Daphnoldines A and B, Two New Yuzurine Alkaloids with a Four-Ring System, from the Fruits of *Daphniphyllum oldhami*

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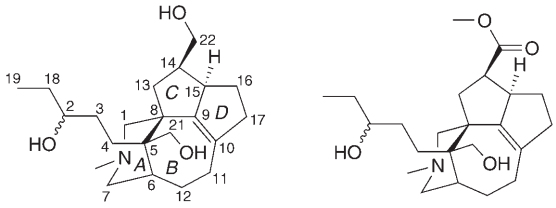
Two new yuzurine alkaloids, daphnoldines A (**1**) and B (**2**), possessing a rare four-ring system, were isolated from the fruits of *Daphniphyllum oldhami*. Their structures were established on the basis of extensive spectroscopic analyses. Daphnoldine A (**1**) is the first example of *Daphniphyllum* alkaloids bearing a hydroxymethyl group at C(14).

Introduction. – The complex and structurally diversified *Daphniphyllum* alkaloids have been attractive projects for natural products and organic chemistry [1–3]. The previous work carried out by our group on the alkaloids of the genus *Daphniphyllum* led to a series of novel skeleton *Daphniphyllum* alkaloids with highly complex polycyclic systems [4]. As a part of our continuing search for structurally unique and biogenetically interesting *Daphniphyllum* alkaloids, we have investigated the alkaloid constituents of *Daphniphyllum oldhami*, an evergreen shrub widely distributed in South China [5]. Two new yuzurine alkaloids, daphnoldines A (**1**) and B (**2**) with a rare four-ring system, were obtained from the fruits of *D. oldhami*, together with ten known ones: paxdaphnine A, paxdaphnine B [6], daphnezomic acid [7], yuzurimine-B [8], oldhamiphylline A [9], methyl 17-hydroxyhomodaphniphyllate [10], methyl homodaphniphyllate [11], daphnigracine, daphnigraciline [12], and yuzurine [13]. Daphnoldine A (**1**) is the first example of a *Daphniphyllum* alkaloid bearing a CH_2OH group at C(14). Here, we report the isolation and structure elucidation of these two new alkaloids.[1])



1[1]) **2**

[1]) Arbitratry atom numbering; for systematic names, see in the *Exper. Part.*

Results and Discussion. – Daphnoldine A (**1**), obtained as a white solid, showed a *pseudo*-molecular ion peak at *m/z* 364 ($[M+H]^+$) in the ESI mass spectrum, in accord with the molecular formula $C_{22}H_{37}NO_3$, as established by HR-ESI-MS (*m/z* 364.2836 ($[M+H]^+$, calc. 364.2852) indicating five degrees of unsaturation. The ^{13}C-NMR spectrum (*Table*) and the DEPT spectrum revealed the following signals: two Me groups (δ(C) 10.3, 46.2), a tetrasubstituted C=C bond (δ(C) 132.3, 148.7), two quaternary C-atoms (δ(C) 40.7, 46.5), twelve CH_2 and four CH groups. Among them, two low-field *triplet*s (δ(C) 63.0, 66.9) were assigned to CH_2OH groups. One Me group (δ(C) 46.2) and two CH_2 groups (δ(C) 55.5, 62.3) were linked to the N-atom, based on their chemical shifts in the ^{13}C-NMR. Besides one degree of unsaturation ascribed to the C=C bond, the remaining degrees of unsaturation must be accounted for by the presence of four rings in compound **1**.

By comparison of the NMR data of **1** with those of daphnigraciline [12], which was also isolated from the same plant, both compounds were similar, with the exception that compound **1** is lacking the hemiketal and the COOMe groups present in daphnigraciline, but has instead a CH_2-O (δ(C) 63.0) and a H–C–O (δ(C) 73.4) group. To determine the structure of compound **1**, 2D-NMR experiments (including ^{1}H,^{1}H-COSY, HMQC, and HMBC) were employed. Analysis of the 2D-NMR spectra established three fragments[1]) **a** (C(19)–C(18)–C(2)–C(3)–C(4)), **b** (C(7)–C(6)–C(12)–C(11)) and **c** (C(13)–C(14)–C(15)–C(16)–C(17), C(14)–C(22)) as shown with bold bonds in *Fig. 1*. Fragment **a**, the aliphatic chain with five C-atoms, was attached to C(5) based on the correlation between H_b–C(4) and C(5) in the HMBC spectrum. The connection between C(5) and C(6) in fragment **b** could be deduced by the correlation of H–C(6) to C(5) in the HMBC. In fragment **c**, a CH_2-OH group (δ(C) 63.0, C(22)) was attached to C(14), according to the correlations of H_a–C(22) to C(14) and C(15) in the HMBC spectrum. Furthermore, another CH_2-OH group (δ(C) 66.9) was supposed to be attached to C(5) based on the correlation of CH_2(21) to C(5) in the HMBC. The HMBC correlations of H_β–C(11) and H_β–C(17) to C(10) suggested that C(10) connected the fragments **b** and **c**. The connection between C(1) and C(13) *via* C(8) was exhibited by HMBC correlations of H_β–C(1) and H_α–C(13) to C(8), and H_α–C(13) to C(1). The above data could be explained by the new CH_2-OH group (C(22)) and the aliphatic chain with five C-atoms (fragment **a**) in **1**, which are different from other yuzurine type alkaloids. Therefore, the gross structure of daphnoldine A (**1**) was elucidated as indicated.

The relative configuration of **1** was deduced from ROESY correlations, as shown in the computer-generated 3D drawing (*Fig. 2*). The configuration of the CH_2-OH group (C(22)) as β-oriented was revealed by the NOE correlation between H_b–C(22) and H_a–C(21). However, the relative configuration at C(2) remains to be established.

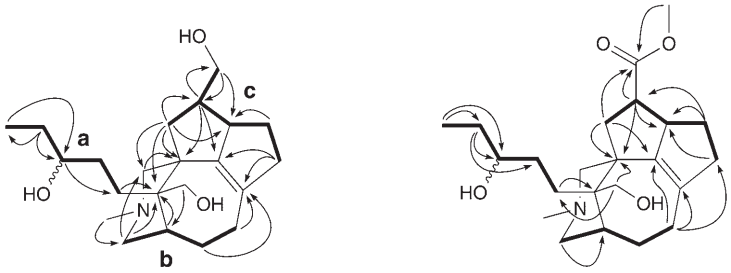
Daphnoldine B (**2**) was obtained as a light yellow solid and has the molecular formula $C_{23}H_{37}NO_4$ according to the HR-ESI-MS (*m/z* 392.2809 ($[M+H]^+$, calc. 392.2801), indicating six degrees of unsaturation. All 23 C-atom signals were observed in the ^{13}C-NMR spectrum (*Table*), and the DEPT spetrum revealed the presence of three Me groups (δ(C) 9.9, 46.3, 51.7), two sp^2 quaternary C-atoms (δ(C) 133.3, 147.8), two sp^3 quaternary C-atoms (δ(C) 40.8, 47.7), one CO group (δ(C) 178.5), eleven CH_2 and four H–C groups. The ^{1}H-NMR and ^{13}C-NMR also indicated that the two CH_2

Table. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2** *at 400 and 100 MHz, resp.* Measured in $CDCl_3$; δ in ppm, J in Hz.

	1[1])		**2**[1])	
	δ(H)	δ(C)	δ(H)	δ(C)
H_α–C(1)	2.62–2.68 (*m*)	55.5 (*t*)	2.62–2.69 (*m*)	56.3 (*t*)
H_β–C(1)	2.55–2.59 (*m*)		2.51–2.60 (*m*)	
H–C(2)	3.64–3.69 (*m*)	73.4 (*d*)	3.41–3.48 (*m*)	73.9 (*d*)
H_a–C(3)	1.48–1.54 (*m*)	29.2 (*t*)	1.86–1.94 (*m*)	28.4 (*t*)
H_b–C(3)	1.24–1.27 (*m*)		1.14 (*dt*, J = 10.0, 2.4)	
H_a–C(4)	1.94–1.97 (*m*)	21.6 (*t*)	1.85–1.94 (*m*)	24.2 (*t*)
H_b–C(4)	1.64–1.67 (*m*)		1.72–1.81 (*m*)	
C(5)		40.7 (*s*)		40.8 (*s*)
H–C(6)	1.82–1.86 (*m*)	36.6 (*d*)	1.77–1.86 (*m*)	36.6 (*d*)
H_α–C(7)	2.32–2.36 (*m*)	62.3 (*t*)	2.29–2.36 (*m*)	62.9 (*t*)
H_β–C(7)	2.37–2.41 (*m*)		2.37–2.43 (*m*)	
C(8)		46.5 (*s*)		47.7 (*s*)
C(9)		148.7 (*s*)		147.8 (*s*)
C(10)		132.3 (*s*)		133.3 (*s*)
H_α–C(11)	2.54–2.58 (*m*)	26.9 (*t*)	2.45–2.58 (*m*)	27.2 (*t*)
H_β–C(11)	1.88–1.94 (*m*)		1.88–1.94 (*m*)	
H_α–C(12)	1.70–1.74 (*m*)	27.0 (*t*)	1.33–1.41 (*m*)	29.9 (*t*)
H_β–C(12)	1.52–1.57 (*m*)		1.22–1.26 (*m*)	
H_α–C(13)	1.47–1.56 (*m*)	28.7 (*t*)	2.94–3.03 (*m*)	38.7 (*t*)
H_β–C(13)	1.38–1.43 (*m*)		1.47–1.56 (*m*)	
H–C(14)	2.03–2.09 (*m*)	37.7 (*d*)	2.90–2.96 (*m*)	43.1 (*d*)
H–C(15)	3.21–3.27 (*m*)	53.6 (*d*)	3.43–3.52 (*m*)	55.0 (*d*)
H_α–C(16)	2.67–2.70 (*m*)	38.1 (*t*)	1.71–1.81 (*m*)	26.7 (*t*)
H_β–C(16)	2.63–2.66 (*m*)		1.46–1.54 (*m*)	
H_α–C(17)	2.62–2.68 (*m*)	42.9 (*t*)	2.60–2.68 (*m*)	42.3 (*t*)
H_β–C(17)	2.30–2.37 (*m*)		2.27–2.35 (*m*)	
H_a–C(18)	1.70–1.75 (*m*)	24.3 (*t*)	1.42–1.52 (*m*)	30.7 (*t*)
H_b–C(18)	1.50–1.54 (*m*)		1.42–1.52 (*m*)	
Me(19)	0.94 (*t*, J = 7.4)	10.3 (*q*)	0.93 (*t*, J = 7.4)	9.9 (*q*)
H_a–C(21)	4.18 (*d*, J = 12.0)	66.9 (*t*)	4.28–4.33 (*m*)	65.2 (*t*)
H_b–C(21)	3.54–3.58 (*m*)		3.34–3.43 (*m*)	
H_a–C(22)	3.56–3.62 (*m*)	63.0 (*t*)		178.5 (*s*)
H_b–C(22)	3.38–3.44 (*m*)			
MeO			3.64 (*s*)	51.7 (*q*)
MeN	2.25 (*s*)	46.2 (*q*)	2.14 (*s*)	46.3 (*q*)

groups at δ(C) 56.3 and 62.9, and one Me group at δ(C) 46.3 were flanked by the N-atom.

The ^{13}C-NMR data and the mentioned structural features of **2** were closely related to those of **1** with exception of the replacement of one CH_2–OH group in **1** by a COOMe group in **2**. These data suggested that compound **2** has the same skeleton as **1** with C(22)OOMe attached to C(14), which was confirmed by the correlations of H_β–C(13) and H–C(14) to C(22) in the HMBC. The data of the ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra further justified the structure of **2**, as shown in *Fig. 1*.



1 **2**

Fig. 1. *Key* $^1H,^1H$*-COSY* (—) *and HMBC* (H → C) *correlations of compounds* **1** *and* **2**

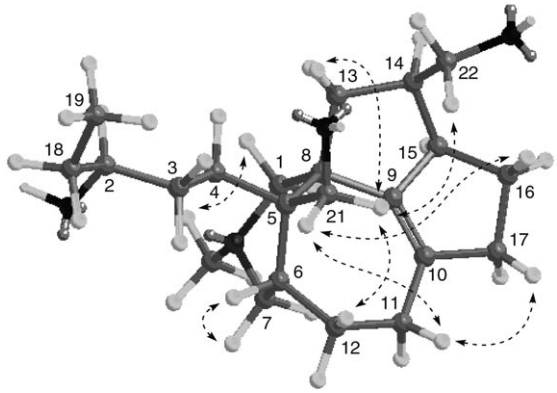


Fig. 2. *Key ROESY correlations of compound* **1**

Furthermore, the relative configurations were elucidated to be the same as in **1** by ROESY data as well as by the similarity of the ^{13}C-NMR chemical shifts of **2** compared to those of **1**. Since the biosynthetic relationships of these two new alkaloids with daphnigraciline are obvious, it can be speculated that the absolute configurations of daphnoldine A (**1**) and daphnoldine B (**2**) are as shown in the formula collection.

Experimental Part

General. MPLC: *Büchi Pump Module C-605*, and *Büchi Pump Manager C-615*. Column chromatography (CC): Silica gel *H* (10–40 μm; *Qingdao Marine Chemical Ltd. Co.*), and *Sephadex LH-20* (40–70 μm, *Pharmacia*). TLC: On silica-gel plates; visualization by *Dragendorff*'s reagent. Optical rotations: *JASCO DIP-370* digital polarimeter. IR Spectra: *Bio-Rad FTS-135* spectrometer, KBr pellets, in cm^{-1}. NMR spectra: *Bruker AM-400* instrument (400/100 MHz), and *Bruker DRX-500* instrument (500/125 MHz); δ in ppm rel. to TMS as internal standard, *J* in Hz. ESI-MS: *Finnigan MAT 90* instrument; in *m*/*z*. HR-ESI-MS: *API Qstar Pulsar LC/TOF* instrument.

Plant Material. The fruits of *Daphniphyllum oldhami* were collected in Xinning of Hunan Province, P. R. China, in October 2006. The material was identified by Prof. *Xun Gong*, and a specimen 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 fruits of *D. oldhami* were extracted with 95% EtOH (20 l) at reflux for 3 h three times. After evaporation of the solvent, the residue was partitioned between AcOEt and sat. tartaric acid aq. soln. The aq. layer was alkalinized to pH 10 with sat. aq. Na_2CO_3 soln. followed by exhaustive extraction with $CHCl_3$ to yield the crude alkaloid fraction (60 g). This was subjected to CC (SiO_2; petroleum ether (PE)/AcOEt 1:0 to 0:1, and then $CHCl_3$/MeOH 1:0 to 0:1) to afford eight fractions (*Fr. 1–8*). *Fr. 2* (15.2 g) was separated by CC (SiO_2; PE/Et_2NH 200:1 to 50:1), and then the major fractions were further purified by CC (SiO_2; PE/Me_2CO) to afford compound **12** (7 mg). *Fr. 3* was separated by CC (SiO_2; PE/Et_2NH 200:1 to 50:1) firstly, and then purified by CC (*Sephadex LH-20*, MeOH) and CC (SiO_2; PE/Me_2CO) to afford **7** (15 mg), **10** (11 mg), and **11** (10 mg). *Fr. 4* was also separated by CC (SiO_2; PE/Et_2NH 100:1–20:1) to afford white solid compound **4** (1 g), and then another fraction purified by CC ($CHCl_3$/MeOH) to afford compound **3** (35 mg). *Fr. 5* was separated by CC (*Sephadex LH-20*, MeOH) and CC (SiO_2; PE/Et_2NH 100:1 to 20:1). Then, compounds **6** (25 mg), **9** (20 mg), and **8** (17 mg) were obtained after purification by CC (SiO_2, $CHCl_3$/MeOH). *Fr. 7* was separated by CC (SiO_2; $CHCl_3$/MeOH) and CC (*Sepahdex LH-20*, H_2O/MeOH 8:2 to 0:1), then the major fraction was further purified by CC (SiO_2, AcOEt) to afford **1** (136 mg) and **2** (61 mg). *Fr. 8* was separated by CC (SiO_2; PE/Et_2NH 50:1 to 5:1), and the major fraction was further subjected to CC (*Sephadex LH-20*, H_2O/MeOH 8:2 to 0:1) and CC (SiO_2, $CHCl_3$/MeOH 25:1) to afford compound **5** (27 mg).

Daphnoldine A (=*(8a*RS,*9*RS,*10a*RS,*11*RS*)-2,3,4,5,6,7,8,8a,9,10-Decahydro-11-(3-hydroxypentyl)-2-methyl-1*H*-4,10a-methanopentaleno[1,6-*cd*]azonine-9,11-dimethanol*; **1**). Colorless, amorphous solid. $[\alpha]_D^{23} = -3.84$ ($c = 0.82$, MeOH). IR (KBr): 3406, 2932, 1458, 1447, 1139, 1113, 1050, 1021, 757. ^{1}H- and ^{13}C-NMR: *Table.* HR-ESI-MS (pos.): 364.2836 ($[M+H]^+$, $C_{22}H_{38}NO_3^+$; calc. 364.2852).

Daphnoldine B (=*Methyl (8a*RS,*9*RS,*10a*RS,*11*RS*)-2,3,4,5,6,7,8,8a,9,10-Decahydro-11-(hydroxymethyl)-11-(3-hydroxypentyl)-2-methyl-1*H*-4,10a-methanopentaleno[1,6-*cd*]azonine-9-carboxylate*; **2**). Light yellow solid. $[\alpha]_D^{23} = -1.48$ ($c = 1.24$, $CHCl_3$). IR (KBr): 3423, 2933, 1712, 1454, 1375, 1203, 1171, 1038, 1020. ^{1}H- and ^{13}C-NMR: *Table.* HR-ESI-MS (pos.): 392.2809 ($[M+H]^+$; $C_{23}H_{38}NO_4^+$, calc. 392.2801).

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