Alkaloids from *Daphniphyllum oldhami*

Shu-Zhen Mu,[†,‡] Jun-Song Wang,[‡] Xiao-Sheng Yang,[†] Hong-Ping He,[‡] Chun-Shun Li,[‡] Ying-Tong Di,[‡] Ye Wang,[†] Yu Zhang,[‡] Xin Fang,[‡] Lie-Jun Huang,[†] and Xiao-Jiang Hao*[,‡]

The Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, Guiyang 550002, People's Republic of China, and State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China.

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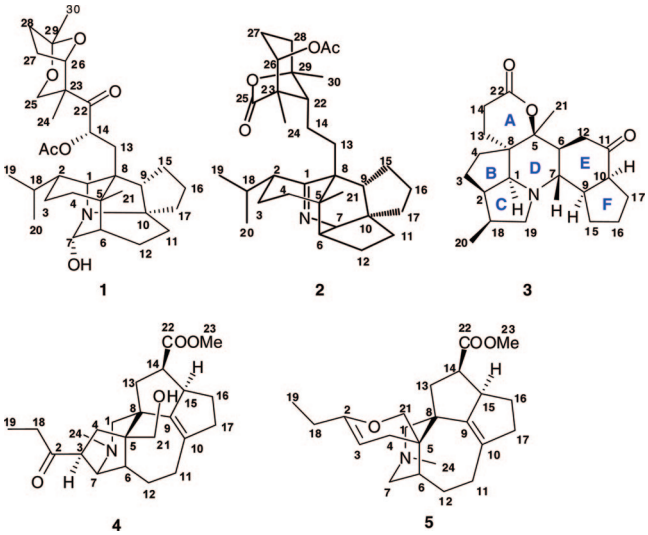
Four new *Daphniphyllum* alkaloids, daphnioldhanines H–K (**1**–**4**), along with 34 known alkaloids, were isolated from *Daphniphyllum oldhami*. The known alkaloid dehydrodaphnigraciline (**5**) is now reported as a natural product. Their structures were elucidated by spectroscopic methods, especially 2D NMR techniques. The effects against platelet aggregation of compounds **1**, **3**, and **5** were evaluated, and **3** showed stronger activity against platelet aggregation induced by PAF. This is the first report of quinolizidine alkaloids from the genus *Daphniphyllum*.

Daphniphyllum oldhami Rosenth. (Daphniphyllaceae) is an evergreen shrub in southern China.[1] Previous studies on this species resulted in the isolation of several *Daphniphyllum* alkaloids.[2,6,7] In the course of our systematic search of alkaloids from *D. oldhami*, 38 alkaloids, including new compounds (**1**–**4**), were isolated from the leaves, roots, and fruit. The first isolation of quinolizidine alkaloids from *D. oldhami* is interesting, implying the existence of two totally different alkaloid biogenetic pathways in this species.[3,4] This paper describes the isolation and structural elucidation of four new *Daphniphyllum* alkaloids (**1**–**4**) and the known alkaloid dehydrodaphnigraciline (**5**),[5,6] the distribution and biogenetic relationship of 38 alkaloids isolated from *D. oldhami*, and the effects against platelet aggregation of compounds **1**, **3**, and **5**.



Results and Discussion

The ESIMS of daphnioldhanine H (**1**) exhibited the pseudomolecular ion peak at *m/z* 544 $[M + H]^+$, and its molecular formula was determined as $C_{32}H_{49}NO_6$ by HRESIMS at *m/z* 544.3654 ($[M + H]^+$, $C_{32}H_{50}NO_6^+$, calcd 544.3638). IR absorption bands at 1739 and 1717 cm^{-1} suggested the presence of carbonyl functionalities. The ^{13}C NMR and DEPT data (Table 1) displayed 32 carbon signals, including two carbonyls and five quaternary, eight methine, 11 methylene, and six methyl carbons. Inspection of the NMR data of **1** (Table 1) indicated that its structure was related to the known alkaloid daphniphylline.[7] Analysis of 2D NMR spectra (HSQC, ^1H–^1H COSY, and HMBC, Figure 1A) of **1** confirmed the above deduction. Compared with daphniphylline, the major difference was the presence of a C-7 hydroxy group (δ_C 81.3, δ_H 5.72) in **1**. The location of the hydroxy group was determined by the HMBC correlations of H-7 to C-1 and C-5 and of H_2-12 to C-7. The relative configuration of **1** was consistent with that of daphniphylline[7] on the basis of the ROESY spectrum, as shown in Figure 1B. The ROESY cross-peaks for H_3-20/H-3b and H-3b/H-7 suggested that H-7 was β-oriented.

The ESIMS of daphnioldhanine I (**2**) showed the pseudomolecular ion peak at *m/z* 510 $[M + H]^+$, and the molecular formula was inferred as $C_{32}H_{47}NO_4$ by HRESIMS at *m/z* 510.3571 ($[M + H]^+$, $C_{32}H_{48}NO_4^+$, calcd 510.3583). IR absorptions implied the presence of two carbonyls (1771 and 1743 cm^{-1}) and an imino (1628 cm^{-1}) group. Its ^{13}C NMR spectrum in $CDCl_3$ (Table 1) displayed 29 carbon signals, including some partially broadened ones, which might be due to conformational exchange in the solvent.

To further elucidate its structure, **2** was treated with TFA and converted to its TFA salt (**2a**).[8a] The ^{13}C NMR spectrum of **2a** in $CDCl_3$ (Table 1) exhibited 32 resonances corresponding to eight quaternary carbons including three sp^2 (δ_C 213.3, 177.8, and 170.0) and five sp^3 (δ_C 85.8, 61.8, 58.1, 52.3, and 50.3) ones and seven sp^3 methine, 11 sp^3 methylene, and six sp^3 methyl (δ_C 24.5, 22.8, 22.0, 21.1, 19.4, and 17.9) carbons, suggesting that **2a** had a similar skeleton to that of daphnezomine N[8b] and daphnioldhanine E.[7] Inspection of the NMR data of **2a** revealed the presence of a carbon (δ_C 69.8, δ_H 4.61) bearing a nitrogen, an iminium carbon (δ_C 213.3), one lactone carbonyl (δ_C 177.8), and one *O*-acetyl group (δ_C170.0; δ_C 21.1, δ_H 2.10). The ^1H–^1H COSY spectrum indicated connectivities of partial structures **a** (C-2 to C-4 and C-18, and C-18 to C-19 and C-20), **b** (C-6 to C-7 and C-12, and C-11 to C-12), **c** (C-15 to C-17 and C-9), **d** (C-13 to C-14), and **e** (C-26 to C-28). The overall connectivity of the five units (**a**–**e**) was based on HMBC correlations, as shown in Figure 2A. The location of the iminium moiety at C-1 was further indicated by HMBC cross-peaks for H-2 to C-1 and H-9 to C-1. Thus, the planar structure of **2a**, the TFA salt of **2**, was unambiguously established. Its relative configuration deduced by the ROESY experiment was the same as that of daphnioldhanin E,[9] as illustrated in a computer-generated 3D drawing (Figure 2B). Thus, the structure of **2** was concluded to be the iminium (C-1 and N-1) form of daphnioldhanine E.[9]

The ESIMS of daphnioldhanine J (**3**) showed the pseudomolecular ion peak at *m/z* 358 $[M + H]^+$, and its molecular formula

* To whom correspondence should be addressed. Tel: +86-871-5223263. Fax: +86-871-5219684. E-mail: haoxj@mail.kib.ac.cn.
† The Key Laboratory of Chemistry for Natural Products of Guizhou Province.
‡ Kunming Institute of Botany, Chinese Academy of Sciences.

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Table 1. ^{1}H NMR and ^{13}C NMR Data of **1**, **2**, and **2a**

	1[a]		**2**[a]		**2a**[b,c]	
no.	δ_H multi, *J* (Hz)	δ_C	δ_H multi, *J* (Hz)	δ_C	δ_H multi, *J* (Hz)	δ_C
1	3.62 (d, 4.4)	65.4		*c*		213.3
2	1.75 (m)	38.0	2.75 (brs)	57.0 (br)	2.87 (brs)	57.2
3a	1.54 (m)	26.3	1.95 (m)	42.9	1.82 (m)	43.9
3b	2.01 (m)		2.33 (m)		2.46 (brs)	
4a	2.24 (m)	40.6	1.24 (m)	36.4	1.33 (m)	36.1
4b	2.31 (m)		1.72 (m)		1.81 (m)	
5		38.9		*c*		58.1
6	1.93 (m)	46.2	2.15 (brs)	50.3	2.23 (brs)	50.3
7	5.72 (brs)	81.3	4.72 (brs)	69.3 (br)	4.61 (brs)	69.8
8		47.0		*c*		61.8
9	2.54 (t, 9.2)	53.6	1.85 (m)	52.3	1.91 (m)	52.4
10		77.5		52.1		52.3
11a	1.60 (m)	29.2	1.63 (m)	39.5	1.66 (m)	39.3
11b	2.10 (m)		2.05 (m)		2.09 (m)	
12a	2.32 (m)	31.4	1.70 (m)	22.9	1.75 (m)	22.8
12b			1.86 (m)		1.92 (m)	
13a	1.54 (m)	30.0	1.98 (m)	30.6	2.07 (m)	30.5
13b	2.66 (dd, 9.2, 2.8)		2.08 (m)			
14a	5.66 (dd, 10.4, 2.8)	73.0	1.66 (m)	24.2	1.67 (m)	24.1
14b			2.30 (m)		2.36 (m)	
15a	1.60 (m)	30.0	1.00 (m)	35.4	1.03 (m)	35.6
15b	2.60 (m)		2.10 (m)		2.10 (m)	
16a	1.46 (m)	25.3	1.75 (m)	25.7	1.79 (m)	25.6
					1.79 (m)	
16b	1.93 (m)					
17a	1.61 (m)	35.8	1.85 (m)	36.8	1.89 (m)	36.6
17b	2.03 (m)					
18	1.46 (m)	30.2	3.02 (brs)	27.5	2.61 (brs)	27.6
19	0.96 (d, 6.4)	21.2	1.01 (d, 6.4)	23.0	1.01 (d, 6.8)	22.8
20	1.13 (d, 6.0)	22.5	1.01 (d, 6.4)	19.8	1.04 (d, 6.8)	19.4
21	1.07 (s)	25.3	1.10 (s)	21.9	1.17 (s)	22.0
22		212.8	1.93 (t, 7.2)	56.1	1.96 (m)	56.0
23		51.0		50.0		50.3
24	0.91 (s)	19.0	1.28 (s)	18.0	1.29 (s)	17.9
25a	4.49 (brd, 13.6)	65.5		176.5		177.8
25b	3.75 (brd, 13.6)					
26	4.57 (d, 6.4)	82.7	4.94 (d, 5.2)	70.3	4.95 (d, 5.2)	70.5
27a	1.98 (m)	24.8	1.86 (m)	25.3	1.89 (m)	25.2
27b	2.00 (m)		2.02 (m)		2.04 (m)	
28a	1.93 (m)	34.0	1.42 (m)	25.8	1.44 (m)	25.6
28b	2.08 (m)		1.62 (m)		1.67 (m)	
29		105.8		84.8		85.8
30	1.47 (s)	24.3	1.53 (s)	24.6	1.55 (s)	24.5
31		170.5		169.2		170.0
32	2.12 (s)	21.2	2.06 (s)	21.1	2.10 (s)	21.1

[a] ^{1}H, ^{13}C NMR data measured at 400 and 100 MHz, respectively, in $CDCl_3$. [b] ^{1}H, ^{13}C NMR data measured at 500 and 125 MHz, respectively, in $CDCl_3$ (containing 1% TFA, v/v). [c] Not observed.

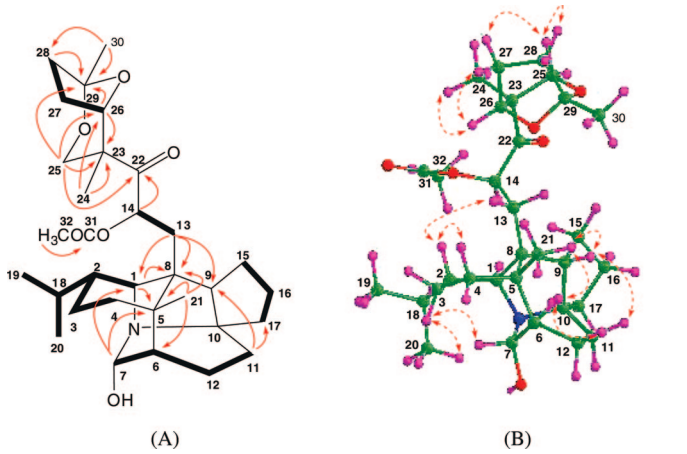


Figure 1. (A) ^{1}H–^{1}H COSY (—) and key HMBC (arrow) correlations of **1**; (B) key ROESY (dashed arrow) correlations of **1**.

was established as $C_{22}H_{31}NO_3$ by HRESIMS at *m/z* 358.2376 ([M + H], $C_{22}H_{32}NO_3^+$, calcd 358.2382), indicating eight degrees of unsaturation. An IR absorption band at 1730 cm^{-1} indicated the presence of a carbonyl group. Besides two carbonyl signals (δ_C 210.9 and 171.2) revealed by the ^{13}C NMR data (Table 3), the remaining 20 carbon signals were ascribed to two sp^3 quaternary, seven sp^3 methine, nine sp^3 methylene, and two methyl carbons. Since the carbonyls accounted for two degrees of unsaturation, the

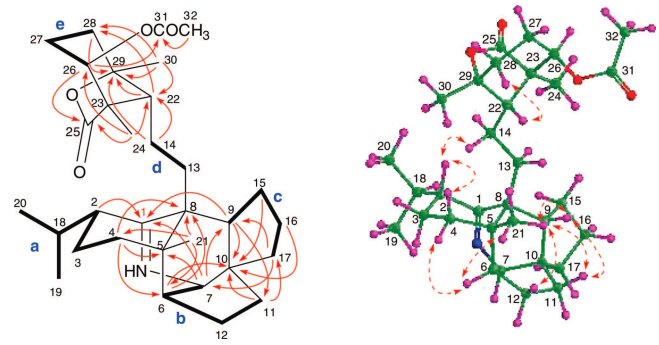


Figure 2. (A) ^{1}H–^{1}H COSY (—) and key HMBC (arrow) correlations of **2a**; (B) key ROESY (dashed arrow) correlations of **2a**.

Table 2. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 HMz) Data of **3** in $CDCl_3$

no.	δ_H multi, *J* (Hz)	δ_C	no.	δ_H multi, *J* (Hz)	
1	3.18 (m)	74.5	12	2.64 (m)	171.2
2	2.68 (m)	48.3	13a	1.66 (m)	53.8
			13b	1.92 (m)	
3	1.56 (m)	22.4	14	2.74 (m)	26.3
4	2.11 (m)	34.1	15	1.88 (m)	26.5
5		84.4	16	2.35 (m)	30.8
6	2.42 (m)	42.6	17	1.71 (m)	33.7
7	3.77 (m)	58.8	18	2.36 (m)	35.2
8		48.8	19	2.50 (m)	59.4
9	2.18 (m)	31.7	20	1.03 (d, 6.5)	14.3
10	2.68 (m)	50.3	21	1.54 (s)	22.8
11		210.9	22		171.2

remaining six degrees of unsaturation suggested that **3** had a hexacyclic backbone.

Comparison of the NMR data of **3** (Table 3) with those of the known alkaloid deoxyisocalyciphylline B,[10] which was also obtained from the leaves of *D. oldhami*, showed that the chemical shifts were similar, except those of the E and F rings. Analysis of 2D NMR data (HSQC, ^{1}H–^{1}H COSY, and HMBC) revealed the planar structure of **3**. The ketone carbonyl was assigned to C-11 (δ_C 210.9) by the HMBC cross-peaks of H_2-12 and H-10 to C-11. The correlations of H-9 with C-6 and C-7 and of H_2-15 with C-7 and C-10 established the connection of C-7, C-15, and C-10 via C-9, which confirmed the planar structure of **3**. The relative configuration of **3** was similar to that of deoxyisocalyciphylline B[8] by the ROESY spectrum. ROESY cross-peaks for H_3-21/H-1, H_3-21/H-6, H_3-21/H-13a, H-1/H-18, H-1/H-2, H-6/H-10, and H-10/H-9 indicated that H_3-21, H-6, H-1, H-18, H-2, H-13a, H-10, and H-9 were α-oriented. H-7 was *β*-oriented by the correlations of H_3-20/H_2-3 and H_2-3/H-7. The aforementioned ROESY correlations also indicated that the A, B, C, D, E, and F rings in **3** had the chair, envelope, envelope, boat, boat, and envelope conformations,respectively.

The ESIMS of daphnioldhanine K (**4**) showed the pseudomolecular ion peak at *m/z* 388 $[M + H]^+$. Its molecular formula was determined to be $C_{23}H_{33}NO_4$ by HRESIMS at *m/z* 388.2492 ($[M + H]^+$, $C_{23}H_{34}NO_4^+$, calcd 388.2487), which was smaller than that of daphnilongerine by a CH_2 unit. The IR spectrum was indicative of the presence of one hydroxy (3435 cm^{-1}) and two carbonyls (1733 and 1708 cm^{-1}). ^{13}C NMR and DEPT data (Table 3) indicated 23 carbon signals including two carbonyl, one tetrasubstituted double bond, two quaternary, five methine, nine methylene, and three methyl carbons. ^{1}H and ^{13}C NMR data of **4** (Table 3) were similar to those of daphnilongerine,[11] except for several different chemical shifts at C-18 (δ_C 35.5, δ_H 2.57 and 2.51), C-19 (δ_C 8.4, δ_H 1.02), and C-20. This revealed that **4** was a daphnilongerine-type *Daphniphyllum* alkaloid,[11] which has an ethyl group connected to C-2 instead of a isopropyl group at C-2 in daphnilongerine.[11] The structure of **4** was further confirmed by 2D NMR spectra (^{1}H–^{1}H COSY, HSQC, and HMBC). The relative configuration of **4** was similar to that of daphnilongerine by the ROESY spectrum.[11] Thus, the structure of daphnioldhanine K was assigned as **4**, which was the second daphnilongerine-type *Daphniphyllum* alkaloid reported.

The known dehydrodaphnigraciline (**5**) was first obtained by the dehydration of daphnigraciline with Ac_2O−AcOH (1:1) in 1975,[5,6] and only limited NMR data were reported. This is now reported as a natural product from *D. oldhami*, and its full ^{1}H and ^{13} C NMR assignments (Table 3) are reported through 2D NMR techniques.

Table 3. ^{1}H NMR and ^{13}C NMR Data of **4**, Daphnilongerine, and **5**

	4[a]		daphnilongerine[a]		**5**[b]	
no.	δ_H multi, *J* (Hz)	δ_C	δ_H multi, *J* (Hz)	δ_C	δ_H multi, *J* (Hz)	δ_C
1a	2.62 (d, 11.6)	60.0	2.62 (brd, 11.7)	60.0	2.36 (brd, 10.5)	62.1
1b	2.05 (d, 11.6)		2.08 (brd, 11.7)		2,10 (brd, 10.5)	
2		214.6		218.1		156.0
3	3.09 (dd, 9.2, 5.6)	46.2	3.25 (dd, 9.2, 5.4)	44.8	4.32 (brd, 5.0)	92.0
4a	1.50 (dd, 13.2, 9.2)	33.7	1.51 (dd, 9.2, 13.4)	34.1	2.22 (m)	27.3
4b	2.23 (m)		2.21 (m)		1.80 (m)	
5		51.4		51.6		36.6
6	1.96 (m)	42.1	2.04 (m)	42.3	1.97 (m)	34.3
7a	2.90 (m)	72.2	2.87 (m)	71.9	2.67 (brd, 12.0)	56.4
7b					2.49 (dd, 12.0, 4.5)	
8		47.6		47.6		46.4
9		145.5		145.6		145.8
10		138.9		138.9		133.7
11a	2.19 (m)	26.4	2.24 (m)	26.4	2.22 (m)	27.2
11b	1.90 (m)		1.93 (m)		1.60 (m)	
12a	2.22 (m)	25.9	2.29 (m)	25.9	2.49 (m)	27.1
12b	1.71 (m)		1.74 (m)		1.83 (m)	
13a	1.63 (m)	39.8	1.65 (dd, 14.0, 8.7)	39.8	1.60 (dd, 14.5, 9.0)	39.9
13b	2.44 (m)		2.45 (dd, 14.0, 6.9)		2.40 (dd, 14.5, 2.5)	
14	2.94 (m)	42.2	2.97 (m)	42.3	2.86 (m)	42.6
15	3.51 (m)	53.6	3.53 (m)	53.7	3.44 (brs)	54.9
16a	1.91 (m)	29.3	1.95 (m)	29.3	1.86 (m)	28.1
16b	1.29 (m)		1.32 (m)		1.26 (m)	
17a	2.56 (m)	41.6	2.59 (m)	41.6	2.33 (m)	42.6
17b	2.31 (m)		2.35 (m)		2.62 (m)	
18a	2.57 (m)	35.5	2.86 (m)	40.9	1.99 (m)	26.6
18b	2.51 (m)					
19	1.02 (t, 7.3)	8.4	1.09 (d, 7.0)	19.4	1.02 (t, 7.5)	11.6
21a	3.85 (d, 11.5)	65.6	3.85 (brd, 11.5)	65.7	4.46 (dd, 11.5, 2.5)	69.6
21b	3.59 (d, 11.5)		3.59 (brd, 11.5)		3.96 (brd, 11.5)	
22		177.5		177.5		175.5
23	3.61 (s)	51.8	3.62 (s)	51.8	3.62 (s)	51.0
24	2.26 (s)	43.0	2.27 (s)	43.1	2.17 (s)	46.6

[a] ^{1}H, ^{13}C NMR data measured at 400 and 100 MHz, respectively, in $CDCl_3$. [b] ^{1}H, ^{13}C NMR data measured at 500 and 125 MHz, respectively, in $CDCl_3$.

Table 4. Alkaloids Isolated from the Leaves, Roots, and Fruit of *Daphniphyllum oldhami*

alkaloidal type	alkaloid name	plant material	ref
yuzurimine-type	daphnioldhanins A, B	leaves	18
	yuzurimine E	leaves	19
	deoxyyurimine	leaves	13
	macrodaphniphyllidine	leaves	13
	yuzurimine B	leaves, fruit	13, 19
daphnilactone B-type	daphnioldhanin C	leaves	18
	daphnilactone B	leaves	13, 20
	zwitterionic alkaloid	leaves	13, 21
	isodaphnilactone B	fruit	20, 22
calyciphylline B-type	daphnioldhanine J	leaves	
	deoxycalyciphylline B	leaves, roots, fruit	8
	deoxyisocalyciphylline B	leaves, roots, fruit	8
	calyciphylline B	leaves	14
secodaphnane-type	methyl homosecodaphniphyllate	leaves	13, 23
	secodaphniphylline	roots	24, 25
	daphnioldhanins D−G	roots	10
	daphnioldhanine I	roots	
daphnane-type	daphnioldhanine H	leaves	
	daphnilongeranin D	leaves	26
	daphnimacropine	leaves	27
	daphmacropodine	roots	13, 29
	codaphniphylline	roots	8, 13
	daphmacrine	roots	13, 29, 31
daphmanidin A-type	daphmanidin A	leaves, roots	16
daphnilongerine-type	daphnioldhanin K	fruit	
yuzurine-type	dehydrodaphnigraciline	fruit	5, 6
	daphnigraciline	fruit	6, 13
	yuzurine	fruit	13
quinolizidine-type	matrine	leaves, roots	31
	sophocarpine	leaves, roots	31
	anagyrine	leaves	32
	camoensine	roots	32
	oxymatrine	roots, fruit	31, 33
	pxysophocaroine	roots, fruit	31, 33

Besides the above four new alkaloids, the other 34 isolated (Table 4) were identified by comparison of physical data with those reported.[6,8,10,13,14,16,18–33]

As shown in Table 4, 38 alkaloids were isolated and identified from the leaves, roots, and fruit of *D. oldhami*, which involved eight different types of *Daphniphyllum* alkaloids (daphnane-type,[13] secodaphnane-type,[13] yuzurimine-type,[13] daphnilactone B-type,[13] yuzurine-type,[13] daphnilongerine-type,[11] calyciphylline B-type,[14] and daphmanidin A-type[15]) and two types of quinolizidine alkaloids (matrine-type[16] and lupine-type[17]). The alkaloids found in the leaves of *D. oldhami* are more diverse than those in the other two plant parts. The proposed biogenetic pathways of these alkaloids are closely related (Scheme 1). Remarkably, quinolizidine alkaloids have been reported for the first time from the genus *Daphniphyllum*, and their biogenetic pathways are quite different from those of the *Daphniphyllum* alkaloids: the former originate from an amino acid,[4] while the latter are generated from six molecules of mevalonic acid via a squalene-like intermediate (Scheme 1).[3] The fact that two entirely different kinds of alkaloids exist in the same species presents a significant argument for further investigation of the phytotaxonomy of the genus *Daphniphyllum*.

The effects against platelet aggregation were evaluated for compounds **1**, **3**, and **5**. Compound **3** at 1.0 mmol/L showed stronger inhibition activity (inhibition [%]: 71.6 ± 16.4 for **3** and 44.9 ± 5.0 for aspirin at 2.4 mmol/L; $n = 5$, $X^- \pm$ SD) in vitro platelet aggregation induced by PAF, and compounds **1** and **5** at 1.0 mmol/L showed negative inhibition activity (inhibition [%]: 10.9 ± 9.3 for **1**, 13.4 ± 8.4 for **5**, and 44.9 ± 5.0 for aspirin at 2.4 mmol/L; $n = 5$, $X^- \pm$ SD).

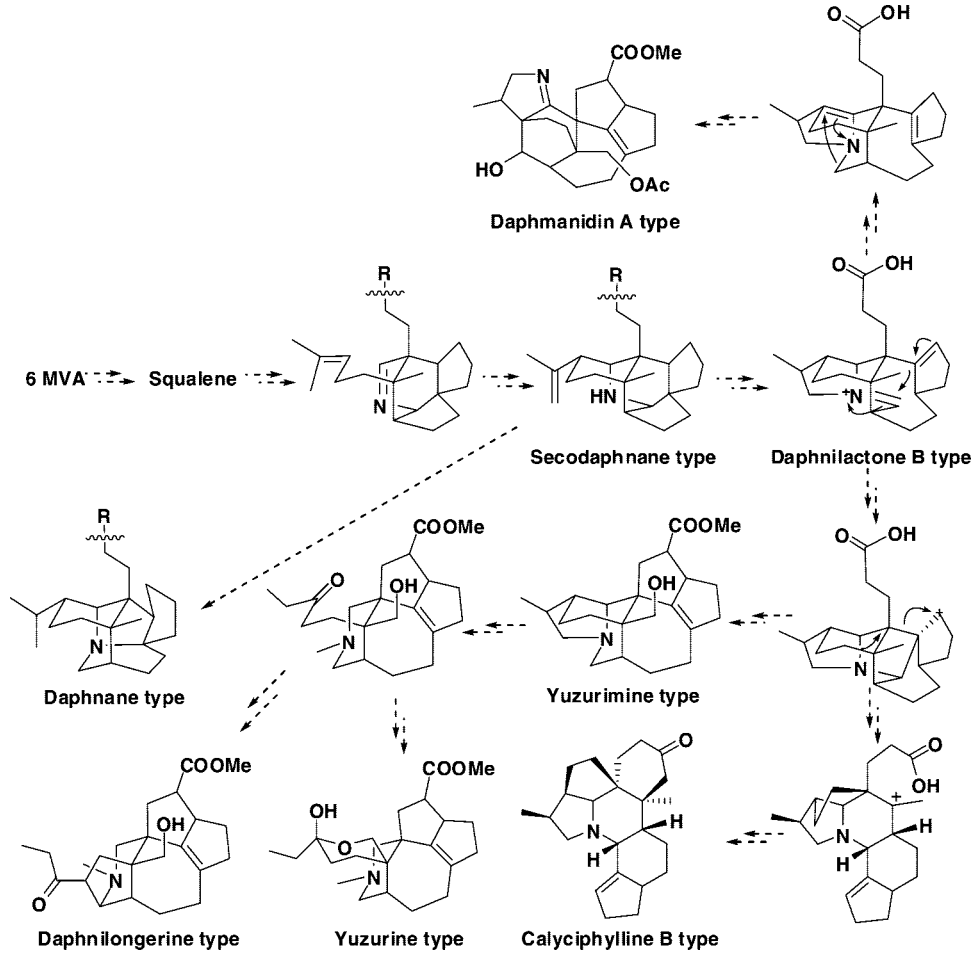
Experimental Section

General Experimental Procedures. Optical rotations were measured on a Horiba SEPA-300 high sensitive polarimeter. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. NMR spectra were obtained on a Bruker AM-400 or DRX-500 NMR spectrometer with TMS as internal standard. ESIMS were measured on a Waters 2695 HPLC-Thermo Finnigan LCQ Advantage ion trap mass spectrometer. HRESIMS was measured by a VG Auto Spec 3000 spectrometer. Column chromatography was carried out on Si gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, People's Republic of China), Si gel H (10–40 μm, Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China), amino Si gel (75–100 μm, Fuji Silysia Chemical LTD, Japan), and Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden). TLC was performed with glass precoated Si gel GF_{254} (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China) plates. Solvents used for extraction and isolation were distilled prior to use.

Plant Material. The leaves, roots, and fruit of *Daphniphyllum oldhami* were collected from Jinping County of Guizhou Province, People's Republic of China, in September 2006, and the plant sample was identified by Prof. Xun Chen of Guizhou Academy of Sciences. A voucher specimen (GY 05032602) was deposited at the Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences.

Extraction and Isolation. The Isolation of Alkaloids 1 and 3 and Known Alkaloids from the Leaves of *D. oldhami.* The powdered fresh leaves of *D. oldhami* (70.0 kg) were percolated with 75% EtOH three times (each time for 3 h) to give a crude extract. After removal of the solvent under reduced pressure, the extract was suspended in 5.0 L of acidic water (adjusted with 3% tartaric acid to about pH 2–3). The acidified suspension was immediately partitioned with EtOAc (4 × 5.0 L) to remove the nonalkaloids. The acidic aqueous phase was adjusted with saturated Na_2CO_3 to pH 10 and partitioned with $CHCl_3$ (4 × 5.0 L) to give the crude alkaloids (101.0 g). The crude alkaloids were subjected to an amino Si gel column chromatography eluted with $CHCl_3$−MeOH (1:0 to 0:1) to give five major fractions (Fr.$\mathbf{L_1}$–Fr.$\mathbf{L_5}$). Fr.$\mathbf{L_2}$ was further separated by column chromatography with amino Si gel and eluted with $CHCl_3$−MeOH (40:1) to yield the alkaloids daphnioldhanin B (15 mg), yuzurimine E (22

Scheme 1. Possible Biogenetic Pathways for Eight Types of *Daphniphyllum* Alkaloids from the Leaves, Roots, and Fruit of *D. oldhami*



mg), deoxycalyciphylline B (30 mg), deoxyyurimine (15 mg), and macrodaphniphyllidine (17 mg), consecutively. Repeated column chromatography of Fr.$\mathbf{L_3}$ over Si gel ($CHCl_3$−MeOH, 30:1; petroleum ether−Et_2NH, 40:1; and petroleum ether−actone, 2:1) gave alkaloids **1** (5 mg), calyciphylline B (5 mg), matrine (15 mg), sophocarpine (18 mg), yuzurimine B (14 mg), daphnilongeranin D (8 mg), and daphnimacropine (5 mg). Fr.$\mathbf{L_4}$ was subjected to column chromatography on Si gel (petroleum ether−Et_2NH, 20:1) and Sephadex LH-20 ($CHCl_3$−CH_3OH, 1:1) to obtain daphnioldhanin A (15 mg), deoxyisocalyciphylline B (15 mg), and daphnilactone B (8 mg). Fr.$\mathbf{L_5}$ was also separated by repeated column chromatography on Si gel (petroleum ether−Et_2NH, 20:1; $CHCl_3$−CH_3OH, 15:1), and Sephadex LH-20 (CH_3OH) to afford daphnioldhanin C (8 mg), daphmanidin A (10 mg), **3** (5 mg), zwitterionic alkaloid (150 mg), anagyrine (10 mg), and methyl homosecodaphniphyllate (8 mg).

Isolation of Alkaloid 2 and Known Alkaloids from the Roots of *D. oldhami*. The powdered fresh roots of *D. oldhami* (20.0 kg) were subjected to the above isolation procedure to obtain the crude alkaloids (18.6 g). The crude alkaloids were divided into four major fractions (Fr.$\mathbf{R_1}$–Fr.$\mathbf{R_4}$) by column chromatography on amino Si gel eluted with $CHCl_3$−MeOH (1:0 to 0:1). Fr.$\mathbf{R_2}$ was then chromatographed on a Si gel column ($CHCl_3$−MeOH, 40:1) to give alkaloids secodaphniphylline (100 mg), daphnioldhanin G (38 mg), daphnioldhanin D (18 mg), daphnioldhanin F (10 mg), and daphnioldhanin E (9 mg). Repeated column chromatography of Fr.$\mathbf{R_3}$ over Si gel ($CHCl_3$−MeOH, 30:1; and petroleum ether−Et_2NH, 30:1) afforded camoensine (9 mg), oxymatrine (8 mg), matrine (9 mg), daphmacrine (6 mg), codaphniphylline (10 mg), and daphmanidin A (11 mg). Fr.$\mathbf{R_4}$ was also separated and purified by repeated Si gel column chromatography with $CHCl_3$−MeOH (15:1) and petroleum ether−Et_2NH (10:1) to give daphmacropodine (58 mg), pxysophocaroine (8 mg), sophocaroine (7 mg), deoxyisocalyciphylline B (10 mg), deoxycalyciphylline B (10 mg), and **2** (5.7 mg).

Isolation of Alkaloids 4 and 5 and Known Alkaloids from the Fruit of *D. oldhami*. The powdered damp-dry fruit of *D. oldhami* (10.0 kg) were also subjected to the above isolation procedure to obtain the crude alkaloids (17.9 g). The crude alkaloids were chromatographed on an amino Si gel column eluted with $CHCl_3$−MeOH (1:0 to 0:1) to give four fractions (Fr.$\mathbf{F_1}$–Fr.$\mathbf{F_4}$). Fr.$\mathbf{F_2}$ was further separated using Sephadex LH-20 column chromatography ($CHCl_3$−CH_3OH, 1:1) and repeated column chromatography over Si gel with petroleum ether−Et_2NH (40:1) and petroleum ether−acetone (10:1) to obtain alkaloids **4** (15 mg), deoxycalyciphylline B (10 mg), and daphnigraciline (10 mg). Fr.$\mathbf{F_3}$ was subjected to repeated column chromatography over Si gel H with petroleum ether−Et_2NH (30:1) and purified by Sephadex LH-20 column chromatography using $CHCl_3$−MeOH (1:1) and MeOH, alternately, to yield alkaloids **5** (8 mg), pxysophocaroine (9 mg), yuzurine (9 mg), and oxymatrine (6 mg). Fr.$\mathbf{F_4}$ was separated and purified by repeated column chromatography on Si gel with $CHCl_3$−MeOH (15:1) and petroleum ether−Et_2NH (20:1), followed by Sephadex LH-20 column chromatography (MeOH), to afford isodaphnilactone B (5.3 mg), yuzurimine B (18 mg), and deoxyisocalyciphylline B (15 mg), consecutively.

Daphnioldhanine H (1): white powder; $[\alpha]^{23}_D$ +11.4 (*c* 0.34, $CHCl_3$); IR (KBr) ν_{max} 3415, 2924, 1739, 1718, and 1639 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESIMS (positive) *m/z* 544 $[M + H]^+$; HRESIMS (positive) *m/z* 544.3654 $[M + H]^+$ (calcd for $C_{32}H_{50}NO_6^+$, 544.3638).

Daphnioldhanine I (2): white powder; $[\alpha]^{21}_D$ −135.1 (*c* 0.29, $CHCl_3$); IR (KBr) ν_{max} 3426, 2932, 1771, 1743, and 1628 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESIMS (positive) *m/z* 510 [M +

H]$^+$; HRESIMS (positive) *m/z* 510.3571 [M + H]$^+$ (calcd for $C_{32}H_{48}NO_4^+$, 510.3583).

Daphnioldhanine J (3): white solid; $[\alpha]^{25}_D$ −5.6 (*c* 0.15, $CHCl_3$); IR (KBr) ν_{max} 3424, 2922, 1730, and 1639 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; ESIMS (positive) *m/z* 358 [M + H]$^+$; HRESIMS (positive) *m/z* 358.2376 [M + H]$^+$ (calcd for $C_{22}H_{32}NO_3^+$, 358.2382).

Daphnioldhanine K (4): white solid; $[\alpha]^{23}_D$ +32.2 (*c* 0.45, $CHCl_3$); IR (KBr) ν_{max} 3435, 2930, 1733, 1708, and 1640 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; ESIMS (positive) *m/z* 388 [M + H]$^+$; HRESIMS (positive) *m/z* 388.2492 [M + H]$^+$ (calcd for $C_{23}H_{34}NO_4^+$, 388.2487).

Dehydrodaphnigraciline (5): white solid; $[\alpha]^{26}_D$ −52.0 (*c* 0.62, $CHCl_3$); IR (KBr) ν_{max} 3439, 2930, 1737, 1679 and 1629 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; ESIMS (positive) *m/z* 372 [M + H]$^+$; HRESIMS (positive) *m/z* 372.2525 [M + H]$^+$ (calcd for $C_{23}H_{34}NO_3^+$, 372.2538).

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Supporting Information Available: The 1D and 2D NMR, HRESIMS, and IR spectra for **1**–**5** and **2a**, and figures of ^{1}H−^{1}H COSY, HMBC, and ROESY correlations for compounds **1**, **3**, **4**, and **5**. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Zhen M.; Min T. L. *Chinese Flora (Zhongguo Zhiwu Zhi)*; Science Press: Beijing, 1980; Vol 45 (1), pp 1–11..
(2) Chen, X.; Zhan, Z. J.; Yue, J. M. *Chem. Biodiversity* **2004**, *1*, 1513–1518.
(3) Heathcock, C. H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14323–14327.
(4) Golebiewski, W. M.; Spenser, I. D. *J. Am. Chem. Soc.* **1976**, *98*, 6726–6727.
(5) Yamamura, S.; Lamberton, J. A.; Irikawa, H.; Okumura, Y.; Toda, M.; Hirata, Y. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1836–1840.
(6) Yamamura, S.; Lamberton, J. A.; Irikawa, H.; Okumura, Y.; Hirata, Y. *Chem. Lett.* **1975**, 923–926.
(7) Morita, H.; Yoshida, N.; Kobayashi, J. *Tetrahedron* **1999**, *55*, 12549–12556.
(8) (a) Di, Y. T.; He, H. P.; Li, C. S.; Tian, J. M.; Mu, S. Z.; Li, S. L.; Gao, S.; Hao, X. J. *J. Nat. Prod.* **2006**, *69*, 1745–1748. (b) Morita, H.; Kobayashi, J. *Tetrahedron* **2002**, *58*, 6637–6641.
(9) Mu, S. Z.; Yang, X. W.; Di, Y. T.; He, H. P.; Wang, Y.; Wang, Y. H.; Li, L.; Hao, X. J. *Chem. Biodiversity* **2007**, *4*, 129–138.
(10) Yang, S. P.; Yue, J. M. *J. Org. Chem.* **2003**, *68*, 7961–7966.
(11) Li, L.; He, H. P.; Di, Y. T.; Gao, S.; Hao, X. J. *Tetrahedron Lett.* **2006**, *47*, 6259–6262.
(12) Chen, X.; Zhan, Z. J.; Yue, J. M. *Helv. Chim. Acta* **2005**, *88*, 854–860.
(13) Kobayashi, J.; Morita, H. In *The Alkaloids*; Cordell, G. A., Ed.; Academic: New York, 2003; Vol*60*, p 165.
(14) Morita, H.; Kobayashi, J. *Org. Lett.* **2003**, *5*, 2895–2898.
(15) Kobayashi, J.; Ueno, S.; Morita, H. *J. Org. Chem.* **2002**, *67*, 6546–6549.
(16) Gonnella, N. C.; Chen, J. *Magn. Reson. Chem.* **1988**, *26*, 185–190.
(17) Mears, J. A.; Mabry, T. J. *Chemotaxonomy of Leguminosae*; Harborne, J. B. , Boulter, D., Turner, B. J., Eds.;Academic Press: London, 1971; pp 73–83..
(18) Mu, S. Z.; Wang, Y.; He, H. P.; Yang, X. W.; Wang, Y. H.; Di, Y. T.; Lu, Y.; Chang, Y.; Hao, X. J. *J. Nat. Prod.* **2006**, *69*, 1065–1069.
(19) El Bitar, H.; Nguyen, V. H.; Gramain, A.; Sevenet, T.; Bodo, B. *J. Nat. Prod.* **2004**, *67*, 1094–1099.
(20) Morita, H.; Yoshida, N.; Kobayashi, J. *Tetrahedron* **2000**, *56*, 2641–2646.
(21) Hao, X. J.; Zhou, J.; Node, M.; Fuji, K. *Yunnan Zhiwu Yanjiu* **1993**, *15*, 203–207.
(22) Yamamura, S.; Terada, Y. *Chem. Lett.* **1976**, 1381–1382.
(23) Heathcock, C. H.; Hansen, M. M.; Ruggeri, R. B.; Kath, J. C. *J. Org. Chem.* **1992**, *57*, 2544–2553.
(24) Sasaki, K.; Hirata, Y. *J. Chem. Soc. B: Phys. Org.* **1971**, 1565–1568.
(25) Toda, M.; Hirata, Y.; Yamamura, S. *Tetrahedron* **1972**, *28*, 1477–1484.
(26) Yang, S. P.; Zhang, H.; Zhang, C. R. *J. Nat. Prod* **2006**, *69*, 79–82.
(27) Kamijo, N.; Nakano, T.; Terao, Y.; Osaki, K. *Tetrahedron Lett.* **1966**, *25*, 2889–2892.
(28) Nakano, T.; Hasegawa, M.; Saeki, Y. *J. Org. Chem.* **1973**, *38*, 2404–2405.
(29) Nakano, T.; Saeki, Y.; Gibbons, C. S.; Trotter, J. *Chem. Commun* **1968**, 600–601.
(30) Gibbons, C. S.; Trotter, J. *J. Chem. Soc. B: Phys. Org.* **1969**, 840–843.
(31) Qiao, L.; Huang, L. R.; Gao, C. Y.; Zhao, Y. Y.; Yang, X. B.; Zhang, L. H. *J. Beijing Med. Univ.* **1994**, *26*, 485–486.
(32) Peter, G. W.; Dalton, F. F. *Phytochemistry* **1982**, *21*, 215–218.
(33) Zhao, Y. Y.; Chong, Q. Y.; Liu, J. B.; Chen, Y. Y.; Lou, Z. C. *Nat. Prod. Res. Dev* **1994**, *6*, 10–13.

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