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# Schoberine B, an alkaloid with an unprecedented straight $C_5$ side chain, and myriberine B from *Myrioneuron faberi*†

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Two novel *Myrioneuron* alkaloids, schoberine B (**1**) and myriberine B (**2**), were isolated from the aerial part of *Myrioneuron faberi*. Schoberine B is the first example among lysine-originating alkaloids to possess a ring-free $C_5$ fragment as a side chain. The structures of **1** and **2** were elucidated using analyses of extensive NMR and MS spectra. **1** and **2** were evaluated *in vitro* against hepatitis C virus (HCV), showing a therapeutic index ($CC_{50}/EC_{50}$) of higher than 36.2 and 15.2, respectively.

*Myrioneuron* alkaloids comprise a family of fascinating lysine-based metabolites produced by plants of the genus *Myrioneuron* R. Br. (Rubiaceae).[1–7] $C_5$ units originating from lysine often incorporate into *Myrioneuron* alkaloids as building blocks, and construct intricate polycyclic ring systems (tricyclic-, tetracyclic-, pentacyclic-, hexacyclic-, and decacyclic-type).[2–7] Besides their structural features, a number of these alkaloids show remarkable antimalarial,[7] and good anti-HCV activities,[4,5] and have attracted great organic synthesis interest.[6–10] The $C_5$ building blocks originating from lysine were produced after decarboxylation, oxidation, and cyclization procedures.[11,12] Afterwards, these reactive $C_5$ intermediates were incorporated into the ring systems of various *Myrioneuron* alkaloids.[13] However, during our ongoing investigation of *Myrioneuron* alkaloids, schoberine B (**1**), possessing a $C_5$ exogenous straight side chain on the tetracyclic system, was obtained (Fig. 1). Described herein are the isolation, structural elucidation, anti-HCV activities, and proposed biosynthetic pathways of **1** and **2**.

The aerial parts of *M. faberi* were collected in October 2011 from Sichuan Province, People's Republic of China. During our continuing investigation of alkaloids from *M. faberi*, compound **1** was obtained as an ultra-trace constituent (2 mg from 30 kg plant material). The purification of **1** using HPLC with a UV-detector was a challenge due to its weak UV absorption, as there is neither n → π* nor π → π* electron transition in the structure of **1**. However, **1** was successfully purified using HPLC with an evaporative light-scattering detector (ELSD). Along with **1**, compound **2**, possessing a pentacyclic system with a C-14 substituted novel carbon skeleton, was obtained and their structures were elucidated *via* extensive NMR and MS spectroscopic data.

Schoberine B (**1**)[14] was isolated as a colorless gum. Its molecular formula $C_{20}H_{36}N_2O$ was established using its $^{13}C$ NMR and HREIMS (*m/z* 320.2836 $[M]^+$, calcd for $C_{20}H_{36}N_2O$, 320.2828) data, indicating four indices of hydrogen deficiency. The $^{13}C$ NMR and DEPT data (Table 1) revealed 20 $sp^3$ carbon signals comprising 5 × CH and 15 × $CH_2$ (two $CH_2$ signals overlapped). Among them, two downfield methines C-13 ($\delta_C$ 83.3) and C-10 ($\delta_C$ 66.0) were recognized as the di-nitrogenated and mono-nitrogenated methine in the *Myrioneuron* alkaloids, respectively.[2,3]

2D NMR (HSQC, $^1H$–$^1H$ COSY, and HMBC experiments) data revealed that **1** possesses three spin-coupling systems: (a) H-13/

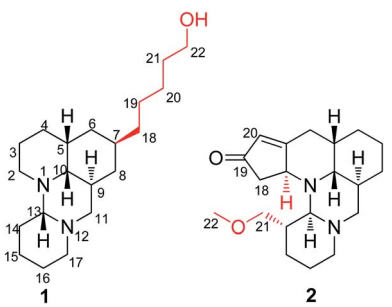


Fig. 1 Structures of compounds **1** and **2**.

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† Electronic supplementary information (ESI) available: 1D and 2D NMR, ESIMS, HREIMS, IR spectra of **1** and **2**, and experimental section. See DOI: 10.1039/c5ra25218k

Table 1 $^{13}C$ NMR and $^{1}H$ NMR spectroscopic data for **1** recorded in pyridine-$d_5$ at 313 K at 150 and 600 MHz, respectively

| No. | $\delta_C$ | $\delta_H$ (mult, $J$ in Hz) | No. | $\delta_C$ | $\delta_H$ (mult, $J$ in Hz) |
|---|---|---|---|---|---|
| 2 | 40.7 t | 2.92 (m) | 13 | 83.3 d | 2.92 (m) |
| 3 | 27.2 t | 1.69 (m), 1.58 (m) | 14 | 30.5 t | 1.75 (m), 1.68 (m) |
| 4 | 26.3[a] t | 1.69 (m), 1.28 (m) | 15 | 25.5 t | 1.70 (m), 1.25 (m) |
| 5 | 36.4 d | 1.97 (m) | 16 | 26.3[a] t | 1.52 (m), 1.46 (m) |
| 6 | 39.3 t | 1.55 (m), 1.13 (m) | 17 | 56.8 t | 2.78 (d, 11.4), 1.86 (td, 12.0, 2.4) |
| 7 | 31.8 d | 1.40 (m) | 18 | 38.1 t | 1.13 (m) |
| 8 | 37.6 t | 1.47 (m), 0.46 (q, 12.0) | 19 | 27.7 t | 1.35 (m) |
| 9 | 27.1 d | 2.19 (m) | 20 | 27.3 t | 1.52 (m) |
| 10 | 66.0 d | 2.52 (dd, 10.8, 4.8) | 21 | 34.2 t | 1.80 (m) |
| 11 | 64.6 t | 2.81 (dd, 10.8, 3.6), 1.68 (m) | 22 | 62.6 t | 3.92 (t, 6.0) |

[a] Overlapped $\delta_C$ values.

$H_2$-14/$H_2$-15/$H_2$-16/$H_2$-17, (b) $H_2$-2/$H_2$-3/$H_2$-4/H-5/$H_2$-6(H-10)/H-7/$H_2$-8/H-9/H-10($H_2$-11) and (c) $H_2$-18/$H_2$-19/$H_2$-20/$H_2$-21/$H_2$-22 (Fig. 2A). The HMBC correlations from $H_2$-2 ($\delta_H$ 2.92, m), H-10 ($\delta_H$ 2.52 dd, 10.8, 34.8 Hz) and H-11b ($\delta_H$ 2.81 dd, 10.8, 3.6 Hz) to C-13, and H-11b to C-17 ($\delta_C$ 56.8) suggested that a and b are neighboring systems. These findings along with the featured nitrogenated carbon atoms C-13, C-10, C-11 ($\delta_C$ 64.6), and C-17 suggested that **1** possesses a tetracyclic moiety accounting for 20 atoms as schoberine; the structure was confirmed using single-crystal X-ray analysis.[5] The remaining five carbon atoms all showed '–$CH_2$–' signals in the DEPT135 spectrum indicating that this $C_5$ moiety is a '–$(CH_2)_5$–'. As there is only one oxygenated methylene ($CH_2$-22, $\delta_C$ 62.6; $\delta_H$ 3.92 t, 6.0 Hz), the '–$(CH_2)_5$–' was predicted to be an *n*-pentanol group, which is supported using the COSY correlation system c (Fig. 2A). Subsequently, the attachment of spin-coupling system c to the tetracyclic moiety was elucidated *via* HMBC correlations from $H_2$-19 ($\delta_H$ 1.35 m), H-6a ($\delta_H$ 1.55 m), H-5 ($\delta_H$ 1.97 m), H-9 ($\delta_H$ 2.19 m), and H-8a (0.46 q, 12.0) to C-7 ($\delta_C$ 31.8). Thus the 2D structure of **1** was established *via* the connection of a, b and c to form a tetracyclic system (rings A, B, C, and D) as shown in Fig. 2A.

The relative configuration of **1** was resolved using its ROESY data and the $^{1}H$–$^{1}H$ vicinal coupling constants. NOE correlations between H-10 ($\delta_H$ 2.52, dd, 10.8, 4.8) and H-8b ($\delta_H$ 0.46, q, 12.0), H-10 and H-11b ($\delta_H$ 1.68, m), and H-10 and H-13 ($\delta_H$ 2.92, m) were observed, which indicated that these four protons were cofacial and were arbitrarily assigned as β-oriented. As H-13/H-10/H-11b took the 1,3,5-*cis*-triaxial position, this required H-9 ($\delta_H$ 2.19, m) to take α-axial (Fig. 2B) and the resulting $J_{H\text{-}10/H\text{-}9}$ = 10.8 Hz (*trans*). The proton at the C-5 stereogenic center ($\delta_H$ 1.97, m) was assigned as β-oriented based on $J_{H\text{-}10/H\text{-}5}$ (4.8 Hz, *cis*). The stereogenic center of C-7 could be resolved using the NOE correlation of H-9/H-7 ($\delta_H$ 1.40, m) (Fig. 2B). Consequently, the relative configuration of **1** was elucidated as 5β, 9α, 7β, 10β, and 13β.

Myriberine B (**2**)[15] was obtained as a white powder. The HREIMS of **2** gave a molecular formula of $C_{20}H_{30}N_2O_2$ (*m*/*z* 330.2300 $[M]^+$, calcd for $C_{20}H_{30}N_2O_2$, 330.2307), corresponding to seven indices of hydrogen deficiency. The $^{13}C$ NMR and DEPT data (Table 2) revealed three $sp^2$ carbon atoms (1 × CH, 2 × qC) and 17 $sp^3$ carbon atoms (6 × CH, 10 × $CH_2$ and 1 × $CH_3$). The IR absorption bands at 1714 and 1626 $cm^{-1}$ along with the UV absorption at 280 nm indicated the existence of an α,β-unsaturated ketone moiety (qC, $\delta_C$ 209.7; CH, $\delta_C$ 128.3; and qC, $\delta_C$ 183.9), and a '$CH_3OCH_2$–' group could be recognized by its C-22 ($CH_3$, $\delta_C$ 59.3) and C-21 ($CH_2$, $\delta_C$ 75.4) signals. Besides the two indices of hydrogen deficiency accounted for by the α,β-unsaturated ketone moiety, the remaining five suggested that alkaloid **2** possesses a pentacyclic system (Fig. 1).

2D NMR (HSQC, $^{1}H$–$^{1}H$ COSY, and HMBC experiments) data revealed that **2** possesses three spin coupling systems, (a) $H_2$-4/H-5/$H_2$-6(H-10)/$H_2$-7/$H_2$-8/H-9/H-10($H_2$-11), (b) H-13/H-14 ($H_2$-21)/$H_2$-15/$H_2$-16/$H_2$-17, and (c) H-2/$H_2$-18 (Fig. 3A). Comparing

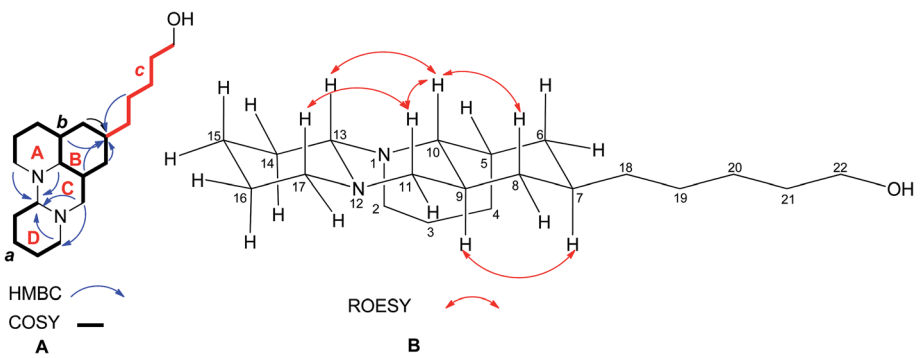



Fig. 2 $^{1}H$–$^{1}H$ COSY (bold) and key HMBC correlations of **1** (H to C) (A); selected ROESY correlations of **1** (B).

Table 2 $^{13}$C NMR and $^{1}$H NMR spectroscopic data for **2** recorded in methanol-$d_4$ at 313 K at 150 and 600 MHz, respectively

| No. | $\delta_C$ | $\delta_H$ (mult, $J$ in Hz) | No. | $\delta_C$ | $\delta_H$ (mult, $J$ in Hz) |
|---|---|---|---|---|---|
| 2 | 57.2 d | 4.31 (br d, 4.2) | 13 | 77.2 d | 3.63 (d, 10.4) |
| 3 | 183.9 s | | 14 | 31.8 d | 2.44 (m) |
| 4 | 30.9 t | 2.72 (t, 13.8), 2.51 (dd, 13.8, 4.2) | 15 | 30.1 t | 1.91 (m), 1.49 (ddd, 16.6, 13.2, 4.0) |
| 5 | 37.2 d | 2.01 (m) | 16 | 29.9 t | 1.82 (m), 1.29 (m) |
| 6 | 32.1 t | 1.71 (m), 1.71 (m) | 17 | 54.9 t | 2.90 (m), 2.90 (m) |
| 7 | 20.9 t | 1.55 (m), 1.55 (m) | 18 | 44.7 t | 2.58 (m), 2.19 (dd, 18.0, 1.8) |
| 8 | 31.2 t | 1.63 (m), 1.07 (m) | 19 | 209.7 s | |
| 9 | 30.1 d | 2.24 (m) | 20 | 128.3 d | 5.90 (s) |
| 10 | 57.3 d | 2.87 (dd, 12.6, 4.8) | 21 | 75.4 t | 3.36 (m), 3.26 (dd, 9.6, 2.4) |
| 11 | 53.0 t | 2.95 (t, 11.4), 2.59 (m) | 22 | 59.3 q | 3.24 (3H, s) |

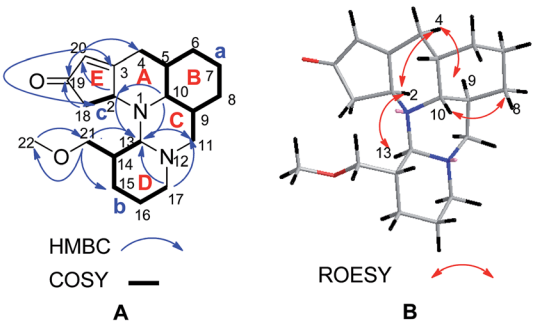



Fig. 3 $^{1}$H–$^{1}$H COSY (bold) and key HMBC correlations of **2** (A), and key ROESY correlations of **2** (B).

the featured and similar four carbon signals C-10 (CH, $\delta_C$ 57.3), C-11 ($CH_2$, $\delta_C$ 53.0), C-13 (CH, $\delta_C$ 77.2), and C-17 ($CH_2$, $\delta_C$ 54.9) in **2** with those in myriberine A (the structure of which has been confirmed using single-crystal X-ray analysis),[2] suggested that these two alkaloids shared the same A, B, C, and D ring system (Fig. 3A). In the HMBC spectrum, cross-peaks of H-10 ($\delta_H$ 2.87, dd, 12.6, 4.8 Hz) to C-2 (CH, $\delta_C$ 57.2) and C-13, and H-2 ($\delta_H$ 4.31, br d, 4.2 Hz) to C-13 indicated that C-2, C-10, and C-13 were linked through N-1 as shown in Fig. 3A. Furthermore, the striking presence of a C-2 sp$^3$ methine signal and a spin system H-2/$H_2$-18 (1H, $\delta_H$ 2.58, m; 1H, $\delta_H$ 2.19, dd, 18.0, 1.8) in **2** implied that C-2 might be incorporated into a new ring, and HMBC correlations from $H_2$-18 to C-2 and C-3, and H-20 ($\delta_H$ 5.90, s) to C-3 and C-4 indicated the attachments of C-18 ($\delta_C$ 44.7) at C-2, and C-20 ($\delta_C$ 128.3) at C-3 ($\delta_C$ 183.9), respectively. The presence of the five-membered ring E with an α,β-unsaturated lactone moiety could be elucidated using the key HMBC correlations of $H_2$-18 and H-20 to C-19 (qC, $\delta_C$ 209.7). Thus the basic pentacyclic fragment of **2**, the same as in myriberine A except for the different olefinic position, was deduced. For the position of the additional '$CH_3OCH_2$–' group, the attachment of C-21 ($\delta_C$ 75.4) at C-14 ($\delta_C$ 31.8) was deduced from HMBC correlations from $H_2$-21 ($\delta_H$ 3.36, m; 3.26, dd, 9.6, 2.4 Hz) to C-13, C-14, and C-15 ($\delta_C$ 30.1) as shown. Finally, the 5/6/6/6/6 pentacyclic planar structure of **2** was constructed as shown in Fig. 3A.

The relative configuration of **2** was also resolved *via* its ROESY spectrum and the $^{1}$H–$^{1}$H vicinal coupling constants. The NOE cross-peak of H-10 ($\delta_H$ 2.87, dd, 12.0, 4.8 Hz)/H-8b ($\delta_H$ 1.07, m) suggested that these two protons have a 1,3-*cis*-diaxial configuration on ring B, requiring H-10/H-9 ($\delta_H$ 2.24, m) to take a *trans* configuration. Proton H-10 was arbitrarily assigned as β-oriented, and H-9 was elucidated as α accordingly. Then, the stereochemistry of the β-oriented H-5 could be established using the $^{1}$H–$^{1}$H vicinal coupling constants of H-10 ($\delta_H$ 2.87, dd, 12.0, 4.8 Hz) because $J_{H\text{-}10/H\text{-}9}$ = 12.0 Hz and $J_{H\text{-}10/H\text{-}5}$ = 4.8 Hz could be calculated. Accordingly, the ROESY cross-peaks of H-9/H-13, and H-13/H-2 indicated these three protons took an α orientation (Fig. 3B). Moreover, the *trans* relationship of H-13/H-14 was supported by the $J_{H\text{-}13/H\text{-}14}$ value (10.4 Hz), and the β-oriented H-14 ($\delta_H$ 2.44, m) was deduced. As a result, the relative configuration of **2** was elucidated as 2α, 5β, 9α, 10β, 13α, and 14α.

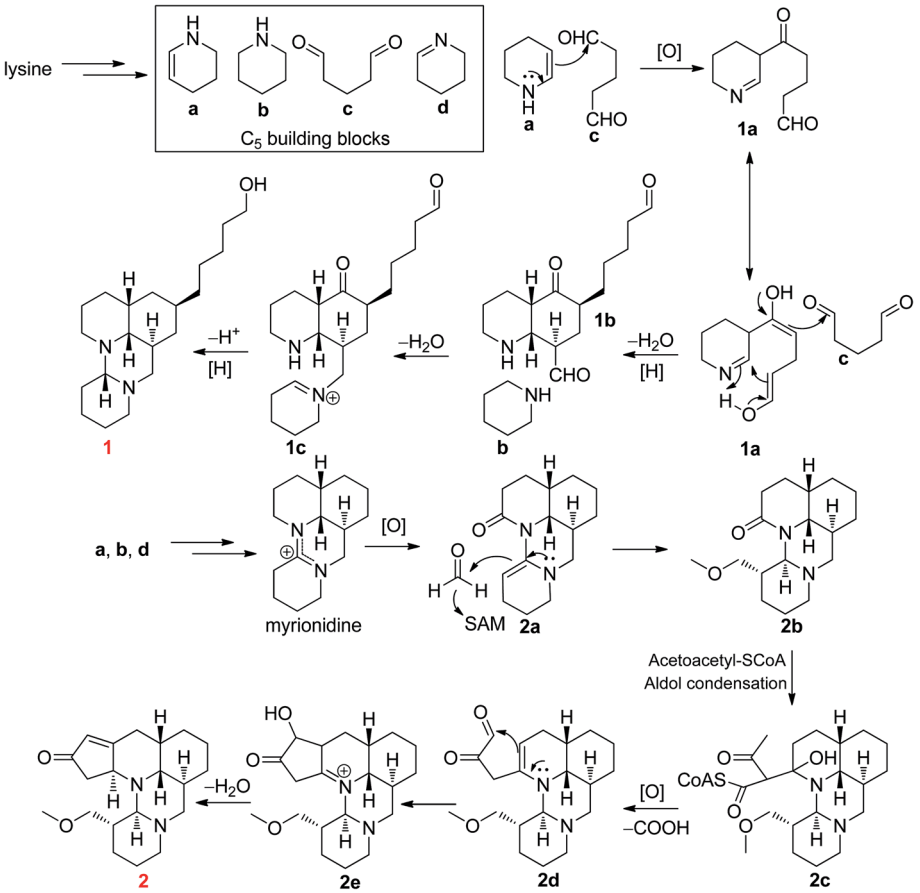
Alkaloids **1** and **2** were evaluated *in vitro* against hepatitis C virus (HCV).[2–5] Alkaloids **1** and **2** exhibited moderate inhibitory activity with a selective index (SI) of higher than 36.2 and 15.2, respectively, as shown in Table 3 (VX-950 was used as a positive control).

A hypothetical biosynthetic pathway for alkaloids **1** and **2** was proposed in Scheme 1. The starting material for the *Myrioneuron* alkaloids biosynthesis was deemed to be lysine.[6,7] The four $C_5$ building blocks **a**, **b**, **c**, and **d** could originate from lysine.[3] The $C_{10}$ intermediate **1a** could result from the condensation between **a** and **c**, which could give the $C_{15}$ intermediate **1b** *via* condensation with another **c**. Then, **1b** could be transformed into the $C_{20}$ framework **1c** through a Mannich reaction with **b**. Consequently, **1** could be reached after a selective reduction from **1c**. Compound **2** might be derived from myrionidine (which was proposed to be established by **a**, **b**, and **d**).[7]

Table 3 The anti-HCV activities of compounds **1** and **2**[a]

| | $CC_{50}$ (μM) | | $EC_{50}$ (μM) | | |
|---|---|---|---|---|---|
| Compounds | Mean | SD | Mean | SD | SI |
| **1** | >100 | 0.00 | 2.76 | 1.09 | >36.2 |
| **2** | 75.67 | 13.86 | 5.12 | 1.31 | 15.2 |
| VX-950 | 47.83 | 1.25 | 0.13 | 0.01 | 370.8 |

[a] $CC_{50}$, the median cytotoxic concentration. $EC_{50}$, the median effective concentration. VX-950, telaprevir.




Scheme 1 Putative biosynthetic pathway of **1** and **2**.

The C-14 substituted intermediate **2b** could be produced from myrionidine and **2a** as described.[5] As a result, with the participation of acetoacetyl-SCoA, **2** could be established through a series of procedures from **2b** as hypothetically suggested in the formation of myriberine A.[2]

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13 P. Erwan and G. Edmond, *Chem.–Eur. J.*, 2015, **21**, 10604–10615.
14 Schoberine B (**1**): colorless gum; $[\alpha]_D^{24}$ −12 (*c* 0.3, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 202 (2.70); IR (KBr) $\nu_{max}$ 3423, 2925, 2854, 1631, 1460, 1384, 1280, 1238, 1182, 1131, 1048 and 977 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, Table 1; positive ESIMS

$m/z$ 321 $[M + H]^+$; positive HREIMS $m/z$ 320.2836 $[M]^+$, calcd for $C_{20}H_{36}N_2O$, 320.2828.

15 Myriberine B (2): amorphous powder; $[\alpha]_D^{24}$ −25 ($c$ 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 280 (2.51), 222 (3.42); CD (0.00066 M, MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 238 (−1.67), 275 (−1.44), 320 (0.68); IR (KBr) $\nu_{max}$ 3424, 2922, 2887, 2847, 1714, 1626, 1463, 1448, 1402, 1381, 1173, 1128, and 983 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, Table 2; positive ESIMS $m/z$ 331 $[M + H]^+$, 353 $[M + Na]^+$; positive HREIMS $m/z$ 330.2300 $[M]^+$, calcd for $C_{20}H_{30}N_2O_2$, 330.2307.

 