Daphhimalenines C and D. New Alkaloids from *Daphniphyllum himalense*

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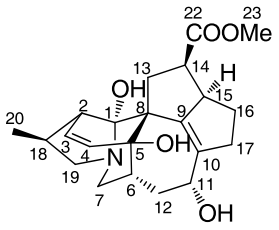
Z. Naturforsch. **2012**, *67b*, 407 – 410 / DOI: 10.5560/ZNB.2012-0004
Received January 2, 2012

Two new *Daphniphyllum* alkaloids, daphhimalenines C and D (**1** and **2**) were isolated from the leaves of *Daphniphyllum himalense*. Their structures were established by spectroscopic methods, especially 2D NMR techniques.

Key words: *Daphniphyllum* Alkaloids, Daphhimalenine C, Daphhimalenine D, *Daphniphyllum himalense*

Introduction

Daphniphyllum alkaloids with highly complex polycyclic systems constitute a group of structurally diverse natural products formed by plants of the genus *Daphniphyllum* [1 – 5]. Their unique ring systems have been attractive targets of total synthesis and biosynthetic studies [2, 3]. Previous chemical investigations on *Daphniphyllum* alkaloids conducted in our group have led to the isolation of a series of novel alkaloids with highly complex polycyclic skeletons [4]. On further investigations on the leaves of *D. himalense* [4a, 4c], two new alkaloids, daphhimalenines C and D (**1**, **2**), were obtained. Herein, we describe the isolation and structure elucidation of the new compounds **1** and **2**.



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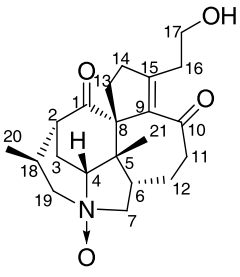

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Results and Discussion

Daphhimalenine C (**1**) was obtained as an optically active, colorless solid. The molecular formula of **1** was established as $C_{22}H_{29}NO_5$ by HR-ESI-MS (m/z = 388.2117, $[M+H]^+$; calcd. 388.2123), with nine degrees of unsaturation. IR absorptions implied the presence of hydroxyl (3405 cm^{-1}) and carbonyl (1707 cm^{-1}) functionalities. The ^{13}C NMR spectra (Table 1) revealed 22 carbon signals, comprising six quaternary carbon atoms (one ester carbonyl group and two olefinic carbon atoms), eight methines (two olefinic carbon atoms), six methylenes, one methyl and one methoxy group. Among them, two methylenes ($\delta_C = 61.5, 55.2$) were ascribed to those bearing the N atom, while one quaternary carbon ($\delta_C = 99.6$) was assigned as an amino ketal carbon. One methine (δ_C = 67.0) and one quaternary carbon atom (δ_C = 73.7) were attributed to those bearing an oxygen atom. Inspection of the NMR data of **1** (Table 1) indicated that its structure was related to the yuzurimine-type alkaloids [1] containing a hexacyclic ring system. Three structural fragments (Fig. 1a): **a** (C-2 to C-4, 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) shown with bold bonds were readily established by using a combination of 2D NMR spectra (including ^1H-^1H COSY, HSQC,

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of-daphhimalenines C (**1**) and D (**2**).

	1 ($CDCl_3$)		**2** (CD_3OD)	
No.	δ_H (mult., J in Hz)	δ_C (mult.)	δ_H (mult., J in Hz)	δ_C (mult.)
1	–	99.6 (s)	–	217.9 (s)
2	2.80 (br s)	45.3 (d)	2.31 (m)	43.1 (d)
3a	5.72 (dd, 10.0, 2.8)	126.9 (d)	2.43 (m)	20.0 (t)
3b	–		2.76 (m)	
4	5.61 (d, 10.0)	137.2 (d)	3.96 (t, 3.0)	90.3 (d)
5	–	73.7 (s)	–	54.3 (s)
6	1.93 (m)	39.5 (d)	3.00 (m)	47.1 (d)
7a	3.04 (dd, 12.0, 5.6)	55.2 (t)	3.08 (dd, 16.0, 8.0)	69.5 (t)
7b	3.20 (d, 12.0)		3.46 (t, 16.0)	
8	–	54.3 (s)	–	71.8 (s)
9	–	142.9 (s)	–	138.9 (s)
10	–	141.6 (s)	–	206.8 (s)
11a	4.04 (t, 3.6)	67.0 (d)	2.28 (m)	37.1 (t)
11b	–		2.28 (m)	
12a	1.88 (ddd, 10.0, 5.6, 4.0)	31.7 (t)	1.82 (m)	19.2 (t)
12b	2.54 (m)		2.12 (m)	
13a	2.66 (m)	39.1 (t)	1.82 (m)	34.9 (t)
13b	2.66 (m)		2.71 (dd, 16.5, 10.0)	
14a	2.98 (ddd, 9.6, 6.4, 3.6)	43.4 (d)	2.46 (m)	37.2 (t)
14b	–		2.62 (m)	
15	3.58 (m)	58.7 (d)	–	158.9 (s)
16a	1.28 (m)	27.8 (t)	2.41 (m)	34.3 (t)
16b	1.95 (t, 7.2)		2.41 (m)	
17a	2.52 (m)	41.2 (t)	3.66 (m)	60.9 (t)
17b	3.11 (m)		3.66 (m)	
18	2.80 (br s)	35.0 (d)	2.55 (m)	32.8 (d)
19a	2.36 (dd, 12.0, 2.0)	61.5 (t)	3.01 (m)	68.0 (t)
19b	3.62 (dd, 12.0, 8.8)		3.60 (dd, 16.5, 9.0)	
20	1.24 (d, 6.4)	19.3 (q)	1.11 (d, 8.5)	19.4 (q)
21			1.51 (s)	23.0 (q)
22	–	178.7 (s)		
23	3.71 (*s*)	51.9 (q)		

and HMBC). The connectivities of components **a**–**c** with the quaternary carbons and heteroatoms were finally established by HMBC experiments. HMBC correlations of H-4, H-6 and H_2-7 to C-5 ($\delta_C = 73.7$) indicated that **1** should have a 21-nor-yuzurimine skeleton, which was identical to the one of daphnezomine T [5]. The major difference was the presence of a hydroxyl group at C-11 ($\delta_C = 67.0$) in **1**, as judged by the HMBC correlations of H-6, H_2-12 and H_2-17 with C-11. Thus, the gross structure of daphhimalenine C was established as **1** shown in Fig. 1a.

The relative stereochemistry of **1** was elucidated by a ROESY spectrum as shown in a computer-generated 3D drawing (Fig. 1b). The correlations of H-3/H-4, H-3/H_3-20, H-4/H-6, H-13a/H-14, and H-14/H-15 indicated a half-chair configuraion of the cyclohexene ring and a chair configuration of the piperidine ring. The small coupling constants of H-11 ($\delta_H = 4.04$, t, $J = 3.6$ Hz) implied that H-11 had a β-orientation [6, 7].

The molecular formula of daphhimalenine D (**2**) was assigned as $C_{21}H_{29}NO_4$ from HR-ESI-MS ($m/z =$ 360.2168 ($[M+H]^+$; calcd. 360.2174), with eight degrees of unsaturation. The IR spectrum was indicative of the presence of hydroxyl (3440 cm^{-1}), ketone carbonyl (1689 cm^{-1}) and α, β-unsaturated ketone carbonyl (1672 and 1630 cm^{-1}) functionalities. The ^{1}H and ^{13}C NMR data (Table 1) of **2** revealed 21 carbon signals corresponding to six quaternary carbon atoms, four methines, nine methylenes and two methyls.

Detailed analysis of the NMR data of **2** and comparison with those of daphniyunnine B [8] indicated that both compounds shared the same backbone skeleton. The down-field shifts of C-4, C-7 and C-19 ($\delta_C = 90.3$, 69.5 and 68.0, resp.) in **2** as compared with those of daphniyunnine B ($\delta_C = 65.4$, 53.6 and 49.8, resp.) indicated that **2** was an N-oxide form of daphniyunnine B, which was further substantiated through 2D NMR experiments, including ^{1}H-^{1}H COSY, HSQC, HMBC, and ROESY spectra.

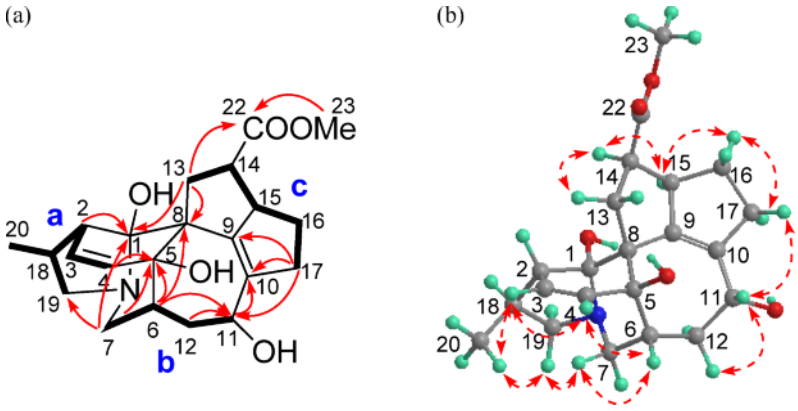

Fig. 1 (color online). (a) ^{1}H-^{1}H COSY (bold) and HMBC (arrow, H → C) correlations of **1**. (b) ROESY correlations of **1**.

The cytotoxic activities of **1** and **2** against the growth of human tumor cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480) by using the MTT method were evaluated [9]. The results indicated that **1** and **2** were inactive against the above cancer cells (IC_{50} > 40 μm).

Experimental Section

General experimental procedures

IR spectra were measured with a Bio-Rad FTS-135 spectrometer from KBr pellets. Optical rotations were obtained from 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 Ltd. Co., Qingdao, P. R. China), Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Lichroprep RP-18 gel (20–45 μm; Merck, Darmstadt, Germany).

Plant material

The leaves of *Daphniphyllum himalense* were collected in Yunnan Province, People's Republic of China, in October 2008. The material was identified by Prof. Heng Li, Kunming Institute of Botany, Chinese Academy of Sciences, and a specimen (KIB 08090418) 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 air-dried and powdered leaves of *D. himalense* (16.0 kg) were extracted with 95 % EtOH, and the crude extract was adjusted to pH = 2 with saturated tartaric acid. The acidic mixture was defatted with petroleum ether (PE) and then extracted with $CHCl_3$. The aqueous phase was basified to pH = 10 with saturated Na_2CO_3 and extracted with $CHCl_3$ to obtain the crude alkaloid fraction (60.0 g). This material was subjected to a silica gel column ($CHCl_3$-MeOH, 1 : 0 → 0 : 1) to obtain four major fractions (F1–F4). Fraction 2 (4.0 g) was eluted with $CHCl_3$-Me_2CO (20 : 1 → 5 : 1) and was further subjected to a Sephadex LH-20 column eluted with MeOH to afford **1** (30.0 mg). Fraction 3 (7.8 g) was subjected to a RP-18 silica gel column (MeOH-H_2O) to give four parts (P1–P4). Part 3 was subjected to silica gel (PE-Me_2CO-Et_2NH, 30 : 1 : 0.1 → 5 : 1 : 0.1) to give **2** (8.0 mg).

Identification

Daphhimalenine C (**1**): Colorless solid. – ^{1}H NMR and ^{13}C NMR data: see Table 1. – $[\alpha]_D^{28}$ = +72.9 (c = 0.35, $CHCl_3$). – IR (KBr): ν = 3405, 2951, 2923, 1707, 1437 cm^{-1}. – MS ((+)-ESI): m/z = 388 $[M+H]^+$. – HRMS ((+)-ESI): m/z = 388.2117 (calcd. 388.2123 for $C_{22}H_{30}NO_5^+$, $[M+H]^+$).

Daphhimalenine D (**2**): Colorless solid. – ^{1}H NMR and ^{13}C NMR data: see Table 1. – $[\alpha]_D^{25}$ = +79.9 (c = 0.20, MeOH). – UV (MeOH): 247 ($\log\varepsilon$ = 3.8). – IR (KBr): ν = 3440, 2923, 1698, 1671, 1630, 1450 cm^{-1}. – MS ((+)-ESI): m/z = 360 $[M+H]^+$. – HRMS ((+)-ESI): m/z = 360.2168 (calcd. 360.2174 for $C_{21}H_{30}NO_4^+$, $[M+H]^+$).

Acknowledgement

This research was supported by the Chinese National Natural Science Foudation (30830114 and 31100259), the Chinese Ministry of Science and Technology (2009CB522300),

and the Young Academic and Technical Leader Raising Foundation of Yunnna Province (2009CI072). We thank Prof. H. Li, Kunming Institute of Botany, Chinese Academy of Sciences (CAS), for the collection and identification of the plant material, and Prof. Y. Li, Kunming Institute of Botany, Chinese Academy of Sciences (CAS), for bioactivity testing.

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