

Daphlongamines I and J. New Yuzurine-type Alkaloids from *Daphniphyllum longeracemosum*

Chun-Shun Li^{a,b}, Ying-Tong Di^a, Jie Guo^a, Qiang Zhang^a, Xin Fang^a, and Xiao-Jiang Hao^a

^a State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China

^b Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, P. R. China

Reprint requests to Prof. Dr. Xiao-Jiang Hao. Fax: +86-871-5223070. E-mail: haoxj@mail.kib.ac.cn

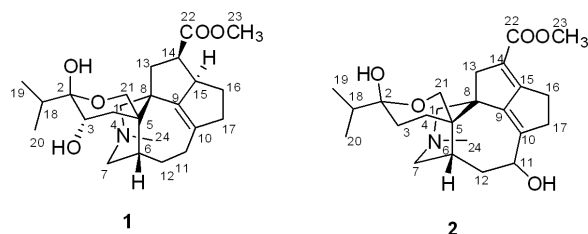
Z. Naturforsch. **2010**, 65b, 1–3; received May 6, 2010

Two new Yuzurine-type *Daphniphyllum* alkaloids, daphlongamines I and J (**1**, **2**), were isolated from the fruits of *Daphniphyllum longeracemosum*. Their structures were established by spectroscopic methods, especially 2D NMR techniques.

Key words: Daphniphyllum Alkaloids, Yuzurine, Daphlongamine, *Daphniphyllum longeracemosum*

Introduction

Daphniphyllum alkaloids are a family of structurally specific and complex metabolites elaborated by trees of genus *Daphniphyllum* [1–3]. The unusual ring systems have attracted great attention for total synthesis [4] as well as biosynthetic studies [5]. In our continuing search for biogenetically and structurally interesting alkaloids [6, 7], two new *Daphniphyllum* alkaloids, daphlongamines I and J (**1**, **2**) were isolated from fruits of *Daphniphyllum longeracemosum*. Herein, we report the isolation and structure determination of **1** and **2**.



Results and Discussion

Daphlongamine I (**1**), a colorless amorphous powder, was indicated to have the molecular formula $C_{24}H_{37}NO_5$ by HR-ESI-MS ($m/z = 420.2743$, $[M+H]^+$, calcd. 420.2749), suggesting seven degrees of unsaturation. The IR absorption bands at 1735 and 3432 cm^{-1} implied the existence of ester carbonyl and hydroxyl groups, respectively. The ^{13}C NMR and DEPT spectra displayed 24 carbon atoms (Table 1), including one carbonyl ($\delta_C = 177.4$), one olefinic bond

($\delta_C = 135.0, 147.7$), three sp^3 quaternary carbons, five methines, nine methylenes, and four methyl groups (one MeO, one MeN). Among them, two methylenes ($\delta_C = 57.5, 62.9$) were ascribed to those connected to the N atom, one quaternary carbon ($\delta_C = 99.4$), one methine ($\delta_C = 65.8$) and one methylene carbon atom ($\delta_C = 63.3$) were deduced to be those bearing the oxygen atom. Detailed analyses of the 1D NMR data suggested that **1** should be a yuzurine-type *Daphniphyllum* alkaloid [1] containing a hexacyclic ring system. The position of the double bond and the hydroxyl group were further established by the analyses of 2D NMR spectra including ^1H - ^1H COSY, HSQC, and HMBC data. The ^1H - ^1H COSY spectrum indicated the presence of four partial fragments: **a** (C-18 to C-19 and C-20), **b** (C-3 to C-4), **c** (C-6 to C-7 and C-12, and C-11 to C-12), and **d** (C-13 to C-17) drawn with bold bonds in Fig. 1A. In the HMBC spectrum, correlations of H-18 with the oxygenated carbon atoms C-2 ($\delta_C = 99.4$) and C-3 ($\delta_C = 65.8$) implied that C-2 and C-3 were substituted by hydroxyl groups of hemiketal and hydroxyl functionalities, respectively. The existence of the double bond between C-9 and C-10 was verified by the HMBC correlations of H-15 to C-9 and C-10, as well as H₂-17 to C-9 and C-10. Thus, the gross structure of daphlongamine I was established as **1** shown in Fig. 1A.

The relative stereochemistry of **1** was elucidated by ROESY data as shown in a computer-generated 3D drawing (Fig. 1B). The ROESY correlations observed between the proton pairs of H-21b/H-6 and

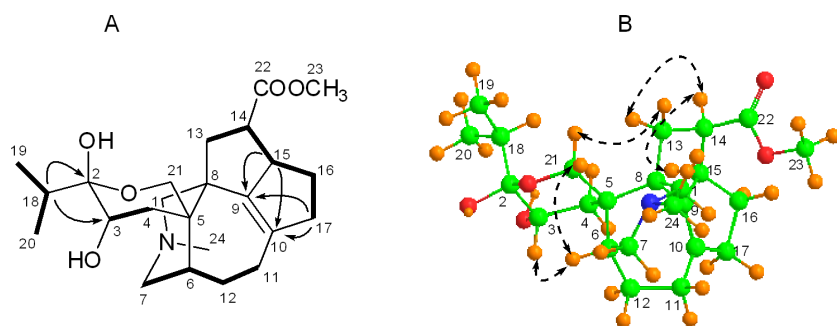


Fig. 1. (A) ^1H - ^1H COSY (bold) and HMBC (arrow, H→C) correlations of **1**. (B) NOESY correlations of **1**.

Table 1. ^1H (400 MHz) and ^{13}C (100 MHz) data of Daphlongamines I (**1**) and J (**2**).

No.	δ_{H} (mult., J in Hz) ^a	δ_{C} ^a	δ_{H} (mult., J in Hz) ^b	δ_{C} ^b
1a	2.76 (1H, m)	57.5 t	2.42 (2H, m)	58.6 t
1b	2.68 (1H, m)			
2	—	99.4 s	—	97.6 s
3	3.85 (1H, dd, 11.2, 5.6)	65.8 d	1.57 (2H, m)	24.5 t
4a	1.45 (1H, t, 12.0)	33.7 t	1.93 (1H, m)	21.5 t
4b	2.23 (1H, m)		1.73 (1H, m)	
5	—	41.9 s	—	34.9 s
6	2.22 (1H, m)	36.4 d	2.43 (1H, m)	33.0 d
7a	2.39 (1H, d, 12.0)	62.9 t	2.96 (1H, m)	54.6 t
7b	2.27 (1H, d, 12.0)		2.74 (1H, m)	
8	—	48.0 s	—	46.0 s
9	—	147.7 s	—	148.9 s
10	—	135.0 s	—	152.7 s
11a	2.46 (1H, m)	28.5 t	4.17 (1H, m)	65.9 d
11b	2.05 (1H, m)			
12a	2.24 (1H, m)	28.6 t	2.06 (1H, m)	34.4 t
12b	1.63 (1H, m)		2.31 (1H, m)	
13a	2.72 (1H, dd, 14.8, 2.8)	41.1 t	2.84 (1H, m)	39.8 t
13b	1.65 (1H, m)		3.20 (1H, m)	
14	2.90 (1H, dt, 9.6, 2.7)	44.0 d	—	118.6 s
15	3.45 (1H, m)	56.4 d	—	168.3 s
16a	1.28 (1H, m)	29.4 t	2.70 (1H, m)	25.7 t
16b	1.83 (1H, m)			
17a	2.55 (1H, m)	43.8 t	2.35 (1H, d, 15.0)	43.6 t
17b	2.32 (1H, m)		3.25 (1H, d, 15.0)	
18	1.97 (1H, dd, 13.9, 6.2)	36.9 d	1.69 (1H, m)	38.8 d
19	0.99 (3H, d, 6.9)	17.0 q	0.92 (1H, d, 7.0)	16.8 q
20	0.97 (3H, d, 6.9)	18.4 q	0.92 (1H, d, 7.0)	16.2 q
21a	4.26 (1H, d, 12.5)	63.3 t	3.76 (1H, d, 9.6)	62.5 t
21b	3.78 (1H, dd, 12.5, 3.0)		3.36 (1H, dd, 9.6, 2.4)	
22	—	177.4 s	—	166.5 s
23	3.61 (3H, s)	51.9 q	3.71 (3H, s)	51.1 q
24	2.21 (3H, s)	47.4 q	2.38 (3H, s)	45.1 q

^a Measured in CD_3OD ; ^b measured in CDCl_3 .

H-6/H-3 suggested that H-6 and C-3 were in a β -orientation. Furthermore, H-14 and H-15 were determined to be in an α -configuration by the correlations between H-13a/H-14, as well as H-14/H-15.

Daphlongamine J (**2**) was obtained as a colorless solid. Its molecular formula was determined as

$\text{C}_{24}\text{H}_{35}\text{NO}_4$ by HR-ESI-MS ($m/z = 418.2743$, calcd. 418.2749), with 8 degrees of unsaturation. Extensive comparison of 1D NMR data of **2** (Table 1) with those of **1** suggested that both compounds were similar, except that there was one more olefinic bond in **2**, which was confirmed by the 2D NMR data (^1H - ^1H COSY, HSQC, and HMBC). In the HMBC spectrum, the observed cross-peaks of H₂-12 with C-11 at $\delta_{\text{C}} = 62.5$, and H-11 with C-9 and C-10 implied that C-11 was substituted by a hydroxyl group, instead of C-3 in **1**. In addition, the conjugated double bonds between C-8/C-9 and C-14/C-15 were suggested by the HMBC correlations of H₂-13 to C-14 and C-15, and H₂-16 to C-9, C-10 and C-15.

The ROESY spectrum suggested that **2** had the same relative stereochemistry as **1**. However, the relative configuration of the hydroxyl functionality at C-11 could not be determined yet.

Experimental Section

General experimental procedures

IR spectra were measured with a Bio-Rad FTS-135 spectrometer from KBr pellets. Optical rotations were obtained on a Perkin-Elmer model 241 polarimeter. ESI and high-resolution mass spectra were recorded on a Finnigan MAT 90 instrument and a VG Auto Spec-3000 spectrometer. 1D and 2D NMR spectra were measured on Bruker DRX-500 or AM-400 spectrometers, using TMS as internal standard, and chemical shifts were recorded as δ values. Column chromatography was performed on silica gel H (10–40 μm ; Qingdao Marine Chemical Factory) and Sephadex LH-20 (40–70 μm , Amersham Pharmacia Biotech AB, Uppsala, Sweden).

Plant material

The Fruits of *Daphniphyllum longeracemosum* were collected in Hekou of Yunnan Province, People's Republic of China, in October 2005. The sample was identified by Prof.

Xun Gong, Kunming Institute of Botany, Chinese Academy of Sciences, and a voucher specimen (KIB 05110021) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The fruits (60 kg) of *D. longeracemosum* were extracted with 95 % EtOH, and the crude extract was adjusted to pH = 2 with 2 % HCl. The acidic mixture was defatted with petroleum ether (PE), which was followed by extraction with CHCl₃. The pH of the aqueous layer was roughly adjusted to pH = 10 with 3 % NaOH, and then exhaustively extracted with CHCl₃ to give the crude alkaloid. The crude extracts were subjected to a silica gel column (CHCl₃/MeOH, 1:0 → 0:1) to obtain six major fractions (F1–F6). Fraction 3 was further chromatographed over a silica gel (300–400 mesh) column (PE/Et₂NH, 50:1 → 10:1) followed by a Sephadex LH-20 column eluted with CH₃OH to afford daphlongamine J (**2**, 7.0 mg). Fraction 4 was subjected to a RP-18 silica gel column (MeOH-H₂O) to give 4 parts (P1–P4). Part 3 was subjected to silica gel (PE/EtOAc/Et₂NH, 50:1:0.2 → 10:1:0.2) followed by Sephadex LH-20 col-

umn chromatography eluted with CH₃OH to isolate daphlongamine I (**1**, 21.0 mg).

Identification

Daphlongamine I (1): Colorless powder. – ¹H NMR and ¹³C NMR data: see Table 1. – $[\alpha]_D^{24} = -35.03$ ($c = 0.59$, CH₃OH). – IR (KBr): $\nu_{\max} = 3432, 2932, 2835, 1734 \text{ cm}^{-1}$. – MS ((+)-ESI): $m/z = 420$ $[M+H]^+$. – HRMS ((+)-ESI): $m/z = 420.2743$ (calcd. 420.2749 for C₂₄H₃₈NO₅⁺, $[M+H]^+$).

Daphlongamine J (2): Colorless solid. – ¹H NMR and ¹³C NMR data: see Table 1. – $[\alpha]_D^{27} = 39.02$ ($c = 0.39$, CH₃OH). – UV (MeOH): $\lambda_{\max}(\log \epsilon) = 295 (2.57)$. – IR (KBr): $\nu_{\max} = 3383, 2928, 2873, 1702, 1628 \text{ cm}^{-1}$. – MS ((+)-ESI): $m/z = 418$ $[M+H]^+$. – HRMS ((+)-ESI): $m/z = 418.2598$ (calcd. 418.2593 for C₂₄H₃₆NO₅⁺, $[M+H]^+$).

Acknowledgements

This research was supported by the National Natural Science Foundation (20672120) of PRC. The authors thank Prof. X. Gong, Kunming Institute of Botany, Chinese Academy of Sciences (CAS), for the identification of the plant material.

- [1] For a review of *Daphniphyllum* alkaloids, see: J. Kobayashi, H. M. Orita in *The Alkaloids*, Vol. 60, (Ed.: G. A. Cordell), Academic Press, New York, **2003**, pp. 165–205, and refs. cited therein.
- [2] a) Q. Zhang, Y. T. Di, C. S. Li, X. F. ang, C. J. Tan, Z. Z. hang, Y. Z. hang, H. P. He, S. L. Li, X. J. Hao, *Org. Lett.* **2009**, *11*, 2357–2359; b) C. S. Li, Y. T. Di, H. P. He, S. G. ao, Y. H. Wang, Y. L. u, J. L. Zhong, X. J. Hao, *Org. Lett.* **2007**, *9*, 2509–2512; c) Y. T. Di, H. P. He, Y. S. Wang, L. B. Li, Y. L. u, J. B. Gong, X. F. ang, N. C. Kong, S. L. Li, H. J. Zhu, X. J. Hao, *Org. Lett.* **2007**, *9*, 1355–1358; d) Y. Zhang, H. P. He, Y. T. Di, S. Z. Mu, Y. H. Wang, J. S. Wang, C. S. Li, N. C. Kong, S. G. Gao, X. J. Hao, *Tetrahedron Lett.* **2007**, *48*, 9104–9107; e) C. S. Li, H. P. He, Y. T. Di, Y. H. Wang, S. Z. Mu, S. L. Li, S. G. ao, Z. L. Gao, X. J. Hao, *Tetrahedron Lett.* **2007**, *48*, 2737–2740.
- [3] a) S. Saito, T. K. ubota, E. F. ukushi, J. K. awabata, H. P. Zhang, J. K. obayashi, *Org. Lett.* **2007**, *9*, 1207–1209; b) Z. Y. Li, P. C. heng, H. G. Xu, Y. M. Yang, S. Y. Peng, Z. Z. Zhao, Y. W. Guo, *Org. Lett.* **2007**, *9*, 477–480; c) C. Q. Fan, S. Y. in, J. J. Xue, J. M. Yue, *Tetrahedron* **2007**, *63*, 115–119; d) W. Zhang, Y. W. Guo, K. K. rohn, *Chem. Eur. J.* **2006**, *12*, 5122–5127.
- [4] a) S. E. Denmark, R. Y. Baiazitov, *J. Org. Chem.* **2006**, *71*, 593–605; b) D. Sole, X. Urbaneja, J. Bonjoch, *Org. Lett.* **2005**, *7*, 5461–5464.
- [5] a) H. Niwa, Y. H. irata, K. T. Suzuki, S. Y. amamura, *Tetrahedron Lett.* **1973**, *14*, 2129–2132; b) K. T. Suzuki, S. O. kuda, H. N. iwa, M. T. oda, Y. H. irata, S. Y. amamura, *Tetrahedron Lett.* **1973**, *14*, 799–802.
- [6] a) C. S. Li, Y. T. Di, S. Z. Mu, H. P. He, Q. Z. hang, X. F. ang, Y. Z. hang, S. L. Li, Y. L. u, Y. Q. Gong, X. J. Hao, *J. Nat. Prod.* **2008**, *71*, 1202–1206; b) Q. Zhang, Y. T. Di, H. Y. Liu, N. C. Kong, S. G. ao, C. S. Li, Y. Z. hang, S. L. Li, X. J. Hao, *Helv. Chim. Acta.* **2008**, *91*, 914–919; c) Y. T. Di, L. L. Liu, C. S. Li, Y. Z. hang, Q. Z. hang, S. Z. Mu, Q. Y. Sun, F. M. Yang, H. Y. Liu, X. J. Hao, *Helv. Chim. Acta* **2008**, *91*, 838–843; d) S. Z. Mu, J. S. Wang, X. S. Yang, H. P. He, C. S. Li, Y. T. Di, Y. W. ang, Y. Z. hang, X. F. ang, L. J. Huang, X. J. Hao, *J. Nat. Prod.* **2008**, *71*, 564–569.
- [7] a) S. Z. Mu, C. S. Li, H. P. He, Y. T. Di, Y. W. ang, Y. H. Wang, Z. Z. hang, Y. L. u, L. Z. hang, X. J. Hao, *J. Nat. Prod.* **2007**, *70*, 1628–1631; b) N. C. Kong, H. P. He, Y. H. Wang, S. Z. Mu, Y. T. Di, X. J. Hao, *J. Nat. Prod.* **2007**, *70*, 1348–1351; c) S. Z. Mu, Y. T. Di, H. P. He, Y. W. ang, Y. H. Wang, L. L. i, X. J. Hao, *Chem. Biodivers.* **2007**, *4*, 129–138; d) N. C. Kong, H. P. He, Y. H. Wang, S. Gao, Y. T. Di, X. J. Hao, *Helv. Chim. Acta* **2007**, *90*, 972–976.