

## Alkaloids from the Leaves of *Daphniphyllum longeracemosum*

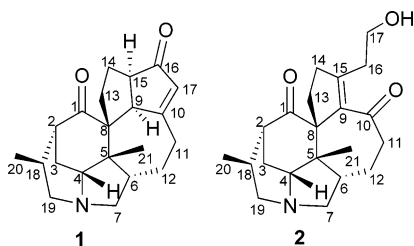
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Two new C-21 *Daphniphyllum* alkaloids, longeracinphyllins A (**1**) and B (**2**), have been isolated from the leaves of *Daphniphyllum longeracemosum*. Longeracinphyllin A (**1**) is a new daphnilongeranin B type alkaloid with a rearranged  $\alpha,\beta$ -unsaturated ketone group, and the structure is supported by X-ray crystal data. Longeracinphyllin B (**2**) is a new *seco*-10,17-daphnilongeranin B type alkaloid.

Plants of the *Daphniphyllum* genus are a prolific source of diverse alkaloids with highly complex polycyclic systems.<sup>1</sup> They were demonstrated to be derived from squalene-like intermediates by isotope tracer experiments and biomimetic total synthesis.<sup>2,3</sup> Recently, many new alkaloids were isolated from this species,<sup>4–8</sup> which have attracted interest as challenging targets for total synthesis<sup>9</sup> as well as biogenetic studies.<sup>1–8</sup> In search for structurally unique and biogenetically interesting *Daphniphyllum* alkaloids, two new C-21-fused alkaloids (**1**, **2**), along with four known ones, calyciphylline A,<sup>4c</sup> longistylumphylline A,<sup>5b</sup> deoxyisocalyciphylline B,<sup>5e</sup> and deoxycalyciphylline B,<sup>5c</sup> have been isolated from the leaves of *D. longeracemosum*. Longeracinphyllin A (**1**) possesses a daphnilongeranin B type skeleton with a rearranged  $\alpha,\beta$ -unsaturated ketone group, and its structure was supported by X-ray crystal data. Longeracinphyllin B (**2**) is a new *seco*-10,17-daphnilongeranin B type alkaloid. Herein, we describe the isolation and structural elucidation of the new alkaloids.



The HRESIMS data ( $[M + H]^+$ ,  $m/z$  326.2113) of compound **1** indicated the molecular formula  $C_{21}H_{27}NO_2$ , corresponding to nine degrees of unsaturation. IR absorption bands at 1694 and 1604  $cm^{-1}$  implied the presence of a conjugated carbonyl group. The  $^{13}C$  NMR and DEPT spectra (Table 2) revealed 21 carbon signals, including one trisubstituted olefin, two carbonyls, two  $sp^3$  quaternary carbons, six  $sp^3$  methines, seven  $sp^3$  methylenes, and two methyls. Two methylenes ( $\delta_C$  58.9,  $\delta_H$  3.07 and 2.52;  $\delta_C$  49.0,  $\delta_H$  2.76 and 2.45) and one methine ( $\delta_C$  67.3,  $\delta_H$  3.29) were attributed to those bearing a nitrogen atom. Since one double bond and two carbonyl groups accounted for three degrees of the unsaturation, **1** was inferred to possess six rings.

Three spin systems: **a** (C-2 to C-4 and C-18, and C-18 to C-20), **b** (C-6 to C-7 and C-11), and **c** (C-15 to C-9 and C-13) were established as shown in Figure 1, by extensive analysis of 2D NMR data of **1**, including  $^1H-^1H$  COSY, HMQC, and HMBC. Long-range HMBC correlations ( $^3J$ ) for H-7a to C-19 ( $\delta_C$  49.0) and for

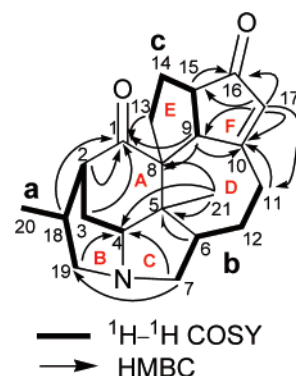


Figure 1. Selected 2D NMR correlations of compound **1**.

H-7b to C-4 ( $\delta_C$  67.3) confirmed that C-4, C-7, and C-19 were connected through a nitrogen atom. The correlations of H-4, H-6, and H<sub>3</sub>-21 to C-5 indicated that C-4 and C-6 ( $\delta_C$  49.8) were connected via the quaternary C-5 ( $\delta_C$  50.7) with an angular C-21 methyl ( $\delta_C$  26.0), which was further confirmed by correlations of H-3 and H-7 to C-5. One carbonyl group ( $\delta_C$  217.0) assigned at C-1 was indicated by the correlations of H-3 and H-18 to C-1. The HMBC correlations of H-9 and H-13 to C-1 and C-8 ( $\delta_C$  63.8) revealed the connectivity of C-1 to C-9 ( $\delta_C$  49.0) and C-13 ( $\delta_C$  30.5). Quaternary carbons C-5 and C-8 were linked by the HMBC correlations of H-4, H-6, and H-21 to C-8. The correlations of H-9, H-11, and H-17 to  $sp^2$  quaternary carbon C-10 ( $\delta_C$  184.0) indicated that C-9 and C-11 ( $\delta_C$  27.3) were attached to C-10 of the  $\Delta^{10(17)}$  double bond. In addition, the other carbonyl group was assigned at C-16 ( $\delta_C$  211.4) on the basis of the HMBC correlations of H-15 and H-17 to C-16, and H-15 to C-17 ( $\delta_C$  131.9). Thus, the planar structure of **1**, possessing a daphnilongeranin B type skeleton with a rearranged  $\alpha,\beta$ -unsaturated ketone group, was unambiguously established as shown in Figure 1.

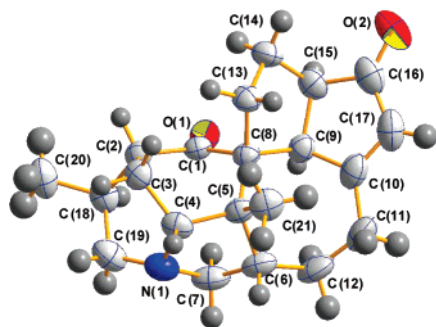
The relative configuration of **1** was elucidated by a ROESY experiment. Correlation of H<sub>3</sub>-21/H-13b indicated that C-13 and C-21 were  $\beta$ -oriented, and correlations of H<sub>3</sub>-21/H-4, H<sub>3</sub>-21/H-6, H-6/H-7b, H-13b/H-14b, H-13a/H-2, H-2/H<sub>3</sub>-20, and H<sub>3</sub>-20/H-19b indicated that H-2, H-4, H-6, H-7b, H-14b, H-19b, and H<sub>3</sub>-20 were cofacial. H-9, H-14a, and H-15 were  $\alpha$ -oriented due to correlations of H-9/H-15 and H-15/H-14a. Thus the rings A/C, A/D, C/D, D/E, and E/F were all *cis*-fused. In addition, ROESY correlation of H-7a/H-18 indicated that **1** adopted a conformation with outside direction of the nitrogen lone pair.

The structure and the relative configuration of **1** were further confirmed by X-ray crystallographic analysis.<sup>10</sup> The ORTEP drawing, with the atom-numbering indicated, is shown in Figure 2. In the crystal structure, rings A and B adopted a twist-boat

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**Figure 2.** ORTEP drawing with atom labeling of **1**. Thermal ellipsoids are shown at the 50% probability level. Labels for H atoms are omitted for clarity.

**Table 1.**  $^1\text{H}$  [ $\delta_{\text{H}}$  ( $J$ , Hz)] NMR Data of Longeraciphyllins A (**1**) and B (**2**) at 300 K

no.	<b>1</b> <sup>a</sup>	<b>2</b> <sup>b</sup>
2	2.01 (1H, m)	2.10 (1H, br d, 3.3)
3a	1.98 (1H, m)	2.08 (1H, br dd, 15.4, 3.8)
3b	2.21 (1H, br dd, 14.7, 4.2)	2.47 (1H, m)
4	3.29 (1H, br d, 4.2)	3.51 (1H, br d, 2.2)
6	2.28 (1H, m)	2.28 (1H, m)
7a	2.52 (1H, m)	2.77 (2H, m)
7b	3.07 (1H, dd, 9.3, 6.8)	
9	4.14 (1H, d, 5.2)	
11a	2.85 (2H, m)	2.28 (1H, m)
11b		2.41 (1H, m)
12a	1.76 (1H, m)	1.78 (1H, m)
12b	2.00 (1H, m)	2.02 (1H, brdd, 14.3, 3.3)
13a	1.86 (1H, m)	1.79 (1H, m)
13b	1.70 (1H, m)	2.67 (1H, br dd, 11.5, 8.3)
14a	1.24 (1H, m)	2.36 (1H, m)
14b	1.83 (1H, m)	2.53 (1H, m)
15	2.69 (1H, dd, 9.8, 5.2)	
16a		2.48 (1H, m)
16b		2.60 (1H, m)
17	6.02 (1H, d, 1.0)	3.67 (2H, m)
18	2.91 (1H, m)	2.80 (1H, m)
19a	2.76 (1H, dd, 14.1, 3.9)	2.54 (1H, m)
19b	2.45 (1H, dd, 14.1, 6.3)	2.79 (1H, m)
20	0.99 (3H, d, 6.8)	0.99 (3H, d, 6.0)
21	1.14 (3H, s)	1.34 (3H, s)

<sup>a</sup> Measured in  $\text{CDCl}_3$ . <sup>b</sup> Measured in  $\text{CDCl}_3/\text{CD}_3\text{OD}$  (1:9).

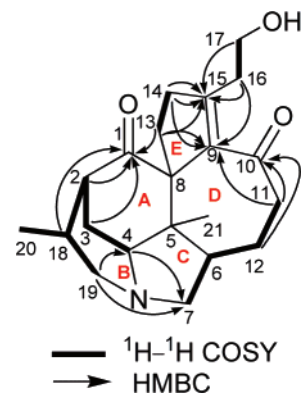
**Table 2.**  $^{13}\text{C}$  [ $\delta_{\text{C}}$ ] NMR Data of Longeraciphyllins A (**1**) and B (**2**) at 300 K

no.	<b>1</b> <sup>a</sup>	<b>2</b> <sup>b</sup>	no.	<b>1</b> <sup>a</sup>	<b>2</b> <sup>b</sup>
1	217.0	220.2	12	24.1	19.5
2	44.1	44.6	13	30.5	34.4
3	20.3	20.5	14	24.8	36.6
4	67.3	66.2	15	49.8	156.0
5	50.7	53.9	16	211.4	33.7
6	49.8	49.4	17	131.9	60.2
7	58.9	54.0	18	34.3	32.3
8	63.8	72.3	19	49.0	50.2
9	48.9	140.0	20	18.6	19.2
10	184.0	208.4	21	26.0	22.6
11	27.3	37.2			

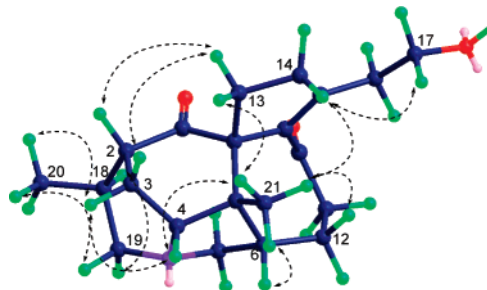
<sup>a</sup> Measured in  $\text{CDCl}_3$ . <sup>b</sup> Measured in  $\text{CDCl}_3/\text{CD}_3\text{OD}$  (1:9).

conformation, ring D a boat conformation, and ring E an envelope conformation, and ring C an envelope conformation, and ring E was almost planar.

The HRESIMS data ( $[\text{M} + \text{H}]^+$ ,  $m/z$  344.2228) of compound **2** suggested the molecular formula  $\text{C}_{21}\text{H}_{29}\text{NO}_3$ , corresponding to eight degrees of unsaturation. The IR absorptions at 1689, 1660, and 1615  $\text{cm}^{-1}$  suggested the presence of a conjugated ketone group. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR (Tables 1 and 2) spectra of **2** were analogous to daphnilongeranin B<sup>5a</sup> except that an oxygen-bearing  $\text{sp}^3$  methylene ( $\delta_{\text{C}}$  60.2) in **2** replaced an  $\text{sp}^3$  methine in the latter. All of these suggested **2** possessed a pentacyclic skeleton with a hydroxymethyl.



**Figure 3.** Selected 2D NMR correlations of compound **2**.



**Figure 4.** Energy-minimized model of **2** illustrating the major ROESY correlations used to define the relative configuration.

The planar structure of **2** was established by 2D NMR (including HMQC,  $^1\text{H}$ - $^1\text{H}$  COSY, and HMBC) analysis, which showed rings A-C of **2** were identical to those of **1**. One ketone group ( $\delta_{\text{C}}$  220.2) was assigned to C-1 on the basis of the correlations of H-2, H-3a, and H-18 with C-1 and C-10 ( $\delta_{\text{C}}$  208.4) and by the HMBC correlations of H-11 and H-12 to C-10. The additional correlations of H-13, H-14, H-16, and H-17 to C-15 ( $\delta_{\text{C}}$  156.0) and of H-11, H-13, H-14, and H-16 to C-9 clearly indicated the presence of a  $\Delta^{9(15)}$  double bond. Thus, the planar structure of **2**, possessing a novel *seco*-10,17-longeraciphyllin A type skeleton, was established as shown in Figure 3.

The configuration of **2** (Figure 4) was analogous to that of **1** due to their similar ROESY correlations, except that ring D adopted a chair conformation, which was suggested by the ROESY correlation between H-12b and H<sub>3</sub>-21. These data were also consistent with the results of a conformational analysis after energy minimization, using MM2 followed by semiempirical methods (AM1).

## Experimental Section

**General Experimental Procedures.** Melting points were obtained on an XRC-1 apparatus and are uncorrected. Optical rotations were carried out on a Perkin-Elmer model 241 polarimeter. UV spectra were obtained in a UV 210A spectrometer. IR spectra were measured in a Bio-Rad FTS-135 spectrometer with KBr pellets. ESI and high-resolution mass spectra were recorded using a Finnigan MAT 90 instrument and VG Auto Spec-3000 spectrometer, respectively.  $^1\text{H}$  and 2D NMR spectra were measured on a Bruker DRX-500 instrument, while  $^{13}\text{C}$  NMR spectra were recorded on a Bruker AM-400 spectrometer. Chemical shifts were reported using residual  $\text{CDCl}_3$  ( $\delta_{\text{H}}$  7.26 and  $\delta_{\text{C}}$  77.0) as an internal standard. Column chromatography was performed on silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, P. R. China), silica gel H (10–40  $\mu\text{m}$ ; Qingdao Marine Chemical Inc.), and amino silica gel (100–75  $\mu\text{m}$ , Fuji Silysia Chemical LTD, Japan), and precolated silica gel GF254 plates (Qingdao Haiyang Chemical Plant, Qingdao, P. R. China) were used for TLC.

**Plant Material.** The leaves of *D. longeracemosum* were collected in Wenshan of Yunnan Province, People's Republic of China, in December 2004. The sample was identified by Prof. Xun Gong, and a

voucher specimen (KIB 05020503) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy Sciences.

**Extraction and Isolation.** Air-dried and powdered leaves of *D. longeracemosum* (11 kg) were extracted with 95% EtOH, and the extract was partitioned between EtOAc and 0.001 N HCl. The aqueous layer was alkalized to pH 10 with 2 N NaOH followed by exhaustive extraction with CHCl<sub>3</sub>. CHCl<sub>3</sub>-soluble materials were roughly separated by an amino silica gel column chromatography, using CHCl<sub>3</sub>/MeOH (from 0:1 to 1:0) as eluent, to give 10 fractions. Fraction 2 was purified by repeated normal silica gel filtration (petroleum ether/Et<sub>2</sub>NH, 10:1) to afford **1** (0.12 g). Fraction 6 was chromatographed over a silica gel column (CHCl<sub>3</sub>/CH<sub>3</sub>OH, 10:1) and then purified by H silica gel (petroleum ether/Et<sub>2</sub>NH, 10:1) to yield **2** (0.05 g).

**Longeraciphyllin A (1):** colorless prisms (CH<sub>3</sub>OH); mp 160–162 °C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> -246.2 (c 0.57, CHCl<sub>3</sub>); UV (CHCl<sub>3</sub>)  $\lambda_{\max}$  242, 226 nm; IR  $\nu_{\max}$  2900, 1694, and 1604 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, see Tables 1 and 2; ESIMS *m/z* 326 [M + H]<sup>+</sup>; HRESIMS *m/z* 326.2113 (calcd for C<sub>21</sub>H<sub>27</sub>NO<sub>2</sub>, 326.2120).

**Longeraciphyllin B (2):** amorphous white powder; [ $\alpha$ ]<sub>D</sub><sup>25</sup> 89.5 (c 0.53, CHCl<sub>3</sub>); UV (CHCl<sub>3</sub>)  $\lambda_{\max}$  247, 200 nm; IR  $\nu_{\max}$  3425, 2900, 1689, 1660, and 1615, 1200, 1175, and 755 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Tables 1 and 2; ESIMS *m/z* 344 [M + H]<sup>+</sup>; HRESIMS *m/z* 344.2228 (calcd for C<sub>21</sub>H<sub>29</sub>NO<sub>3</sub>, 344.2225).

**Acknowledgment.** The authors thank Prof. Xun Gong for collection and identification of the plant material.

**Supporting Information Available:** 1D, 2D NMR, HRESIMS, and IR spectra for compounds **1** and **2** and crystal data of **1**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References and Notes

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- (10) Crystal data for longeraciphyllin A (**1**): C<sub>21</sub>H<sub>27</sub>NO<sub>2</sub>; MW = 325.45; dimensions 0.10 × 0.20 × 0.30 mm; monoclinic system, space group *P*2<sub>1</sub>, *a* = 8.626(2) Å, *b* = 10.240(2) Å, *c* = 10.035(2) Å,  $\beta$  = 103.58–(3). *V* = 863.2(3) Å<sup>3</sup>, *Z* = 2, *d* = 1.252 g/cm<sup>3</sup>, *T* = 295 K. A crystal was used for measurements on a MAC DIP-2030K diffractometer with a graphite monochromator ( $\omega$  scans,  $2\theta_{\max}$  = 27.43°), Mo K $\alpha$  radiation. The total number of independent reflections measured was 1836, of which 1725 were observed ( $|F|^2 \geq 2\delta|F|^2$ ). Final indices: *R*<sub>f</sub> = 0.0490, *wR*<sub>2</sub> = 0.1229, *S* = 1.123, ( $\Delta/\sigma$ )<sub>max</sub> = 0.031, ( $\Delta/\rho$ )<sub>min</sub> = -0.139 e/Å, ( $\Delta/\rho$ )<sub>max</sub> = 0.157 e/Å. The crystal structure of **1** was solved by direct methods using SHELXS-97 (Sheldrich, G. M. University of Gottingen: Gottingen, Germany, 1997) and expanded using difference Fourier techniques, refined by the program and method SHELXL-97 (Sheldrich, G. M. University of Gottingen: Gottingen, Germany, 1997) and the full-matrix least-squares calculations. All H atoms were placed in geometrically idealized positions and constrained to ride on their parent atoms. The absolute configuration of this compound was not determined. Crystallographic data for the structure **1** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (deposition number: 293035). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 ore-mail: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk)).

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