286.2050 $[M]^+$, calcd 286.2045) with seven degrees of unsaturation. The UV absorption maximum at 284 nm and an aborption band at 1632 cm^{-1} in the IR spectrum implied the presence of an α,β-unsaturated ketone moiety. The 18 carbon signals observed in the ^{13}C NMR and DEPT spectra of **1** could be classified into 10 methylenes (two nitrogenated), six methines (two nitrogenated and one olefinic), and two quaternary carbons (a ketone carbonyl and an olefinic) (Table 1). Since one ketone and two olefinic carbons accounted for two out of the seven degrees of unsaturation, the remaining five degrees of unsaturation were assumed to be the presence of a pentacylic system in **1**.

Detailed 2D NMR (HSQC, $^{1}H-^{1}H$ COSY, and HMBC experiments) studies revealed that **1** possessed two spin coupling systems: H_2-20/H-3/H_2-4/H-5/H_2-6(H-10)/H_2-7/H_2-8/H-9/H-10(H_2-11) and H-13/H-14/H-15/H-16/H-17 (Figure 1A). This information, coupled with the similarity of the NMR data of **1** for rings B–D with those of myrionamide,[4b] suggested that both alkaloids shared the same B–D ring systems. Furthermore, the striking presence of a C-3 sp^3 methine signal (δ_H 2.86, m) and a spin system H-3/H_2-20 in **1** implied that C-3 might be incorporated into a new ring. In the HMBC spectrum, cross-peaks of H-10 (δ_H 3.62, dd, 11.2, 5.6 Hz) and H-13 (δ_H 3.77, dd, 9.0, 4.4 Hz) to C-2 (δ_C 175.3) and that of H-13 to C-10 (δ_C 55.8) indicated that C-2, C-10, and C-13 were linked through N-1, as shown in Figure 1A. Moreover, the HMBC correlations of H-3, H_2-4 (δ_H 1.66, m), H-18 (δ_H 5.07, s), and H_2-20 (δ_H 2.52, dd, 16.8, 7.2 Hz; δ_H 2.14, dd, 16.8, 5.2 Hz) to C-2 and that of H-18 to C-3 indicated that

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(7) The twigs and leaves of *M. faberi* Hemsl. were collected in Emei Mountain, Sichuan Province, China, and identified by Prof. X. Gong of Kunming Institute of Botany, Chinese Academy of Sciences. A specimen is deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, CAS, China (No. KIB 20101018). The air-dried and powdered twigs and leaves of *M. faberi* (20 kg) were extracted with 95% EtOH (40 L × 3) under reflux conditions, and the solvent was evaporated in vacuo. The crude extract was partitioned between EtOAc and an acidic liquor pH 2–3. The aqueous layer was then basified to pH 9–10 with saturated Na_2CO_3, followed by exhaustive extraction with $CHCl_3$. The $CHCl_3$-soluble material was subjected to silica gel column chromatography ($CHCl_3$/MeOH, 1:0 → 0:1) to give five major fractions I–V. Fraction I (5.0 g) was chromatographed on a silica gel column eluted with PE/EtOAc (15:1) to give myrionamide (**2**, 50 mg) and schoberine (**3**, 9 mg). Fraction II (3.0 g) was chromatographed on a silica gel column eluted with $CHCl_3$/MeOH (20:1) to give a major alkaloid, which was purified on normal H silica gel (PE/acetone, 10:1 → 1:1), and then by a Sephadex LH-20 gel column eluted with MeOH to afford myriberine A (**1**, 15 mg).

(8) **Myriberine A (1):** colorless crystals (in methanol); mp 145–147 °C; HREIMS at *m*/*z* 286.2050 $[M]^+$ (calcd 286.2045, $C_{18}H_{26}N_2O$); $[\alpha]_D^{20}$ +22.2 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 284 (3.0) nm, CD (0.00039 M, MeOH) λ_{max} ($\Delta\varepsilon$) 263 (7.3), 290 (+16.9); IR ν_{max} (KBr) cm^{-1} 3432, 2921, 2851, 1632, 1554, 1426; ^{1}H and ^{13}C NMR data, see Table 1.

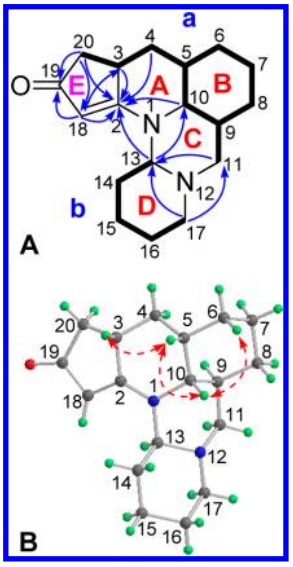

Figure 1. $^1H-^1H$ COSY (A: –), selected HMBC (A: →), and ROESY (B: ↔) correlations of **1**.

C-3, C-18, and N-1 were connected to each other through the quaternary carbon C-2. Finally, demonstration of the presence of the five-membered ring E with the α,β-unsaturated lactone motif was achieved by the key HMBC correlations of H-18 and H_2-20 to C-19 (δ_C 202.2). Accordingly, the planar structure of **1** was constructed as shown in Figure 1A.

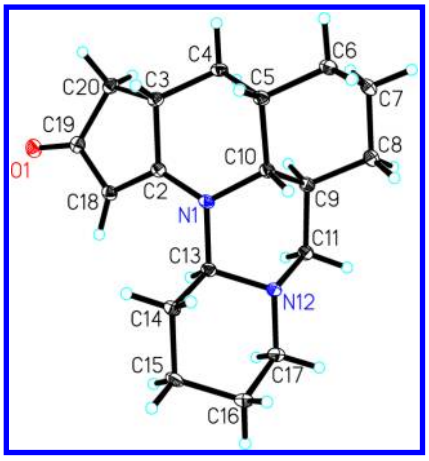

Figure 2. Single-crystal X-ray structure of **1**.

The relative configuration of **1** was elucidated by analysis of the ROESY spectrum (Figure 1B) and the values of the $^1H-^1H$ vicinal coupling constants. The ROESY correlations of H-3/H-5, H-5/H-10, H-10/H-6a indicated that these protons were cofacial and were arbitrarily assigned as β-oriented. The α-orientation of H-9 and H-13, as well as the conformations of rings C and D, were eventually resolved by the single-crystal X-ray diffraction experiments (Figure 2). It is noteworthy that H-13 of **1** possesses an opposite configuration to that of H-10, which is beyond the common expectation for the same configuration of the proton pair H-10/H-13 within the *Myrioneuron* alkaloids category.[4b]

The unprecedented structure, with a unique stereochemistry of the proton pair H-10/H-13 in *Myrioneuron* alkaloids, raised interest to assign the absolute configuration of **1**. Because of the absence of application exciton coupling in the CD spectrum of **1**, as well as no suitable model compounds found for reference, the absolute configuration of **1** cannot be resolved directly by the analysis of its CD spectrum. Thus, attention was turned to theoretical calculation of its ECD spectrum using the time-dependent density functional theory method of the Gaussian 03[9] program package, as this method has been demonstrated to be powerful in determining the absolute configuration of natural products.[10] Since myriberine A must be one of the two enantiomers (3*S*,5*S*,9*S*,10*R*,13*R*)-**1** or (3*R*,5*R*,9*R*,10*S*,13*S*)-**1** according to the relative configuration that was confirmed by the single-crystal X-ray diffraction analysis, the two enantiomers were calculated by the TDDFT method at the B3LYP/aug-CC-pVDZ//B3LYP/6-31G** level in the gas phase and at the B3LYP/aug-CC-pVDZ//B3LYP/6-31G** and B3LYP/6-311++G**//B3LYP/6-31G** levels with the IEFPCM model in methanol solution (see the Supporting Information, Figures S2 and S4). As illustrated in Figure 3, the calculated ECD curve for (3*S*,5*S*,9*S*,10*R*,13*R*)-**1** resembled the CD spectrum recorded for **1**, which is opposite to that calculated for (3*R*,5*R*,9*R*,10*S*,13*S*)-**1**.

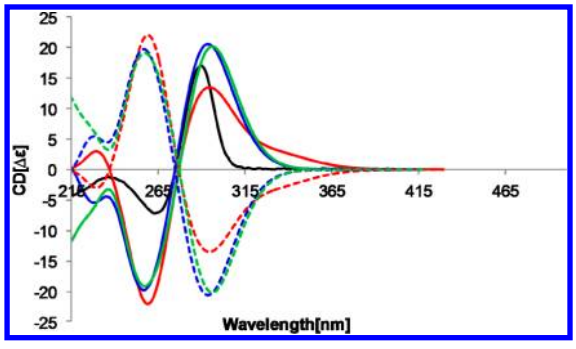

Figure 3. ECD spectrum of (3*S*,5*S*,9*S*,10*R*,13*R*)-**1** (solid) and its enantiomer (3*R*,5*R*,9*R*,10*S*,13*S*)-**1** (dash): esperimental ECD (black), calculated ECD at the B3LYP/aug-CC-pVDZ level in the gas phase (red), at the B3LYP-SCRF/aug-CC-pVDZ//B3LYP/6-31G** and B3LYP-SCRF/6-311++G**//B3LYP/6-31G** levels with the IEFPCM model in MeOH (blue and cyan, respectively).

To provide comprehension of the origin of the experimentally observed ECD of **1** at the molecular level,

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molecular orbital (MO) analysis at the B3LYP-SCRF/6-31G**//B3LYP/6-31G** level with the IEFPCM model in MeOH was then carried out. The electronic transitions from MO76, MO77, and HOMO78 to LUMO79 involving the $\pi-\pi^*$ transition of the α,β-unsaturated ketone in the cyclopentenone system predominantly contribututes to the negative rotatory strength at 268 nm, which can be assigned to the experimentally observed CE at 263 nm. The next rotatory strengths at 285 nm are given by the electronic transitions from MO77 and HOMO78 to LUMO79 involving the $\pi-\pi^*$ and n-π^* excitations, which are associated with another diagnostic positive CE at 290 nm in the experimental ECD of **1** (see the Supporting Informaiton, Table S2, Figure S3).

Compound **1** represents the first *Myrioneuron* alkaloid featuring an unprecedented pentacyclic system conjugated between the parent compound and a C_3 unit. Myriberine A (**1**) was tested for cytotoxicity against A-549, MCF-7, SMMC-7721, SW-480, and HL-60 human cancer cell lines using the MTT method[11] with *cis*-platin as the positive control. Alkaloid **1** exhibited no cytotoxicity against all the five cells with $IC_{50} > 40\,\mu M$. Moreover, attention was focused on anti-HCV bioassay inspired by the structural similarity to the matrine alkaloids,[12] Alkaloid **1** showed inhibitory effects on the hepatitis C virus (HCV) life cycle with a good therapeutic index (CC_{50}/EC_{50}) of greater than 12.0 in vitro (see the Supporting Information, Table S3).[12] The results indicated that the anti-HCV activity should not be due to the cytotoxicity.

Acknowledgment. This project was supported financially by the National Natural Science Foundation of China (No. 30830114), the National Basic Research Program of China (973 Program, 2009CB522300), the Young Academic and Technical Leader Raising Foundation of Yunnan Province (2010CI047), and the Foundation of Chinese Academy of Sciences to Dr. Y. Zhang. The calculation part was supported by the HPC Center of Kunming Institute of Botany, CAS.

Supporting Information Available. HREIMS, IR, CD, 1D and 2D NMR spectra, isolation procedures, bioactivity assay, detailed computational methods, and the X-ray crystallographic data (CIF) of **1** are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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