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# Three new alkaloids from *Myrioneuron faberi*

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ABSTRACT

*Myrioneuron* alkaloids are a class of fast growing natural products elaborated by plants of the genus *Myrioneuron*. Three new alkaloids secomyrionamide (**1**), isomyrionine (**2**), and 12-oxomyriberine A (**3**) were isolated from *Myrioneuron faberi*. **1** and **2** presenting the simplest two members of alkaloids derived from *M. faberi*, and possessing tricyclic $C_{15}$ carbon framework, which might furnish precursors for myrionamide and bring new insight into biosynthesis pathway of myrionamide. Structures of **1**–**3** were elucidated on basis of NMR and MS spectra. Compounds **2** and **3** were tested for inhibitory activity to the hepatitis C virus (HCV), and showed weak activity.



*Myrioneuron* alkaloids were firstly reported in 2002 isolated from *Myrioneuron nutans*, and altogether ten interesting structures were reported to date from this plant.[1–7] $C_5$ fragment such as piperidine was commonly found in *Myrioneuron* alkaloids, and its lysine origination was suggested.[1,6] Structurally, *Myrioneuron* alkaloids hold various polycyclic ring systems (tricyclic-, tetracyclic-, pentacyclic-, hexacyclic-, and decacyclic- types) which has attached great interests from total synthesis.[5–10]

Since 2013, a number of structural fascinating alkaloids were obtained from *Myrioneuron faberi* and *Myrioneuron tonkinensis*.[11–16] In our continuing investigation of chemical constitutes from *M. faberi*, two tricyclic new alkaloids secomyrionamide (**1**) and isomyrionine (**2**) were obtained, which might furnish intermediates on biogenetical route of tetracyclic *Myrioneuron* alkaloids myrionamide[14] (Fig. 1). Myrionamide was previously proposed to accomplish its structure by the final oxidation step at C-2,[7] while the opened ring C and oxidized C-2 of **1** and **2** suggested another possible biosynthesis pathway of myrionamide (Scheme 1). Meanwhile, another new structure 12-oxomyriberine A (**3**) was also isolated. Reported herein, is the isolation, structural elucidation, and anti-HCV bioassay of these alkaloids.

The aerial parts of *M. faberi* were collected in October 2011 from Sichuan Province, People's Republic of China. The air-dried, powdered leaves and stems (30 kg) of *M. faberi* were extracted with 95% EtOH. The saccharides were then removed by macro-porous resin to obtain the crude alkaloids (223 g). The crude alkaloids was subjected to normal phase silica gel chromatography (200–300 mesh; $CHCl_3$/MeOH, 20:1 → 0:1) and obtained four fractions (Fr 1–4). From Fr.1, compounds **1**–**3** (Fig. 1) were finally obtained

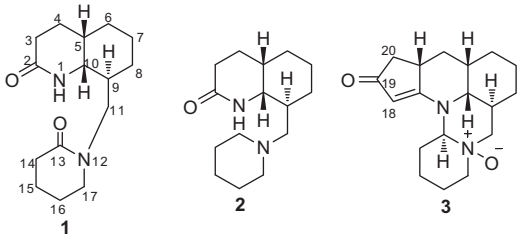
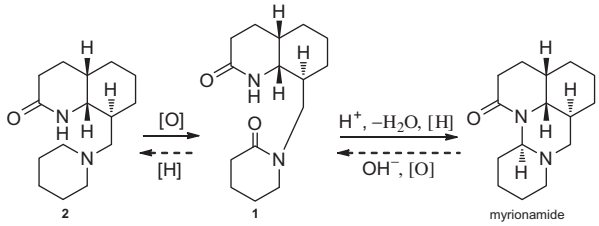


**Figure 1.** Structures of **1**–**3**.




**Scheme 1.** Hypothetical biosynthetic relationships of **1**, **2**, and myrionamide.

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**Table 1**
$^{13}$C NMR data for **1**–**3** ($\delta$ in ppm)

| No. | **1**[a] | **2**[b] | **3**[c] |
|---|---|---|---|
| 2 | 171.3 | 175.1 | 177.9 |
| 3 | 31.4 | 31.0 | 39.8 |
| 4 | 23.9 | 23.8 | 28.3 |
| 5 | 33.1 | 33.4 | 34.3 |
| 6 | 29.7 | 29.8 | 22.9 |
| 7 | 21.3 | 21.0 | 20.6 |
| 8 | 29.1 | 29.6 | 28.4 |
| 9 | 40.2 | 37.0 | 32.1 |
| 10 | 55.5 | 57.5 | 56.9 |
| 11 | 50.2 | 63.2 | 63.1 |
| 13 | 171.1 | 55.5 | 85.9 |
| 14 | 33.0 | 25.2 | 27.2 |
| 15 | 21.9 | 29.7 | 31.1 |
| 16 | 23.9 | 25.2 | 23.0 |
| 17 | 49.9 | 55.5 | 70.8 |
| 18 | | | 105.4 |
| 19 | | | 201.8 |
| 20 | | | 41.4 |

[a] Recorded at 100 MHz and 323 K in pyridine-$d_5$.
[b] Recorded at 150 MHz and 313 K in $CD_3OD$.
[c] Recorded at 100 MHz and 291 K in pyridine-$d_5$.

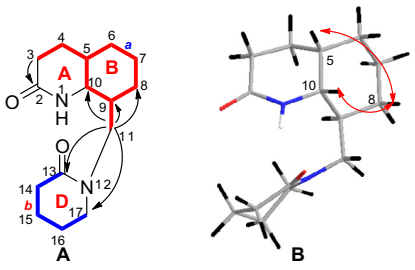


**Figure 2.** (A) $^1$H–$^1$H COSY (bold) and key HMBC correlations of **1** and (B) selected ROESY correlations of **1**.

by HPLC system after repeated purification by Si and sephadex columns. The structures of **1**–**3** were elucidated via 1D and 2D NMR, MS, and HREIMS spectroscopic data.

Secomyrionamide[17] (**1**, 7 mg) was obtained as white powder. The HREIMS of **1** gave a molecular formula of $C_{15}H_{24}N_2O_2$ (*m*/*z* 264.1842 $[M]^+$, calcd. for $C_{15}H_{24}N_2O_2$, 264.1838), corresponding to five devices of hydrogen deficiency. The $^{13}$C NMR and DEPT data (Table 1) revealed two carbonyl groups ($\delta_C$ 171.3 and $\delta_C$ 171.1) and 13 $sp^3$ carbon atoms (3 × CH and 10 × $CH_2$). Besides the two devices of hydrogen deficiency accounted by that two carbonyl groups, the rest three indicated that alkaloid **1** possesses a tricyclic system (Fig. 1).

2D NMR (HSQC, $^1$H–$^1$H COSY, and HMBC experiments) data revealed that **1** possesses two spin coupling systems: *a*, $H_2$-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 *b*, $H_2$-14/$H_2$-15/$H_2$-16/$H_2$-17 (Fig. 2A, Table 2). In the HMBC spectrum, correlations from $H_2$-11 ($\delta_H$ 3.81, dd 14.0, 5.5 Hz; $\delta_H$ 3.24, dd 14.0, 5.0 Hz) to C-8 ($\delta_C$ 29.1), C-9 ($\delta_C$ 40.2), C-10 ($\delta_C$ 55.5), C-13 ($\delta_C$ 171.1), and C-17 ($\delta_C$ 49.9) showed ring D was connected to ring B through $CH_2$-11, and the linkage between spin coupling systems *a* and *b* was also elucidated. HMBC correlations from $H_2$-3 ($\delta_H$ 2.47, ddd 17.5, 6.0, 4.0 Hz; $\delta_H$ 2.36, m) to C-2 ($\delta_C$ 171.3) suggested another carbonyl was located at C-2. Therefore, the 2D structure of **1** was elucidated as shown in Figure 1.

The relative configuration of **1** was resolved via its ROESY spectrum. The NOE cross-peak of H-10 ($\delta_H$ 3.10, m)/H-8b ($\delta_H$ 1.11, qd 10.0, 4.5 Hz) required these two protons to be 1,3,-cis-diaxial configuration on ring B, which suggested H-9 ($\delta_H$ 1.86, m) to take an opposite orientation to H-10 and H-8b. Proton H-10 was arbitrarily assigned as β-oriented, and H-9 was elucidated as α accordingly. The β-oriented H-5 could be established by NOE correlation of H-5 ($\delta_H$ 1.98, m)/H-8b (Fig. 2B). As a result, the relative configuration of **1** was elucidated as 5β, 9α, and 10β.

**Table 2**
$^1$H NMR data for **1**-**3** ($\delta$ in ppm and *J* in Hz)

| No. | **1**[a] | **2**[b] | **3**[c] |
|---|---|---|---|
| 3 | 2.47 (ddd, 17.5, 6.0, 4.0) | 2.36 (m) | 2.66 (m) |
| | 2.36 (m) | | |
| 4 | 1.84 (m) | 2.04 (m) | 1.60 (m) |
| | 1.40 (m) | 1.63 (m) | 1.26 (m) |
| 5 | 1.98 (m) | 2.04 (m) | 1.97 (m) |
| 6 | 1.52 (m) | 1.79 (m) | 1.66 (m) |
| | 1.38 (m) | 1.61 (m) | 1.52 (m) |
| 7 | 1.44 (m) | 1.53 (m) | 1.29 (m) |
| | 1.34 (m) | | 1.21 (m) |
| 8 | 1.60 (m) | 1.66 (m) | 1.36 (m) |
| | 1.11 (qd, 10.0, 4.5) | 1.15 (m) | 0.92 (m) |
| 9 | 1.86 (m) | 2.11 (m) | 2.99 (m) |
| 10 | 3.10 (m) | 3.20 (dd,12.0, 4.2) | 3.47 (dd, 13.0, 6.5) |
| 11 | 3.81 (dd, 14.0, 5.5) | 2.93 (m) | 3.56 (m) |
| | 3.24 (dd, 14.0, 5.0) | 2.71 (dd, 12.0, 3.6) | 2.95 (m) |
| 13 | | 2.93 (m) | 5.07 (m) |
| 14 | 2.40 (m) | 1.79 (m) | 2.29 (m) |
| | | | 1.73 (m) |
| 15 | 1.58 (m) | 1.79 (m) | 1.50 (m) |
| 16 | 1.58 (m) | 1.79 (m) | 2.07 (m) |
| 17 | 3.15 (m) | 2.93 (m) | 3.71 (m) |
| | 3.09 (m) | | 3.60 (m) |
| 18 | | | 6.09 (s) |
| 20 | | | 2.50 (dd, 16.5, 7.0) |
| | | | 2.15 (dd, 16.5, 5.0) |

[a] Recorded at 500 MHz and 333 K in pyridine-$d_5$, based on HSQC.
[b] Recorded at 600 MHz and 313 K in $CD_3OD$, based on HSQC.
[c] Recorded at 500 MHz and 291 K in pyridine-$d_5$, based on HSQC.

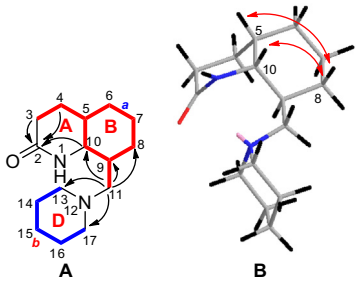


**Figure 3.** (A) $^1$H-$^1$H COSY (bold) and key HMBC correlations of **2** and (B) selected ROESY correlations of **2**.

Isomyrionine[18] (**2**, 3 mg) was isolated as colorless gum. Its molecular formula $C_{15}H_{26}N_2O$ was established by its $^{13}$C NMR and HREIMS (*m*/*z* 250.2045 $[M]^+$, calcd. for $C_{15}H_{26}N_2O$, 250.2045) data, indicating four indices of hydrogen deficiency. The existence of a carbonyl carbon was revealed by $\delta_C$ 175.1 (Table 1), which accounted for one index of hydrogen deficiency and suggested **2** possessing a tricyclic framework. Although alkaloid **2** shared the same molecular formula with myrionine[6], their NMR data was not in agreement, and the reported $^{13}$C NMR assignments of $\delta_C$ 57.9 for C-10 and $\delta_C$ 35.6 for C-9 in myrionine should be interchanged.

2D NMR (HSQC, $^1$H–$^1$H COSY, and HMBC experiments) data of **2** revealed that two spin coupling systems: *a*, $H_2$-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 *b*, $H_2$-13($H_2$-17)/$H_2$-14($H_2$-16)/$H_2$-15 could be figured out (Fig. 3A, Table 2). The HMBC correlations from $H_2$-3 ($\delta_H$ 2.36, m), $H_2$-4 ($\delta_H$ 2.04, m; $\delta_H$ 1.63, m) and H-10 ($\delta_H$ 3.20, dd 12.0, 4.2 Hz) to C-2 ($\delta_C$ 175.1) suggested that

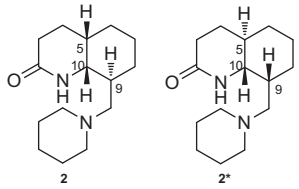

**Figure 4.** Two possible relative configuration types of **2**.

the carbonyl group was located at C-2. In addition, HMBC correlations from $H_2$-11 ($\delta_H$ 2.93, m; $\delta_H$ 2.71 dd, 12.0, 3.6 Hz) to C-8 ($\delta_C$ 29.6), C-9 ($\delta_C$ 37.0), C-10 ($\delta_C$ 57.5), and C-13/17 ($\delta_C$ 55.5) suggested the attachment of ring D to ring B through C-11. These findings revealed that the 2D structure of **2** resembled to myrionamide except for the opening of ring C.

The relative configuration of **2** was resolved by its ROESY data and the $^1H$–$^1H$ vicinal coupling constants. H-10 (dd 12.0, 4.2 Hz) indicated that $J_{H\text{-}10/H\text{-}5}$ = 4.2 Hz (*cis*) and $J_{H\text{-}10/H\text{-}9}$ = 12.0 Hz (*trans*), or $J_{H\text{-}10/H\text{-}5}$ = 12.0 Hz (*trans*) and $J_{H\text{-}10/H\text{-}9}$ = 4.2 Hz (*cis*), which suggested two possible relative configuration as **2** or **2**$^*$ as shown in Figure 4. Further analysis of its ROESY spectra showed that NOE correlations between H-10 and H-8b ($\delta_H$ 1.15, m), and H-8b and H-5 ($\delta_H$ 2.04, m) were observed, (Fig. 3B) which was indicative of the cofacial protons of H-10, H-8b, and H-5. Thus the relative configuration of **2** was elucidated as 5β, 9α, and 10β as shown in Figure 1.

12-oxomyriberine A[19] (**3**, 5 mg) was isolated as colorless gum. Its molecular formula $C_{18}H_{26}N_2O_2$ was established by its $^{13}C$ NMR and HREIMS (*m*/*z* 302.2000 $[M]^+$, calcd. for $C_{18}H_{26}N_2O_2$, 302.1994) data, indicating seven indices of hydrogen deficiency. The $^{13}C$ NMR and DEPT data (Table 1) revealed three $sp^2$ carbon atoms (1 × CH, 2 × qC) and 15 $sp^3$ carbon atoms (5 × CH and 10 × $CH_2$). The IR absorption bond at 1641 and 1548 $cm^{-1}$ along with the UV absorption at 282 nm suggested the α,β-unsaturated ketone moiety (qC, $\delta_C$ 201.8; CH, $\delta_C$ 105.4; and qC, $\delta_C$ 177.9). As two devices of hydrogen deficiency accounted by the α,β-unsaturated ketone moiety, the rest five ones predicted a pentacylic system in compound **3** (Fig. 1).

One feature of **3** is its α,β-unsaturated ketone moiety, which resembles to previously reported myriberine A[11] and myriberine B.[15] Detailed analysis of the HSQC and HMBC spectrum of **3** revealed that the NMR data of **3** showed similarity to myriberine A except for the low field moved carbon signals ($CH_2$-11, $\delta_C$ 63.1; CH-13, $\delta_C$ 85.9; and $CH_2$-17, $\delta_C$ 70.8). As the molecular formula of **3** showed one additional oxygen atom compared to myriberine A, the 2D structure of **3** was elucidated as the N-12 oxide of myriberine A (Fig. 1).

The relative configuration of **3** was resolved by the $^1H$–$^1H$ vicinal coupling constants and its ROESY data. The coupling constants of H-10 and H-20 was in good agreement with that of myriberine A which indicated their same relative configuration of H-3, H-5, H-9, and H-10. NOE correlation between H-13 and H-18 was observed to suggest the α-oriented H-13. Consequently, the relative configuration of **3** was elucidated as 3β, 5β, 9α, 10β, and 13α as in myriberine A.

Alkaloids **2** and **3** were evaluated against hepatitis C virus (HCV) in vitro using a previously reported method.[13,14] As a result, **2** and **3** showed weak inhibitory activity with selective index (SI) of higher than 36.2 and 15.2, respectively. (VX-950 as a positive control) The bioactivity of **1** has not yet been examined.

## Acknowledgments

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.07.039. These data include MOL files and InChiKeys of the most important compounds described in this article.

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17. Secomyrionamide (**1**): white powder; $[\alpha]_D^{24}$ −14 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 205 (3.30); $^1H$ and $^{13}C$ NMR data, see Tables 2 and 1; positive ESIMS *m*/*z* 265 $[M+H]^+$, 287 $[M+Na]^+$; positive HREIMS *m*/*z* 264.1842 $[M]^+$, calcd for $C_{15}H_{24}N_2O_2$, 264.1838.
18. Isomyrionine (**2**): colorless gum; $[\alpha]_D^{23}$ −27 (*c* 0.3, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 204 (3.06); $^1H$ and $^{13}C$ NMR data, see Tables 2 and 1; positive ESIMS *m*/*z* 251 $[M+H]^+$, 273 $[M+Na]^+$; positive HREIMS *m*/*z* 250.2045 $[M]^+$, calcd for $C_{15}H_{26}N_2O$, 250.2045.
19. Oxomyriberine A (**3**): colorless gum; $[\alpha]_D^{24}$ 144 (*c* 0.2, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 282 (3.55); IR (KBr) $\nu_{max}$ 3423, 2928, 1641, 1548, 1384, 1289, 1227, and 1163 $cm^{-1}$; ECD (0.0002 M, MeOH) $\lambda_{max}$ (Δε) 282 (+29), 264 (−10); $^1H$ and $^{13}C$ NMR data, see Tables 2 and 1; positive ESIMS *m*/*z* 303 $[M+H]^+$, 325 $[M+Na]^+$ positive HREIMS *m*/*z* 302.2000 $[M]^+$, calcd for $C_{18}H_{26}N_2O_2$, 302.1994.