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New findings of cyclohexane-fused octahydroquinolizine alkaloids from *Myrioneuron faberi*

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ABSTRACT

Cyclohexane-fused octahydroquinolizine (COHQ) alkaloids present an unwonted carbon framework among alkaloids isolated from genus *Myrioneuron*, which featured with bridge-ring and hemiacetal groups. Continued chemical investigation of *Myrioneuron faberi* led to the isolation of four COHQ related structures, β-myrifabral C (**1**), α-myrifabral C (**2**), β-myrifabral D (**3**), and α-myrifabral D (**4**). **1** and **2** were inseparable hemiacetal epimers (cluster A), as did **3** and **4** (cluster B). The structures of **1**–**4** were elucidated on basis of MS and NMR spectra. In vitro, cluster A showed moderate inhibition activity against hepatitis C virus (HCV) replication with therapeutic index (CC_{50}/EC_{50}) of 74.0.

Myrioneuron alkaloids are a class of fast growing natural products elaborated by plants of the genus *Myrioneuron*. The lysine-based origination of *Myrioneuron* alkaloids was suggested,[1–3] and the reactive C_5 units derived from lysine constructed intricate polycyclic ring systems (tri-, tetra-, penta-, hexa-, and decacyclic types).[4–10] A number of these structural distinctive alkaloids exhibited antimalarial,[2] antimicrobial,[7] and anti-HCV activities,[7–11] and attracted great organic synthesis interests.[1,2,12–15] Recent phytochemical investigation of *M. faberi*, *M. effusum*, and *M. tonkinensis* resulted in several interesting carbon frameworks,[7–11,16–18] which bring new insights into the biogenetical pathway and structural diversity of *Myrioneuron* alkaloids.

Cyclohexane-fused octahydroquinolizine (COHQ) alkaloids are a group of metabolites obtained from *M. faberi*.[8] Its unique octahydroquinolizine (OHQ) core was differed from other *Myrioneuron* alkaloids, and also featured with a bridge-ring and a hemiacetal groups. However, COHQ alkaloids show no exception with other typical *Myrioneuron* alkaloids, they all holding 'n × C_5′ carbon frameworks (C_{10}, C_{15}, C_{20}, and C_{35}).[3,7]

During our ongoing investigation of structurally unique and biologically interesting *Myrioneuron* alkaloids, two C_{20} COHQ structures (**1** and **2**) and two C_{15} ones (**3** and **4**) were obtained. **1** And **2** presented a new carbon skeleton bearing a '—$(CH_2)_5$—' straight chain attached to the previously reported C_{15} COHQ carbon framework (myrifabral A)[8] through carbon-carbon bond. **3** and **4** presented C-6 epimer of myrifabral A. These findings to some degree broadened our understanding of structural diversity of *Myrioneuron* alkaloids. In this work, we report the isolation, structure elucidation, bioactivity, and hypothesized biogenetical pathway of **1**–**4**.

Cluster A (**1** and **2**)[19] was obtained as colorless gum. The NMR spectrum of Cluster A exhibited a mixture of two compounds' resonance signals (S1.1–1.6 in Supporting Information), and the downfield '—CH—' signals ($\delta_{C\text{-}13}$ = 98.2 and $\delta_{C\text{-}12}$ = 80.7 in **1**; $\delta_{C\text{-}13}$ = 92.2 and $\delta_{C\text{-}12}$ = 72.6 in **2**) (Tables 1 and 2) suggested it to be a pair of hemiacetal epimers as myrifabral A.[8] Because **1** and **2** are isomers, their molecular formula $C_{20}H_{35}NO_3$ was revealed by HRESIMS data (*m*/*z* found 337.2635 for $[M]^+$; calcd. for $C_{20}H_{35}NO_3$, 337.2617). The molecular formula $C_{20}H_{35}NO_3$ indicated that **1** and **2** possessing four devices of hydrogen deficiency. The ^{13}C NMR, DEPT and HSQC spectra of the mixture of **1** and **2** (S1.2 and S1.4 in Supporting Information) revealed that there are 40 carbon signals comprising 2 × (14 × CH_2, 5 × CH, 1 × qC) carbon atoms (Fig. 1). In addition, the typical nitrogenated carbon atoms in COHQ alkaloids (C-2, C-6, and C-16, see Table 1), and highfield quaternary carbon atoms ($\delta_{C\text{-}11}$ = 32.1 in **1**; $\delta_{C\text{-}11}$ = 32.6 in **2**) were observed. The above featured NMR data and the four devices of hydrogen deficiency indicated **1** and **2** possessed the COHQ carbon framework.

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Table 1
^{13}C NMR data for **1**–**4** (δ in ppm).

No.	**1**[a]	**2**[a]	**3**[b]	**4**[b]
2	57.1 (CH_2)	57.1 (CH_2)	54.8 (CH_2)	54.7 (CH_2)
3	26.4 (CH_2)	26.4 (CH_2)	25.9 (CH_2)	26.1 (CH_2)
4	30.4 (CH_2)	30.4 (CH_2)	22.6 (CH_2)	22.5 (CH_2)
5	37.2 (CH)	37.3 (CH)	29.8 (CH_2)	29.9 (CH_2)
6	70.3 (CH)	70.8 (CH)	64.1 (CH)	64.5 (CH)
7	35.3 (CH)	35.0 (CH)	40.2 (CH)	40.0 (CH)
8	20.7 (CH_2)	20.5 (CH_2)	19.2 (CH_2)	19.4 (CH_2)
9	21.4 (CH_2)	21.5 (CH_2)	25.4 (CH_2)	25.4 (CH_2)
10	29.5 (CH_2)	28.6 (CH_2)	29.2 (CH_2)	28.4 (CH_2)
11	32.1(qC)	32.6 (qC)	31.9 (qC)	32.4 (qC)
12	80.7 (CH)	72.6 (CH)	74.3 (CH)	66.4 (CH)
13	98.2 (CH)	92.2 (CH)	98.2 (CH)	92.3 (CH)
14	30.6 (CH_2)	27.4 (CH_2)	30.7 (CH_2)	31.2 (CH_2)
15	34.2 (CH_2)	29.3 (CH_2)	35.9 (CH_2)	27.6 (CH_2)
16	69.1(CH_2)	69.6 (CH_2)	62.2 (CH_2)	62.9 (CH_2)
17	32.0 (CH_2)	32.0 (CH_2)		
18	26.6 (CH_2)	26.6 (CH_2)		
19	27.2 (CH_2)	27.2 (CH_2)		
20	34.2 (CH_2)	34.2 (CH_2)		
21	62.4 (CH_2)	62.4 (CH_2)		

[a] Recorded at 294 K.
[b] Recorded at 300 K.

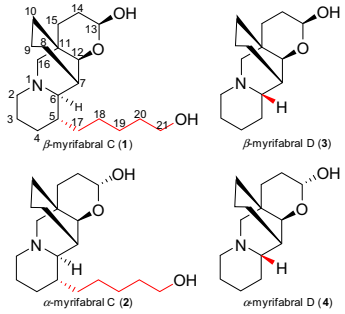


Fig. 1. Structures of **1**–**4**.

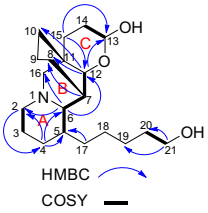


Fig. 2. ^{1}H–^{1}H COSY and Key HMBC correlations of **1**.

The 2D structure of **1** was confirmed by 2D NMR (^{1}H–^{1}H COSY, HSQC, and HMBC) spectrums. Related to the downfield NMR signals, four ^{1}H–^{1}H spin fragments were figured out: H_2-2/H_2-3, H-5/H-6/H-7/H-12, H-13/H_2-14, and H_2-21/H_2-20 (Fig. 2). The location of CH_2-4 was elucidated by HMBC correlations from H_2-2 (δ_H 2.70, m; 1.67, m) to C-4 (δ_C 30.4), and H_b-4 (δ_H 0.84, m) to C-5 (δ_C 37.2) and C-6 (δ_C 70.3) (Fig. 2). Then the linkage of rings A and B through C-6 and N-1 was suggested by HMBC correlations from H-6 (δ_H 1.74, m) to C-2 (δ_C 57.1), C-12 (δ_C 80.7), and C-16 (δ_C 69.1). The existence of ring C and hemiacetal group was revealed by HMBC correlations from H-12 (δ_H 3.20, d, 3.0 Hz) to C-13 (δ_C 98.2), H_2-15 (δ_H 1.25, m) to C-12 and C-13. Finally, the bridge ring over CH-7 and qC-11 can be elucidated by HMBC correlations from H-7 (δ_H 2.16, br s) to C-8 (δ_C 20.7), as well as H-12 to C-8 and C-10 (δ_C 29.5). Thus a C_{20} planar fragment harboring COHQ moiety of **1** was elucidated as in myrifabral A, whose structure was confirmed by single crystal X-ray analysis.[8]

The rest five carbon atoms in structure of **1** all showed '—CH_2—' signals in DEPT135 spectrum indicating that this C_5 moiety to be a

Table 2
^{1}H NMR data for **1**–**4** Recorded in prydine-d_5 at 600 MHz, assigned based on HSQC.

No.	**1**[a]	**2**[a]	**3**[b]	**4**[b]
2	2.70 (m)	2.70 (m)	2.79 (m)	2.79 (m)
	1.67 (m)	1.67 (m)	2.41 (m)	2.41 (m)
3	1.53 (m)	1.53 (m)	1.66 (m)	1.66 (m)
	1.26 (m)	1.26 (m)	1.29 (m)	1.29 (m)
4	1.76 (m)	1.76 (m)	1.61 (m)	1.58 (m)
	0.84 (m)	0.84 (m)	1.23 (m)	1.23 (m)
5	1.53 (m)	1.53 (m)	1.60 (m)	1.60 (m)
			1.23 (m)	1.23 (m)
6	1.74 (m)	1.79 (m)	2.50 (m)	2.53 (m)
7	2.16 (br s)	2.06 (br s)	1.84 (m)	1.71 (m)
8	2.04 (m)	1.99 (m)	2.29 (m)	2.29 (m)
	1.62 (m)	1.62 (m)	1.50 (m)	1.50 (m)
9	2.73 (m)	2.78 (m)	2.10 (m)	2.10 (m)
	1.48 (m)	1.54 (m)	1.35 (m)	1.35 (m)
10	2.24 (m)	2.28 (m)	2.24 (m)	2.24 (m)
	1.21 (m)	1.21 (m)	0.99 (m)	1.03 (dd, 12.6, 5.4 Hz)
12	3.20 (d, 3.0 Hz)	4.20 (d, 2.4 Hz)	3.88 (m)	4.73 (d, 4.2 Hz)
13	5.14 (m)	5.75 (d, 2.4 Hz)	5.20 (dd, 9.6, 2.4 Hz)	5.70 (d, 3.0 Hz)
14	2.02 (m)	2.09 (m)	1.99 (m)	2.08 (m)
	1.88 (m)	1.78 (m)	1.87 (ddt, 13.2, 4.8, 2.4 Hz)	1.22 (m)
15	1.25 (m)	1.88 (m)	1.56 (m)	2.07 (m)
		1.10 (dd, 12.6, 4.2 Hz)	1.40 (m)	1.78 (m)
16	2.59 (m)	2.59 (m)	2.66 (d, 11.4 Hz)	2.74 (d, 11.4 Hz)
	1.80 (m)	1.98 (m)	2.38 (d, 11.4 Hz)	2.38 (d, 11.4 Hz)
17	1.41 (m)	1.41 (m)		
	1.00 (m)	1.00 (m)		
18	1.41 (m)	1.41 (m)		
19	1.78 (m)	1.78 (m)		
20	1.77 (m)	1.77 (m)		
21	3.90 (dd, 14.4, 6.8 Hz)	3.90 (dd, 14.4, 6.8 Hz)		

[a] Recorded at 300 K.
[b] Recorded at 313 K.

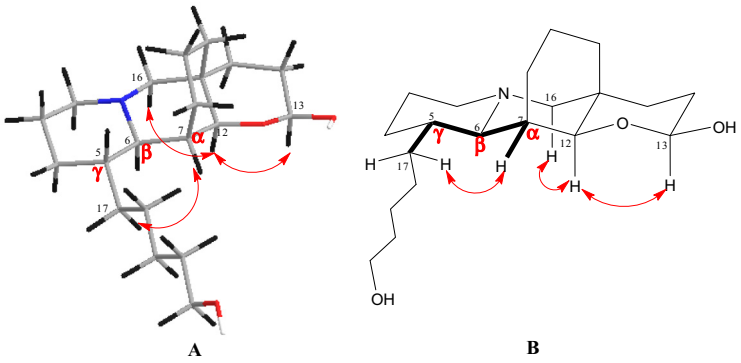


Figure 3. Key ROESY correlations of **1**. (A) showed in 3D sticks mode; (B) showed in Haworth mode (Bolded bonds marked the γ-gauche effect structure).

'—$(CH_2)_5$—'. One of these methylenes (CH_2-21, δ_C 62.4; δ_H 3.90, dd, 14.4, 6.8 Hz) was oxygenated, and that '—$(CH_2)_5$—' was recognized as a *n*-pentanol group as in schoberine B.[10] Compared with C-5 in myrifabral A ('—CH_2—', δ_C 30.6), C-5 in **1** ('—CH—', δ_C 37.2) indicated that *n*-pentanol group was directly bonded to C-5, which can be supported by HMBC correlations from H-17b (δ_H 1.00, m) to C-5. As a result, the 2D structure of **1** was established as shown in Fig. 2.

The relative configuration of **1** was resolved by its ROESY data and substituent effects. The NOE correlations of H-16a (δ_H 2.59, m)/H-12/H-13 (δ_H 5.14, m) revealed these protons are co-facial (deemed as α-oriented), which required the bridge ring to be β-oriented (Fig. 3). According to the β-oriented bridge ring, the orientation of H-7 can be elucidated as α. The fluctuations of NMR data of C-3, C-4, C-5, C-6, and C-7 compared with that of myrifabral A were probably resulted from the substituent on C-5. Among them, $\delta_{C\text{-}7}$ in **1** was 5.1 ppm to lower frequency compared to that of $\delta_{C\text{-}7}$ in myrifabral A, which suggesting the γ-gauche effect[21] caused by co-facial H-7 and *n*-pentanol group (Fig. 3B) and indicating the α-oriented *n*-pentanol group. The 5α configuration can also be supported by NOE correlation between H-7 and H-17b (δ_H 1.00, m). The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of other positions on COHQ core matched well with that of myrifabral A suggesting that rings A and B on **1** possessed the same *trans* relationship as in myrifabral A, required the α-oriented H-6. Thus the relative configuration of **1** was elucidated as 5α, 6α, 7α, 12α, and 13α.

Accordingly, the structure of **2** was elucidated as the 13-epimer of **1** (Fig. 1). The NMR data of **1** and **2** can be distinguished by ROESY correlation between H-12 and H-13 (detected in **1**, negative in **2**) (S1.6 in Supporting Information). The population ratio of **1**:**2** as 1:0.66 in pyridine-d_5 was calculated by ^{1}H NMR spectrum integrals.

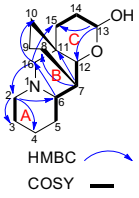


Figure 4. ^{1}H–^{1}H COSY and Key HMBC correlations of **3**.

Cluster B (**3** and **4**)[20] was obtained as amorphous powder. Their molecular formula was established as $C_{15}H_{25}NO_2$ (identical to myrifabral A) by HRESIMS spectrum (*m*/*z* 252.1957 $[M+H]^+$, calcd. for $C_{15}H_{26}NO_2$, 252.1958), corresponding to four devices of hydrogen deficiency. The ^{13}C and DEPT NMR spectra of **3** and **4** presented 30 resonance signals comprising 2 × (10 × CH_2, 4 × CH, and 1 × qC) carbon atoms (Fig. 1). In which the downfield '—CH—' signals ($\delta_{C\text{-}13}$ = 98.2 in **3**; $\delta_{C\text{-}13}$ = 92.3 in **4**) (Table 1) matched the double-oxygenated methenyl in hemiacetal epimers. The above mentioned data highly resembled to that of myrifabral A despite their NMR data differs at some positions, (Tables 1 and 2) which suggests **3** and **4** to be isomers of myrifabral A.

To unveil the 2D structure of **3**, its 2D NMR (^{1}H–^{1}H COSY, HSQC, and HMBC) spectrums were analyzed. Similar to **1**, four ^{1}H–^{1}H spin fragments: H_2-2/H_2-3, H-5/H-6/H-7/H-12, H-13/H_2-14, and H_2-21/H_2-20 can be figured out (Fig. 4). HMBC correlations from H-2 (δ_H 2.79, m; 2.41, m) to C-6 (δ_C 64.1), H-6 (δ_H 2.50, m) to C-16 (δ_C 62.2), and H-16a (δ_H 2.66, d, 11.4 Hz) to C-2 (δ_C 54.8) suggests rings A and B were linked by N-1 and C-6 (Fig. 4). Existence of ring C can be supported by HMBC correlations from H-16a to C-15 (δ_C 35.9), H-7 (δ_H 1.84, m) to C-11 (δ_C 31.9), and H-13 (δ_H 5.20, dd, 9.6, 2.4 Hz) to C-12 (δ_C 74.3) and C-15 (δ_C 35.9). The bridge ring over CH-7 and qC-11 was supported by HMBC correlations from H-16a to C-10 (δ_C 29.2), and H-7 to C-8 (δ_C 19.2). Consequently, the 2D structure of **3** was elucidated as the same as myrifabral A (Fig. 4).

The relative configuration of **3** was elucidated by its ROESY spectrum and substituent effects. The NOE correlations of H-16a/H-12 (δ_H 3.88, m)/H-13 were detected as in the case of **1**, which indicating β-oriented bridge ring and α-oriented H-7, H-12, and H-13 (Fig. 5). The ^{13}C NMR data of **3** (Table 1) mainly differed from myrifabral A at C-2 ($\Delta\delta_C$ −2.4), C-4 ($\Delta\delta_C$ −4.2), C-6 ($\Delta\delta_C$ −2.3), C-9 ($\Delta\delta_C$ +4.1), and C-12 ($\Delta\delta_C$ −6.3), and we deduced

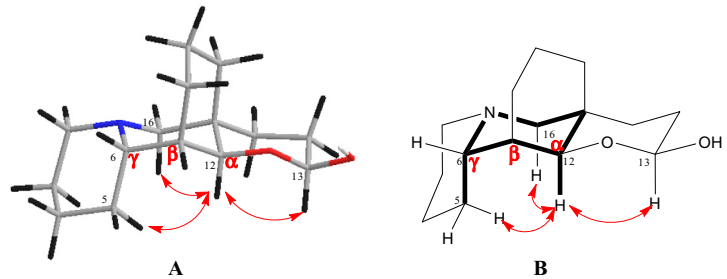
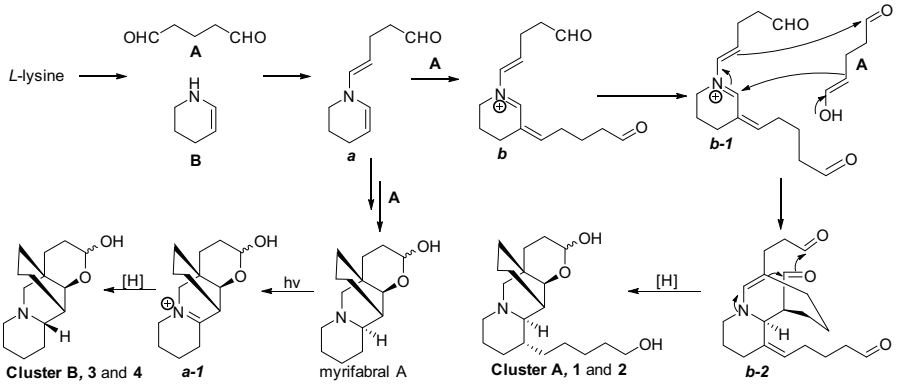


Figure 5. Key ROESY correlations of **3**. (A) showed in 3D sticks mode; (B) showed in Haworth mode (Bolded bonds marked the γ-gauche effect structure).



Scheme 1. Hypothesized biogenetical pathway of **1**–**4**.

that it was resulted from their opposite H-6 orientation. The β-oriented H-6 could be supported by: 1) the lower frequency shifted $\delta_{C\text{-}12}$ ($\Delta\delta_C$ −6.3) caused by γ-gauche effect[21] between axial bond of C_6-C_5 and H-12 of **3** (Fig. 4B); 2) the NOE correlation of H-5a (δ_H 1.60, m)/H-12 (δ_H 4.73, d, 4.2 Hz) of **4**. As a result, the relative configuration of **3** was elucidated as 5α, 6β, 7α, 12α, and 13α. By the same manner, the structure of **4** was elucidated as the 13-epimer of **3** (Fig. 1). The ROESY spectra of **3** and **4** showed difference at the NOE correlation spot between H-12 and H-13 (detected in **3**, negative in **4**) (S2.6 in Supporting Information).

Previously reported β-myrifabral A and α-myrifabral A were proved to be two pairs of enantiomers by chiral column analysis on HPLC after chemical modification.[8] Cluster A (**1** and **2**) and cluster B (**3** and **4**) were directly subjected to four different types of chiral column analysis on HPLC, but there was no evidence supported they are enantiomers. Alkaloids **1** and **2** were evaluated against hepatitis C virus (HCV) in vitro as previously reported method,[8,9] and showed moderate inhibition activity against hepatitis C virus (HCV) replication with therapeutic index (CC_{50}/EC_{50}) of 74.0 (VX-950 as a positive control).

A hypothesized biogenetical pathway of **1**–**4** starting from *L*-lysine similar to myrifabrals A and B was suggested (Scheme 1). Reactive C_5 units **A** and **B** could be derived from L-lysine.[4] Condensation of **A** and **B** then yield intermediate ***a***, which could incorporate unit A to afford myrifabral A as previously suggested pathway. Then an imine intermediate ***a*-*1*** could be resulted from ultraviolet radiation,[22] and further reduced to cluster B. On pathway to cluster A, the C-15 intermediate ***b*-*1*** could be achieved from ***a***, and condensed with one unit **A** to establish ***b*-*2***. Consequently, cluster A could be achieved from ***b*-*2*** through condensation and reduction procedures.[8]

Acknowledgment

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A. Supplementary data

Supplementary data (1D and 2D NMR, ESIMS, and HRESIMS spectra for **1**–**4**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.11.001. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

1. Pham VC, Jossang A, Chiaroni A, Sévenet T, Bodo B. *Tetrahedron Lett.* 2002;43:7565–7568.
2. Pham VC, Jossang A, Grellier P, et al. *J Org Chem.* 2008;73:7565–7573.
3. Gravel E, Poupon E. *Nat Prod Rep.* 2010;27:1630–1680.
4. Erwan P, Edmond G. *Chem Eur J.* 2015;21:10604–10615.
5. Pham VC, Jossang A, Sévenet T, Nguyen VH, Bodo B. *Eur J Org Chem.* 2009;74:1412–1416.
6. Pham VC, Jossang A, Sévenet T, Nguyen VH, Bodo B. *Tetrahedron.* 2007;63:11244–11249.
7. Cao MM, Huang SD, Di YT, et al. *Org Lett.* 2014;16:528–531.
8. Cao MM, Zhang Y, Li XH, et al. *J Org Chem.* 2014;79:7945–7950.
9. Cao MM, Zhang Y, Huang SD, et al. *J Nat Prod.* 2015;78:2609–2616.
10. Cao MM, Zhang Y, Peng ZG, Jiang JD, Gao YJ, Hao XJ. *RSC Adv.* 2016;6:10180–10184.
11. Huang SD, Zhang Y, Cao MM, et al. *Org Lett.* 2013;15:590–593.
12. Pham VC, Jossang A, Chiaroni A, Sévenet T, Nguyen VH, Bodo B. *Org Lett.* 2007;9:3531–3534.
13. Nocket AJ, Weinreb SM. *Angew Chem Int Ed.* 2014;53:14162–14165.
14. Nocket AJ, Feng YQ, Weinreb SM. *J Org Chem.* 2015;80:1116–1129.
15. Song DP, Wang ZS, Mei RM, et al. *Org Lett.* 2016;18:669–671.
16. Cao MM, Zhang Y, Huang SD, Peng ZG, Jiang JD, Hao XJ. *Tetrahedron Lett.* 2016;57:4021–4023.
17. Li XH, Zhang Y, Zhang JH, et al. *J Nat Prod.* 2016;79:1203–1207.
18. Zhang JH, Guo JJ, Yuan YX, et al. *Fitoterapia.* 2016;112:217–221.
19. Colorless gum; $[\alpha]_D^{23}$ −27 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 204 (2.81); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m*/*z* 338, $[M+H]^+$; positive HRESIMS *m*/*z* found 337.2635 for $[M]^+$; calcd. for $C_{20}H_{35}NO_3$, 337.2617 $[M]^+$.
20. Amorphous powder; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m*/*z* 252 $[M+H]^+$; positive HRESIMS *m*/*z* 252.1957 $[M+H]^+$, calcd. for $C_{15}H_{26}NO_2$, 252.1958 $[M+H]^+$.
21. Barfield M, Yamamura SH. *J Am Chem Soc.* 1990;112:4747–4758.
22. Ahmed SA, Khairou KS, Asghar BH, Muathen HA, Nahas NMA, Alshareef HF. *Tetrahedron Lett.* 2014;55:2190–2196.