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Myrifabine, the First Dimeric *Myrioneuron* Alkaloid from *Myrioneuron faberi*

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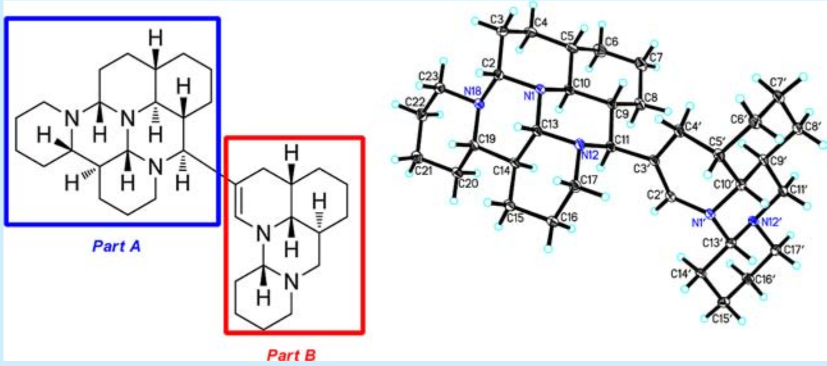
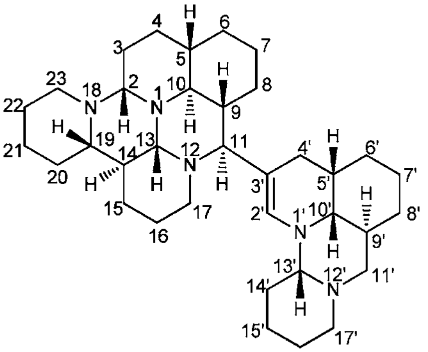
S *Supporting Information*



ABSTRACT: One *Myrioneuron* alkaloid, myrifabine (**1**), the first example of a dimer with 12 chiral centers embraced in a decacyclic novel skeleton, was isolated from *Myrioneuron faberi*. Its structure was elucidated by spectroscopic data and single-crystal X-ray diffraction. The antimicrobial and cytotoxic activities of **1** were evaluated in vitro.

Myrioneuron alkaloids are a newly discovered family of lysine-based structurally diverse natural products[1] with various polycyclic structures (tricyclic-, tetracyclic-, pentacyclic-, and hexacyclic-type) elaborated by plants of the genus *Myrioneuron* R. Br. (Rubiaceae).[2] Their polycyclic skeletons have attracted great interest as challenging targets for total synthesis,[2a−c] and their bioactivities as significant antimalarial, inhibition on KB cell proliferation, and anti-HCV were reported previously.[2c−f] Herein, we report **1**, the most complicated structure of *Myrioneuron* alkaloid so far, as well as its biological activity and hypothesized biogenetic pathway.

Myrifabine (**1**), its molecular formula $C_{35}H_{55}N_5$, was established by HREIMS (m/z 545.4472 $[M]^+$, calcd. for $C_{35}H_{55}N_5$, 545.4457) indicating 11 degrees of unsaturation. The ^{13}C NMR and DEPT spectra of **1**, displayed 35 carbon signals classified as two sp^2 carbon atoms and 33 sp^3 carbon atoms (12 × CH and 21 × CH$_2$). The two sp^2 carbon atoms (CH, δ_C 127.6 and qC, δ_C 105.3) suggested the existence of one double bond, and the methine (CH, δ_C 127.6) should be closer to an electron-donating group because of its desheilding effect. As there are no oxygen atoms in the structure, three downfield sp^3 methines (δ_C 78.4, 79.3, and 80.2) were deduced as typical dinitrogenated methine as in the cace of other *Myrioneuron* alkaloids.[2] Since one double bond accounted for 1 out of 11 degrees of unsaturaion, the remaining 10 degrees of unsaturation were assumed for the presence of a decacyclic system as shown (Figure 1).



myrifabine (**1**)

Figure 1. Structure of myrifabine (**1**).

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Table 1. ^{1}H and ^{13}C NMR Data for 1 Recorded in Prydine-d_5 at 400 and 100 MHz, Respectively

no.	δ_C	$\delta_{H\text{-}a}$ (mult, J)	$\delta_{H\text{-}b}$ (mult, J)	no.	δ_C	$\delta_{H\text{-}a}$ (mult, J)	$\delta_{H\text{-}b}$ (mult, J)
2	78.4	3.18 (m)		2′	127.6	6.39 (s)	
3	28.0	2.08 (m)	1.66 (m)	3′	105.3		
4	26.0	1.30 (m)	1.23 (m)[a]	4′	20.8	2.11(d, 8.8)	
5	42.7	1.35 (m)		5′	31.0	2.26 (m)	
6	32.5	1.46 (m)[a]	1.04 (m)	6′	30.3	1.57 (m)	1.57 (m)
7	25.4	1.45 (m)[a]	1.39 (m)	7′	19.7	1.46 (m)[a]	1.29 (m)
8	29.2	1.87 (m)[a]	1.00 (m)	8′	30.0	1.36 (m)	0.86 (m)
9	42.2	1.68 (m)[a]		9′	31.5	1.88 (m)	
10	59.0	2.88 (m)		10′	63.1	2.62 (m)[a]	
11	62.8	3.14 (d, 10.0)		11′	63.2	2.70 (dd, 11.2, 3.6)	1.62 (m)
13	79.3	3.69 (d,10.4)		13′	80.2	2.92 (m)	
14	30.2	2.20 (m)		14′	24.7	1.75 (m)	1.23 (m)[a]
15	18.9	1.65 (m)[a]	1.03 (m)	15′	29.0	1.85 (m)[a]	
16	28.0	1.92 (m)	0.88 (m)	16′	25.9	1.47 (m)[a]	1.24 (m)
17	49.2	3.27 (d, 14.0)	2.61 (m)	17′	55.6	2.62 (m)[a]	1.80 (m)
19	67.6	1.53 (m)[a]		22	24.5	1.65 (m)[a]	1.09 (m)
20	26.2	1.53 (m)[a]		23	49.5	2.88 (m)[a]	1.54 (m)
21	29.1	1.68 (m)[a]	1.21 (m)				

[a] Overlapped.

In the HSQC, ^{1}H–^{1}H COSY, and HMBC spectra of **1** (Table 1), easily recognized CH-2′ (δ_C 127.6, δ_H 6.39, s) was set as a starting point. Key HMBC correlations of H-2′ (δ_H 6.39, s) to C-10′ (δ_C 63.1) and C-13′ (δ_C 80.2), H-13′ (δ_H 2.92, m) to C-10′, C-11′ (δ_C 63.2), and C-17′ (δ_C 55.6) showed similar connection manner as in the case of myriberine A.[2f] The two ^{1}H–^{1}H COSY spin subunits ***a*** and ***b*** were recognized, and these two subunits being linked through N-1′ and N-12′ was indicated by HMBC correlations from H-17b′ (δ_H 1.80, m) to C-11′, and H-11a′ (δ_H 2.70, dd, 11.2, 3.6) to C-13′. Further comprehensive analysis of 2D NMR indicated one fragment of **1** has the same planar structure as dehydroschoberine,[3] which was artificially deemed as *Part B*. (Figure 2).

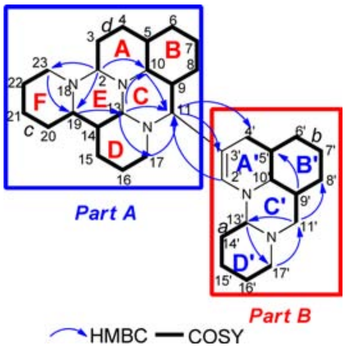

Figure 2. ^{1}H–^{1}H COSY and key HMBC correlations of **1**.

Besides *Part B*, the remaining six rings and eight chiral centers were assigned in *Part A*. Some featured ^{13}C NMR signals of *Myrioneuron* alkaloids: dinitrogened C-2 (δ_C 78.4) and C-13 (δ_C 79.3) could be recognized in *Part A*. One vague face of *Part A* containing one tetracyclic framework could be further suggested by HMBC correlations from H-13 (δ_H 3.69, d, 10.4) to C-10 (δ_C 59.0), C-17 (δ_C 49.2), and C-11 (δ_C 62.8); H-10 (δ_H 2.88, m) to C-11; H-17 (δ_H 3.27, d, 14.0) to C-11 as in other tetracyclic[2c] and pentacyclic[2f] *Myrioneuron* alkaloids. The remaining C_5N fragment (ring F) was inferred as a common piperidine ring as in other lysine-based alkaloids,[1] and its attachment to that tetracyclic framework was indicated by the HMBC correlations of H-2 (δ_H 3.18, m) to C-19 (δ_C 67.6) and C-23 (δ_C 49.5); H-23b (δ_H 1.54, m) to C-19 and H-19 (δ_H 1.53, m) to C-13 as well as the presence of spin coupling system of ***c***. The hexacyclic ring system in *Part A* was thus tentatively assigned as shown in Figure 2. In addition, the connection between *Parts A* and *B* through C_{11}–$C_{3'}$ single bond could be supported by HMBC correlations from H-2′ to C-11, H-11 (δ_H 3.14, d, 10.0) to C-3′ (δ_C 105.3), C-2′ (δ_C 127.6), and C-4′ (δ_C 20.8) (Figure 2). However, after reviewing the corresponding references as well as the insufficiency of overlapping proton signals, we have not found any structure or fragment showing similar NMR signals to *Part A*.

In order to unveil *Part A*, compound **1** was carefully recrystallized from acetone. Fortunately, single crystals suitable for X-ray diffraction experiment were obtained, and the data analysis showed it to be orthorhombic crystals with space group of *P*212121.[4] As a result, *Part A* and the full view of **1** as a heterodimer was finally unfolded as shown in Figure 3. The absolute configuration of **1** was analyzed by using Hooft methods,[5] and the results indicated that its absolute structure had been correctly assigned. The probability that the structure of **1** is inverted is 7.1 × 10^{-9} was also caculated by using Hooft methods.

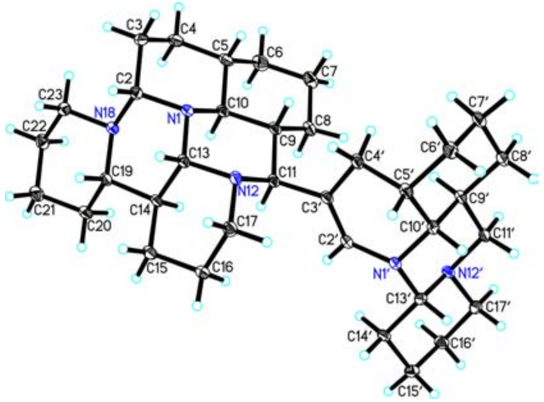

Figure 3. X-ray crystal structure of **1**.

It is noteworthy that **1** features an unusual *trans*-decahydroquinoline (*trans*-DHQ) motif in *Part A* compared to the *cis*-decahydroquinoline (*cis*-DHQ) motif in most reported *Myrioneuron* alkaloids, as well as up to 12 chiral centers on a decacyclic novel skeleton.

A hypothesized biosynthetic pathway of **1** starting from L-lysine[2c,6] was suggested as shown in Scheme 1. Basic C_5 building bricks could be supplied by L-lysine through decarboxylation, oxidation, and cyclization.[7] Condensation of **2b** and **2d** followed by reduction and cyclization could give two key intermediates **3a** and **3b**, respectively. **4a** could be built up with **3a** and **2b** and then assembled with **2a** by similar reaction reported to establish *Part A*.[6] On the other branch, **4b** could be formed by condensation reaction between **3b** and **2c**. *Part B* could be afforded after a series of double-bond migration, nucleophilic attack, and oxidation reactions. Finally, alkaloid **1** could be built up with parts A and B through intermolecular nucleophilic attack and deprotonation.

The antimicrobial and cytotoxicities of myrifabine (**1**) were evaluated in vitro by double-dilution[8] and MTT methods,[9] respectively. As shown in Tables 2 and 3, **1** showed moderate

Scheme 1. Hypothetical Biosynthetic Pathway of 1

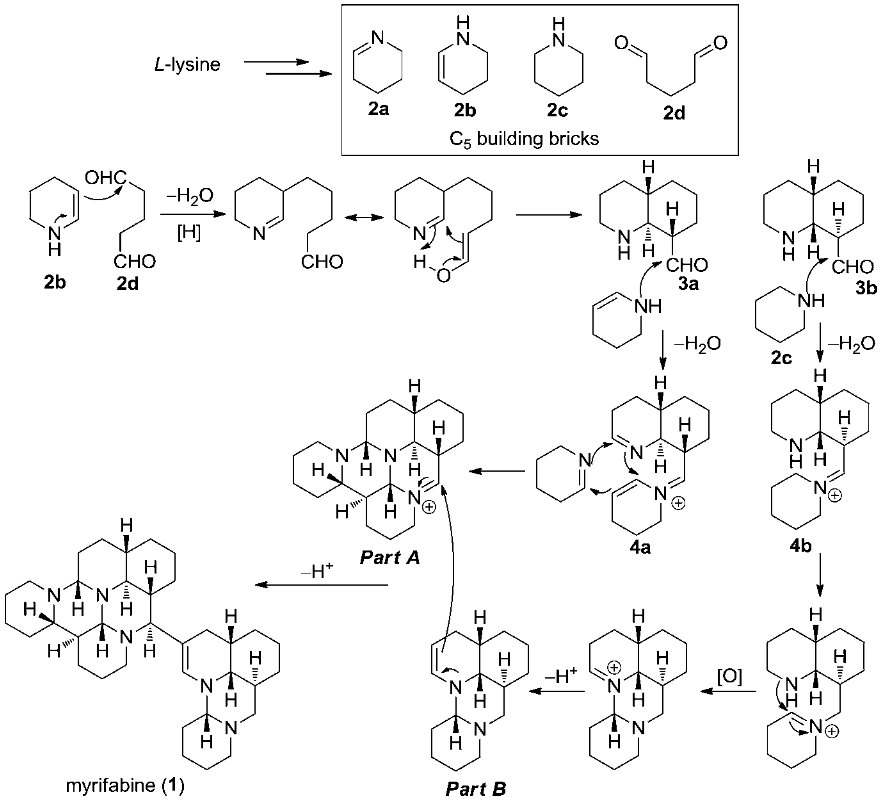

Table 2. Minimum Inhibitory Concentration (μg/mL) of 1 in Vitro (Vancomycin Hydrochloride As Positive Control)

	ATCC25913	MRSA092	MRSA098
1	25.07	12.54	6.32
vancomycin hydrochloride	3.12	1.56	1.56

Table 3. Cytotoxicity of 1 (IC_{50} in μM) in Vitro (Cisplatin As Positive Control)

	HL60	SMMC7721	A549	MCF7	SW480
1	19.1	21.2	19.0	16.4	>40
cisplatin	3.1	10.2	9.1	17.5	12.0

activities against *Staphylococcus aureus* (ATCC25913) and methicillin-resistant *Staphylococcus aureus* (MRSA082 and MRSA098), with MIC values ranging from 6.32 to 25.07 μg/mL, while **1** showed weak cytotoxicities against five human tumor cell lines with IC_{50} values in the range 16.4−21.2 μM.

■ ASSOCIATED CONTENT

Supporting Information

MS, HREIMS, IR, UV, ECD, NMR spectra, isolation procedures, bioactivity assay, and the X-ray crystallographic data (CIF) of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(4) Myrifabine (**1**): colorless crystals (in acetone); mp 161−163 °C; HREIMS at *m/z* 545.4472 $[M]^+$ (calcd. 545.4457, $C_{35}H_{55}N_5$); $[\alpha]_D^{20}$ −57.0° (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 233 (2.98) nm, CD (0.0012 M, MeOH) λ_{max} ($\Delta\varepsilon$) 223 (−6.8); IR ν_{max} (KBr) cm^{-1} 3441, 2925, 2851, 1631; ^1H and ^{13}C NMR data, see Table 1. Crystal data

for **1**: $C_{35}H_{55}N_5$, M = 545.84, orthorhombic, a = 9.2966(5) Å, b = 12.0684(7) Å, c = 27.4671(16) Å, $\alpha = 90.00°$, $\beta = 90.00°$, $\gamma = 90.00°$, V = 3081.7(3) Å^3, T = 100(2) K, space group P212121, Z = 4, μ(Cu Kα) = 0.525 mm^{-1}. The final R_1 values were 0.0420 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1127 ($I > 2\sigma(I)$). The final R_1 values were 0.0434 (all data). The final $wR(F^2)$ values were 0.1138 (all data). The goodness of fit on F^2 was 1.060. The Hooft parameter is 0.06(15) for 2240 Bijvoet pairs. Bijvoet Coverage = 91. P2(true) = 1.00. P3(true) = 0.983. P3(rac-twin) = 0.017. P3(false) = 0.7 × 10^{-8}. Correlation coefficient = 0.998. Deposited to Cambridge Crystallographic Data Center with No. CCDC 968886.

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