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Longeracemine, an unprecedented C-7/C-9 bonding alkaloid from *Daphniphyllum longeracemosum*†

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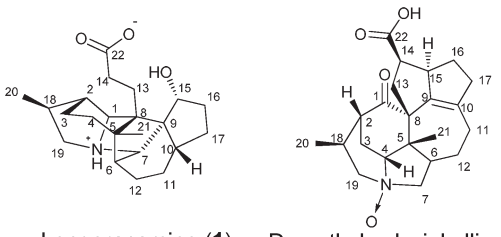
Qiang Zhang,[ab] Yu Zhang,[a] Ting-Quan Yang,[a] Ying-Tong Di*[a] and Xiao-Jiang Hao*[a]

A phytochemical investigation of the fruits of *Daphniphyllum longeracemosum* K. Rosenthal led to the isolation of a novel fused-hexacyclic alkaloid, longeracemine (1), along with a new one, demethyl calyciphylline A (2). Their structures were established on the basis of comprehensive spectra associated with quantum chemical analysis. Bioassay firstly indicated that *Daphniphyllum* alkaloid 1 can stimulate wheat shoot elongation.

Daphniphyllum alkaloids are a family of natural products with complex and diverse structures produced by *Daphniphyllum* trees.[1] As early as 1973, Suzuki and Yamamura traced the origin of these alkaloids to squalene.[2,3] Twenty years later, the fortuitous discovery of an efficient cascade reaction from dihydrosqualene dialdehyde to *proto*-daphniphylline elucidated the biogenetic mechanism for these alkaloids.[4] The particularly concise biogenesis has attracted widespread interest in the areas of phytochemistry[1] and total synthesis[5–7] in recent years. *D. longeracemosum* was distinguished by its production of diverse alkaloid skeletons.[8–14] To search for structurally unique and biogenetically interesting alkaloids from *D. longeracemosum*, its alkaloid components were investigated thoroughly. As a result, a novel alkaloid, longeracemine (**1**), along with a new one, demethyl calyciphylline A (**2**), was isolated from the fruits of the title plant. To the best of our knowledge, **1** is the first C-7/C-9 bonding alkaloid found in Daphniphyllaceae family. In this paper, we describe the isolation, structure elucidation, and bioassay results of **1** and **2**.

The air-dried fruits (60 kg) of *D. longeracemosum* were extracted with 95% EtOH, and the crude extract was pretreated with general acid–alkali treatments to enrich the alkaloids.[11,12] The extract was then exhaustively extracted with chloroform and *n*-BuOH. The *n*-BuOH fraction was separated on silica gel column eluted with acetone, methanol, and methanol/diethylamine (20 : 1). The third fraction was further separated over ion-exchange resin and C_{18} silica gel to afford **1** (36 mg) and **2** (19 mg).



Longeracemine (**1**) Demethyl calyciphylline A (**2**)

Longeracemine (**1**) was obtained as white amorphous powder.[15] Its molecular formula ($C_{22}H_{33}NO_3$) was established by HR-ESI-MS, requiring seven degrees of unsaturation. IR absorption band at 1574 cm^{-1}, associated with δ_C 181.6 ppm, suggested the presence of a carboxyl acid salt (COO^-). The ^{13}C NMR and DEPT (Table 1) spectra revealed 22 carbon signals due to two methyls, nine methylenes, seven methines, and four quaternary carbons. Apart from one degree of unsaturation due to the carboxyl group, the rest indicated that **1** possesses a hexacyclic system.

Extensive analysis of the 2D NMR spectra (HSQC, ^1H-^1H COSY, TOCSY, and HMBC) revealed the framework of **1**. All protons directly bonded to the carbon atoms were assigned on the base of the HSQC data. Three fragments (a, b, and c in Fig. 1) were deduced from COSY and TOCSY interaction pairs. Further detailed HMBC analysis established the connections of the fragments, the quaternary carbons, the nitrogen atom and the isolated methyl group. The HMBC correlations of H-19/C-1, H-19/C-7, and H-7/C-1 indicated that C-1, C-7, and C-19 are linked to the nitrogen atom, while those of H_3-21 to C-4, 5, 6, and 8 disclosed that Me-21 and C-4, 6 and 8 were connected to the quaternary carbon C-5. In HMBC spectrum, the cross-peaks of H-6, 10, 16 with C-9, and those of H-10 and H-15 with C-8 introduced the linkages of C-7, 8, 10, 15 to the spiral carbon C-9.[16] The HMBC correlation of H-1/C-8 indicated the connection between C-1 and C-8. Furthermore, fragment **c** (C-13 to C-14) was extended from C-8 and the carboxyl group from C-14 by the HMBC correlations between H-1/C-13 and

[a]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, PRC. E-mail: haoxj@mail.kib.ac.cn; diyt@mail.kib.ac.cn; Fax: (86) 871-6522-3070; Tel: (86) 871-6522-3263*

[b]*Research Centre for Natural Medicinal Chemistry, College of Science, Northwest A&F University, Yangling 712100, Shanxi, China*

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Table 1 NMR data for **1** and **2**[a]

	1		**2**[b]	
	δ_H (mult. Hz)	δ_C	δ_H (mult. Hz)	δ_C
1	3.96 (d, 5.7)	73.4		215.1
2	2.23 (m)	38.4	2.54 (m)	43.6
3	1.54 (m)	17.9	2.52 (m)	19.3
	1.30 (m)		1.97 (m)	
4	1.54 (m)	36.7	4.21 (brs)	87.3
	1.42 (m)			
5		42.2		62.6
6	2.29 (d, 5.9)	39.2	2.84 (m)	49.5
7	3.51 (s)	67.4	3.83 (dd, 13.5, 7.6)	72.1
			3.31 (m)	
8		53.5		52.5
9		72.3		140.8
10	2.45 (m)	40.3		140.6
11a	1.84 (m)	26.0	2.16 (m)	27.0
11b	1.22 (m)		2.16 (m)	
12a	1.84 (m)	24.9	1.90 (m)	29.8
12b	1.70 (m)		1.90 (m)	
13a	1.83 (m)	25.7	2.59 (m)	41.4
13b	1.54 (m)		2.40 (dd, 13.9, 7.8)	
14a	2.24 (m)	36.2	2.77 (m)	42.6
14b	2.03 (m)			
15	4.18 (br. s)	74.4	3.37 (m)	53.7
16a	1.65 (m)	35.1	1.94 (m)	28.3
16b	1.43 (m)		1.46 (m)	
17a	1.84 (m)	34.5	2.83 (m)	41.6
17b	1.42 (m)		2.75 (m)	
18	2.64 (m)	42.0	2.56 (m)	32.3
19a	3.17 (dd, 11.2, 6.6)	49.8	3.78 (dd, 16.0, 6.7)	65.4
19b	2.96 (t, 11.2)		3.46 (m)	
20	0.97 (d, 6.6)	11.4	1.20 (d, 6.7)	18.9
21	1.09 (s)	24.1	1.56 (s)	27.5
22		181.6		177.8

[a] δ_H 400MHz, δ_C 100 MHz in CD_3OD. [b] 2% (v/v) acetic acid was added to dissolve compound **2**.

H_2-13/C-22. Thus, the planar structure was elucidated as **1**, with a novel 5/6/5/5/6/5 skeleton.

The relative configuration of **1** (Fig. 2) was established by ROESY correlations in combination with molecular modelling studies. As shown in Fig. 2, the observed correlations of H_3-21/H-10 and H_3-21/H_b-11 indicated that they are on the up-side of molecule and were arbitrarily assigned as β-orientation. The ROE

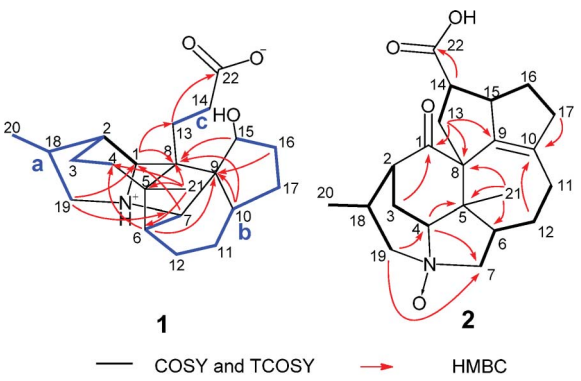
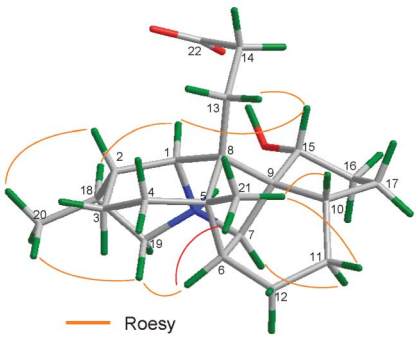


Fig. 1 Selected COSY and HMBC correlations of **1**.



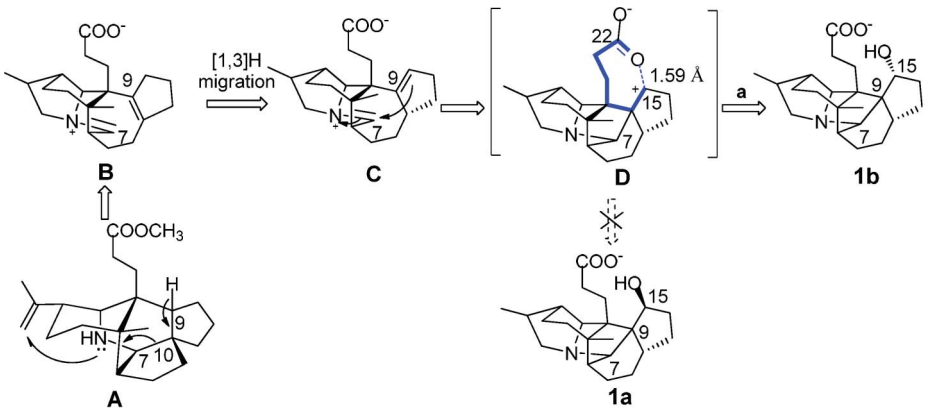
Proton pairs	Distances in H-15β epimer	Distances in H-15α epimer	ROE correlations
H1/H15	2.50	2.93	Yes
H13a/H-15	3.63	2.53	Yes

B

Fig. 2 A) Selected ROESY correlations of **1**. B) The calculated distances of the key proton pairs in the two possible DFT-optimized structures (**1a** and **1b**) are listed.

correlations of H_a-11/H-7 indicated that both protons are α-oriented. The ROE correlations of Me-21/H-6, H-6/H_a-19, H_a-19/H_3-20, and H_3-20/H-2 showed that H-6, H-2 and Me-20 were β-configuration, whereas H-1 and H-18 were assigned as α-configuration judging from ROESY correlation of H-18/H-1. Moreover, although ROESY correlations of H-15 with H-13 and H-1 were observed, the orientation of H-15 could not be determined unambiguously. Therefore, DFT calculation at the B3LYP/6-311G+(d,p) level in Gaussian 03[17] were conducted on the two possible epimers of **1** corresponding to α (**1a**) and β (**1b**) orientation of H-15, respectively. The calculated distances of the proton pairs of the H-15β epimer of **1** are fully consistent with the corresponding ROESY data, and thus H-15 was assigned as the β-orientation (Fig. 2B). Therefore, the relative configurations of all the chiral centers of **1** were determined and in agreement with its presumed biosynthetic origin, which will be discussed in detail later.

The absolute configuration of **1** was assigned on the basis of comprising the experimental optical rotation (OR) with theoretical values. The *ab initio* calculation for OR, especially B3LYP/aug-cc-pvDZ, is a sophisticated method to give high reliable OR value in theory to determine absolute configuration for natural products with complex and rigid skeletons.[18] The 3D models of **1** was subject to a conformational search under MMFF94S by Conflex 6.8, a conformational searching tool independent of initial models.[19] The searching results gave only one stable configuration, and it was further optimized at the B3LYP/6-311G+(d,p) level using Gaussian 03 package. The ORs were calculated at the B3LYP/aug-cc-pvDZ level with a PCM (MeOH) solvent model. The resulting stereochemistry gave a consistent OR direction and approximate value (−58.4) to the experimental one (−61.8). Thus, the absolute configuration of **1** was assigned as 1*S*, 2*R*, 5*S*, 6*S*, 7*R*, 8*S*, 9*S*, 10*S*, 15*R*, and 18*S*. The identified configuration is consistent to the biosynthetic origin (Scheme 1), (–)-homosecodaphniphyllate (**A**), which chirality was secured by asymmetric total synthesis previously.[20]



Scheme 1 Proposed biogenetic pathway of Longeracemine (**1**).

A plausible biogenetic pathway of **1** is proposed as shown in Scheme 1. Longeracemine might be generated from homosecodaphniphylline-type alkaloid through daphnilactone B-type one as follows. The homosecodaphniphylline-type one, homodaphniphyllate (**3**), was isolated from the title plant, and identified by comparison of ^{1}H and ^{13}C NMR data with those of authentic samples (see supplementary information). The key intermediate N,7-9,10-diene **C** should be generated by fragmentation of C-7/C-10 bond of the alkaloid **A** followed by 1,3-hydrogen shift. Then C-7 should bind to the nearest C-9 to create finally the tetrahydropyrrole ring in **1**. Generally, both epimers (**1a** and **1b**) of **1** could be formed from the intermediate **D** as shown in Scheme 1. However, only one of them with α-hydroxyl at C-15 was isolated, which disagreed with the proposed biosynthesis pathway. Then, chemical computation was employed to model the key intermediate with cation at C-15 (**D**) (for detail see supplementary information†). In the most stable conformation of **D**, side chain was curved, and a new cycloheptane-like moiety, including C-8, C-13, C-14, C-22, O-1(oxygen atom of carbonyl group), C-15 and C-9, was formed. In the moiety, the distance between the C-15 cation and the O-1 is only 1.59 Å. Obviously, lone pair of oxygen in the carbonyl group helped to stabilize positive charge at the C-15.[20] Moreover, this interaction disfavored attack of the hydroxyl group from the upside. Therefore, only one product with hydroxyl group at α position should be formed, which was also the only epimer that could be detected from the metabolites of *n*-BuOH part *via* HPLC/MS analysis (200 nm). All this data supported the proposed biogenetic pathway.

HR-ESI-MS of demethyl calyciphylline A (**2**) indicated its molecular formula $C_{22}H_{29}NO_4$.[21] Its planar structure was identified by its ^{1}H and ^{13}C NMR data (Table 1) and the assignment for those data was based on the key correlations in 2D NMR (HSQC, ^{1}H-^{1}H COSY and HMBC) as shown in Fig. 1. The compound could be also considered as demethylation from calyciphylline A or *N*-oxydation from daphnilongeranin C.[22,23] The similarities of ^{13}C NMR and ROESY correlations among the three compounds were further supported that compound **2** possesses the same relative configuration as calyciphylline A or daphnilongeranin C.

Compounds **1** and **2** were bioassayed for their plant growth regulating function according to the previous reported protocol.[24] Both compounds were bioassayed on wheat seed at 200 and 50 ppm to estimate the average percentage (three repetitions) of germination and seedling growth with respect to the control, glyphosate. **1** can stimulate the shoot elongation of wheat by 437.5% and 394.4% at 200 and 50 ppm ($\mu g\ ml^{-1}$) with respect to the blank.

Conclusions

In summary, longeracemine (**1**) was the firstly found novel C-7/C-9 bonding alkaloid in *Daphniphyllum* alkaloids family. Along with it, a new calyciphylline-A type alkaloid (**2**) was also isolated from the same plant *Daphniphyllum longeracemosum*. Their structures including absolute configuration were identified on the basis of spectra analysis associated with quantum chemical approach. Moreover, we firstly found that *Daphyniphyllum* alkaloid possess plant growth regulating activity.

Acknowledgements

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Notes and references

1 J. Kobayashi and T. Kubota, *Nat. Prod. Rep.*, 2009, **26**, 936–962.
2 H. Niwa, Y. Hirata, K. T. Suzuki and S. Yamamura, *Tetrahedron Lett.*, 1973, **14**, 2129–2132.
3 K. T. Suzuki, S. Okuda, H. Niwa, M. Toda, Y. Hirata and S. Yamamura, *Tetrahedron Lett.*, 1973, **14**, 799–802.
4 C. H. Heathcock, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 14323–14327.

5 B. Darses, I. N. Michaelides, F. Sladojevich, J. W. Ward, P. R. Rzepa and D. J. Dixon, *Org. Lett.*, 2012, **14**, 1684–1687.
6 I. Coldham, A. J. M. Burrell, H. D. S. Guerrand and N. Oram, *Org. Lett.*, 2011, **13**, 1267–1269.
7 M. E. Weiss and E. M. Carreira, *Angew. Chem., Int. Ed.*, 2011, **50**, 11501–11505.
8 Y.-T. Di, H.-P. He, Y. Lu, P. Yi, n. L. Li, L. Wu and X.-J. Hao, *J. Nat. Prod.*, 2006, **69**, 1074–1076.
9 C.-S. Li, Y.-T. Di, S.-Z. Mu, H.-P. He, Q. Zhang, X. Fang, Y. Zhang, S.-L. Li, Y. Lu, Y.-Q. Gong and X.-J. Hao, *J. Nat. Prod.*, 2008, **71**, 1202–1206.
10 S. P. Yang, H. Zhang, C. R. Zhang, H. D. Cheng and J. M. Yue, *J. Nat. Prod.*, 2006, **69**, 79–82.
11 C.-S. Li, Y.-T. Di, H.-P. He, S. Gao, Y.-H. Wang, Y. Lu, J.-L. Zhong and X.-J. Hao, *Org. Lett.*, 2007, **9**, 2509–2512.
12 C. Li, H. He, Y. Di, Y. Wang, S. Mu, S. Li, S. Gao, Z. Gao and X. Hao, *Tetrahedron Lett.*, 2007, **48**, 2737–2740.
13 L. Li, H.-P. He, Y.-t. Di, S. Gao and X.-j. Hao, *Tetrahedron Lett.*, 2006, **47**, 6259–6262.
14 Q. Zhang, Y. T. Di, C. S. Li, X. Fang, C. J. Tan, Z. Zhang, Y. Zhang, H. P. He, S. L. Li and X. J. Hao, *Org. Lett.*, 2009, **11**, 2357–2359.
15 Longeracemine: white amorphous; $[\alpha]_D^{17}$ −61.8 (*c* 0.51, MeOH); IR (KBr) ν_{max}: 3421, 1574, 1392, 1460 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); positive FABMS and ESIMS *m*/*z* 360 $[M + H]^+$; HRESIMS *m*/*z* 360.2537 (calcd for $[M + H]^+$ 360.2539).
16 For the chemical shift of the spiral carbon, refer to: C. Q. Fan, S. Yin, J. J. Xue and J. M. Yue, *Tetrahedron*, 2007, **63**, 115–119.
17 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. G. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, *GAUSSIAN 03 (Revision D.01)*, Gaussian, Inc., Wallingford, CT, 2004.
18 S.-D. Zhao, L. Shen, D.-Q. Luo and H.-J. Zhu, *Curr. Org. Chem.*, 2011, **15**, 1843–1862.
19 H. Goto, K. Ohta, T. Kamakura, S. Obata, N. Nakayama, T. Matsumoto and E. Ōsawa, CONFLEX, Conflex corp., Tokyo-Yokohama, Japan, 2004.
20 C. H. Heathcock and J. A. Stafford, *J. Org. Chem.*, 1992, **57**, 2566–2574.
21 Demethyl calyciphylline A: white amorphous; $[\alpha]_D^{25}$ −134.8 (*c* 0.7, MeOH); IR (KBr) ν_{max}: 3422, 2929, 1687, 1434, 1084 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 372 $[M + H]^+$; HRESIMS *m*/*z* 372.2178 (calcd for $[M + H]^+$ 372.2174).
22 H. Morita and J. Kobayashi, *Org. Lett.*, 2003, **5**, 2895–2898.
23 H. Takatsu, H. Morita, Y. C. Shen and J. Kobayashi, *Tetrahedron*, 2004, **60**, 6279–6284.
24 G. Bruce Williamson and D. Richardson, *J. Chem. Ecol.*, 1988, **14**, 181–187.