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Daphlongeranines A and B, Two Novel Alkaloids Possessing Unprecedented Skeletons from *Daphniphyllum longeracemosum*

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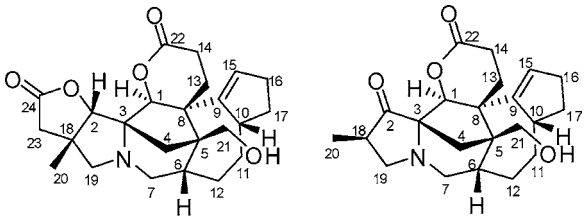
ABSTRACT

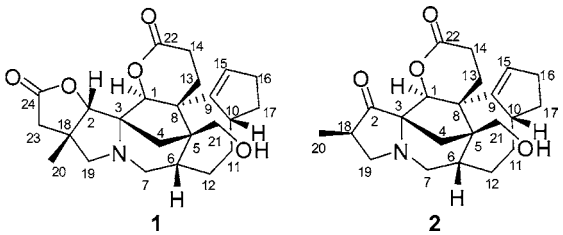


Daphlongeranine A (1) **Daphlongeranine B (2)**

Two novel alkaloids, daphlongeranines A (1) and B (2), with an unprecedented ring system, were isolated from the fruits of *Daphniphyllum longeracemosum*. Their unique structures and relative stereochemistries were established on the basis of spectroscopic and single-crystal diffraction analysis. The proposed biosynthetic pathway was also discussed. Compounds 1 and 2 showed weak inhibition on platelet aggregation.

Daphniphyllum alkaloids, elaborated by trees of the genus *Daphniphyllum* (Daphniphyllaceae), are a group of structurally diversified alkaloids with polycyclic skeletons,[1−3] which have attracted great interest as challenging projects for total synthesis[4] and biosynthetic research.[5]

In our continuing search for structurally unique and biogenetically interesting *Daphniphyllum* alkaloids,[2,6] two novel alkaloids, daphlongeranines A (**1**) and B (**2**), with an unprecedented hepta- and hexacyclic ring system, respectively, were isolated. Herein, we present the isolation and structural elucidation of **1** and **2**.



1 **2**

The air-dried and powdered fruits of *D. longeracemosum* Rosenth. were extracted with 95% EtOH, and the crude extract was adjusted to pH 2 with 2% HCl. After extracting with petroleum ether (PE) and $CHCl_3$, the aqueous layer,

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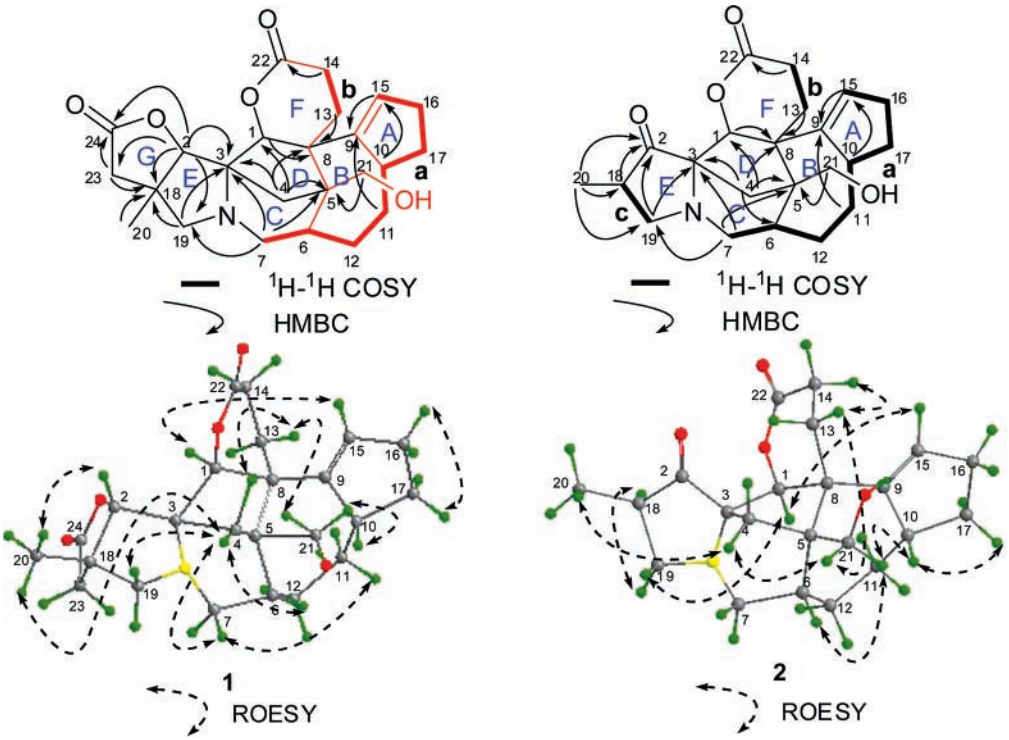

Figure 1. Key two-dimensional NMR correlations for daphlongeranines A (**1**) and B (**2**).

alkalinized to pH 10 with 3% NaOH, was then extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to silica gel column chromatography ($CHCl_3$/MeOH, 1:0 → 0:1), from which a fraction ($CHCl_3$/MeOH, 20:1) was chromatographed over a series of silica gel columns to afford daphlongeranines A (**1**, 0.00035%) and B (**2**, 0.00025%).

Daphlongeranine A (**1**)[7] was isolated as a block crystal (PE/acetone 2:1). Its molecular formula was demonstrated as $C_{24}H_{31}NO_5$ by HR-ESI-MS (*m*/*z* 414.2284, $[M + H]^+$, calcd 414.2280), with ten degrees of unsaturation. The IR spectrum suggested the presence of a hydroxyl (3426 cm^{-1}) and two carbonyl groups (1774 and 1737 cm^{-1}). All 24 carbon signals observed in the ^{13}C NMR and DEPT spectrum could be classified into one trisubstituted olefin, two carbonyls, four sp^3 quaternaries, four sp^3 methines, eleven sp^3 methylenes together with one methyl. Among them, two methylenes (δ_C 64.2, δ_H 2.88 and 2.70; δ_C 45.8, δ_H 3.10 and 2.32) and one quaternary carbon (δ_C 72.7) were ascribed as attaching to a nitrogen atom, whereas one methylene (δ_C 64.4, δ_H 3.94 and 3.60) and two methines (δ_C 92.8, δ_H 4.26; δ_C 91.2, δ_H 4.58) were ascribed as bearing oxygen atoms. Besides the three degrees of unsaturation ascribed to two carbonyl groups and one olefin, seven degrees of unsaturation could only be assigned to the presence of a heptacyclic skeleton.

The structure of **1** was elucidated to be composed of two moieties (Figure 1) by analyses of 2D NMR ($^1H-^1H$ COSY, HSQC, and HMBC). The $^1H-^1H$ COSY spectrum revealed connectivity of two structural fragments: **a** (C-6 to C-7 and C-12, C-10 to C-12 and C-17, C-15 to C-17), and **b** (C-13 to C-14) drawn with bold bonds in Figure 1. Furthermore, HMBC correlations proved the presence of the right moiety, as shown in Figure 1 (in red), containing two fused ring systems (ring A and B) with a double bond between C-9 and C-15, a propionate at C-8, an oxomethylene at C-5, and a methylene at C-6, which were the common structural units in yuzurimine- and daphnilactone-type alkaloids.[1]

In the left moiety, HMBC correlations of H-7α (δ_H 2.32) and H_2-19 (δ_H 2.88 and 2.70) with C-3 (δ_C 72.7) and of H_2-7 with C-19 (δ_C 64.2) indicated that C-3, C-7, and C-19

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(7) Daphlongeranine A (**1**): light yellow block crystals (PE/acetone 2:1); mp 227−229 °C; $[\alpha]^{22}_D$ 142.7 (*c* 0.16, $CHCl_3$); IR (KBr) ν_{max} 3426, 2925, 1774, 1737, 1638, 1457, 1191, 755 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESI-MS *m*/*z* 414 $(M + 1)^+$; HR-ESI-MS *m*/*z* 414.2284 ($[M + H]^+$; calcd for $C_{24}H_{31}NO_5$, 414.2280).

connected to each other through the nitrogen atom. Correlation signals of H_2-4 to C-3 (^2J) and C-5 (^2J), as well as of H_2-7 to C-5 (^3J), demonstrated the existence of a six-membered heterocyclic ring C (C-3, C-4, C-5, C-6, C-7, and nitrogen). Meanwhile, the formation of ring D was similarly verified by correlations of H-1 to C-8 and of H_2-4 to C-1 and C-8, respectively. HMBC cross-peaks of H-2 (δ_H 4.26) with C-3, C-18, and C-19, respectively, along with the correlations of H_2-19 to C-18 indicated the existence of ring E (C-2, C-3, nitrogen, C-19, and C-18). The presence of the five-membered lactone ring G (C-2, C-18, C-23, C-24, and oxygen) and assignment of the methyl (C-20, δ_C 24.7) at C-18 were achieved by the ^3J correlations of H-2 to C-23 and C-24 and the ^2J correlations of H_2-23 to C-18 and C-24, as well as of H_3-20 to C-2 (^3J).

However, the correlation between H-1 and C-22 could not be observed in the HMBC spectrum; therefore, the formation of δ-lactone ring F was eventually resolved by the X-ray experiment. Thus, the gross structure of daphlongeranine A (**1**), possessing a unique heptacyclic ring system with two lactones, was established as shown in Figure 1.

The relative stereochemistry of **1** was elucidated by analysis of the ROESY spectrum as shown in Figure 1 and was further confirmed by an X-ray crystallographic study.[8] In the crystal structure (Figure 2), H-2, H-6, and H-10 took

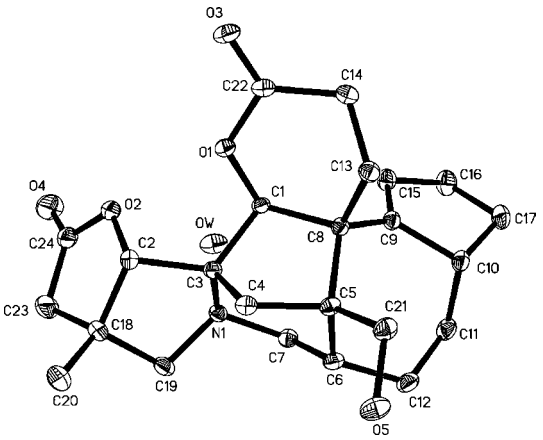


Figure 2. X-ray crystal structure of **1** (ORTEP drawing).

the β configuration, and H-1 was α facial. The rings A, D, E, and G took an envelope conformation; the rings B and C took a chair conformation; and the ring F took a twist-boat conformation.

Daphlongeranine B (**2**)[9] was obtained as needle crystals (PE/acetone 2:1). Its molecular formula, $C_{22}H_{29}NO_4$, was established by HR-ESI-MS (*m*/*z* 372.2174, $[M + H]^+$, calcd 372.2174), corresponding to nine degrees of unsaturation. The IR spectrum indicated the absorption of carbonyl (1755 cm^{-1}) and hydroxyl (3439 cm^{-1}) groups.

Extensive comparison of ^1H NMR, ^{13}C NMR, and DEPT spectra of compound **2** with **1** (see Table 1) suggested that

Table 1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR Data of Daphlongeranines A (**1**)[a] and B (**2**)[b]

no.	δ_H[a]	δ_C[a]	δ_H[b]	δ_C[b]
1	4.58 (1H, d, 1.6)	91.2	4.67 (1H, d, 9.2)	85.0
2	4.26 (1H, s)	92.8	---	211.4
3	---	72.7	---	72.7
4a	1.66 (1H, dd, 2.0, 12.0)	32.5	2.36 (1H, m)	39.3
4b	1.52 (1H, d, 12.0)		1.43 (1H, m)	
5	---	52.3	---	52.2
6	2.10−2.04 (1H, m)	35.1	2.25 (1H, m)	40.1
7α	2.32 (1H, m)	45.8	2.58 (1H, m)	50.6
7β	3.10 (1H, t, 14.0)		2.73 (1H, dd, 4.8, 11.2)	
8	---	49.2	---	50.0
9	---	151.1	---	151.1
10	2.56−2.52 (1H, m)	44.9	2.57 (1H, m)	44.7
11	1.52−1.37 (2H, m)	30.2	1.65−1.49 (2H, m)	31.5
12	1.30 (2H, m)	24.8	1.92 (1H, m)	25.8
			1.52 (1H, m)	
13a	1.90−1.81 (1H, m)	25.8	2.00 (1H, m)	24.9
13b	1.30−1.25 (1H, m)		1.34 (1H, m)	
14a	2.35−2.28 (1H, m)	26.4	2.42−2.27 (2H, m)	25.9
14b	2.19−2.15 (1H, m)			
15	5.38 (1H, s)	126.0	5.29 (1H, s)	126.9
16α	2.10−2.04 (1H, m)	29.1	2.17 (1H, m)	29.3
16β	2.33−2.29 (1H, m)		2.43 (1H, m)	
17α	1.52−1.50 (1H, m)	34.8	1.61 (1H, dd, 6.8, 12.4)	34.8
17β	1.89−1.81 (1H, m)		1.96 (1H, m)	
18	---	42.2	2.51 (1H, m)	39.8
19α	2.70 (1H, d, 9.2)	64.2	2.92 (1H, t, 9.0)	54.4
19β	2.88 (1H, d, 9.2)		2.78 (1H, dd, 1.4, 9.0)	
20	1.18 (3H, s)	24.7	1.16 (1H, d, 7.6)	16.1
21a	3.94 (1H, d, 10.8)	64.4	4.07 (1H, d, 11.0)	64.8
21b	3.60 (1H, d, 10.8)		3.77 (1H, d, 11.0)	
22	---	173.7	---	171.6
23α	2.55 (1H, d, 18.0)	43.9	---	---
23β	2.45 (1H, d, 18.0)			
24	---	177.5	---	---

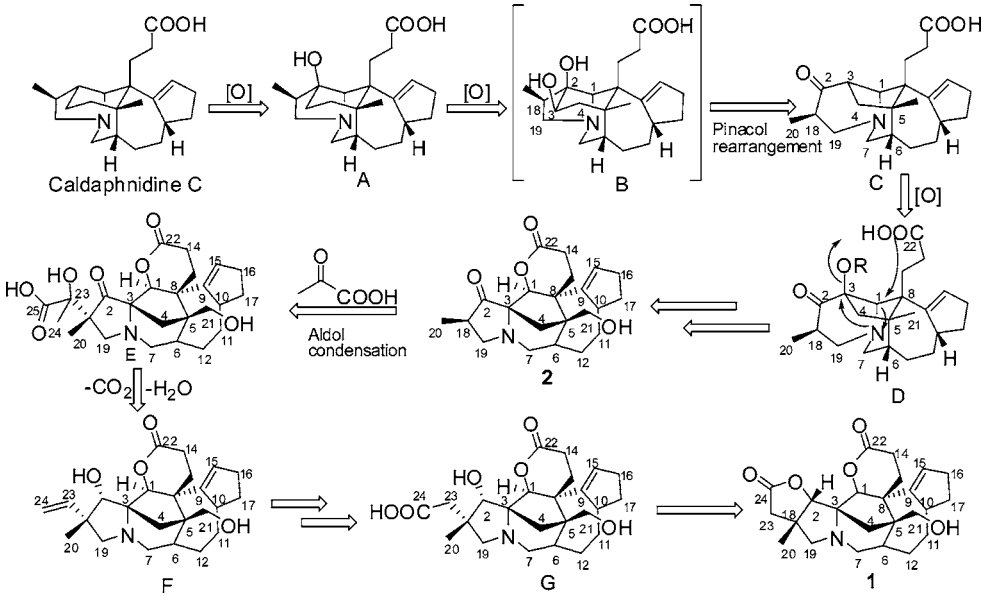
[a] NMR data for **1** (in $CDCl_3$:CD_3OD, 9:1). [b] NMR data for **2** (in $CDCl_3$).

2 had a skeleton partially similar to **1**. The presence of rings A−D and F in alkaloid **2** was further demonstrated by 2D NMR ($^1H-^1H$ COSY, TOCSY, HSQC, and HMBC) data similar to compound **1**, as shown in Figure 1. The $^1H-^1H$ COSY and TOCSY spectra (Figure 1) of **2** implied the presence of unit **c** (C-18 to C-19), and HMBC correlations of H_3-20 to C-2 (^3J), C-18 (^2J) and C-19 (^2J), H-18 and H-19α to C-2 established the formation of ring E with C-20 at C-18. Thus, the gross structure of **2** with a skeleton similar to **1** was assigned as shown in Figure 1.

(8) Crystallographic data of **1**: $C_{24}H_{31}NO_5$, M = 413.51, monoclinic, space group $P21$, a = 13.576(1) Å, b = 8.049(2) Å, c = 10.772(1) Å, β = 109.893(4)°, V = 1106.9(2) Å^3, Z = 2, crystal dimensions 0.25 × 0.40 × 0.60 mm^3 were used for measurements on a MAC DIP-2030K diffractometer with a graphite monochromator (ω scan, 2θ max = 50.0°), Mo Kα radiation. The total number of independent reflections measured was 2124, of which 2093 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: R_f = 0.0381, wR2 = 0.1026 ($w = 1/\sigma|F|^2$), S = 1.050. The crystal structure (**1**) was solved by the direct method SHELXS-97, expanded using geometrical calculations and difference Fourier techniques, and refined by least-squares calculations. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Date Centre (deposition number: CCDC 634546). Copies of this data can be obtained free of charge via http://www.ccdc.cam.ac.uk/deposit (or from Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K., facsimile: (44) 01223 336033, e-mail: deposit@ccdc.cam.ac.uk).

(9) Daphlongeranine B (**2**): colorless needle crystals (PE/acetone 2:1); mp 241−243 °C; $[\alpha]^{17}_D$ 47.2 (*c* 0.41); IR (KBr) ν_{max} 3439, 2931, 1755, 1637, 1455, 1052, 756 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESI-MS *m*/*z* 372 $(M + 1)^+$; HR-ESI-MS *m*/*z* 372.2174 ($[M + H]^+$; calcd for $C_{22}H_{29}NO_4$, 372.2174).

Scheme 1. Plausible Biosynthetic Pathway of **1** and **2**



The relative configuration of compoud **2**, as shown in Figure 1, was deduced from its ROESY spectrum and the comparison of NMR data with **1**. Correlations of H_2-21/H_2-13, H-4a, H-6, and H-10, and H-15/H-1 indicated that alkaloid **2** had the same configuration as **1**. Meanwhile, the NOE correlation of H-4a/H-20 implied that Me-18 (C-20) was in β configuration.

Due to the structural similarity between compounds **1** and **2**, the latter should be the precursor of the former. Therefore, a plausible biogenetic pathway for them is proposed as shown in Scheme 1. The biosynthetic precursor of both **1** and **2** could be admitted to caldaphnidine C,[10] which should produce a key intermediate C through a pinacol rearrangement.[2c] The oxidation of intermediate C at C-3 followed by further rearrangement might produce daphlongeranine B (**2**) through the formation of a C-3−N bond[11] and the following esterification. After an aldol-type condensation reaction with pyruvic acid and subsequent dehydration and decarboxylation, daphlongeranine B (**2**) will eventually convert to daphlongeranine A (**1**).

Compounds **1** and **2** showed weak inhibition on vitro platelet aggregation induced by ADP, PAF, and AA. At 240 μM concentration, the inhibition of aggregation for **1** was 38.4 ± 17.2%, 37.5 ± 14.7%, and 19.8 ± 7.0% and for **2** was 38.7 ± 10.5%, 28.3 ± 9.4%, and 24.0 ± 11.2%, respectively.

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Supporting Information Available: Experimental procedures, 1D and 2D NMR spectra for compounds **1** and **2**, and X-ray crystallographic data of compound **1** (PDF and CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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