ORGANIC LETTERS

2009
Vol. 11, No. 23
5414−5417

Daphhimalenine A, a New Alkaloid with an Unprecedented Skeleton, from *Daphniphyllum himalense*

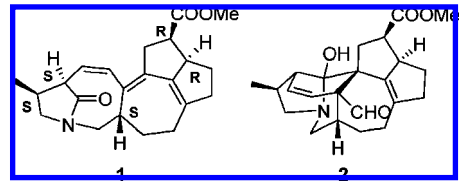
Yu Zhang,[†,‡] Ying-Tong Di,[†] Qiang Zhang,[†] Shu-Zhen Mu,[§] Cheng-Jian Tan,[†] Xin Fang,[†] Hong-Ping He,[†] Shun-Lin Li,[†] and Xiao-Jiang Hao*[,†]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, P. R. China, Graduate School of Chinese Academy of Sciences, Beijing 100039, P. R. China, and The Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, Guiyang 550002, P. R. China

haoxj@mail.kib.ac.cn

Received September 30, 2009

ABSTRACT



Daphhimalenine A (1), a novel alkaloid with a rearrangement C-21 skeleton, containing a unique 1-azabicyclo[5.2.1]decane ring system, was isolated from the leaves of *Daphniphyllum himalense*, along with biogenetically related alkaloids daphhimalenine B (2) and daphnezomine T. Their structures were established on the basis of spectroscopic data, and the absolute configuration of 1 was assigned by computational methods. A plausible biosynthetic pathway of 1 was also proposed.

Daphniphyllum alkaloids are a family of structurally diverse natural products with complex polycyclic systems elaborated by plants of the genus *Daphniphyllum*.[1] Their unique structural features have attracted great attention to the total synthesis[2] and biosynthetic research.[3] In recent years, a series of novel *Daphniphyllum* alkaloids have been isolated and identified, which may be explained by the unique biogenetic process involving repeated fissions of C−C or C−N bonds followed by rearrangements and recyclization.[1]

Daphniphyllum himalense (Benth.) Muell.-Arg (Daphniphyllaceaae), an evergreen tree, is distributed mainly in the Yunnan Province of China.[4] Up to now, the chemical constituents of *D. himalense* have not been investigated. In our further search for structurally unique and biogenetically interesting *Daphniphyllum* alkaloids,[5,6] two biogenetically related new alkaloids, daphhimalenines A(**1**) and B (**2**), and the recently reported alkaloid daphnezomine T (**3**)[7] were

[†] Kunming Institute of Botany.
[‡] Graduate School of the Chinese Academy of Sciences.
[§] The Key Laboratory of Chemistry for Natural Product of Guizhou Province.

(1) (a) Kobayashi, J.; Kubota, T. *Nat Prod. Rep*. **2009**, *26*, 936–962, a nd references therein. (b) Dong, M.; Zhang, M. L.; Shi, Q. W.; Gu, Y. C.; Kiyota, H. *Curr. Org. Chem*. **2009**, *13*, 646–682.

(2) (a) Wallence, G. A.; Heathcock, C. H. *J. Org. Chem*. **2001**, *66*, 450–454. (b) Heathcock, C. H. *Proc. Natl. Acad. Sci. U.S.A*. **1996**, *93*, 14323–14327. (c) Heathcock, C. H.; Joe, D. *J. Org. Chem*. **1995**, *60*, 1131–1142. (d) Heathcock, C. H. *Angew. Chem., Int. Ed*. **1992**, *31*, 665–681.

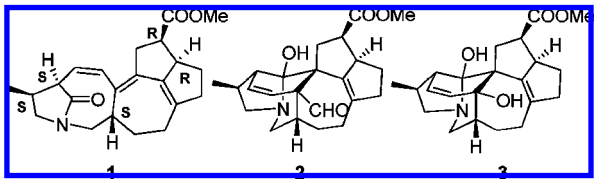
(3) (a) Niwa, H.; Hirata, Y.; Suzuki, K. T.; Yamamura, S. *Tetrahedron Lett*. **1973**, *4*, 2129–2132. (b) Suzuki, K. T.; Okuda, S.; Niwa, H.; Toda, M.; Hirata, Y.; Yamamura, S. *Tetrahedron Lett*. **1973**, *14*, 799–802.

(4) Zhen, M., Min, T. L. In *Chinese Flora (Zhongguo Zhiwu Zhi*); Science Press: Beijing, 1980; Vol. 45 (1), pp 1−11.

(5) (a) Zhang, Y.; He, H. P.; Di, Y. T.; Mu, S. Z.; Wang, Y. H.; Wang, J. S.; Li, C. S; Kong, N, C.; Gao, S.; Hao, X. *J. Tetrahedron Lett*. **2007**, *48*, 9104–9107. (b) Li, C. S.; Di, Y. T.; He, H. P.; Gao, S.; Wang, Y. H.; Lu, Y.; Zhong, J. L.; Hao, X. *J. Org. Lett*. **2007**, *9*, 2509–2512. (c) Di, Y. T.; He, H. P.; Wang, Y. S.; Li, L. B.; Lu, Y.; Gong, J. B.; Fang, X.; Kong, N. C.; Li, S. L.; Zhu, H. J.; Hao, X. *J. Org. Lett*. **2007**, *9*, 1355–1358. (d) Mu, S. Z.; Wang, Y.; He, H. P.; Yang, X. W.; Wang, Y. H.; Di, Y. T.; Lu, Y.; Chang, Y.; Hao, X. J. *J. Nat. Prod*. **2006**, *69*, 1065–1069.

10.1021/ol902262g CCC: $xxxx
Published on Web 11/11/2009

isolated from the leaves of *D. himalense*. Among them, daphhimalenine A (**1**) contains a 1-azabicyclo[5.2.1]decane ring system due to its unique cleavage of the C-1/C-8 bond. This paper describes the isolation and structural elucidation of the new compounds and a plausible biosynthetic pathway for their generation.



The air-dried and powdered leaves of *D. himalense* were extracted with 95% EtOH, and the crude extract was partitioned between EtOAc and an acidic liquor at pH 3. The aqueous layer was then basified to pH 10 with saturated Na_2CO_3, followed by exhaustive extraction with $CHCl_3$. The $CHCl_3$-soluble material was subjected to repeated column chromatography on silica gel and semipreparative HPLC to afford daphhimalenines A (**1**, 12 mg) and B (**2**, 16 mg) and daphnezomine T (**3**, 10 mg).

Daphhimalenine A (**1**)[8] was obtained as an optically active ($[\alpha]_D^{19}$ −28.1) light yellow oil. The molecular formula of **1** was established as $C_{22}H_{27}NO_3$ by HRESIMS data (*m/z* 376.1897 $[M + Na]^+$, calcd 376.1888), with 10 degrees of unsaturation. IR absorptions implied the presence of an ester carbonyl (1732 cm^{-1}) and a lactam (1687 cm^{-1}) functionalities. All 22 carbon signals observed in the ^{13}C NMR and DEPT spectra of **1** could be classified into three double bonds, two carbonyls, five sp^3 methines, seven sp^3 methylenes, one methyl together with one methoxy group. Among them, two methylenes (δ_C 51.6, 48.6) and one carbonyl carbon (δ_C 183.8) were ascribed as bearing a nitrogen. Since two carbonyl groups and three double bonds accounted for five out of 10 degrees of unsaturation, the remaining five degrees of unsaturation were assumed for the presence of a pentacyclic system in **1**.

The $^1H-^1H$ COSY revealed that **1** possessed three fragments: **a** (C-2 to C-4, C-2 to C-18, and C-18 to C-19 and C-20), **b** (C-6 to C-7 and C-12, and C-11 to C-12), and **c** (C-13 to C-17), as shown in Figure 1. Further detailed

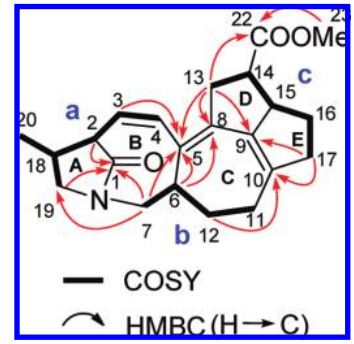


Figure 1. $^1H-^1H$ COSY and key HMBC correlations of **1**.

HMBC studies established the connectivity of the three fragments (**a**−**c**), quaternary carbons, and a nitrogen atom. HMBC correlations of H-7α to C-1 (δ_C 183.8) and C-19 (δ_C 51.6) and of H-19β to C-7 (δ_C 48.6) indicated that C-1, C-7, and C-19 were connected to each other through the nitrogen atom. Moreover, HMBC cross-peaks of H-2 to C-1 and of H-3 and H-6 to C-5 established the connectivity of fragments **a** and **b**, which constitute a 1-azabicyclo[5.2.1]decane ring with a methyl at C-18 and a carbonyl at C-1. HMBC correlations of H_2-13 to C-5, C-8, and C-9, along with the correlations of H_2-17 to C-9 and C-10 and of H_2-12 to C-10, led to the connection of fragments **b** and **c** with C-5, C-8, C-9, and C-10, which also indicated the presence of the seven-membered ring C with the conjugated $\Delta^{5,8}$ and $\Delta^{9,10}$ double bonds. A methoxy group was attached to C-22 by HMBC correlations of H_3-23 and H_2-13 to C-22. Thus, the gross structure of **1** was assigned as shown in Figure 1, having a unique fused 5/8/7/5/5 ring system containing a 1-azabicyclo[5.2.1]decane ring (N-1, C-1-C-7, C-18, and C-19) with a ketone at C-1, a methyl at C-18, and a double bond between C-3 and C-4, a cyclohepta-1,3-diene ring, a cyclopentene ring, and a cyclopentane ring with a methoxy carbonyl at C-14 as shown in Figure 1.

The relative configuration of **1** was deduced from the analysis of its ROESY correlations in combination with molecular modeling studies. As shown in Figure 2A, correlations of H_3-20 with H-3 and H-19β, together with H-4 with H-13β, indicated that CH_3-20, H-19β, H-13β, and double bond C-3−C-4 were cofacial and were arbitrarily assigned as β-oriented, which implied the α-orientation of H-2 accordingly. Correlations of H-19β/H-7β and H-7β/H-6 implied that H-6 was β-oriented. The ROESY correlation of H-13α/H-15 indicated α-orientation of H-15. However, the orientation of H-14 was impossible to determine by ROE methods as its correlations with other protons are both limited and unreliable. Thus we turned our attention to coupling constant analysis and molecular modeling. The two lowest-energy conformers of epimers (Figure 2B) of **1**, correspond-

(6) (a) Zhang, Y.; Di, Y. T.; Mu, S. Z.; Li, C. S.; Zhang, Q.; Tan, C. J.; Zhang, Z.; Fang, X.; Hao, X. J. *J. Nat. Prod.* **2009**, *72*, 1325–1327. (b) Zhang, Q.; Di, Y. T.; Li, C. S.; Fang, X.; Tan, C. J.; Zhang, Z.; Zhang, Y.; He, H. P.; Li, S. L.; Hao, X. J. *Org. Lett.* **2009**, *11*, 2357–2359. (c) Kong, N, C.; Zhang, Y.; Gao, S.; Lu, Y.; Zheng, Q. T.; Sun, Q. Y.; Yang, F. M.; Di, Y. T.; Hao, X. J. *Tetrahedron Lett.* **2009**, *50*, 957–959. (d) Li, C. S.; Di, Y. T.; Zhang, Q.; Zhang, Y.; Tan, C. J.; Hao, X. J. *Helv. Chim. Acta* **2009**, *92*, 653–659. (e) Zhang, Y.; Di, Y. T.; Liu, H. Y.; Li, C. S.; Tan, C. J.; Zhang, Q.; Fang, X.; Li, S. L.; Hao, X. J. *Helv. Chim. Acta* **2008**, *91*, 2153–2158. (f) Li, C. S.; Di, Y. T.; Mu, S. Z.; He, H. P.; Zhang, Q.; Fang, X.; Zhang, Y.; Li, S. L.; Lu, Y.; Gong, Y. Q.; Hao, X. J. *J. Nat. Prod.* **2008**, *71*, 1202–1206. (g) Mu, S. Z.; Wang, J. S.; Yang, X. S.; He, H. P.; Li, C. S.; Di, Y. T.; Wang, Y.; Zhang, Y.; Fang, X.; Huang, L. J.; Hao, X. J. *J. Nat. Prod.* **2008**, *71*, 564–569. (h) Di, Y. T.; Liu, L. L.; Li, C. S.; Zhang, Y.; Zhang, Q.; Mu, S. Z.; Sun, Q. Y.; Yang, F. M.; Liu, H. Y.; Hao, X. J. *Helv. Chim. Acta* **2008**, *91*, 838–843. (i) Tan, C. J.; Di, Y. T.; Wang, Y. H.; Wang, Y.; Mu, S. Z.; Gao, S.; Zhang, Y.; Kong, N, C.; He, H. P.; Zhang, J. X.; Fang, X.; Li, C. S.; Lu, Y.; Hao, X. J. *Tetrahedron Lett.* **2008**, *49*, 3376–3379.

(7) Kubota, T.; Suzuki, T.; Ishiuchi, K.; Kuhara, T.; Kobayashi, J. *Chem.Pharm. Bull.* **2009**, *57*, 504–507.

(8) **Daphhimalenine A (1):** light yellow oil; $[\alpha]_D^{19} = -28.1$ (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.03), 281 (3.98) nm; IR (KBr) ν_{max} 3431, 2929, 1732, 1687, 1640, 1437, 1202, 1166, and 1139 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 354 $[M + H]^+$; HRESIMS *m/z* 376.1897 (calcd for $[M + Na]^+$ 376.1888) .

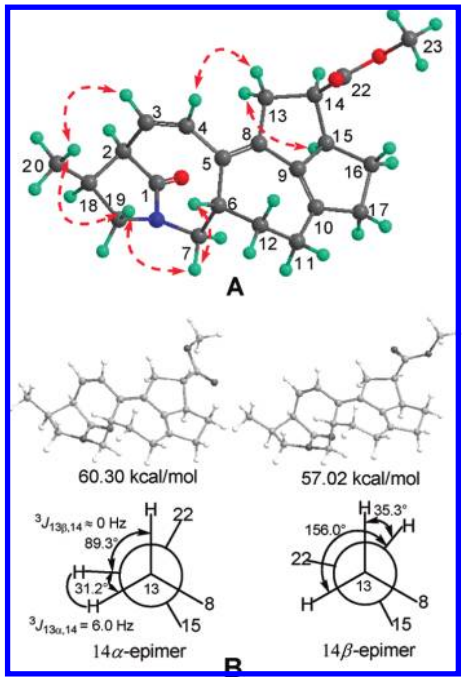


Figure 2. (A) Key ROESY correlations of **1**. (B) Two lowest-energy conformers (A,B) of epimeric form of daphhimalenine A (**1**), corresponding to the α- and *β*-orientations of H-14, analyzed by MMFF945 force field calculation followed by minimization and clustering analysis (upper) and rotation models for the C-13-C-14 bond of the two epimers of **1** (lower).

ing to the α- and *β*-orientations of the observed of H-14, were generated after conformational searching by computer modeling (MMFF945 force field calculations for energy minimization with CONFLEX 6.2). In A, the dihedral angles between H-14 and H-13α and H-13*β* were 31.2° and 89.3°, respectively, whereas in B, the dihedral angles were 156.0° and 35.3°. With a consideration of the coupling constants between the two protons (6.0 and near 0) in **1**, A is in more agreement with the experimental result. Therefore, the H-14 was assigned as α-oriented. Furthermore, the calculation results also suggested the eight-membered ring B takes a chair-half-chair conformation.

Subsequently, for the assignment of the absolute configuration of daphhimalenine A (**1**), the optical rotation (OR) value of **1** was calculated by using the density functional theory (DFT) methods[6b,9] in the Gaussian 03 program package.[10] The "self-consistent reaction field" method (SCRF) was employed to perform the OR caculation of the most stable conformer of **1** in MeOH solution at the B3LYP/6-311G++(d,p)//B3LYP/6-311G(d, p) level. The calculated OR value (−29.2) for **1** is close to its experimental values (−28.1), which indicated that the absolute configuration of daphhimalenine A was elucidated as shown in structure **1** (for more details see Supporting Information). Furthermore, its absolute configuration, i.e., (6*S*,14*R*,15*R*)-**1**, is consistent with calyciphylline J, the other structurally related *Daphniphyllum* alkaloid, whose configuration was assigned by the PGME method.[11]

Daphhimalenine B (**2**)[12] was isolated as a light yellow oil, and the molecular formula was established as $C_{23}H_{29}NO_4$ by HRESIMS (*m*/*z* 384.2190, $[M + H]^+$) with 10 degrees of unsaturation. The ^1H and ^{13}C NMR data of **2** (see Table 1) were strikingly similar to those of yuzurimine C,[13] with the exception of the replacement of the tertiary oxygenated carbon atom at C-2 (δ_C 80.1) in yuzurimine C by a methine carbon atom resonating at δ_C 46.5 in the ^{13}C NMR spectrum of **2**. All the NMR data implied that **2** was the 2-deoxy form of yuzurimine C, which was confirmed by 2D NMR (HSQC, $^1H-^1H$ COSY, HMBC, and ROESY) experiments.

The coexistence of the three structure-related alkaloids (**1**−**3**) in the same plant implies that the unprecedented

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR Data of Daphhimalenines A (**1**)[a] and B (**2**)[b]

	1		**2**	
	δ_H[a]	δ_C[a]	δ_H[b]	δ_C[b]
1		183.8 (s)		103.3 (s)
2	2.78 (overlapped)	49.4 (d)	3.05 (m)	46.5 (d)
3	5.30 (dd, 6.0, 12.5)	122.4 (d)	5.85 (dd, 10.5, 3.0)	127.0 (d)
4	6.29 (d, 12.5)	133.2 (d)	6.16 (dd, 10.5, 2.0)	130.1 (d)
5		129.4 (s)		48.3 (s)
6	3.11 (m)	40.2 (d)	2.18 (m)	38.2 (d)
7α	4.19 (t, 12.0)	48.6 (t)	3.64 (m)	54.4 (t)
7*β*	2.73 (dd, 12.0, 4.0)		3.28 (m)	
8		134.4 (s)		56.4 (s)
9		142.0 (s)		142.0 (s)
10		134.7 (s)		140.4 (s)
11α	2.21 (br s) (overlapped)	26.9 (t)	1.94 (m)	25.8 (t)
11*β*	2.21 (br s) (overlapped)		1.70 (m)	
12α	1.81 (m) (overlapped)	26.3 (t)	2.23 (d, 16.5)	25.0 (t)
12*β*	1.81 (m) (overlapped)		2.62 (m)	
13α	2.47 (ddd, 17.0, 6.0, 3.0)	39.7 (t)	2.55 (dd, 15.5, 9.5)	41.7 (t)
13*β*	2.66 (br d, 17.0)		2.33 (dd, 15.5, 3.0)	
14	2.89 (t, 7.5)	43.9 (d)	3.08 (m)	43.0 (d)
15	3.44 (br s)	55.5 (d)	3.87 (br s)	58.2 (d)
16α	1.94 (m)	28.6 (t)	1.97 (m)	29.8 (t)
16*β*	1.29 (m)		1.27 (overlapped)	
17α	2.78 (overlapped)	42.0 (t)	2.37 (d, 7.5)	43.2 (t)
17*β*	2.36 (m)		2.64 (m)	
18	2.40 (m)	30.4 (d)	3.18 (m)	32.4 (d)
19α	3.21 (dd, 9.0, 7.0)	51.6 (t)	4.19 (dt, 12.0, 3.5)	61.6 (t)
19*β*	3.15 (br t, 9.0)		2.69 (dd, 12.0, 3.5)	
20	1.09 (d, 7.0)	12.9 (q)	1.26 (d, 7.5)	18.2 (q)
21			10.37 (s)	206.1 (s)
22		175.2 (s)		175.8 (s)
23	3.60 (s)	51.0 (q)	3.61 (s)	51.5 (q)

[a] NMR data for **1** (in $CDCl_3$). [b] NMR data for **2** [in $CDCl_3$ (containing 1% TFA, v/v)].

(9) (a) Ge, H. M.; Zhu, C. H.; Shi, D. H. L.; Zhang, D.; Xie, D. Q.; Yang, J.; Ng, S. W.; Tan, R. X. *Chem.—Eur. J.* **2008**, *14*, 376–381. (b) Karna, S. P.; Dupuis, M. *J. Comput. Chem.* **1991**, *12*, 487–504. (c) Kondru, R. K.; Peter, W.; Beratan, D. N. *J. Am. Chem. Soc.* **1998**, *120*, 2204–2205. (d) Liu, D. Z.; Wang, F.; Liao, T. G.; Tang, J. G.; Steglich, W.; Zhu, H. J.; Liu, J. K. *Org. Lett.* **2006**, *8*, 5749–5752.

(10) Frisch, M. J. et al. *Gaussian 03,revision D.01*;, Gaussian, Inc.: Wallingford, CT, 2005 (see Supporting Information for complete reference).

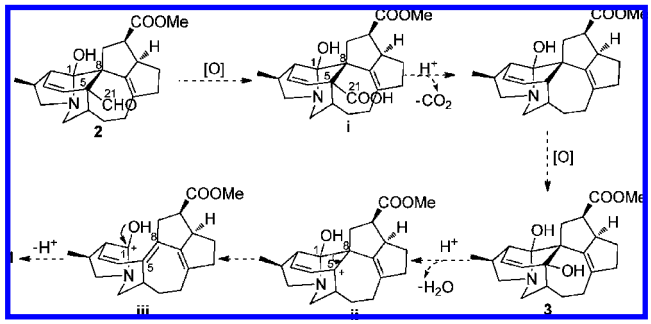
(11) Saito, S.; Yahata, H.; Kubota, T.; Obara, Y.; Nakahata, N.; Kobayashi, J. *Tetrahedron* **2008**, *64*, 1901–1908.

(12) **Daphhimalenine B (2):** Light yellow oil; $[\alpha]_D^9 = +81.8$ (*c* 0.22, MeOH); IR (KBr) λ_{max} 3439, 2935, 1715, 1670, 1437, 1203, and 1136 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 384 $[M + 1]^+$; HRESIMS *m*/*z* 384.2174 (calcd for $[M + H]^+$ 384.2190).

(13) Yamamura, S.; Irikawa, H.; Okumura, Y.; Hirata, Y. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2120–2123.

pentacyclic skeleton of daphhimalenine A (**1**) should be a further modification of two yuzurimine-type alkaloids daphhimalenine B (**2**) and daphnezomine T (**3**). Therefore, a possible pathway to **1** is suggested in Scheme 1. Transformation of **2** underwent oxidation to give intermediate **i**, and then oxidative decarboxylation of **i** and subsequent oxidation yielded **3**. After dehydration, **3** could further convert to the key carbonium intermediate **ii**, which finally underwent a carbocation rearrangement to afford 1-azabicyclo[5.2.1]decane ring system in daphhimalenine A (**1**).

Scheme 1. Biogenetic Pathway Proposed for Compounds **1**−**3**



The cytotoxic activities of **1** and **2** against growth of tumor cell lines [A549 (human lung adenocarcinoma) and HL-60 (human promyelocytic leukemia)] were evaluated. However, the results indicated that **1** and **2** were inactive against the above cancer cells (50% effective dose of clonal inhibition (ED_{50}) > 10 μg/mL).

Acknowledgment. This work was financially supported by the Ministry of Science and Technology (2009CB522303), National Natural Science Foundation (20672120 and 30800092), and West Doctor Foundation of Chinese Academy of Sciences (2908065112w1), P. R. China. The authors thank Prof. Heng Li, Kunming Institute of Botany, Chinese Academy of Sciences (CAS), for the collection and the identification of the plant material.

Supporting Information Available: Experimental section, optical rotation calculation, 1D and 2D NMR spectra and ESIMS for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL902262G