Secophnane-Type Alkaloids from *Daphniphyllum oldhami*

by **Shu-Zhen Mu**[a][b][c], **Xian-Wen Yang**[d], **Ying-Tong Di**[e], **Hong-Ping He**[e], **Ye Wang**[a], **Yue-Hu Wang**[e], **Ling Li**[f], and **Xiao-Jiang Hao***[e]

[a]) The Key Laboratory of Chemistry for Natural Product of Guizhou Province and Chinese Academy of Sciences, Guiyang 550002, P. R. China

[b]) Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, P. R. China

[c]) Graduate University of Chinese Academy of Sciences, Beijing 100049, P. R. China

[d]) School of Pharmacy, Second Military Medical University, Shanghai 200433, P. R. China

[e]) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, P. R. China (phone: 086-871-5223263; fax: 086-871-5219684; e-mail: haoxj@mail.kib.ac.cn)

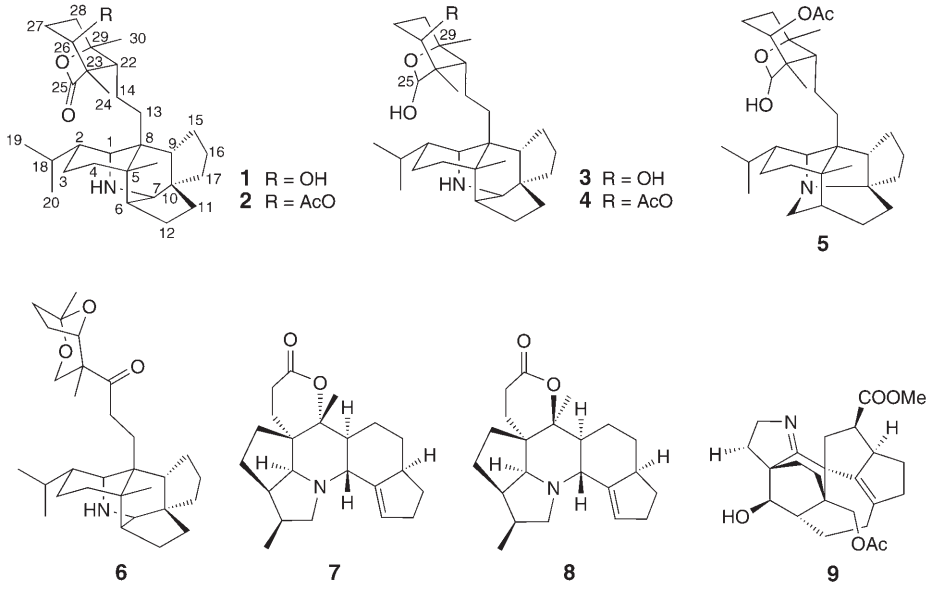
[f]) Yunnan Pharmacological Laboratories of Natural Products, Kunming Medical College, Kunming 650031, P. R. China

Four new alkaloids, daphnioldhanins D–G (**1**–**4**, resp.), together with five known alkaloids, daphmacropodine (**5**), secodaphniphylline (**6**), deoxycalyciphylline B (**7**), deoxyisocalyciphylline B (**8**), and daphmanidin A (**9**), were isolated from the roots of *Daphniphyllum oldhami*. Their structures were elucidated on the basis of spectroscopic data and chemical methods. Compound **1** at 2.0 μM showed potent antioxidant activity against H_2O_2-induced impairment in PC12 cells.

Introduction. – The skeletal types of alkaloids from the *Daphniphyllum* genus are structurally diverse and fascinating [1][2]. In recent years, a number of new *Daphniphyllum* alkaloids were reported [2–9]. These alkaloids with unique complex polycyclic systems led to focus on their total synthesis, biosynthetic pathway, and bioactivity [6][7].

We previously reported some novel alkaloids from the above genus [8][9]. In our continuing research work, four new *Daphniphyllum* alkaloids **1**–**4** of secophnane-type, as well as known compounds **5**–**9** were isolated from the roots of *D. oldhami*. In this paper, we describe the isolation and structural elucidation of **1**–**4**, and their evaluation for antioxidant activity.

Results and Discussion. – 1. *Structure Elucidation*. Daphnioldhanin D (**1**) was obtained as an optically active, colorless solid. The molecular formula of **1** was determined as $C_{30}H_{47}NO_3$ by positive-ion HR-ESI-MS (*m/z* 470.3639 ($[M+H]^+$; calc. 470.3634)), with eight degrees of unsaturation. The IR absorptions at 3424 and 1768 cm^{-1} implied the presence of an OH group and of a γ-lactone group, respectively. The ^{13}C-NMR spectrum of **1** showed 30 signals due to six quaternary C-atoms, eight CH, eleven CH_2, and five Me groups. The 1D-NMR data (*Table 1*) suggested that **1** had the same fused-pentacyclic backbone (N, C(1) to C(21)) as the known alkaloid caldaphnidine D [4] accounting for five degrees of unsaturation as five rings, belonging to a secophnane-type *Daphniphyllum* alkaloid [1c], which was confirmed by the



interpretation of 2D-NMR data (*Fig. 1*). Other resonances including one lactone C=O unit (δ(C) 179.1), one sp^3 CH (δ(C) 56.5), two sp^3-quaternary C-atoms (δ(C) 50.4 and 86.1), one oxygenated CH unit (δ(C) 68.9), two sp^3-CH_2 units (δ(C) 25.5 and 28.6), and two Me groups (δ(C) 18.0 and 24.5) corresponded to those of the side chain (C(13), C(14), and C(22) to C(30)). Thus, the remaining three degrees of unsaturation were assumed to indicate the presence of two rings and one C=O group. Analysis of ^{1}H,^{1}H-COSY and HMBC spectra revealed that the side chain possessed a cyclohexane ring with one OH group at C(26) (δ(C) 68.9) and two Me groups (δ(C) 18.0 and 24.5) at C(23) (δ(C) 50.4) and C(29) (δ(C) 86.1), and a lactone ring (C(22), C(23), C(25), and

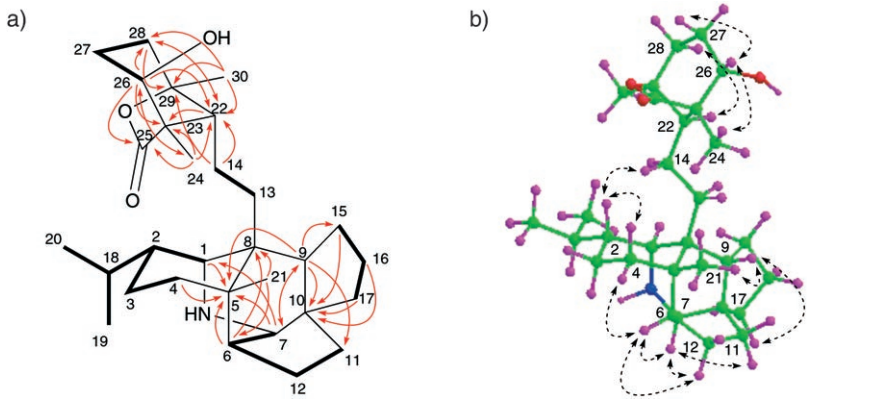


Fig. 1. a) *^{1}H,^{1}H-COSY* (▬) *and key HMBC correlations* (H→C) *of* **1**. b) *Key ROESY correlations* (H ----› H) *of* **1**

Table 1. *^{1}H- and ^{13}C-NMR Data of Compounds* **1** *and* **2**. δ in ppm, J in Hz.

Position	**1**[a])		**2**[a])	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	3.06 (br. *s*)	47.9	3.09 (br. *s*)	47.7
H–C(2)	0.83–0.86 (*m*)	43.2	0.81–0.84 (*m*)	43.2
CH_2(3)	1.51–1.56 (*m*)	20.8	1.51–1.56 (*m*)	20.5
H_a–C(4)	1.17–1.20 (*m*)	39.0	1.19–1.22 (*m*)	39.0
H_b–C(4)	1.54–1.57 (*m*)		1.53–1.56 (*m*)	
C(5)	–	36.6	–	36.6
H–C(6)	1.91–1.93 (*m*)	47.4	1.91–1.94 (*m*)	47.3
H–C(7)	2.56 (*d*, *J*=4.0)	59.7	2.57 (*d*, *J*=4.4)	59.6
C(8)	–	36.7	–	36.7
H–C(9)	1.68–1.72 (*m*)	53.7	1.68–1.72 (*m*)	54.1
C(10)	–	50.8	–	50.4
H_a–C(11)	1.48–1.50 (*m*)	39.8	1.43–1.48 (*m*)	40.0
H_b–C(11)	1.63–1.67 (*m*)		1.64–1.71 (*m*)	
H_a–C(12)	1.40–1.45 (*m*)	22.8	1.41–1.44 (*m*)	22.8
H_b–C(12)	1.56–1.62 (*m*)		1.55–1.60 (*m*)	
H_a–C(13)	1.38–1.42 (*m*)	33.3	1.40–1.43 (*m*)	33.3
H_b–C(13)	1.26–1.32 (*m*)		1.30–1.35 (*m*)	
H_a–C(14))	2.03–2.10 (*m*)	21.6	1.84–1.92 (*m*)	21.5
H_b–C(14)	1.39–1.43 (*m*)		1.41–1.45 (*m*)	
H_a–C(15)	1.75–1.80 (*m*)	29.9	1.72–1.79 (*m*)	30.3
H_b–C(15)	1.55–1.60 (*m*)		1.57–1.63 (*m*)	
H_a–C(16)	1.72–1.77 (*m*)	26.7	1.72–1.78 (*m*)	26.6
H_b–C(16)	1.41–1.46 (*m*)		1.41–1.45 (*m*)	
H_a–C(17)	1.69–1.74 (*m*)	36.1	1.67–1.74 (*m*)	36.0
H_b–C(17)	1.51–1.56 (*m*)		1.53–1.58 (*m*)	
H–C(18)	1.50–1.55 (*m*)	28.6	1.49–1.55 (*m*)	28.6
Me(19)	0.90 (*d*, *J*=6.4)	21.1	0.90 (*d*, *J*=6.4)	21.1
Me(20)	0.89 (*d*, *J*=6.4)	21.1	0.88 (*d*, *J*=6.4)	21.1
Me(21)	0.76 (*s*)	21.1	0.75 (*s*)	21.1
H–C(22)	1.64–1.68 (*m*)	56.5	1.67–1.71 (*m*)	56.2
C(23)	–	50.4	–	50.0
Me(24)	1.28 (*s*)	18.0	1.16 (*s*)	17.5
C(25)	–	179.1	–	177.4
H–C(26)	3.72 (*d*, *J*=4.5)	68.9	4.85 (*d*, *J*=5.3)	70.0
H_a–C(27	1.82–1.88 (*m*)	25.5	1.79–1.82 (*m*)	25.6
H_b–C(27)	1.58–1.63 (*m*)		1.61–1.67 (*m*)	
H_a–C(28)	1.92–1.96 (*m*)	28.6	1.92–2.01 (*m*)	25.4
H_b–C(28)	1.74–1.79 (*m*)		1.74–1.80 (*m*)	
C(29)	–	86.1	–	85.6
Me(30)	1.43 (*s*)	24.5	1.45 (*s*)	24.7
C(31)	–	–	–	169.8
Me(32)	–	–	2.05 (*s*)	21.1

[a]) Recorded at 400 MHz for ^{1}H-NMR and measured at 100 MHz for ^{13}C-NMR in $CDCl_3$.

C(29)), as shown in *Fig. 1,a*. In combination with the HMBC correlations of H–C(14) to C(22), Me(24) to C(22), and Me(24) to C(23), the combinational formula of **1** was finally elucidated as shown in *Fig. 1,a*.

The relative configuration of **1** was deduced from ROESY correlations as shown in a computer-generated 3D drawing (*Fig. 1,b*). ROESY Correlations of H_b–C(4)/H–C(2) and H–C(2)/H_b–C(14) suggested that H–C(2), H_b–4, and the side chain at C(8) are β-oriented, and the cyclohexane ring (C(1) to C(5) and C(8)) assumes a chair form (*Fig. 1,b*). The relative configurations at C(5), C(6), C(7), C(9), and C(10), including the *cis*-ring junction at C(9) and C(10), were elucidated by ROESY correlations of H–C(6)/H–C(7), H_a–C(4)/H–C(6), H–C(7)/H_a–C(12), H–C(7)/H_a–C(11), H–C(9)/Me(21), and H–C(9)/H_a–C(11). A chair form of the six-membered ring (C(22), C(23), and C(26) to C(29)) was verified by a ROESY correlation of H–C(22)/H_b–C(28) as shown in *Fig. 1,b*.

Daphnioldhanin E (**2**) has the molecular formula $C_{32}H_{49}NO_4$, deduced by positive-ion HR-ESI-MS (*m/z* 512.3748 ($[M+H]^+$; calc. 512.3739)), indicating nine degrees of unsaturation. The IR absorption band at 1770 cm^{-1} implied the presence of a γ-lactone unit. ^{1}H- and ^{13}C-NMR data, as well as the HSQC spectrum of **2** provided evidence that **2** possessed 32 ^{13}C signals, including seven quaternary C-atoms, eight CH, twelve CH_2, and six Me groups. The 1D-NMR data of **2** were similar to those of **1** (*Table 1*), suggesting the same basic skeleton for the two alkaloids. Compared with compound **1**, the main difference was the presence of an AcO group in **2**, instead of an OH group in **1**. The location of the AcO group at C(26) (δ(C) 70.0, δ(H) 4.85, *d*, $J=5.3$ Hz) was determined by the HMBC cross-peaks between H–C(24) at δ(H) 1.16 (*s*) and C(26) at δ(C) 68.9. Compound **2** is proposed to be 26-*O*-acetyldaphnioldhanin D, named daphnioldhanin E. Compound **2** was hydrolyzed in basic MeOH to give an alkaloid which was identified as daphnioldhanin D (**1**) by co-TLC, ESI-MS, and ^{1}H-NMR data, and $[\alpha]_D$ value.

Daphnioldhanin F (**3**) was assigned the molecular formula $C_{30}H_{49}NO_3$ by positive-ion HR-ESI-MS (*m/z* 472.3797 ($[M+H]^+$; calc. 472.3790)). The IR spectrum implied the presence of an OH (3419 cm^{-1}) group. The ^{13}C-NMR data (*Table 2*) indicated the presence of five quaternary C-atoms, nine CH, eleven CH_2, and five Me groups. Comparison of the NMR data of **3** with those of **1** and daphnezomine D [2o], indicated that **3** had the same pentacyclic backbone (N, C(1) to C(21)) as **1** and a similar fragment (C(22) to C(30)) including a cyclohexane ring with an OH group at C(26), and two Me groups at C(23) and C(29), and a hemiacetal ring (C(22), C(23), C(25), and C(29)) (*Fig. 2*) as daphnezomine D, which was confirmed by the analysis of 2D-NMR data. The linkage of the backbone and the fragment to C(14) were deduced by HMBC correlations of CH_2(14) with C(22), Me(24) with C(22), and Me(24) with C(23). The relative configuration of this side chain was elucidated by ROESY correlations H–C(25)/H–C(27a), H–C(25)/H–C(26), and H–C(22)/H–C(28b) [20]. Thus, the structure of **3** was established and named daphnioldhanin F.

Daphnioldhanin G (**4**) was obtained as an amorphous powder. The molecular formula was established as $C_{32}H_{51}NO_4$ by positive-ion HR-ESI-MS (*m/z* 514.3889 ($[M+H]^+$; calc. 514.3896)). The IR spectrum of **4** showed strong absorption bands at 3362 cm^{-1} for an OH group and at 1745 cm^{-1} for an ester C=O group. ^{1}H- and ^{13}C-NMR data of **4** (*Table 2*) and the HSQC spectrum indicated that **4** had 32 ^{13}C

Table 2. *[1]H- and [13]C-NMR Data of Compounds* **3** *and* **4**. δ in ppm, J in Hz.

Position	**3**[a])		**4**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	3.51 (br. *s*)	51.0	3.09 (br. *s*)	47.9
H–C(2)	1.23–1.28 (*m*)	43.1	0.86–0.92 (*m*)	43.1
CH_2(3)	1.73–1.78 (*m*)	20.9	1.51–1.57 (*m*)	20.6
H_a–C(4)	1.28–1.35 (*m*)	39.1	1.17–1.22 (*m*)	39.1
H_b–C(4)	1.70–1.75 (*m*)		1.54–1.60 (*m*)	
C(5)	–	37.7	–	36.7
H–C(6)	2.13–2.19 (*m*)	46.3	1.91–1.94 (*m*)	47.6
H–C(7)	3.06 (*d*, J=3.9)	59.4	2.56 (*d*, J=4.2)	59.8
C(8)	–	37.8	–	36.8
H–C(9)	1.91–1.96 (*m*)	54.6	1.69–1.74 (*m*)	54.0
C(10)	–	50.1	–	50.2
H_a–C(11)	1.69–1.74 (*m*)	41.0	1.44–1.50 (*m*)	40.0
H_b–C(11)	1.80–1.84 (*m*)		1.62–1.72 (*m*)	
H_a–C(12)	1.78–1.87 (*m*)	23.8	1.40–1.45 (*m*)	22.9
H_b–C(12)	1.55–1.64 (*m*)		1.56–1.62 (*m*)	
H_a–C(13)	1.50–1.56 (*m*)	34.7	1.37–1.43 (*m*)	34.0
H_b–C(13)	1.39–1.46 (*m*)		1.29–1.34 (*m*)	
H_a–C(14)	2.12–2.18 (*m*)	21.8	1.80–1.87 (*m*)	20.7
H_b–C(14)	1.28–1.34 (*m*)		1.29–1.34 (*m*)	
H_a–C(15)	2.01–2.07 (*m*)	31.0	1.78–1.82 (*m*)	30.4
H_b–C(15)	1.26–1.32 (*m*)		1.61–1.64 (*m*)	
H_a–C(16)	1.77–1.83 (*m*)	27.0	1.86–1.90 (*m*)	25.8
H_b–C(16)	1.20–1.25 (*m*)		1.58–1.62 (*m*)	
H_a–C(17)	1.87–1.93 (*m*)	36.4	1.67–1.75 (*m*)	36.2
H_b–C(17)	1.66–1.72 (*m*)		1.54–1.58 (*m*)	
H–C(18)	1.52–1.58 (*m*)	29.3	1.50–1.54 (*m*)	28.7
Me(19)	1.01 (*d*, J=6.5)	21.1	0.91 (*d*, J=7.0)	21.2
Me(20)	1.08 (*d*, J=6.5)	21.2	0.89 (*d*, J=7.0)	21.3
Me(21)	0.92 (*s*)	21.5	0.77 (*s*)	21.1
H–C(22)	1.50–1.54 (*m*)	52.8	1.54–1.59 (*m*)	51.4
C(23)	–	52.0	–	50.5
Me(24)	1.11 (*s*)	18.0	1.00 (*s*)	16.6
H–C(25)	4.65 (*s*)	101.0	4.82 (*s*)	99.2
H–C(26)	3.51 (br. *s*)	72.4	4.74 (*d*, J=5.0)	73.4
H_a–C(27	1.82–1.88 (*m*)	29.5	1.86–1.90 (*m*)	25.6
H_b–C(27)	1.59–1.66 (*m*)		1.58–1.62 (*m*)	
H_a–C(28)	1.60–1.66 (*m*)	28.8	1.55–1.61 (*m*)	27.6
H_b–C(28)	1.21–1.25 (*m*)		1.30–1.34 (*m*)	
C(29)	–	85.5	–	84.6
Me(30)	1.29 (*s*)	26.7	1.33 (*s*)	26.5
C(31)	–	–	–	170.3
Me(32)	–	–	2.05 (*s*)	21.2

[a]) Recorded at 500 MHz for ^{1}H-NMR and measured at 125 MHz for ^{13}C-NMR in $CDCl_3$. [b]) Recorded at 400 MHz for ^{1}H-NMR and measured at 100 MHz for ^{13}C-NMR in $CDCl_3$.

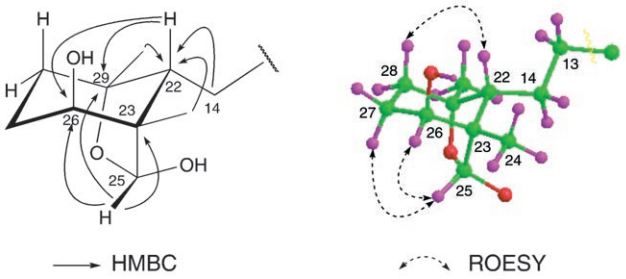


Fig. 2. *Key ROESY and key HMBC correlations of the side chain* (C(22)–C(30)) *in* **3**

signals, including six quaternary C-atoms, and nine CH, eleven CH_2, and six Me groups. The 1D-NMR data of **4** were similar to those of **3**, suggesting that the two alkaloids possessed the same secophnane-type skeleton. Detailed analysis of the 2D-NMR data, including the HSQC, ^{1}H,^{1}H-COSY, and HMBC spectra (*Fig. 3*), confirmed the above deduction. By comparing with **3**, one AcO group (δ(C=O) 170.3 and δ(Me) 21.2) at C(26) (δ(C) 73.4) in **4**, indicating that **4** was the 26-*O*-Ac derivative of **3**. Compound **4** was hydrolyzed in MeOH to give an alkaloid which was identified as natural daphnioldhanin F (**3**) by co-TLC, the spectral data, and the $[\alpha]_D$ value. In addition, oxidation of **4** with pyridinium chlorochromate (PCC) afforded daphnioldhanin E (**2**).

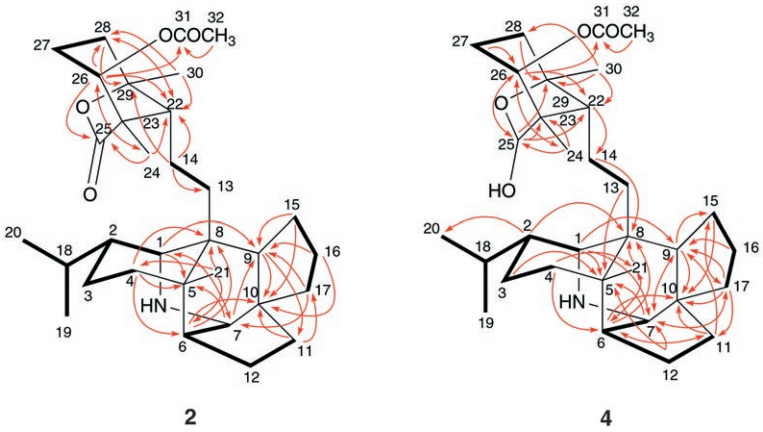


Fig. 3. *^{1}H,^{1}H-COSY* (▬) *and key HMBC correlations* (H→C) *of* **2** *and* **4**

The known alkaloids daphmacropodine (**5**) was identified by its spectral data (ESI-MS, and ^{1}H- and ^{13}C-NMR), and ^{13}C-NMR spectrum of **5** was recorded for the first time. The known alkaloids secodaphniphylline (**6**), deoxycalyciphylline B (**7**), deoxyisocalyciphylline B (**8**), and daphmanidin A (**9**) were identified on the basis of their reported spectral data (EI-MS, ^{1}H- and ^{13}C-NMR) [2i][4f][6g][9c].

2. *Biological Studies.* Antioxidant activities of compounds **1**–**4** at four concentrations (0.4, 2.0, 10.0, and 50.0 μM) were tested for antioxidant effects by MTT method and DPPH assay according to the reported protocols [10–12]. Compound **1** at 2.0 μM promoted significantly the viability of cells (viability [%]: 55.2 ± 5.0 for **1**, 40.4 ± 4.6 for

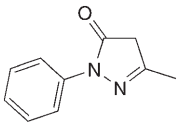


Fig. 4. *The structure of edaravone as positive control*

model, and 60.0 ± 5.5 for edaravone (see *Fig. 4*); $n = 5$, $\overline{X} \pm SD$), whereas compounds **2**–**4** were found to be inactive at all four concentrations in H_2O_2-induced impairment in PC12 cells (*Table 3*). In DPPH radical scavenging activity assay, compounds **1**–**4** showed inactive ($IC_{50} \gg 100$ μM). The main difference in structure between compound **1** and compounds **2**–**4** is that the former possesses a γ- lactone ring and an OH−C(26) in the side chain. The above results implied that the lactone ring and the OH−C(26) in **1** may be the active functional groups for antioxidant activity.

Table 3. *Antioxidant Effects of Compounds* **1**–**4** *against H_2O_2-Induced Impairment in PC12 Cells* ($n = 5$, $\overline{X} \pm$ SD)

Group	Concentration [μM]	Viability [%][a])
Control		100***
Model[b])		40.4 ± 4.6
Edaravone[c])	10.0	48.3 ± 5.9
	2.0	52.0 ± 9.8
	0.4	60.0 ± 5.5**
	0.08	46.2 ± 6.5
1	50.0	20.2 ± 2.4
	10.0	46.3 ± 4.6
	2.0	55.2 ± 5.0**
	0.4	46.3 ± 4.4
2	50.0	28.1 ± 0.9
	10.0	32.6 ± 4.7
	2.0	40.1 ± 3.2
	0.4	38.9 ± 1.1
3	50.0	26.7 ± 2.3
	10.0	44.6 ± 3.2
	2.0	42.6 ± 5.5
	0.4	43.8 ± 4.4
4	50.0	23.3 ± 0.6
	10.0	35.7 ± 4.0
	2.0	43.0 ± 3.8
	0.4	41.9 ± 2.8

[a]) **: $P < 0.01$, ***: $P < 0.001$, compared to model. [b]) Negative control. [c]) Positive control.

The authors are grateful to Dr. *Xun Chen* of Guizhou Academy of Science for the identification of the plant materials.

Experimental Part

General. All solvents used for extraction and isolation were distilled prior to use. Petroleum ether for chromatography had a b.p. range of 60–90°. Column chromatography (CC) was performed on silica gel (200–300, 300–400 mesh; *Qingdao Haiyang Chem. Ind. Co. Ltd*. P. R. China), silica gel *H* (10–40 μm; *Qingdao*). Fractions were monitored by TLC, and spots were visualized by spraying with *Dragendoff*'s reagent. Optical rotations: *JASCO DIP-370* Digital Polarimeter. IR Spectra: *Bio-Rad FTS-135* spectrometer, KBr discs; in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker AM-400* and *DRX-500* spectrometers; chemical shifts δ in ppm rel. to residual solvent signals, *J* in Hz. ESI-MS and HR-ESI-MS: *VG Autospec-3000* spectrometers, in *m*/*z*.

Plant Material. Plants of *D. oldhami* were collected in Jinping Country of Guizhou Province, P. R. China, in August 2005, and identified by Prof. *Xun Chen* of Guizhou Academy of Sciences. A voucher specimen (GY 05080601) was deposited in the Herbarium of the Key Laboratory of Chemistry for Natural Product of Guizhou Province and Chinese Academy of Sciences.

Extraction and Isolation. The air-dried roots of *D. oldhami* (15.0 kg) were percolated three times with 95% EtOH to give a crude extract. The extract was concentrated to dryness under reduced pressure, followed by partitioning between AcOEt and 3% tartaric acid. The aq. phase was adjusted to pH *ca*. 9 with sat. Na_2CO_3 and extracted with $CHCl_3$ to give crude alkaloids (13.0 g). The crude alkaloids were subjected to a silica-gel CC with $CHCl_3$/MeOH (1:0→0:1) to obtain six major fractions (*Fr.*) *A*–*F*. *Fr. C* (3.4 g), eluted with $CHCl_3$/MeOH 50:1, was separated and purified by repeated CC on silica gel with $CHCl_3$/MeOH 40:1 and petroleum ether/Et_2NH (20:1→4:1) to afford **1** (25 mg), **2** (20 mg), **7** (11 mg), **8** (15 mg), and **9** (8 mg). *Fr. D* was subjected to repeated CC over silica gel *H* with petroleum ether/acetone/Et_2NH (15:3:1→15:5:1), assisted by CC over silica gel with petroleum ether/Et_2NH (100:1→20:1) to give **3** (8 mg), **4** (40 mg), **5** (100 mg), and **6** (125 mg).

Basic Hydrolyses of Daphnioldhanins E and G (**2** and **4**, resp.). Alkaloid **2** or **4** (5.0 mg) was dissolved in 2.5 ml of MeOH, and then 0.05 g of NaOH was added. The mixture was stirred at r.t. for 3 h. After removal of the MeOH under reduced pressure, the resulting alkaloid was subjected to a silica-gel CC with $CHCl_3$/MeOH 20:1 to afford **1** (2.5 mg) or **3** (3.0 mg)).

Oxidation of Daphnioldhanin G (**4**). A soln. of pyridinium chlorochromate (PCC) (14 mg) in CH_2Cl_2 (2.0 ml) was added to the soln. of **4** (4 mg) in CH_2Cl_2 (1.5 ml), and the mixture was stirred for 2 h at r.t. Then, the black mixture was diluted with 20 ml of Et_2O, filtered. The Et_2O layer was washed with sat. NaCl soln., dried (Na_2SO_4), and evaporated. The crude residue was purified by CC (SiO_2; $CHCl_3$/MeOH 40:1) to give **2** (2 mg).

Daphnioldhanin D (**1**). Colorless, amorphous powder. $[\alpha]_D^{26} = -22.8$ ($c = 0.82$, $CHCl_3$). IR (KBr): 3424, 2942, 2867, 1768, 1628, 1452, 1381, 1261, 1151, 1075, 1040, 965, 922. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 470.7 ($[M+H]^+$). HR-ESI-MS: 470.3639 ($[M+H]^+$, $C_{30}H_{48}NO_3^+$, calc. 470.3634).

Daphnioldhanin E (**2**). Colorless, amorphous powder. $[\alpha]_D^{20} = -16.1$ ($c = 0.83$, $CHCl_3$). IR (KBr): 3441, 2937, 2866, 1770, 1629, 1452, 1381, 1226, 1103, 1070, 1035, 964, 921. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 512.7 ($[M+H]^+$). HR-ESI-MS: 512.3748 ($[M+H]^+$, $C_{32}H_{50}NO_4^+$, calc. 512.3739).

Daphnioldhanin F (**3**). Colorless, amorphous powder. $[\alpha]_D^{28.7} = -48.6$ ($c = 0.60$, $CHCl_3$). IR (KBr): 3419, 2943, 2871, 1588, 1452, 1384, 1282, 1215, 1126, 1062, 1031, 960, 921. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 472.7 ($[M+H]^+$). HR-ESI-MS: 472.3797 ($[M+H]^+$, $C_{30}H_{50}NO_3^+$, calc. 472.3790).

Daphnioldhanin G (**4**). Colorless, amorphous powder. $[\alpha]_D^{26} = -52.0$ ($c = 0.34$, $CHCl_3$). IR (KBr): 3417, 2935, 2867, 1745, 1638, 1450, 1377, 1241, 1169, 1126, 1081, 1025, 965, 918. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 514.7 ($[M+H]^+$). HR-ESI-MS: 514.3889 ($[M+H]^+$, $C_{32}H_{52}NO_4^+$, calc. 514.3896).

Daphmacropodine (**5**). Colorless, amorphous powder. $[\alpha]_D^{26} = +5.0$ ($c = 1.15$, $CHCl_3$). IR (KBr): 3362, 2935, 2867, 1740, 1638, 1453, 1377, 1240, 1170, 1120, 1079, 1030, 957, 925. ^{13}C-NMR (400 MHz, CD_3OD, in ppm): 172.0 (C(31)); 100.4 (C(25)); 85.1 (C(29)); 75.0 (C(26)); 60.9 (C(1)); 56.0 (C(22)); 52.5 (C(9)); 51.6 (C(10)); 51.4 (C(23)); 48.7 (C(6)); 44.6 (C(2)); 41.7 (C(7)); 40.2 (C(4)); 38.0 (C(5)); 37.8 (C(8)), 37.0 (C(13), C(17)); 35.4 (C(14)); 31.8 (C(27)); 29.7 (C(18)); 28.8 (C(28)); 27.6 (C(3)); 26.9 (C(15)); 26.7 (C(30)); 23.9 (C(11), C(16)); 21.8 (C(32)); 21.7 (C(21)); 21.6 (C(12), C(20)); 21.4 (C(19)); 17.3 (C(24)). ESI-MS: 514.6 ($[M+H]^+$).

Antioxidant Activity. PC12 Cells were obtained from Kunming Institute of Zoology, Chinese Academy of Sciences, and maintained in a H_2O-saturated atmosphere of 5% CO_2 at 37°. Cells were seeded into 96-well plates in RPMI 1640 medium (*Invitrogen Corp.*, Grand Isband, NY, USA) with 10% characterized Newborn Bovine Serum (*Lanzhou National Hyclone Bio-engineering Co. Ltd.*, Lanzhou, P. R. China), 100 U/ml penicillin, and 100 μg/ml streptomycin. Experiments were carried out 24 h after cells were seeded according to the reported protocol [10]. After incubation different concentrations of compounds **1**–**4** with cells for 2 h, freshly prepared H_2O_2 (*Sigma-Aldrich Chemie GmbH*, D-Steinheim) in phosphate-buffered saline (PBS) was added to continue incubation for 1 h with the final concentration of 200 μM. The assay for cell viability was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; *Sigma*) reduction [12]. Briefly, MTT soln. (0.5 mg/ml) in PBS was added and the incubation was continued for 4 h. Finally, 100 μl of soln. containing 5% i-BuOH, 10% SDS (*Sigma*), and 0.004% HCl was added. The mixtures were kept overnight, and the index of cell viability (% of control) was calculated by measuring the optical density of the color produced by MTT dye reduction with a microplate reader (*Bio-Rad* model *680*, Hercules, CA, USA) at 570 nm.

The DPPH method was used to determine free radical-scavenging potential of each sample [11]. 100 μl of each compound (five different concentrations ranging from 0.16 to 100.0 μM) was added to 100 μl of DPPH soln. (0.1 mM in EtOH). The absorbance was measured with a *Spectra MAX 340* microplate reader (*Molecular Devices*, Menlo Park, CA, USA) at 517 nm after 30 min of reaction at 37°. The percentage of radical scavenging activity (RSA [%]) was calculated using the following equation: $RSA\,[\%] = [(A_C - A_S)/A_C] \times 100\%$, where A_C is the absorbance of the control and A_s is the absorbance of the samples at 517 nm. The IC_{50} values denote the concentration of sample required to scavenge 50% DPPH free radicals.

REFERENCES

[1] a) S. Yamamura, Y. Hirata, in 'The Alkaloids', Ed. R. H. F. Manske, Academic Press, New York, 1975, Vol. 15, p. 41; b) S. Yamamura, in 'The Alkaloids', Ed. A. Brossi, Academic Press, New York, 1986, Vol. 29, p. 265; c) J. Kobayashi, H. Morita, in 'The Alkaloids', Ed. G. A. Cordell, Academic Press, New York, 2003, Vol. 60, p. 165.

[2] a) H. Morita, N. Ishioka, H. Takatsu, T. Iizuka, Y. Obara, N. Nakahata, J. Kobayashi, *J. Nat. Prod.* **2006**, *69*, 418; b) T. Kubota, Y. Matsuno, H. Morita, T. Shinzato, M. Sekiguchi, J. Kobayashi, Tetrahedron **2006**, *62*, 4743; c) H. Morita, N. Ishioka, H. Takatsu, T. Shinzato, Y. Obara, N. Nakahata, J. Kobayashi, *Org. Lett.* **2005**, *7*, 459; d) H. Takatsu, H. Morita, S. Ya-Ching, J. Kobayashi, *Tetrahedron* **2004**, *60*, 6279; e) H. Morita, H. Takatsu, S. Ya-Ching, J. Kobayashi, *Tetrahedron Lett.* **2004**, *45*, 901; f) H. Morita, J. Kobayashi, *Org. Lett.* **2003**, *5*, 2895; g) J. Kobayashi, H. Takatsu, Y. C. Shen, H. Morita, *Org. Lett.* **2003**, *5*, 1733; h) H. Morita, H. Takatsu, J. Kobayashi, *Tetrahedron* **2003**, *59*, 3575; i) J. Kobayashi, S .Ueno, H. Morita, *J. Org. Chem.* **2002**, *67*, 6546; j) H. Morita, N. Yoshida, J. Kobayashi, *J. Org. Chem.* **2002**, *67*, 2278; k) H. Morita, J. Kobayashi, *Tetrahedron* **2002**, *58*, 6637; l) J. Kobayashi, Y. Inaba, M. Shiro, N.Yoshida, H. Morita, *J. Am. Chem. Soc.* **2001**, *123*, 11402; m) H. Morita, N. Yoshida, J. Kobayashi, *J. Org. Chem.* **2000**, *65*, 3558; n) H. Morita, N. Yoshida, J. Kobayashi, *Tetrahedron* **2000**, *56*, 2641; o) H. Morita, N. Yoshida, J. Kobayashi, *Tetrahedron* **1999**, *55*, 12549.

[3] A. Jossang, H. E. Bitar, V. C. Pham, T. Sévenet, *J. Org. Chem.* **2003**, *68*, 300.

[4] a) H. Zhang, S. P. Yang, C. Q. Fan, J. Ding, J. M. Yue, *J. Nat. Prod.* **2006**, *69*, 553; b) S. P. Yang, H. Zhang, C. R. Zhang, H. D. Cheng, J. M. Yue, *J. Nat. Prod.* **2006**, *69*, 79; c) Z. J. Zhan, C. R. Zhang, J. M. Yue, *Tetrahedron* **2005**, *61*, 11038; d) X. Chen, Z. J. Zhan, J. M. Yue, *Helv. Chim. Acta* **2005**, *88*, 854; e) Z. J. Zhan, S. P. Yang, J. M. Yue, *J. Org. Chem.* **2004**, *69*, 1726; f) S. P. Yang, J. M. Yue, *Org. Lett.* **2004**, *6*, 1401; g) S. P. Yang, J. M. Yue, *J. Org. Chem.* **2003**, *68*, 7961.

[5] H. El Bitar, V. H. Nguyen, A. Gramain, T. Sévenet, B. Bodo, *Tetrahedron Lett.* **2004**, *45*, 515; H. El Bitar, V. H. Nguyen, A. Gramain, T. Sévenet, B. Bodo, *J. Nat. Prod.* **2004**, *67*, 1094.

[6] a) G. A. Wallace, C. H. Heathcock, *J. Org. Chem.* **2001**, *66*, 450; b) C. H. Heathcock, *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14323; c) C. H. Heathcock, D. Joe, *J. Org. Chem.* **1995**, *60*, 1131; d) C. H.

Heathcock, J. C. Kath, R. B. Ruggeri, *J. Org. Chem.* **1995**, *60*, 1120; e) C. H. Heathcock, *Angew. Chem.* **1992**, *104*, 675; f) C. H. Heathcock, *Angew. Chem.*, *Int. Ed.* **1992**, *31*, 665; g) C. H. Heathcock, J. A. Stafford, *J. Org. Chem.* **1992**, *57*, 2566.

[7] R. B. Ruggeri, M. M. Hansen, C. H. Heathcock, *J. Am. Chem. Soc.* **1988**, *110*, 8734.

[8] X. J. Hao, J. Zhou, M. Node, K. Fuji, *Yunnan Zhiwu Yanjiu* **1993**, *15*, 203.

[9] a) Y. T. Di, H. P. He, H. Y. Liu, Z. Z. Du, J. M. Tian, X. W. Yang, Y. H. Wang, X. J. Hao, *Tetrahedron Lett.* **2006**, *47*, 5329; b) Y. T. Di, H. P. He, Y. Lu, P. Yi, L. Li, L. Wu, X. J. Hao, *J. Nat. Prod.* **2006**, *69*, 1074; c) S. Z. Mu, Y. Wang, H. P. He, X. W. Yang, Y. H. Wang, Y. T. Di, Y. Lu, Y. Chang, X. J. Hao, *J. Nat. Prod.* **2006**, *69*, 1065; d) L. Li, H. P. He, Y. T. Di, S. Gao, X. J. Hao, *Tetrahedron Lett.* **2006**, *47*, 6259; e) L. Li, H. P. He, Y. T. Di, J. M. Tian, X. J. Hao, *Helv. Chim. Acta* **2006**, *89*, 1459.

[10] R. Wang, J. Zhou, X. C. Tang, *Mol. Brain Res.* **2002**, *107*, 1.

[11] M. S. Blois, *Nature* **1958**, *181*, 1199.

[12] M. C. Alley, D. A. Scudiero, A. Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemaker, M. R. Boyd, *Cancer Res.* **1988**, *48*, 589.

Received September 19, 2006