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

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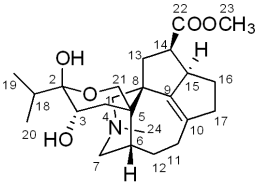
Two new Yuzurine-type *Daphniphyllum* alkaloids, daphlongamines I and J (**1**, **2**), were isolated from the fruits of *Daphniphyllum longeracemosum*. Their structures were established by spectroscopic methods, especially 2D NMR techniques.

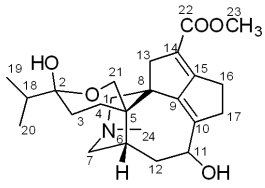
*Key words:* Daphniphyllum Alkaloids, Yuzurine, Daphlongamine, *Daphniphyllum longeracemosum*

## 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**.




**1**




**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 $H_2$-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

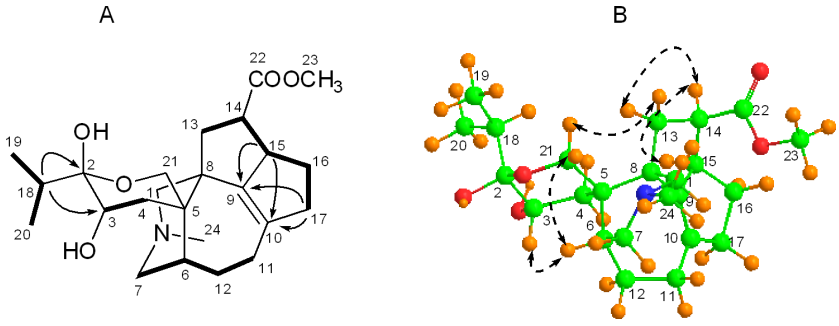



Fig. 1. (A) $^1$H-$^1$H COSY (bold) and HMBC (arrow, H→C) correlations of **1**. (B) NOESY correlations of **1**.

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

| No. | $\delta_H$ (mult., $J$ in Hz)[a] | $\delta_C$[a] | $\delta_H$ (mult., $J$ in Hz)[b] | $\delta_C$[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) | 41.1t | 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 $\beta$-orientation. Furthermore, H-14 and H-15 were determined to be in an $\alpha$-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 $C_{24}H_{35}NO_4$ 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 ($^1$H-$^1$H COSY, HSQC, and HMBC). In the HMBC spectrum, the observed cross-peaks of $H_2$-12 with C-11 at $\delta_C$ = 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 $H_2$-13 to C-14 and C-15, and $H_2$-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 $\delta$ values. Column chromatography was performed on silica gel H (10 – 40 $\mu$m; Qingdao Marine Chemical Factory) and Sephadex LH-20 (40 – 70 $\mu$m, 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 $CHCl_3$. The pH of the aqueous layer was roughly adjusted to pH = 10 with 3 % NaOH, and then exhaustively extracted with $CHCl_3$ to give the crude alkaloid. The crude extracts were subjected to a silica gel column ($CHCl_3$/MeOH, $1:0 \rightarrow 0:1$) to obtain six major fractions (F1 – F6). Fraction 3 was further chromatographed over a silica gel (300 – 400 mesh) column (PE/$Et_2NH$, $50:1 \rightarrow 10:1$) followed by a Sephadex LH-20 column eluted with $CH_3OH$ to afford daphlongamine J (**2**, 7.0 mg). Fraction 4 was subjected to a RP-18 silica gel column (MeOH-$H_2O$) to give 4 parts (P1 – P4). Part 3 was subjected to silica gel (PE/EtOAc/$Et_2NH$, $50:1:0.2 \rightarrow 10:1:0.2$) followed by Sephadex LH-20 column chromatography eluted with $CH_3OH$ to isolate daphlongamine I (**1**, 21.0 mg).

*Identification*

*Daphlongamine I (**1**)*: Colorless powder. – $^1$H NMR and $^{13}$C NMR data: see Table 1. – $[\alpha]_D^{24} = -35.03$ ($c$ = 0.59, $CH_3OH$). – IR (KBr): $\nu_{max}$ = 3432, 2932, 2835, 1734 $cm^{-1}$. – MS ((+)-ESI): $m/z$ = 420 $[M+H]^+$. – HRMS ((+)-ESI): $m/z$ = 420.2743 (calcd. 420.2749 for $C_{24}H_{38}NO_5^+$, $[M+H]^+$).

*Daphlongamine J (**2**)*: Colorless solid. – $^1$H NMR and $^{13}$C NMR data: see Table 1. – $[\alpha]_D^{27} = 39.02$ ($c = 0.39$, $CH_3OH$). – UV (MeOH): $\lambda_{max}(\log \varepsilon)$ = 295 (2.57). – IR (KBr): $\nu_{max}$ = 3383, 2928, 2873, 1702, 1628 $cm^{-1}$. – MS ((+)-ESI): $m/z$ = 418 $[M+H]^+$. – HRMS ((+)-ESI): $m/z$ = 418.2598 (calcd. 418.2593 for $C_{24}H_{36}NO_5^+$, $[M+H]^+$).

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